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EDITORIAL

Screening for diabetic retinopathy—is the use of artificial intelligence and cost-effective fundus imaging the answer?

Aravind R. Sosale[1]

Published online: 28 February 2019

Vision loss among people with diabetes is a major problem. While most know the importance of evaluating a patient for complications, retinopathy screening gets left out by most treating physicians for want of expertise and training needed.

IDF atlas of 2017 [1] estimates that India has 72.9 million people with diabetes. Twenty percent of 24.4 million IGT add up to this number each year and along with 42.2 million undiagnosed cases quoted in the Atlas, takes the total close to 120 million. This figure is likely to double by 2045 as per experts. Really an epidemic that can be called the tsunami of diabetes!

IDF Atlas of 2017 also goes on to say that diabetic retinopathy (DR) is the leading cause of blindness and 1 out of every 3 patients has DR, and 1 out of 10 go on to develop "vision threatening' DR. International Association for Prevention of Blindness (IAPB) estimates 45 million to have vision threatening DR. Meta-analysis published in diabetes care [2] mentions that 1 out of 39 blind people had blindness due to DR and 1 out of 52 visually impaired persons has impaired vision due to DR. Figures are truly "eye opening" and frightening!

In India, prevalence of DR is close to 35.12% in people with diabetes after 5 years of duration of diabetes [3]. CINDI (chronic complications in newly diagnosed patients with type 2 diabetes in India) [4] published in 2014 reported that 6.1% of newly diagnosed type 2 patients have established DR at diagnosis. CINDI 2 in 2016 [5] looked at complications in newly detected T2 DM patients below the age of 40 years (young onset type 2 diabetes) and reported that 5.1% as the study population had established DR at diagnosis. These figures coming from national level survey data clearly tell us that India has a huge problem in terms of prevention of blindness due to DR.

Who should screen for DR?

With hardly 1000 trained retina vitreous surgeons and about 3000 trained medical retina specialists in India, it is an impossible task to screen and treat all patients with retinal problems. So, the onus of screening all patients for DR falls on us physicians who treat them. As most treating physicians do not work in a set-up that has an in-house ophthalmologist, referral is a standard practice. Referring our cases to an ophthalmologist is not solving the problem as close to 70% of the patients do not reach them. Patients' vision is normal as DR is asymptomatic till the last stages and the seriousness of going blind never really hits a patient. Taking off from work, fixing appointment, additional cost, and the issue of not able to drive back after dilatation of the pupil are some of the reasons why those referred rarely stick to their retinal exam schedule.

Most diabetes treating doctors are not trained in direct or indirect ophthalmoscopy, a skill that needs training but hardly given during college days. A very small number of trained ophthalmologists specialize in vitreous-retinal surgery and medical retina as sub-specialties in ophthalmology is growing by the day. Not all eye specialists can treat DR, a fact which is not known to many physicians.

Rationale for early detection of DR at physicians level

There is an urgent need to improve methods for screening for DR as majority of the patients do not have symptoms till advanced macular edema or vitreous hemorrhage occurs. The efficacy of laser photocoagulation and/or vascular endothelial growth factor (VEGF) inhibitors in preventing visual loss from PDR and DME is well established in randomized trials. Early detection through screening programs and appropriate referral for therapy are important to preserve vision in individuals with diabetes. Most importantly, detection of DR helps a physician to tighten glycemic control, hypertension

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control, and control of dyslipidemia; to ensure smoking cessation; and to look for early markers of diabetic nephropathy. Most patients do take their condition more seriously after knowing that the complication has started.

Methods of screening for DR available at present include direct and indirect ophthalmoscopic examination by a trained doctor. Some centers with in-house ophthalmology department will also use "high-end" fundus cameras for capture of images and these cameras are also used to perform fundus fluorescein angiography for further evaluation. Since a treating physician in every city pan-India will not be able to invest so much for evaluation of DR, usage of cost-effective fundus cameras and tele-ophthalmology is an option.

In tele-ophthalmology, fundus images are sent to reading centers via Internet and trained doctors read the images and grade them as no DR, mild NPDR, mod NPDR, and severe NPDR and PDR. Diabetic macular edema (DME) is also graded as per internationally accepted norms. Unfortunately, India does not have "dedicated" centers for tele-ophthalmology unlike other countries. Very few hospitals in India have specialized set-ups as they have many satellite centers to cater to. Most doctors who work in this field are not trained retinal specialists but do this as a spare job. Experience, doctor-fatigue, image quality, pixel size, reader screen type, and Internet issues are some of the reasons for poor-quality grading. Medical errors are the 3rd leading cause of death in the USA which reported an interview in NEJM Catalyst attributable to cognitive errors and not because of bad clinicians [6].

The kappa statistic is frequently used to test interrater reliability in the field of tele-ophthalmology. Many studies [7, 8] have been done to assess agreement among a group of ophthalmic care providers, including ophthalmologists and trained nonphysician personnel, in the interpretation of one-field, two-field, and even three-field digital fundus images for diabetic retinopathy screening. There is only fair agreement among all readers. Most studies conclude by saying "The agreement of the evaluation of retinopathy grades did not correlate with periods of experience as ophthalmologists. The intra-rater agreement of the evaluation of retinopathy grades was 60–70%. These results suggested that the reliability of the evaluation of retinopathy grades was not high among ophthalmologists." Even in some of our own studies (under publication), the Kappa score was only "fair" to "moderate" among five highly trained retina vitreous specialists while reading both two-field and three-field non-mydriatic and mydriatic images. Thus clearly, there is a need to look beyond tele-ophthalmology.

Era of artificial intelligence to give us diagnosis

Software algorithms that use artificial intelligence (AI) to aid in the diagnosis of retinal images are being developed worldwide [9]. Deep learning is considered as a fourth industrial revolution. It is based on learning features from the data. It processes large amount of data and extracts meaningful patterns from them. CNN (convolutional neural networks) algorithm teaches itself by analyzing a labeled training set of expert graded images and provides a diagnostic output.

Deep neural networks can detect subtle changes, patterns, or abnormalities that may be possibly at times be overlooked by human experts. The AI devices provide a screening decision without requiring an ophthalmologist to interpret the retinal images, hence can be used by physicians who may not normally be involved in eye care. Many AI softwares are available in the western world, but IDX is the only one to have the FDA approval in 2018. Most companies use cloud based "on-line" software support for making the diagnosis and hence need Internet support. India has the highest mobile usage in the world, but Internet still poses a problem. Medios is a Singapore-based company that has developed an AI-based solution and has partnered with Remidio Innovative Solutions Pvt. Ltd. that manufactures cost-effective, FDA-approved fundus camera aptly named "Remidio FOP (Fundus on phone). The Medios AI based software works "Offline" and does not need Internet. iPhone fitted on the back of the camera has inbuilt DR detection software and diagnosis is made in less than 20 seconds. PDF report shows Lesions as heat maps indicating presence of DR that needs "referral". AI also checks the quality of the images before making the diagnosis and poor-quality images are discarded to avoid false reporting.

How good are the cost-effective cameras and AI support is the question that comes to all our minds? Among many cost-effective cameras available in the Indian market, Remidio FOP is the only FDA-approved cost-effective camera. This camera has been compared with top end cameras like Topcon and Zeiss FF 450 cameras in separate studies [10, 11] and is found to have high sensitivity and specificity and has substantial agreement with conventional retinal photography. Moreover, studies conclude that "the rate of "ungradable" images was acceptably low and image quality was marginally better with Remidio FOP." This camera can be used as a "hand-held" device or can be mounted on a table top. Remidio FOP takes both mydriatic and non-mydriatic images of high quality. Most importantly any Lay person can be trained to take fundus images within a day.

Even though IDF recommends non-mydriatic fundus imaging for screening of DR, it is important for physicians to know that age of the patient, duration of diabetes, cataract, and pupil size can interfere with the quality of image in non-mydriatic fundus imaging. A single drop of tropicamide can be used to solve the problem. Chances of developing acute angle closure glaucoma are 0.006% as per most studies [12].

AI support for making the diagnosis with Remidio FOP has been tested in India extensively and many studies are ongoing. Fundus images of 301 patients with diabetes were run on the Medios Software at Dr. Mohan's Diabetes specialties Hospital at Chennai. The images were graded by an ophthalmologist

according to the International Diabetic Retinopathy Classification System. The ophthalmologist was blinded to the diagnosis of the AI. The AI reported the images as REFER (mod, severe NPDR, PDR, and DME) or NO REFER (mild NPDR or no DR). The diagnosis of the AI was compared to that of the ophthalmologist. Results presented at APTOS July 2018 conference at Singapore (under publication) showed sensitivity for referable DR (mod, severe NPDR and PDR) as 96.6% (95% CI 92.1–98.9) and for severe NPDR and PDR showed was 100% (95% CI 94.8–100). In another study done by Prof. S Natarajan and colleagues from Aditya Jyot Eye Hospital, Mumbai was presented at the 2018 RSSDI conference (under publication). DR screening at Mumbai Municipal Corporation Dispensary done by his group used the AI to make the diagnosis and sensitivity for any DR was 85.2% and for referable DR (mod, severe PDR and DME) was 100%.

Our own team at Diacon Hospital in association with Retina Institute of Karnataka has two large studies. First study took three images per eye (mydriatic) and compared the AI diagnosis with that of two senior retina vitreous surgeons. Total of six images per patient from 304 patients were analyzed and results are being presented at ATTD 2019 conference at Berlin during February. Sensitivity and specificity for referable DR (mod NPDR and more severe or the presence of DME) was 98.84 (95% CI 97.62–100%) and 86.73% (95% CI 82.87–90.59%). Sensitivity for sight threating DR (severe NPDR, PDR or the presence of DME) was 100%. Sensitivity and specificity for any DR (mild, mod NPDR and more severe) was 86.78% (95% CI 97.62–100%) and 95.45% (95% CI 82.87–90.59%). This study is under publication.

To our knowledge, the largest study from India using an offline AI (SMART STUDY) on a portable cost-effective FOP camera is being presented at ADA 2019 at San Francisco from our team. The results of AI diagnosis using non-mydriatic retinal images of 900 patients are compared to the diagnosis of 5 ophthalmologists (considered as ground truth).

The results from various studies above help us as doctors in adopting newer technology in clinical care. The aim is to use technology that shows consistent, reliable, and accurate results across multiple studies.

Conclusion

Deep learning and artificial intelligence are here to assist us in screening for DR in all our cases. The Remidio FOP is portable, easy to use, FDA-approved, and captures non-mydriatic good quality images comparable to the best of the fundus cameras. Medios AI software for DR screening works offline on the iPhone, gives immediate reports, highlights lesions (heat maps) and produces PDF reports. Studies show that offline AI algorithm by Medios can be used for screening for diabetic retinopathy in primary care. The sensitivity of the Medios AI with mydriatic images exceeds the FDA-mandated superiority end points. This can open new doors to make DR screening more accessible. Larger and multi-center studies will further validate the results of our efforts.

The use of AI promises to reduce cognitive work load for physicians and ophthalmologists thus improving care, diagnostic accuracy while improving clinical and operational efficiency.

Who should bell the cat? We all as treating physicians must take responsibility for screening for DR.

If we do not screen for DR, our patients are likely to go blind.

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SHORT ARTICLE

The Ahmedabad Declaration, 2018: the family and diabetes

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Abstract
The family is the basic social unit and should be taken as the basic unit of engagement and intervention in diabetes care as well. Family members not only contribute to diabetes care and support but are also prone to all effects arising from this. Shared genetic and environmental factors lead to a higher prevalence of diabetes in family members, and open a window of opportunity for diabetes prevention as well. The Ahmedabad Declaration of 2018, titled "The Family and Diabetes" has been released during the Annual Conference of the Research Society for Study of Diabetes India (RSSDI). It supports the theme of World Diabetes Day 2018–2019 and lends its voice towards strengthening the family in our quest for optimal diabetes control.

Keywords Community · Compassion fatigue · Diabetes · Diabetes self-management education (DSME) · Diabetes self-management support (DSMS) · Family · Society · Prevention

Introduction

The dictionary defines a family in various ways [1]. The noun family is a basic social group consisting of parents and their children. The word can be used as an adjective (diabetes is a family disease) and as an idiom (diabetes is common in women in the family way). The Institute for Patient- and Family-Centered Care (IPFCC) defines family members as two or more persons who are related in any way biologically, legally, or emotionally [2]. This allows a more inclusive concept of family, including nuclear, extended and kinship network members. It is also in concordance with Indian customs, where an entire village, community, caste, or tribe may be considered family.

If family is the basic social unit, it stands to reason that it should be considered the basic unit of intervention in a syndrome such as diabetes, which has multiple social ramifications.

The Indian family

The attitudes, wishes, and needs of family members of persons living with diabetes were analyzed in the multinational second Diabetes Attitude, Wishes and Needs (DAWN2) study [3]. The DAWN2 study documented that there was a significant impairment of psychological well-being, diabetes-related distress, and negative impact on the life of these family members.

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The frustration of family members in not being able to help their loved ones manage diabetes, and in dealing with hypoglycemia, was also observed. In spite of these challenge, most family members reported willingness to increase their involvement in diabetes care [4].

India was one of the 17 countries involved in DAWN2. Indians living with diabetes reported their family and "other people in the community," as being most supportive, as compared to peers from other countries. Indians with diabetes were the second most likely (after China) to have supportive friends, and third most likely (after China and Algeria) to encounter supportive people at work or school. This highlights the positive contribution of the family to diabetes care in India [5].

The Indian family does pay a high price for this contribution: the Indian family reported the third highest level of diabetes-related distress among all 17 countries (better only than Algeria and Turkey). Indian family members also reported a significant impact on their own leisure (rank 17/17), work or studies (17/17), physical health (15/17), emotional well-being (15/17), financial situation (11/17), and relationship with family/friends/nears (11/17). They were also the third most likely country to mention lack of any positive impact on their own health [5].

Yet, all these challenges did not prevent India from scoring the best on the chances of likely depression and third best on psychological well-being, among family members in all 17 DAWN2 countries. Another surprising effect was that Indian family members tended not to participate in formal diabetes education activities (rank 12/17). Even if they did do so, they rarely found these activities useful (17/17). Therefore, perhaps, they relied on other sources of knowledge and assistance (2/17) [5].

DAWN2 highlighted what has been termed "the Indian family paradox": though Indian family members had high levels of diabetes distress, they were able to maintain overall psychological well-being and were least likely to develop depression. Simultaneously, though they exhibited high levels of involvement in diabetes care, Indian family members were less likely to participate in formal educational activities and even less likely to find them useful. This, perhaps, prompts them to rely on other source of education, information, and support [5].

The family as a cause

The family acts as a cause of diabetes into two ways: through inheritance and through environmental factors. While type 2 diabetes is a multifactorial syndrome, a positive family history is noted in a significant proportion of seen in type 1 diabetes as well [6]. Monogenic forms of diabetes such as MODY (maturity onset diabetes of the young) are characterized by their strong family history [7]. Family history also informs the risk of developing specific complications, such as nephropathy and coronary artery disease, in diabetes.

Apart from the genetic aspect of causation, psychosocial and other environmental factors related to the family also play a role in etiopathogenesis of diabetes. Spouses of persons with diabetes have been documented to have a higher risk of developing the condition [8]. Shared dietary, physical activity, and stress-related and lifestyle-related characteristics may contribute to this.

The family as a victim

Though diabetes may be defined in terms of an individual's biomedical dysfunction, it impacts the life of the entire family. The management of diabetes may have to accept changes in their daily routine as well. Members of the family also have to shoulder responsibility for motivation and for care of the person with diabetes: this may lead to psychological stress, including compassion fatigue and burnout [9].

Diabetes-related expenditure can also affect the family economy and precipitate financial catastrophe [10]. Diversion of limited family funds, from "non-essential" items to diabetes care, may influence intra-family ties negatively. Family members who assist in tasks such as glucose monitoring and injection administration are prone to needle stick injuries and blood-borne disease [11].

The family as therapy

Family therapy is an accepted method of management in psychology. The same holds true for chronic diseases such as diabetes which has a strong psychosocial dimension [12]. The prevalence of diabetes in dysfunctional or broken families is documented to be high. Fostering of a strong and functional family unit can provide peace of mind and create a support system. This, in turn, facilitates the achievement of comprehensive biopsychosocial health while caring for persons with diabetes.

The family as support

The family acts as a great support in the management of diabetes (Table 1). A supportive family can join in diabetes education, assist in self-case and self-management activities, and encourage adherence to prescribed therapy. Multiple interventional trials have documented the benefit of family-based interventions often culture sensitized in improving diabetes-related outcomes [13–15].

Table 1 The role of family in diabetes care

- Active support and care
  - Educational support
    - Accessing knowledge and information
    - Filtering and minimizing hearsay
  - Culinary support
    - Ensuring diabetes-friendly diet at home
    - Avoiding 'culinary cruelty'
  - Biomedical support
    - Injecting insulin
    - Assisting in SMBG
- Health hazards for family members
  - Compassion fatigue
  - Burnout
  - Needle stick injuries
  - Financial burnout
- Health benefit for family members
  - Health literacy
  - Social/emotional skill enhancement
  - Self discipline
    - Diet
    - Physical activity
- Advocacy for diabetes care

The family as an opportunity

The family of a person with diabetes represents a high-risk group of people from the perspective of development of diabetes [16]. This implies that such family should be viewed as a target for prevention of diabetes. This should be viewed as an opportunity for good health rather than as a cause for dejection. Apart from managing the individual with diabetes, the health care system should take the opportunity to screen his or her family members for diabetes, inculcate healthy lifestyle habits, and start timely pharmacotherapy if indicated. Regular screening and educational intervention for family members should be ensured.

The family as an impediment

Many cultures view chronic "disease," such as type 1 diabetes, as a social stigma or "shame," and ostracize individuals who develop the condition [17]. Family "honor," viewed as worthiness and respectability of the family, is perceived to be dependent on the health of its members. It is not uncommon to encounter situations where marital prospects of siblings of children living with type 1 diabetes have been affected by their "dishonorable" family history. Lack of diabetes literacy in the community can thus become an impediment to diabetes care [14].

Limitations and caveats

One must always be respectful of individual preferences. Some persons may not wish to disclose their diabetes status to a few or all family members for a variety of reasons. Self-disclosure followed by family involvement should be encouraged but must not be forced, in such cases. Well-meaning, but ill-informed family members may share hearsay related to diabetes, which may lead to suboptimal health care seeking and acceptance. Family involvement, therefore, must be accompanied by proper medical education on an ongoing basis.

The family as the future

The diabetes epidemic is gradually converting to an endemic, which shows no signs of abating [18]. Some Indian states, such as Punjab, have a higher rate of diabetes than of impaired glucose tolerance [19], thus reporting a high-endemicity index. As health policymakers begin to understand the implications of this endemic, they will have to tailor their diabetes-care strategies to include more sustainable family- and community-based interventions. This will help ensure that optimal results are achieved in prevention and treatment of diabetes. The family, therefore, should be, and will be, the basic unit of intervention in diabetes care.

Resolution

Keeping this in mind at Ahmedabad, we, as clinicians, researchers, and public health specialists, involved in diabetes care, resolve to:

- Spread awareness about the importance of the family in achieving optimal diabetes care.
- Support individuals with diabetes to communicate their needs and concerns to their family members.
- Strengthen the family in its capacity to prevent and manage diabetes and its complications.
- Sensitize health care professionals to actively involve family member in diabetes care
- Suggest to health policymakers to proactively create programs for education, screening, and treatment of family members of persons with diabetes.
- Solicit support from allied professions, including family medicine, community medicine/public health, and mental health, in improving activity targeting family members of persons with diabetes in health promotion activities.
- Start social marketing campaigns to improve treatment/follow-up adherences and end social stigma or ostracization against persons with diabetes.

Action plan

To achieve this resolution, we propose the following action plan for all diabetes care providers:

At micro-level

- Encourage persons with diabetes to be accompanied by family members and involve them in clinical consultations
- Provide diabetes screening and preventative facilities to family members
- Offer educational and psychological support to family members

At meso-level

- Form peer support groups of family members living with diabetes
- Create formal, professionally reviewed, educational tools for family members
- Create platforms where family members can articulate their wishes, needs, and concern to diabetes care providers

At macro-level

- Consider a national or state level promotive health program focusing on family members of persons with diabetes
- Use family members as advocates of timely diabetes care
- Involve family members of persons with diabetes in creation of patient-oriented guidelines and recommendations

Summary

The Ahmedabad Declaration of 2018 is issued on the occasion of the Annual Conference of the Research Society for Study of Diabetes in India (RSSDI). The Ahmedabad Declaration supports the efforts of the International Diabetes Federation (IDF), World Diabetes Day 2018–2019 with the theme "The Family and Diabetes", over the next year, we hope that this Declaration will facilitate active involvement of the family in diabetes care, as well as encourage actionable focus on the health of these family members as well.

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REVIEW ARTICLE

The ongoing epidemic of diabetes mellitus in India: genetics or lifestyle?

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Abstract
India is estimated to have the second highest number of cases of diabetes mellitus in the world after China. Recent epidemiological evidence indicates that people of lower socioeconomic group in India are equally or even more susceptible to diabetes. Family history is a very strong risk factor for developing type 2 diabetes mellitus; the lifetime risk is nearly 40% for individuals who have one parent affected and approaches 70% if both parents are affected. Genome-wide association studies identified more than 50 genetic variants associated with type 2 diabetes mellitus, but these risk alleles identified to date could only explain less than 10% of the observed heritability. Acquisition of the same unhealthy lifestyle from the parents could be the major reason for the observed heritability that genetics could not explain. The global age-standardised prevalence of diabetes has nearly doubled since 1980, rising from 4.7 to 8.5% in the adult population. If genes are responsible for this doubling of prevalence, the responsible gene pool should also amplify to the same extent in the population. The Hardy–Weinberg law states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences, making genetics as the etiology for this ongoing epidemic less likely. Indians have a tendency to become metabolically obese and develop type 2 diabetes mellitus with normal weight; thus, body mass index cut-off for overweight and obesity is kept lower in Indians. Primary and secondary prevention strategies should be more emphasised at the community level. Physical activity recommended is at least 150 min/week. All adults should decrease the amount of time spent in daily sedentary behaviour. Dietary modifications by reducing carbohydrate intake and increasing the intake of proteins, green leafy vegetables, fruits, and nuts should be promoted.

Keywords Diabetes mellitus · Heritability of diabetes · Lifestyle · Thin fat Indian · Central obesity

Introduction

India is home for 69.2 million people living with diabetes mellitus (DM) and is estimated to have the second highest number of cases of DM in the world after China as per the 2015 data [1]. Though historically a disease of the affluent, recent epidemiological evidence indicates that people of lower socioeconomic group in India are equally or even more susceptible to type 2 diabetes mellitus (T2DM) [2]. DM represents one of the most serious threats to India's public health in the twenty-first century in terms of health and economic tolls, considering the projected increase in prevalence and increasing incidence at younger age. A targeted approach to prevention may be the best tool to curtail the growth of this diabetic epidemic.

Family history in type 2 diabetes mellitus

Family history is a very strong risk factor for developing T2DM; the lifetime risk is nearly 40% for individuals who have one parent with T2DM and approaches 70% if both parents are affected [3–5]. Concordance rate of T2DM in monozygotic twins is about 70% and in dizygotic twins is 20–30% [6]. Prospective studies have demonstrated that family history in a first-degree relative is associated with a twofold increase in the risk of future T2DM [7, 8].

Genetic basis

The strong familial association observed in T2DM is the reason to suggest a genetic basis for the aetiology. The ability to

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interrogate the entire genome was made possible by two key advances, the completion of the Human Genome project and the International HapMap project [9–12]. Genome-wide association studies (GWAS) have identified more than 50 genetic variants associated with T2DM to date, but these risk alleles could only explain less than 10% of the observed heritability of T2DM [13]. GWAS mostly identifies single-nucleotide polymorphisms (SNPs), but there can still be many more relatively uncommon variants associated with diabetes.

Epidemiological data on DM do not support a genetic basis for the current global epidemic. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980 [14]. The global prevalence (age-standardised) of diabetes has nearly doubled since 1980, rising from 4.7 to 8.5% in the adult population [14]. In other words, the prevalence of diabetes mellitus has doubled after one generation, i.e. over a span of 35 years. If genes are responsible for this doubling of prevalence, the prevalence of specific alleles responsible for T2DM should also double in the population. The Hardy–Weinberg law states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences. It is therefore unlikely that the gene pool responsible for diabetes doubles after one generation. A genetic basis has also been postulated for the higher prevalence of T2DM among South Asians when compared to Europeans. However, a recent systematic review that compared the risk alleles of SNPs that predispose to T2DM between South Asians and Europeans revealed no substantial difference to indicate that South Asians possess a greater genetic risk [15].

Explanation for the observed heritability

If genes could explain only less than 10% of the observed heritability, then what could be causing the familial preponderance of T2DM? The most plausible explanation is the acquisition of the same unhealthy diet and lifestyle from the parents. Eating behaviours evolve during the early years of life; children learn what, when, and how much to eat through direct experiences with food and by observing the eating behaviours of others. A child's relationship with food is set by the family, and he/she would mimic their diet and lifestyle practices. The child would also be exposed to the metabolic effects of the same from a very early age [16]. Many societies perceive larger infants as healthy and a sign of successful parenting. Therefore, feeding strategies in these societies are designed to increase children's food intake and promote weight gain. However, when these strategies persist in environments with abundance of food, they tend to promote unhealthy diets, accelerated weight gain, and obesity.

Epigenetics is another cause that may contribute to the observed heritability of T2DM. Environment can influence the expression of the genome by methylation of DNA, post-translational modification of histones, and activation of microRNAs. These modifications in DNA can be transmitted through the germline, producing a phenotypical change that is heritable. The unfavourable metabolic milieu as a consequence of bad diet and lifestyle can thus be transferred to the next generation by epigenetics [16]. A nested case-control study of DNA methylation among 25,372 participants in the London Life Sciences Prospective Population (LOLIPOP) found a 2.5 times higher T2DM incidence among Indian Asians than Europeans after 8-year follow-up, even after adjustment for adiposity, physical activity, family history of type 2 diabetes, and baseline glycemic measures. Using epigenome-wide association analyses, DNA methylation markers at five loci were found to be associated with future type 2 diabetes incidence, and methylation score was higher among Indian Asians [17]. This study provides new insights into the role of epigenetics as a risk factor for T2DM.

Thin-fat Indian concept

India has a lower obesity rate but a prevalence of metabolic abnormalities, such as hyperglycemia, low HDL-cholesterol, and elevated triacylglycerol, that are comparable to or even higher than western countries [14, 18–20]. In other words, Indians have a tendency to become metabolically obese even with normal weight. The anthropometric and biochemical profile of Indian diabetics are different from the western population. Indians have a smaller body size and thinner limbs, which is suggestive of smaller muscle mass. However, in spite of being thin, they are centrally obese (Fig. 1), with a higher waist-to-hip ratio (WHR) and tend to have higher visceral fat and profoundly higher rates of insulin resistance and metabolic syndrome than Europeans [21, 22]. This phenotype has been termed as thin-fat Indian. Mechanisms underlying this central obesity risk among South Asians remain unclear. Even though family studies have shown that central obesity is heritable in South Asians [23, 24], in a recent study among 10,318 South Asians, risk allele frequencies were not higher in South Asians compared to Europeans at known WHR loci, and genome-wide and exome-wide analyses showed no new associations between genetic variants and WHR. This study argues against an important contribution for genetic variants underlying the increased risk of central obesity in South Asians [25].

The bad metabolic profile among Indians is due to a relatively higher proportion of visceral fat. The metabolically inert superficial subcutaneous adipose tissue compartment in the lower extremities is less in Indians compared to Europeans. When energy excess induces obesity, the storage capacity of

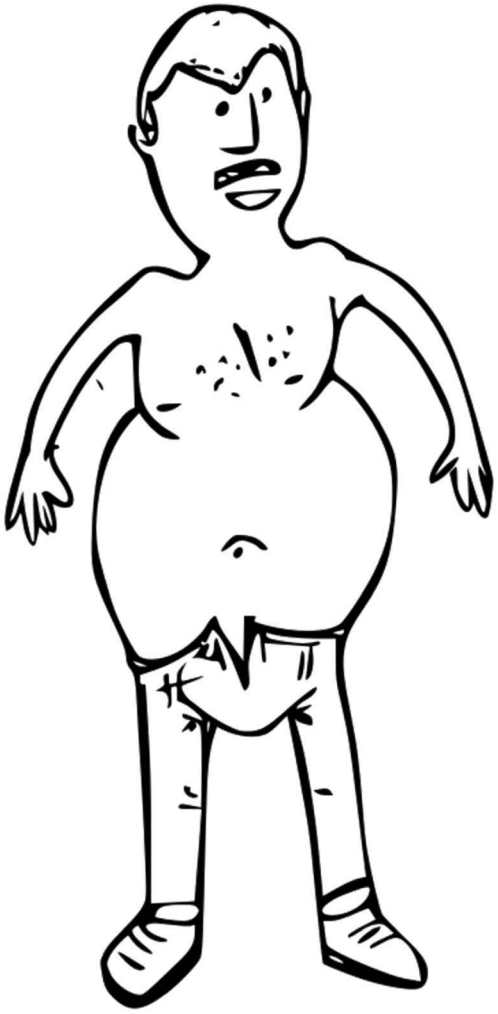

Fig. 1 An illustration of the 'thin-fat Indian' having central obesity with lower skeletal muscle mass and thin and short extremities

the superficial subcutaneous adipose tissue compartment is rapidly exhausted and fat accumulates in the visceral depots, which is metabolically harmful [26, 27]. Considering the above facts, body mass index (BMI) cut-off for overweight and obesity needs to be tailored for the Indian population. (Table 1). The World Health Organization (WHO) in 2004 recommended that for many Asians, for public health action, 23–27.4 kg/m^2 is overweight and 27.5 kg/m^2 or higher is obesity, as against the international cut-off of 25 and 30 kg/m^2, respectively [28]. Guidelines for obesity and overweight based on BMI for Asian Indians were relooked in 2008, and a consensus was developed through discussions by the Prevention and Management of Obesity and Metabolic Syndrome group. This consensus categorised overweight as a BMI of 23.0–24.9 kg/m^2, and obesity as a BMI ≥ 25 kg/m^2 [29].

Waist-hip ratio (WHR) is a simple measure of central obesity. It is the dimensionless ratio of the circumference of the waist to that of the hips [30]. Central obesity usually correlates well with the amount of the visceral fat [31]. Considering the bad metabolic consequences associated with central obesity, WHR would be a better method than BMI to assess cardiovascular and diabetes risk [32]. This is particularly true in Asian Indians due to high prevalence of central obesity with BMI being in normal range. Central obesity is defined as WHR above 0.90 for males and above 0.85 for females [30].

Diabetes in low body weight group (BMI < 18 kg/m2)

A study of 9873 patients with T2DM in India revealed that 3.5% were lean with a BMI < 18.5 kg/m^2 [33]. It was also highlighted that HbA1c, fasting and postprandial blood glucose levels, and microvascular complications were higher among those in the lean group [33]. The major pathophysiology in this group appears to be rapid beta cell failure as opposed to insulin resistance, as highlighted by the fact that they do not have central obesity, have a higher prevalence and early initiation of insulin use, and have biochemical parameters consistent with lower insulin resistance [34–37]. The pathogenesis for this type of T2DM is yet to be elucidated.

Environmental contaminants in the pathogenesis of type 2 diabetes mellitus

Environmental contaminants like pesticides might also play an important role in the pathogenesis of diabetes in India, especially in the rural population involved in agriculture. A meta-analysis of 13 studies revealed pesticide exposure as an increased risk of T2DM; odds ratio of 1.61 (95% CI 1.37–1.88) [38]. A recent study among 3080 people from rural India indicated a high prevalence of diabetes (18.3%) among the

Table 1 Body mass index cut-off for overweight and obesity

	International cut-off (kg/m^2)	South Asians (WHO* 2004) (kg/m^2)	Indian population (PMOMS** group 2008) (kg/m^2)
Over weight	25.0–29.9	23.0–27.4	23.0–24.9
Obesity	≥ 30.0	≥ 27.5	≥ 25.0

*World Health Organization

**Prevention and Management of Obesity and Metabolic Syndrome group

people directly exposed to organophosphate insecticides while it was threefold lesser (6.2%) among the indirectly exposed group. There was a strong association between plasma organophosphate residues and HbA1c levels in this study. Gut microbial degradation of organophosphate insecticides inducing glucose intolerance via gluconeogenesis was the possible mechanism suggested [39]. Many more contaminants in the environment and food can be involved in the pathogenesis of T2DM in India, but is yet to be investigated.

Urban-rural differences in India

National data show the prevalence of diabetes to be double in urban areas than that of rural areas. In the multicentric study conducted by Indian Council Of Medical Research during 2003–06, the prevalence of diabetes among men and women was 11.4 and 10.3% in urban areas and 6.2 and 5.7% in rural areas, respectively [40]. Interestingly, in the same study, the data from the state of Kerala showed a paradoxically higher prevalence of diabetes in rural than urban dwellers; 19% in rural men and 22% in rural women compared to 12% in urban men and 17% in urban women [41]. This contrasting data from Kerala may be due to the rising rural household food consumption mainly because of the effective public distribution system (PDS) [42]. Food items supplied through PDS at subsidised rates are predominantly cereals [43]. Pulses, vegetables, and fruits do not come under PDS. Also, over the past few decades, there is a huge shift from manual labour to physically less demanding office jobs among rural Keralites. As a consequence, their diet has carbohydrates in excess of their energy demands, but is deficient in pulses, animal proteins, fruits, and vegetables.

Microvascular complications among Indian diabetics

Increased incidence of diabetes at a younger age and increased longevity due to modern health care facilities will give an opportunity for a large number of diabetic individuals to develop various microvascular and macrovascular complications. It was observed that Indians with T2DM develop microvascular complications much faster, when compared to the western population [44]. Reasons that can be postulated for this rapid progression are:

- Late diagnosis of cases, as screening programmes to detect diabetes mellitus are in a nascent stage in India [45].
- Poor glycemic control in the initial years of diabetes due to irregular treatment and switching to alternative systems of medicine [46].

The UK Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT) have demonstrated that patients with intensive glycemic control in the initial years developed less microvascular complications on long-term follow-up [47, 48]. The lack of strict initial glycemic control may be one of the important factors responsible for faster progression to microvascular complications.

- Lack of incorporation of HbA1c testing in the routine diabetic care.

HbA1c is the gold standard test around the world for monitoring glycemic control, but the usual practice in India is to target fasting blood sugar (FBS) and postprandial blood sugar (PPBS). HbA1c can be grossly deranged even if FBS and PPBS are in control, as lunch or dinner is usually the largest meal among most Indians. Blood sugar surge following lunch and dinner are thus never addressed. In the Delhi Diabetes Community (DEDICOM) survey conducted among urban diabetics from middle- and high-income groups, only 13% of respondents had undergone HbA1c testing and only 21.7% had heard of HbA1c testing [49].

- Late initiation of Insulin therapy due to financial constraints, needle phobia, and fear of bad reputation among physicians [50].

Prevention strategies

Dietary modifications and increase in the physical activity are the two crucial components in preventing T2DM. Dietary modifications cannot simply be delivered by giving a patient a diet sheet in a one-size-fits-all approach. Dietary modifications that are too complex to follow or deviate grossly from the routine pattern followed for long years are difficult to adhere to; poor compliance will be the end result. It is more practical if one is allowed to continue the same dietary pattern if it is not grossly unbalanced, but simultaneously reducing excess carbohydrate intake in each meal and increasing intake of pulses, vegetables, and fruits. Many vegetable side dishes and curries used in India often feature potato and other tubers, which are carbohydrate-heavy, and hence should be addressed in the advised dietary modifications. Dividing the meals into frequent small meals is also helpful by preventing severe escalations of postprandial blood sugar. There is a popular belief that diet based on wheat is better than rice for diabetes. Even though food items made from wheat will produce early satiety [51], and have a glycemic index slightly less than rice [52], what ultimately matters is the amount of carbohydrate ingested. Meat and fish are good sources of protein, and there is no theoretical harm in regular intake, but a practical risk is

that one will tend to consume carbohydrate (cereals) when taste-enhancing non-vegetarian side dishes are used. All adults, particularly those with type 2 diabetes, should decrease the amount of time spent in daily sedentary behaviour. Prolonged sitting should be interrupted with bouts of light physical activity such as walking, leg extensions, or overhead arm stretches every 30 min [53]. Physical activity recommended is at least 150 min/week (30 min/day for 5 days a week). This is particularly important in populations at high risk and with prediabetes [53].

Diabetes awareness in India

Knowledge and awareness regarding diabetes are grossly inadequate in India. In the Chennai Urban Rural Epidemiology Study (CURES-9), only 22.2% of the whole population and 41.0% of the known diabetic subjects were aware that diabetes could be prevented. Knowledge of the role of obesity and physical inactivity in causing diabetes was very low, with only 11.9% of study subjects reporting these as risk factors for diabetes. Only 19.0% of the study population knew that diabetes could cause complications [54]. In the Indian Council of Medical Research India Diabetes Study (Phase I) conducted in representative samples of four geographical regions of India—Chandigarh, Tamil Nadu, Jharkhand, and Maharashtra, only 43.2% of the overall study population had heard about a condition called diabetes. Among the general and diabetic population who knew about diabetes, only 56.3 and 63.4%, respectively, were aware that diabetes could be prevented [55]. These studies underscore the need for conducting large-scale diabetes awareness and education programmes.

Does genomic profiling to assess type 2 diabetes risk improve health outcomes?

There is clear evidence available from diabetes prevention studies that prevention works equally better in individuals with heightened genetic risk and in individuals with no such risk, an argument to emphasise lifestyle modification as the means for all to prevent the development of diabetes, irrespective of the genetic risk status [56, 57]. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group discourages clinical use of genetic testing until further evidence supports improved clinical outcomes [58].

Conclusions

Lifestyle factors play a far greater role in the ongoing epidemic of diabetes when compared to genetic risk. This epidemic is occurring in a genetic background that has been relatively static for centuries, whereas lifestyle is undergoing rapid change. However, a potential gene environment interaction cannot be ruled out. Risk alleles identified to date through genome-wide association studies have been able to explain only less than 10% of the observed heritability. Acquisition of the same unhealthy lifestyle from parents could be the major reason for the observed heritability that genetics could not explain. High-calorie, low-activity lifestyle by India's growing middle class are the major causes for the current diabetic epidemic in India. More emphasis should be given for epidemiological studies and formulation and implementation of community level preventive strategies.

Author contribution All authors contributed equally to the work, participating in collection of the data and writing the manuscript and approving the final version of it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors. Informed consent was obtained from all individual participants included in the survey.

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REVIEW ARTICLE

A critical review of red lesion detection algorithms using fundus images

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Abstract
There are two types of clinical signs of diabetic retinopathy: red lesions and bright lesions. Red lesions which include microaneurysms are early signs of diabetic retinopathy. Microaneurysms are clinically important initial qualities of retinopathy. Their number increases with the severity of retinopathy. Hemorrhages are also signs of diabetic retinopathy which appear after microaneurysms. This work has concentrated on the review of the development of several techniques to automate the detection and classification of suggestive features of red lesions of first retinopathy progress.

Keywords Microaneurysms · Hemorrhages · Red lesions · Diabetic retinopathy

Introduction

Microaneurysms (MAs) and hemorrhages (HEs) are small red lesions which appear on retinal imaging as the early lesions in diabetic retinopathy (DR). Several image distractions such as pigmentation, uneven illumination, broken capillaries, and lighting variation make red lesion identification and segmentation a challenging problem. A wide variety of novel algorithms have been presented to address this specific research problem. The retinal image segmentation structure plays a vital role as a non-intrusive diagnostic tool in modern ophthalmology.

Main attributes of red lesions

The most common appearance of MAs is the small circular dark stain on the surface retina. In color fundus pictures, MAs have small drawings reddish-isolated patterns. MAs are hard to distinguish from noise and from typically low contrast of the picture because of pigmentation variation. For candidate MA extractions, the occurrence of MAs near thin vessels but not, in fact, on the vessels tends to mislead. The main characteristic point in the detection step of MAs is its diameter. MAs have also been characterized as Gaussian-like structures and also structures similar to a cliff in the space of radon of a local window. MAs appear usually in windows that contain other structures without layers having high pixel values and with a highly noisy condition. HEs can occur due to the leakage of weak capillaries or from ruptured microaneurysms found in any part of the retina. Varying in appearance, HEs have irregular boundaries and several shapes such as dot, flame, and blot with uneven or indistinct edges. The size of the red lesions is an important factor to be considered in the automated DR detection system for the decision-making process related to the severity of disease which is needed for timely treatment. The main challenge in the automated DR detection system is due to the infrequent occurrence of large HEs, their highly variable appearance, and shape modeling related to it. Red lesions are shown in Fig. 1.

The most commonly applied approach for red lesion detection is shown in Fig. 2 which includes the given sequence of operations.

Literature survey for the detection of red lesions

Three primary categories of red lesion detection are the image-based approaches, pixel-based approaches, and approaches based on lesions. Their main purposes are as follows:

1. Image-based approaches—detection of images with red lesions

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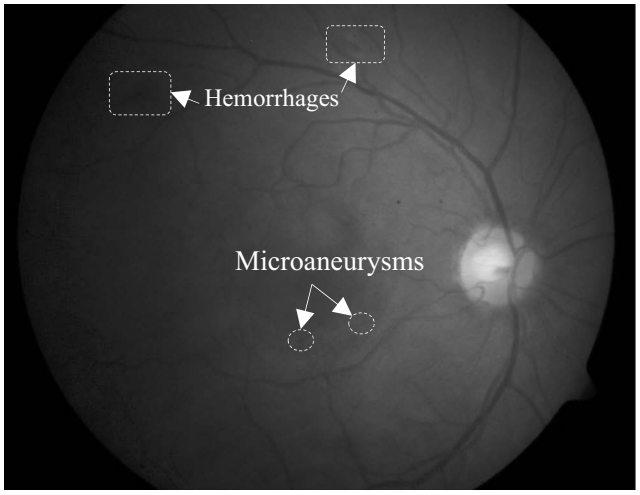

Fig. 1 A typical fundus image that contains microaneurysms and hemorrhages

2. Pixel-based approaches—aimed at finding the location of red lesions on the retina
3. Lesion-based approaches—concentrate on the extraction of candidate lesions and their counts

For the above-mentioned three categories, the related methods that were employed and suggested by different researchers are briefly elaborated as follows:

Mathematical morphology

Lay et al. [1] in 1983 introduced the first algorithm for the detection of MAs in fluorescent angiographies. In order to remove MAs but to preserve piecewise linear vessels, morphological opening with linear structuring elements using top hat transform in different directions was performed and then the difference image was computed to extract MA candidates. A similar approach of prefiltering was also applied by Oien et al. [2]. As a first approach of morphological operation, the hit-and-miss transform was applied for MA detection in color retinal images. Morphology with pixel classification was applied for the extraction of lesion candidates. The pixel classification using K-nearest neighbor (KNN) classifier required manually marking each image pixel as reference standard model followed by extraction of 68 features of each mark candidate to be further classified as lesions or non-lesions by Spencer et al. [3]. The study of Spencer and Frame proposed a method based on bilinear top-hat transformation. A matched filter along with rule-based classification as a more advanced image correction procedure in MA detection was used to discriminate linear and circular segments of MAs [4]. Bounding box closing-based morphological top-hat operation was presented, and any dark object with maximum diameter less than a given size was retained by Walter and Klein [5]. A lesion detection algorithm using multiscale morphological processing was proposed by Zhang and Fan et al. [6]. After that, vessels were removed through scale-based lesion validation. For the segmentation of retinal lesions, Karnowski et al. [7] adopted a morphological reconstruction method in which ground-truth data at a variety of scales was used to separate nuisance blobs from true lesions in the segmentation process. To separate the machine-segmented results into nuisance and actual lesion classes, ground-truth data was used to design post-processing classifiers.

A preprocessing module for shade correction and dynamic range normalization was proposed by Spencer et al. [3]. Matched filter approaches were given for the detection of MA candidates. The limitation of computational time required for the initial detection of red lesions based on a hybrid approach of morphology and pixel classification, which combines the prior works, by Spencer and Frame was overcome in the work of Balasubramanian et al. [8]. The proposed algorithm of Matei et al. [9] consists of sequential operations of linear filtering morphology and thresholding to identify DR signs as a presence of specific red lesions such as MAs and HEs. A neural network approach was described by Kamel et al. [10] for automatic detection of MAs in retinal angiograms. The advantage of this approach was that it is able to distinguish between MA and non-MA regions. Learning vector quantization classified it into their desired classes.

A computer-aided system proposed by Jaafar et al. [11] automatically identified red lesions from retinal fundus images. The separation of red lesions from their background and other artifacts results through image preprocessing and morphology operations. Morphological operation along with a classification step was the work related to Goldbaum et al. [12]. Top-hat morphological filtering followed by thresholding of extracted features was the basis of the algorithm proposed by Yang et al. [13]. A series of experiments consisting of the grayscale morphology method, complex active contour model, Naïve-Bayes classifier trained through appropriate feature set, and adaptive boosting approach were applied to result in HE candidates by Harangi and Hajdu [14].

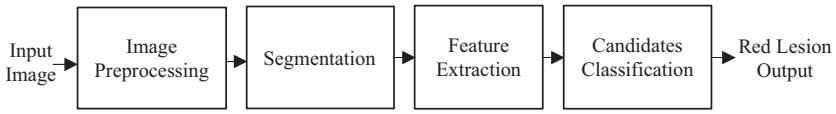

Fig. 2 General scheme for red lesion detection

Classification

An approach developed by Niemeijer et al. [15] introduced segmentation step based on pixel classification as hybrid methods. The number of false positives in the detection of red lesions such as MA and HE detection was possibly reduced by accurate thick-and-thin blood vessel extraction. The hybrid strategy combines the method of Spencer and Frame in the detection step for which there is no information about the size and shape of HEs. The contribution of this paper was to use pixel classification as a candidate detection system for red lesions. The second contributions have to add a large number of new features to those proposed by Spencer-Frame. To find the underlying candidate, region-growing algorithm was used and feature classification algorithm was used to separate MAs from other spurious objects [16]. Bayesian, Mahalanobis, and KNN type of statistical classifiers were tested for the detection of the salient features of the DR images by Ege et al. [17]. Out of these, the Mahalanobis classifier showed the best result in detecting red lesions. Kahai et al. [18] was described as a decisive facilities system of the early discovery of DR in terms of the presence of MAs. Three types of classification approaches namely Bayesian framework using likelihood ratio test, maximum a posteriori detector, and Bayes detector were proposed. The KNN method for candidate segmentation as well as classification criteria-based closing was employed by Walter [19]. The feature set based on radon space was derived by Giancardo et al. [20] without applying any preprocessing on retina images and without considering previous knowledge related to other structures of fundus images [20]. In summary, the method does not require vessel segmentation and it has strength through the machine learning approach which keeps the image preprocessing at a minimum while identifying MAs. A fully rule-based approach was exposed by Giancardo et al. [21] compressing radon space which considerably improved MA detection. Combined advantages of pixel-based classification and morphological-based detection were employed by Kande et al. [22]. To separate enhanced red lesion segments from the background, local entropy thresholding algorithm was described. After that, morphological top-hat transformation was used to suppress the vascular network, and then, to classify the red lesions from other dark regions, the support vector machine (SVM) classifier was applied.

For detecting MAs as primary signs of DR, color normalization and contrast enhancement as preprocessing steps on fundus images were proposed in the literature related to Osareh et al. [23]. Fuzzy c-means clustering algorithm was applied for image segmentation followed by classification of candidate regions into bright lesions and non-lesions. Li et al. [24] presented splat feature classification algorithm for HE detection. This specified that the classification stage results in all lesion candidates that are true positives with the lowest number of true-negative candidates. The main difficulty occurs when the color and gradient information are not enough for identifying true lesions. Niemeijer et al. [25] presented the supervised learning automated DR system, which is the further development of the red lesion detection system including MAs and HEs both with a newly added large set of features. The similar approach based on pixel classification and mathematical morphology was employed for automated microaneurysm detection by Kande et al. [26]. The technique by Usher et al. [27] presented the extraction of MAs using a similar neural network approach for pixel classification and feature analysis. In this technique, after preprocessing, recursive region growing and adaptive intensity thresholding with moat operator along with edge enhancement operator was adopted. Garcia et al. [28] presented an automatic method based on a set of features extracted from the properties of red lesions and used binary logistic regression to select the subset, which distinguished between MAs or HEs and the retinal background. For obtaining the final segmentation of red lesions, a multilayer perceptron (MLP) was subsequently used. The inadequacy of this proposed method was improved again by Garcia et al. [29] in which the set of features selected by using logistic regression and region-growing method. MLP, radial basis function (RBF), SVM, and these three neural networks combination based on majority voting scheme was employed for final segmentation of red lesions. The SVM classifier was used for the extraction of MAs in retinal fundus images by Wenhua et al. [30]. This method applied generalizes histogram algorithms for enhancement followed by removal of an object, which is too large to be a red lesion. This result was given as the input to the SVM for getting the final extraction of MAs. The rejection-based strategy was proposed by Ram et al. which progressively lowers the number of clutter responses, to formulate MA detection as a problem of target detection from clutter, where the probability of occurrence of the target is considerably smaller compared to the clutter [31]. Nagayoshi et al. [32] introduced a normalization process in addition to the Usher method which was further development of the algorithm proposed by Usher. Gardner et al. employed an artificial neural network to classify image regions as a vessel, HEs, or another lesion type. Feature evaluation on a large number basis followed by classification performed by heuristic rules was a basic algorithm described in the literature by Hipwell et al. To identify candidate lesions, classification based segmentation was proposed in the literature by Grisan et al. [33] using a higher-level entity which performs the collection of pixels with similar color and spatial location, i.e., splat.

Splat features derived were based on individual splat distribution in response to splat area, solidity, orientation, shape features, and extent by Li et al. [24] The fundus image consisting of an optic disc and blood vessels was detected through a novel minimum-intensity maximum-solidity

(MinIMaxS) algorithm. An optimal classifier, which ensures high specificity for bright lesion classification as well as high sensitivity for red lesion classification, was selected by comparing evaluation result of various feature-based classifiers such as SVM, KNN, AdaBoost, Gaussian mixture model (GMM), and its combination as classifiers [34]. Several features used in the red lesion detection scheme are given in Table 1.

Wavelet transformation

The solution for the detection of MAs was presented by Quellec et al. [35] by combining wavelet transformation with template matching followed by thresholding. Lesion template matching in the wavelet domain was proposed by Quellec et al. [35] using the sum of the squared errors as criteria on some decomposition sub-bands. The evaluation of results of this method was given on a manually segmented retinal image database for different mother wavelets.

Domain knowledge-based approaches

The density analysis approach was planned in the work of Hatanaka et al. [36] which pulls out the optic nerve head and detects hemorrhage candidates. This method was less sensitive as compared with the use of a morphological filter. To increase red lesion detection accuracy and to decrease the number of false positives for MA and HE extraction, accurate blood vessel location knowledge plays a vital role as described by Larsen et al. A procedure consisting of a series of six stages was offered by Hatanaka et al. [37] which included image digitalization first, then image normalization followed by optic nerve head extraction. Further, HE candidate extraction was included along

Table 1 Description of various features for red lesion detection

	Feature description
1	Area of the candidates
2	Half range area
3	Perimeter of the candidates
4	MAs candidate depth
5	MAs candidate boundary energy
6	Standard deviation—green, normalized blue, filter output, HSV, opening image, preprocessed image, RGB, HIS
7	Aspect ratio
8	Eccentricity
9	Variance—minor axis, major axis, red, green, saturation
10	Smoothed energy function peaks
11	Rotational inertia of normalized green
12	Compactness
13	Circularity
14	Length—major axis, minor axis, region
15	Solidity
16	Orientation
17	Distance—OD center, vascular region
18	Euler number
19	Diameter
20	Edge strength
21	Pixel intensity (minimum/maximum)—saturation, red, intensity, green, shade-corrected image, region-growing seed
22	Energy
23	Mean—green and normalized green, intensity, red and normalized red, minor and major axis cross section, filter output, energy, energy difference, neighbor pixels gradient value, HSV, shade-corrected image and normalized shade-corrected image, opening image, top-hat transform, preprocessed image, circular region around object center, HIS, object color, RGB
24	Average—filter response, correlation coefficients
25	Region size
26	Elongatedness
27	Peak of energy function
28	Entropy
29	Homogeneity
30	Third moment value
31	Compactness
32	Ratio—major axis variance vs minor axis variance
33	Region width
34	Grey level contrast
35	Second moment
36	Complexity
37	Color contrast
38	Number of holes

with false-positive elimination through proper blood vessel detection. Finally, feature set analysis was used to provide HE detection by the removal of false positives in retinal images. Moreover, successful HE detection relies on proper extraction and removal of the vascular structure. Lee et al. employed image-processing techniques along with pattern recognition to detect MAs and HEs using retinal images [38]. In retinal fluorescein angiograms, an image is sub-sampled by a factor of four for each dimension and the fovea localized by correlating with two-dimensional circularly symmetric triangular function, in which modeled gross sheading the macula was used by Cree et al. for MA detection and localization [39]. MA detection were carried out by a three-stage approach as described by Huang and Yan [40]. First, through image enhancement, the image was divided into different sub-regions. Next, MAs were detected based on local adaptive thresholding. Finally, resultant output was given based on prior knowledge, which incorporated that MAs cannot occur on the vessels, optic disc, and hard exudates. The limitation of this approach lies in inaccuracy result related to classification features which are provided in post-analysis steps. The approach used by Sherif et al. [41] described that those MA candidates was rejected where independent vessel detection was successful. Circular hough transform was used for the detection of circularity of MAs it proves to be an effective method for detecting red features in an image. A two-stage approach was used for the detection where the first step selects candidate lesions as a form of connected region and the second step refines retentive selection as the true lesions. The local gray-level information was employed by Grisan et al. [33] for initial segmentation as well as for obtaining a measure of region homogeneity by using the spatial density of segmented pixels. For characterization of MAs from other objects, the different intensity and shape feature were proposed by Raman et al. [42]. To measure and follow up the degeneration in retinal images, inverse segmentation technique was proposed by Kose et al. [43]. Zhang et al. [44] reported a method for detecting HEs by using background estimation algorithm.

Filtering

Feature analysis-based automated MA technique was presented by Hatanaka et al. [45]. After preprocessing the extraction of the candidate, regions for MAs were done through a double-ring filter with the addition of texture filters. Multiscale filtering and dynamic thresholding algorithm were invoked to detect MAs by Zhang et al. [46]. The double-ring filter approach was employed by Mizutani et al. 2009 [47]. for extraction of candidate regions of the image after applying image preprocessing methods. Mendonca et al. [48] proposed automated detection of red lesions based on a set of matched filters along with region-growing algorithm to demarcate MA candidates. Another approach was developed by Agurto et al. [49] based on the extraction of AM-FM features of retinal images, subsequently applied as dimensionally reduction and hierarchical clustering to assign DR severity grade the use of partial least squares method was proposed. The result of orientation matched filtering, thresholding, and eigen space image analysis was presented to encapsulate the MA profile extraction [50].

Region growing

Sinthanayothin et al. [51] described a recursive region-growing technique with moat operator for extraction of MAs and HEs. Streeter et al. [52] also proposed a similar approach of region-growing algorithm for MA detection. After preprocessing, thresholding and region-growing algorithm were used to select the candidate seed image. The region-growing approach was presented by Serrano et al. [53] for detecting MAs in order to analyze the fluorescein angiogram. Fleming et al. [54] employed local contrast normalization with local vessel detection as an initial preprocessing stage followed by a technique using a watershed region-growing transform approach for the detection of MAs in fluorescein angiograms. KNN classifier was applied to classify the MA candidates from local vessel detection. The results of this method relied on cross validation of the training set instead of excruciating the data set into training and test. The algorithm was performed by Marino et al. using correlation filters, region growing and classification approach [55].

Lalonde et al. [56] employed RetsoftPluse software as a tool for fundus image analysis. An automatic computer-added screening system based on the processing of digital fundus images was required for improving the clinical practice which handles the issue of manual grading which was slow and resources demanding [57]. The Diaretdb diabetic retinopathy database along with evolution protocol was provided in [58]. The actual grading scheme for diagnostics was performed manually by the proper medical protocols established in the work related to [59]. Table 2 shows the DR severity according to the rules. (Usher 2002) is cited in the body but its bibliographic information is missing. Kindly provide its bibliographic information. Otherwise, please delete it from the text/body.Request you to please delete the same.

Table 2 Diabetic retinopathy grading criteria

Grading	0	1	2	3
Stage	(No DR)	Mild	Moderate	Severe
Number of MAs	0	1 to 5	5 to 15	> 15
Number of HEs	0	0	≤ 5	> 5

MAs microaneurysms, *HEs* hemorrhages

Table 3 Comparative study of various red lesion detection algorithms

Author	Method	Classifier	SN	SP	Acc
Gardner et al. 1996 [60]	Preprocessing with edge detection filter ($n = 179$)	NN	–	–	73.8
Spencer et al. 1996 [61]	Recursive region growing (RRG)	–	82	86	–
Hipwell et al. 2000 [62]	Discrimination function, matched filter	–	81	93	–
Ege et al. 2000 [17]	Image intensity threshold based on estimate of b/c image intensity levels ($n = 134$) MAs	Mahalanobis	–	–	69
Ege et al. 2000 [17]	Image intensity threshold based on estimate of b/c image intensity levels ($n = 134$) HEs	Mahalanobis	–	–	83
Cree 2000	Integrated automated analyzer region-growing algorithm	–	82	84	–
Yang et al. 2001 [13]	Top-hat transform, CFAR threshold and region growing	Rule based	90	80	–
Sinthanayothin 2002 [51]	Recursive region growing (RRG)	–	77.5	88.7	–
Larsen et al. 2003 [63]	Detection of red dots ($n = 200$)	–	96.7	71.4	–
Streeter and Cree 2003 [52]	Enhancement, morphology	–	56	–	–
Usher 2004 [27]	RRGS, adaptive intensity thresholding	NN	95.1	46.3	–
Lalonde et al. 2004 [56]	Retsof tPlus software	–	90	75	–
Niemeijer et al. 2005 [15]	Spencer-frame methodology	KNN	100	87	–
Grisan and Ruggeri 2005 [64]	Pixel wise and object wise classification	Bayesian MAP, linear discriminant	71	99	83
Zhang et al. 2006 [6]	Multiscale morphological processing ($n = 30$)	–	84.1	89.2	–
Fleming et al. 2006 [54]	Watershed retinal region growing ($n = 1441$)	KNN	85.4	83.1	–
Kahai et al. 2006 [18]	Decision support system, binary hypothesis testing	Bayesian	100	67	–
Walter 2007 [65]	Diameter closing and automatic threshold ($n = 94$)	KNN	88.5	–	–
Garcia et al. 2008 [28]	29 features related to shape and color of image regions (lesion-based criterion)	MLP	86.1	71.4	–
Garcia et al. 2008 [28]	29 features related to shape and color of image regions (image-based criterion)	MLP	100	60	80
Quellec et al. 2008 [66]	Wavelet transformation, template matching ($n = 32$)	–	89.6	–	–
Karnowski et al. 2008 [7]	Morphological reconstruction method ($n = 86$)	–	90	90	–
Balasubramanian et al. 2008 [8]	Automatic seed generation ($n = 63$)	KNN, GMM	87	95.5	–
Kande et al. 2009 [22]	Local entropy thresholding, morphology ($n = 80$)	SVM	96.2	99.5	–
Mizutani et al. 2009 [47]	Double-ring filter	ANN	65	–	–
Dupas 2010 [67]	Morphology, thresholding ($n = 94$)	–	88.1	–	–
Kande et al. 2010 [26]	Matched filtering, morphology	SVM	100	91	–
Garcia et al. 2010 [29]	Morphological and region growing	MLP, RBF, SVM	86	52	–
Antal 2010 [68]	Globally optimal combination of the preprocessing	–	99	–	–
Langroudi and Sadjedi 2010 [69]	Morphology	–	92.5	81.5	–
Jaafar et al. 2011 [11]	Morphological technique	Rule based	89.7	98.6	–
Wenhua et al. 2012 [30]	Histogram algorithms	SVM	–	–	90
Kose et al. 2012 [43]	Discrimination function, matched filter	–	95.1	99.3	–
Giancardo 2013 [70]	Radon transform ($n = 169$)	Nearest neighbor	–	–	70.8
Giancardo 2013 [70]	Radon transform (n = 169)	Naive Bayes (Gaussian)	–	–	82.4
Giancardo 2013 [70]	Radon transform ($n = 169$)	SVM	–	–	83.3
Tang et al. 2013 [71]	A variety of filter bank, interactions with neighboring splats, shape, texture information	KNN	–	–	96
Azar 2013 [72]	Thresholding, match filtering ($n = 100$) (MAs)	Minimum distance	–	–	60
Azar et al. 2013 [72]	Thresholding, match filtering ($n = 100$) (HEs)	Minimum distance	–	–	94
Roychoudhari et al. 2014 [34]	Minimum-intensity maximum-solidity algorithm,	Hierarchical	80	85	–
Manjiri 2014 [73]	Preprocessing, histogram equalization	–	–	–	99.6
Angadi 2014 [74]	Dual tree complex WT, Log Gabor features	SVM	91.1	91.16	91.4
Varsanyi 2014 [75]	Morphology, region growing, skeletonization	Rule based	–	–	60HR
Manoj K 2015 [76]	Morphology and thresholding	–	–	–	95
V Das 2015 [77]	Shannon and Tsallis entropy thresholding ($n = 25$)	Naïve Bayes	58.2	–	–
Bharali 2015 [78]	Morphology, filtering and region growing ($n = 561$)	–	97.3	98.9	–
Mane 2015 [79]	Matched filtering modified approach ($n = 89$)	SVM	96.4	100	96.6
Shan 2016 [80]	Stacked sparse auto encoder, deep learning strategies	Softmax	91.5	91.6	91.4
Mohd. 2016 [81]	Bottom-hat filtering with gamma correction	–	–	–	84.2
Mumtaz 2017 [82]	Scale-based method, global thresholding	–	84	87	89

HR hit ratio, *SN* sensitivity, *SP* specificity, *ACC* accuracy, *n* number of images, *WT* wavelet transform

Issues for red lesion detection

There are several issues for accurate red lesion extraction in the diabetic screening scheme.

- The low contrast of red lesions in an image with different resolutions
- Bright lesions closer to lesion candidates
- Digital fundus photographs available with different sizes and variable compressed image formats
- Uneven illumination due to lighting artifacts
- Subsequent background structures in an image
- Irregular shape pattern and complex structures

Research application of red lesion detection

The characterization of retinal structures to avoid difficulties of explicit feature segmentation in an automated DR system is required. Characterization is related to structural variation in the retina. Standard grading criteria are related to exact measurement of true MA and HE candidates as shown in Table 2. However, manual assessment of the images and grading of the disease by making measurement in fundus images are prone to make mistakes and quite difficult for perfect evaluation. This leads to the need of a fully automated system for segmentation and measurement of red lesions by the system initially and then rechecking by professional graders to provide appropriate count and evaluation of the course of retinopathy disease. Table 3 illustrates the comparative study of various red lesion detection algorithms.

Conclusion

Numerous recent works have the emphasis to solve this particular problem including an online challenge for red lesion detectors. Therefore, the extraction of small red lesions is still an open issue. To tackle the MA detection problem, the procedure is as follows: first, the enhancement of the MA structure was given by the extraction of the green channel of fundus images. In the next candidate extraction coarse level steps, all MA-like objects have segmented in the image. Finally, a fine-level algorithm has removed false candidates using a supervised classifier. The basic morphology for identification of candidate MAs is related to its limited range of size and fairly uniform shape. The solution for false MA detection has been given by the need of efficient vessel segment removal, but the problem is removing the detected vessels also removes other objects from the image, which might be true MAs. Thus, minimizing false MA detection also results in the reduction of true MA detection. This tradeoff always exists in automatic red lesion detection. The main issue related to HE detection is its similarity in color with the fundus background and its irregular size and shape that easily mix with the background. It is hard to detect as compared to other types of bright or red lesions which belong to definite characteristics in fundus image analysis.

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ORIGINAL ARTICLE

A study of eye care service utilization among diabetic patients visiting a tertiary care hospital in Coastal Karnataka, southern India

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Abstract
The purpose of this study was to understand the eye care service utilization of diabetic patients visiting a tertiary care center. This was a cross-sectional study with a study period of 2 months conducted at a tertiary health care teaching hospital in South India. Diabetic patients visiting the hospital during the study period were subjected to a systematic questionnaire-based interview. The details of their ophthalmological evaluation were accessed from their records. A total of 230 diabetic patients were interviewed. Most of them (91%) were above the age group of 40 years, and 84% were literate. Around 73.2% were residing near the center with easy accessibility to health care. Although almost 90% of the patients were regularly visiting their physician for diabetes control, only 67% had undergone an ophthalmological evaluation and only 58% had been screened for retinopathy. Despite the availability of eye care facilities to a literate diabetic population with reasonable knowledge regarding ocular implications of the disease, the utilization of the services was inadequate. An integrated approach to diabetes and teleophthalmology services may help in making the comprehensive evaluation more convenient and thus begetting more compliance from the patient.

Keywords Diabetes mellitus · Ophthalmology · Retinopathy · Delivery of health care · India

Introduction

Diabetes mellitus affects around 285 million people worldwide. This is expected to increase to 439 million by 2030 according to the International Diabetes Federation [1, 2], of which more than 69.9 million would be affected in India [3].

Diabetes is an important cause of adult-onset blindness due to retinopathy and macular edema [4]. Diabetic retinopathy (DR) alone accounts for 2.6% of global blindness [5]. It is estimated that 95% of type 1 diabetics and 60% of type 2 diabetics with the disease duration longer than 20 years show signs of DR [2].

The prevalence of DR in diabetics in South India has been shown to be around 10.3% [6] in a rural population and 18% in an urban population [7]. In Central India, it is reported to be prevalent in around 9.6% of diabetics in the rural population [8]. The overall prevalence obtained through a questionnaire-based survey of ophthalmologists across urban and rural parts of the country has been reported to be 21.27% of the diabetics screened by them [9].

The early detection and treatment of DR is vital in avoiding permanent visual loss [10]. However, being almost asymptomatic in the initial and sometimes advanced stages, it is often overlooked during the management of a diabetic patient.

The lack of knowledge regarding the burden of DR has been addressed by increasing education programs among medical and paramedical personnel who form the primary care providers for diabetic patients.

Although various governmental and other health care programs have succeeded to a certain extent in the delivery of technology and expertise in tackling this preventable form of blindness, the actual success of their initiative lies in the active utilization of these services by the beneficiary.

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There have been many population and hospital-based studies addressing either the awareness or prevalence of DR in India [11]. However, the actual utilization of available resources by the diabetic patients has not been studied.

The objectives of this study were to understand the eye care seeking attitudes of patients visiting a tertiary care center for their diabetes management and to document the clinical profile of their associated retinopathy status.

Methods

This cross-sectional study was conducted at a tertiary health care center in the state of Karnataka, India, between April and June 2016. Institutional ethical committee clearance was obtained before the initiation of the study. The study adhered to the guidelines of the Declaration of Helsinki. A total of 230 patients suffering from diabetes were included in the study. A convenient sampling was used to select the study subjects. Diabetes mellitus was diagnosed on the basis of history of being treated for the same by either oral hypoglycemic agents or insulin or dietary modification regime. Patients who were diagnosed to have diabetes within a month prior to the study, gestational diabetes, systemically or psychologically unstable patients were excluded from the study. After obtaining a written informed consent, subjects were interviewed using a semi-structured questionnaire. A single interviewer obtained the responses after communicating the questions, in the participants' own language. The patient records were accessed to document their place of residence, confirm the presence of diabetes mellitus, level of visual acuity, and fundus evaluation findings. The presence of DR was considered to be present if the same was recorded in our hospital records. As per our hospital protocol, this was based on dilated fundus evaluation by direct and indirect ophthalmoscopy including biomicroscopy and fundus photography. The retinopathy was graded as per the International Clinical Diabetic Retinopathy and Diabetic Macular Edema severity scale as no retinopathy changes, mild nonproliferative diabetic retinopathy (mild NPDR), moderate nonproliferative diabetic retinopathy (moderate NPDR), severe nonproliferative diabetic retinopathy (severe NPDR), and proliferative diabetic retinopathy (PDR) [12]. The data collected was analyzed using SPSS version 15.0. For statistical analysis, data was summarized using frequency and percentage for categorical variables.

Results

Out of 230 study subjects, there were 115 males and 115 females. Most (93.1%) of the study subjects were above the age group of 40 years. Around 31.6% patients were from neighboring districts of Shivamogga and Dakshina Kannada, with 63.9% patients having secondary education or more (Tables 1 and 2). Majority (81.6%) were suffering from diabetes mellitus for over 2 years.

Out of 230 patients, 223 (96.9%) patients were on some form of antidiabetic medication. Majority (89.5%) were being treated by a physician specialized in internal medicine. When asked about knowledge regarding blood sugar, about 99.5% patients claimed to have knowledge regarding their normal blood sugar levels. However, when asked about ocular involvement in diabetes, only 76.1% were aware about ocular involvement and only 57% knew about potential blindness that can occur due to diabetes. These findings suggest that patients are more aware about normal blood sugar levels compared to ocular involvement due to diabetes. On the contrary, awareness regarding diabetic renal complications was known to 85.2% of the participants. This implies a gap in the knowledge related to ocular involvement in diabetes. Nearly 80% of the patients who were aware of diabetic complications attributed their source of information to the physicians. However, this included renal, foot, and eye complications in general. The magnitude of the ocular complications was probably not specified. To strengthen this evidence, it was found that only 67% had undergone visual acuity testing and only 58% had undergone dilation of their pupils and evaluation of fundus by an ophthalmologist before the study. It was striking to note that out of 58% who had undergone fundus evaluation, sight-threatening retinopathy, including all patients with macular edema and all patients with proliferative retinopathy, was seen in 35 patients. The appropriate modality of therapy was initiated for these patients at our center. Despite having suffered the disease for over 10 years, only 47.3% were reviewing at least once a year, for DR screening. Among those evaluated for retinopathy, 61% having proliferative retinopathy, 42% having nonproliferative retinopathy, and 47% having no signs of retinopathy were following up with their ophthalmologist for screening at least once a year (Table 3).

Table 1 Demographic details

Demographic details	Number (percentile)
Gender	
Male	115 (50)
Female	115 (50)
Age group	
< 40 years	16 (6.9)
41–60 years	103 (44.7)
> 61 years	111 (48.2)
Residence	
Same district as the hospital	96 (41.7)
Other districts	134 (58.3)

Table 2 Educational status and awareness of diabetic eye disease

Education and Diabetic eye disease awareness					
		Diabetic Eye Disease			Total
		Aware	Not aware	Unsure	
Education					
Illiterate	Count	22	12	2	36
	% within education	61.1%	33.3%	5.6%	100.0%
	% of total	9.6%	5.2%	0.9%	15.7%
Primary	Count	24	19	4	47
	% within education	51.1%	40.4%	8.5%	100.0%
	% of total	10.4%	8.3%	1.7%	20.4%
Secondary	Count	98	14	2	114
	% within education	86.0%	12.3%	1.8%	100.0%
	% of total	42.6%	6.1%	0.9%	49.6%
Graduate or above	Count	31	2	0	33
	% within education	93.9%	6.1%	0.0%	100.0%
	% of total	13.5%	0.9%	0.0%	14.3%
Total	Count	175	47	8	230
	% within education	76.1%	20.4%	3.5%	100.0%
	% of total	76.1%	20.4%	3.5%	100.0%

Discussion

In our study, we assessed the demographic profile of the diabetic patients visiting a tertiary care center with facilities for comprehensive diabetic care including treatment for advanced retinopathy. We also attempted to assess the eye care-seeking attitude of these patients.

Our cohort had 93.1% above the age of 40 years, with 63.9% having completed secondary schooling. Almost all were on some form of antidiabetic treatment and reviewed with their treating physician on a regular basis. They were also well aware of their blood sugar levels. The good literacy level and keen awareness regarding their systemic diabetes status ought to have been reflected in their awareness and attitude towards other complications of the disease including diabetic retinopathy. Shukla et al. have reported distance and commute to the health care facility as barriers to utilization of eye care services [13]. A lack of awareness and cost of the therapy for retinopathy have also been implicated as barriers in a western population [14]. However, despite referrals by their physician and easy accessibility of eye care facilities, almost one in four patients had no knowledge regarding the association of diabetes with retinopathy. Interestingly, although 67% had visited an eye care provider for refractive correction, only 58% had undergone a fundus evaluation for retinopathy.

The strategy for combating DR as suggested in the VISION 2020 program, includes the setting up of screening or referral systems, improving the education of diabetic patients regarding retinopathy, and establishing facilities for accurate diagnosis and management [15]. Our study reveals that despite the availability of adequate resources for screening as well as management, there is a deficit in actual utilization of the resources. A similar scenario is observed in population-based epidemiological surveys on diabetic retinopathy. Hussain et al. found only around 9% had undergone a fundus evaluation for DR screening despite over 50% being aware of the existence of such a complication [16].

This deficit in eye care-seeking attitude of the patients suffering from diabetes may be attributed to either incomplete knowledge or deficiencies in the delivery of the services.

Table 3 Severity of retinopathy and the pattern of follow-up with ophthalmologist

Diabetic retinopathy (DR) stage	Number of patients undergoing ophthalmic screening < 1 year	Number of patients undergoing ophthalmic screening > 1 year	Never reviewed
Not evaluated	33	21	43
No DR	31	23	11
Nonproliferative DR	21	27	2
Proliferative DR	11	7	0

Almost half of the patients in our study were not aware of the potential of DR to cause irreversible blindness in the late stages. The knowledge of the presence of a relatively asymptomatic initial stage along with a sight-threatening later stage may motivate patients to comply with the regular screening schedules advised. Muñoz et al. reported similar deficit in the accuracy of information provided to their study population owing to language and other sociocultural barriers [17].

In the absence of a proper understanding of the consequences, a reluctance among diabetic patients to visit various departments in a large hospital for screening of asymptomatic conditions is but expected. An integrated diabetic care clinic at the primary care level itself may make the utilization of health care resources more convenient to the patient. The availability of opinions from various specialists on the basis of investigation reports mediated through a familiar primary care physician or health care worker may improve the diabetic care delivery. Such integrated diabetic care clinics have been found to be as effective as hospital visits [18].

As an ocular fundus evaluation by a specialist ophthalmologist in screening of DR has been reported to be more accurate, screening at such centers may be facilitated by teleophthalmology [19, 20]. Here, with the help of fundus image acquisition using nonmydriatic fundus cameras and the Internet to transmit the images for assessment by an ophthalmologist at a base center, ophthalmic screening of diabetics can be completed at such a community peripheral center [21]. The patients needing further management in the form of lasers or surgery may then be referred to the tertiary care hospital.

The National Program for Control of Blindness, an initiative of the Indian Government, has in recent years recognized DR as one of the leading causes of blindness and is supporting the awareness, screening, and management of this condition. It has also provided encouragement developing teleophthalmological services in India [22]. Other initiatives such as the VISION 2020 ICO Guidelines for Diabetic eye care in India will further help management of DR in the community [23].

Although ours was a hospital-based study, population-based studies on the actual attitudes of diabetic patients would be more accurate in identifying and rectifying deficiencies in the present system.

To conclude, there is an enormous burden of diabetes-related complications including retinopathy foreseen in the near future. There are various national and international programs addressing the issue through attempts at increasing facilities for early detection and management. However, proper and complete understanding of the complications, if left untreated, needs to be communicated to the patients. This would improve their attitude towards seeking eye care at the appropriate time. This endeavor can be further encouraged by making the facilities conveniently accessible, with the help of advances in technology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was conducted in accordance with the ethical standards of the Institutional ethical and research committee and with the 1964 Helsinki declaration and its later amendments. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Modelling and developing diabetic retinopathy risk scores on Indian type 2 diabetes patients

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Abstract

The objective was to develop diabetic retinopathy (DR) risk scores and compute prevalence and incidence probabilities of DR in Indian type 2 diabetes mellitus patients. A double sample of size 388 was collected from the R.G. Centre for Diabetes and Endocrinology, J.N.M.C., A.M.U., Aligarh, India, randomly distributed among training and test sets. DR risk scores of Iran and China were administered on Indian training set. Since prevalence probabilities of DR calculated by Logit model were unacceptable, thus actual data of Iranian and Chinese studies were simulated from their variable characteristics. Ridge regression was selected as optimal by regularization and cross-validation techniques. The yearly incidences of DR from ridge probabilities were determined using absorbing Markov chain. Receiver operating characteristic (ROC) curve and Hosmer Lemeshow test were exerted for model discrimination and calibration. Furthermore, these outcomes were implemented on the test sample. Out of 284 training sample patients, 23 had DR currently. Iranian score with an area of 0.815 (95% CI 0.765–0.859) was the better fit. Ridge coefficients acquired from Chinese simulated data contented the Indian data, providing accurate probabilities and an area of 0.784 (95% CI 0.731–0.830). Validating on test data, ROC curves for current, 1 year and 2 years prediction resulted in areas of 0.819, 0.811 and 0.686. Iranian score and simulated Chinese ridge coefficients for prevalence of DR were the best fit on Indian type 2 diabetes patients. Markov two-state model can be applied to forecast yearly incidence of DR.

Keywords Diabetic retinopathy · Logistic regression · Regularization · Ridge regression · Risk score · Type 2 diabetes mellitus

Abbreviations

BMI	Body mass index
BSF	Blood sugar fasting
DBP	Diastolic blood pressure
DR	Diabetic retinopathy
HbA1c	Glycosylated haemoglobin
HDL-C	HDL cholesterol
HTN	Hypertension
ICMR	Indian Council of Medical Research
LDL-C	LDL cholesterol
PP	Post prandial blood sugar
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
T2DM	Type 2 diabetes mellitus (used only in tables)
TC	Total cholesterol
TG	Triglycerides
VLDL-C	VLDL cholesterol

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Introduction

Presently, predicting the risk of developing complications due to diabetes is immensely necessary in India. Over 69 million diabetic patients were recorded in 2015 [1], which is expected to go over 80 million over the next decade [2–4]. Indian Institute of Public Health (IIPH) predicts this number to go up

to 120 million by the year 2030 [5]. A single Indian risk score for complications related to type 2 diabetes mellitus has never been investigated, barring two simple risk scores for incidence of type 2 diabetes mellitus that considered urban or southern populations, respectively [6, 7]. Diabetic retinopathy (DR) is the topmost reason for visual impairment across countries [8]. Assistance in early prediction of a complication will allow for better control of the disease, as the presence of DR indicates dysfunction of various organs [9, 10].

Only two DR models (Iranian and Chinese) have been developed worldwide, which we decided to validate on Indian patients [11, 12]. Possessing prediction models for prior assessment holds an advantage over expensive fundus photography. Thus, only the patients in the high risk criteria from the predictive algorithm should be followed up for fundus examination. Such algorithms can be applied and used to spread awareness about disease complications. Mild, background, grade I, II, III and IV DR were taken as the event of interest. DR risk factors according to the Mayo Clinic (USA) consist of duration of diabetes, blood sugar level, high blood pressure, high cholesterol, tobacco use, pregnancy and race. Apart from running a logistic regression, both Hosseini et al. and Wang et al. created scores for their study making it more applicable in hospitals [11, 12]. The Iranian score is hypothesized to have a better fit on Indian patients.

The main objective of this study was to validate the two aforementioned models on Indian patients based on scores but also to come up with probabilities assisting in the prediction of annual, biannual or triannual risk of developing DR. To our knowledge, no previous work regarding validation of DR scores on Indian type 2 diabetes mellitus patients has ever been done and also no prior research has attempted to estimate prevalence and incidence probabilities of DR. In view of the above, we have thoroughly examined and completed our study.

Material and methods

Study population

With margin of error taken as 3.5%, a double sample of size 388 was determined and randomly collected from the Rajiv Gandhi Centre for Diabetes and Endocrinology, J.N.M.C., A.M.U., Aligarh, India. This sample was divided into two parts: 284 patients in the Indian training set and 104 patients in the Indian test set. The required data of sample individuals was collected through one to one interaction with the patients and also from the recorded patient files. The Rajiv Gandhi centre was chosen due to the considerable number of villages in the vicinity of Aligarh City, since a large number of urban and rural patients visit the centre for treatment on a daily basis. This provides more diversity in the sample and brings about 400 patients weekly, resulting in a higher reliable model.

Procedures

Collected variables include sex, age, height, weight, BMI, blood sugar fasting (BSF), post prandial blood sugar (PP), glycosylated hemoglobin (HbA1c), diastolic blood pressure (DBP), systolic blood pressure (SBP), smoking, alcohol habits, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C, physical activity, duration of diabetes, diet control, hypertension (HTN), history of antihypertensive drug treatment, family history of diabetes, waist circumference, hip circumference, medications, history of DR and central obesity.

After documentation of demographic, anthropometric and individual habitual data, BSF, PP and lipid profiles were estimated by Biochemistry Analyzer, Lab Life Chem Master, India. HbA1c was measured using D10 Instrumentation, Bio-Rad, USA.

Statistical analysis

The impact of these factors is extremely significant on diabetes and DR and thus has been included in the study. Any missing data was replaced with the respective mean value of that variable. Scores were calculated for every patient in our Indian training set by summing each score for each category of the variables in which the patient lies (Eq. 1). This was justified by plotting a receiver operating characteristic (ROC) curve.

$$\left.\begin{aligned}\text{Hosseini's risk score} &= \Big(\text{Age}\ (15(\text{if } 40{-}49), 25(\text{if } 50{-}59), 25\ (\text{if}{\geq}60)) + \text{Duration}\ \Big(20(\text{if } 2{-}4),\\ &\qquad 35(\text{if } 5{-}9), 50(\text{if} = 10)\Big) + \text{HbA1c}(5(\text{if } 7{-}11), 10(\text{if} > 11) + 5\ \text{if female})\Big)\\ \text{Wang's risk score} &= \Big(\text{Age}\ (5(\text{if } 45{-}64), 4(\text{if}{\geq}65)) + \text{Duration}\ \Big(9(\text{if } 1{-}5), 10(\text{if } 5{-}10), 14(\text{if } 10{-}15),\\ &\qquad 20(\text{if}{\geq}15)\Big) + \text{Central Obesity}\ (2\ \text{if yes}) + \text{HTN Treatment}\ (7\ \text{if yes})\Big)\end{aligned}\right\} \quad (1)$$

$$\left.\begin{aligned}\text{Hosseini's partial probabilities} = \Big(&\text{Age } (0.471 \text{ (if } 40\text{–}49), 0.739 \text{ (if } 50\text{–}59), 0.763 \text{ (if}\geq 60))+\\ &\text{Duration } (0.638 \text{ (if } 2\text{–}4), 1.017 \text{ (if } 5\text{–}9), 1.781 \text{ (if}\geq 10))+\\ &\text{HbA1c } (0.187 \text{ (if } 7\text{–}9), 0.237 \text{ (if } 9\text{–}11), 0.348 \text{ (if} > 11))+\\ &\text{BMI } (0.347 \text{ if} > 25) + (0.216 \text{ if female})\Big)\\ \text{Wang's partial probabilities } = \Big(&\text{Age } (0.49 \text{ (if } 45\text{–}64), 0.449 \text{ (if}\geq 65)) + \text{Duration } (0.891 \text{ (if } 1\text{–}5),\\ &1.039 \text{ (if } 5\text{–}10), 1.383 \text{ (if } 10\text{–}15), 1.982 \text{ (if}\geq 15)\Big)+\\ &\text{Central Obesity } (0.213 \text{ if yes}) + \text{HTN Treatment } (0.698 \text{ if yes})\Big)\end{aligned}\right\} \quad (2)$$

Apart from scores, knowing the probabilities of developing DR can be very useful. The individuals' probabilities were calculated from Eq. 2 and Eq. 3. Area under ROC curve was applied to validate the results. However, getting appropriate probabilities from Eqs. 2 and 3 was not as straightforward. This application gave either close to 0 or close to 1 probabilities (Fig. 1 and Supplementary Fig. 1). The rest of the study rectifies the probabilities as the current method is not able to determine it.

$$p(y=1) = \frac{e^{\beta_0+\beta_i x_i}}{1+e^{\beta_0+\beta_i x_i}} \quad (3)$$

Since the authors will not provide us with their original data, we decided to simulate the data. From the percentages, means, standard deviations and other characteristics presented for each variable by Hosseini et al. and Wang et al., we tried to get as close to their real data as possible. R and SPSS version 20 software packages were used for all analysis and computations. Two samples of size 3734 and 1869 with variables and their parameters given in [11, 12] were simulated. Simulation for continuous variables was implemented using $x = \mu + z\sigma$. This option removed the boundation of a specific distribution for each risk factor. However, duration of diabetes and triglyceride factors best fitted the lognormal distribution [13, 14]. Wan et al. has given a method to estimate mean and standard deviation if only median and interquartile ranges are available [15].

Logistic regression by enter method was performed on the two simulated samples. New regression coefficients have been presented in Supplementary Table 1. These coefficients slightly differ from the original results because they have been obtained from a simulated data. Variables were not divided into categories and therefore, one regression coefficient is sufficient for one variable. Triglyceride, HbA1c and diastolic blood pressure were found to be non-significant for Iranian simulated sample, whereas diet control, central obesity, hypertension and regular physical activity were non-significant for Chinese simulated sample. Looking at Supplementary Table 1, the Chinese section, 95% confidence intervals for the odds ratios have not been shown here as they came out to be extremely high. The area under ROC curve was determined. Our areas for scores and probabilities after simulation were acceptable but the curve for probabilities was still the main concern. The spread of probabilities was not uniform and mostly focused on extreme values. The explanation was overfitting. Overfitting occurs when there is low bias and high variance in the model [16] and arises when the model fits the training data well but not the test data. Figure 1 and Supplementary Fig. 2 depict that the models are trying extremely hard to fit every single data point. To overcome this hindrance, regularization techniques must be applied. This introduces another term that brings in more features with the objective function. Suppose the objective function is to minimize the error term in a regression model (Eq. 4). An extra term known as the penalty variable is added which is the sum of squares of coefficients multiplied by a tuning parameter 'λ'.

$$\left.\begin{aligned}&\min|Y_i - f(X_i)|^2\\ &\min \sum_{i=1}^{n} |Y_i - f(X_i)|^2 + \lambda\|f\|^2\end{aligned}\right\} \quad (4)$$

Optimizing 'λ' is the task we need to solve looking at the trade-off between the prediction accuracy of training sample and prediction accuracy of the test sample. Using cross-validation [17] by *glmnet* package in R software, most suited 'λ' and regularization technique can be singled out from ridge, lasso and elastic net regression based on the smallest mean squared error. Such type of techniques can be applied for logistic regression by stating the family as binomial in the R command. In this case both simulated Chinese and Iranian data achieved the least amount of mean squared error for ridge regression.

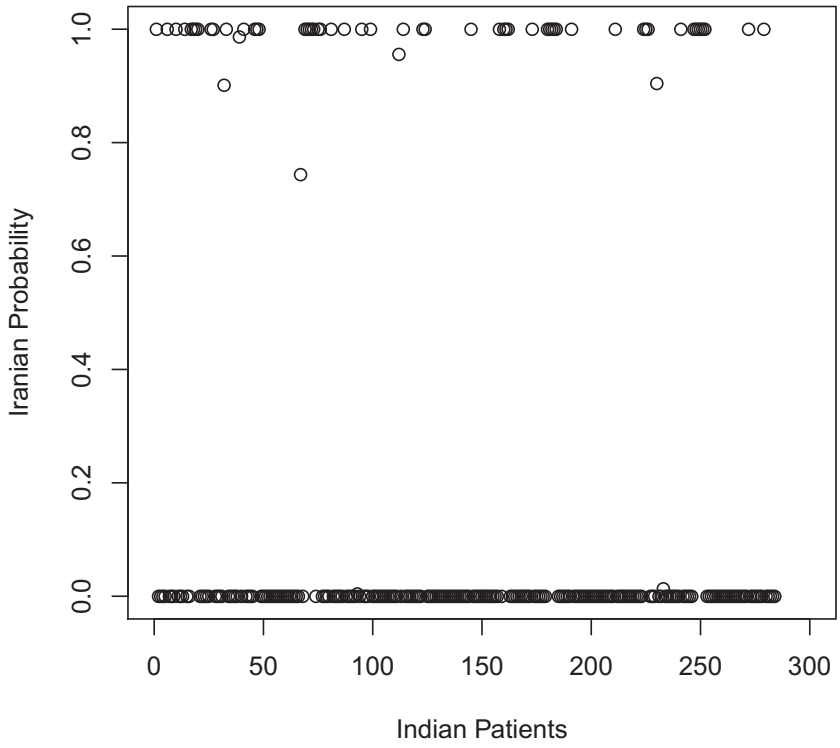

Fig. 1 Scatter plot showing spread of probability of Iranian original coefficients on Indian patients

Selection of 'λ' is depicted in Fig. 2. Unlike ridge, lasso and elastic net techniques remove non-significant variables from the model [18]. Since ridge retains all variables, coefficient values for full model are given in Table 1. Hence, it tries to push the coefficients for many variables to zero as seen in Fig. 3.

Table 1 Coefficients obtained from Iranian and Chinese simulated data by applying ridge regression

Iranian ridge coefficients			Chinese ridge coefficients		
Variables	Beta-coefficient	OR	Variables	Beta-coefficient	OR
Sex: females	− 1.358	0.257	Sex: females	0.329	1.390
Age	0.097	1.102	Age	− 0.00013	1.000
Duration of diabetes	0.193	1.213	Duration of diabetes	0.3089	1.362
HbA1c %	− 0.175	0.839	HbA1c %	− 0.0038	0.996
BMI	− 0.383	0.682	BMI	− 0.0635	0.938
TC	− 0.019	0.981	Waist circumference	− 0.00104	0.999
BSF	0.016	1.016	History of antihypertensive drugs: yes	0.4802	1.616
SBP	0.061	1.063	TG	0.0269	1.027
DBP	− 0.029	0.971	Diet control: yes	0.13537	1.145
Constant	2.547	12.769	Family history of diabetes: yes	0.11835	1.126
			Physical activity: yes	0.01221	1.012
			TC	− 0.000597	0.999
			HDL-C	0.00369	1.004
			LDL-C	0.0011205	1.001
			BSF	− 0.000331	1.000
			SBP	0.01658	1.017
			DBP	− 0.012719	0.987
			Hypertension: yes	0.3643329	1.440
			Constant	− 9.453043	0.000078

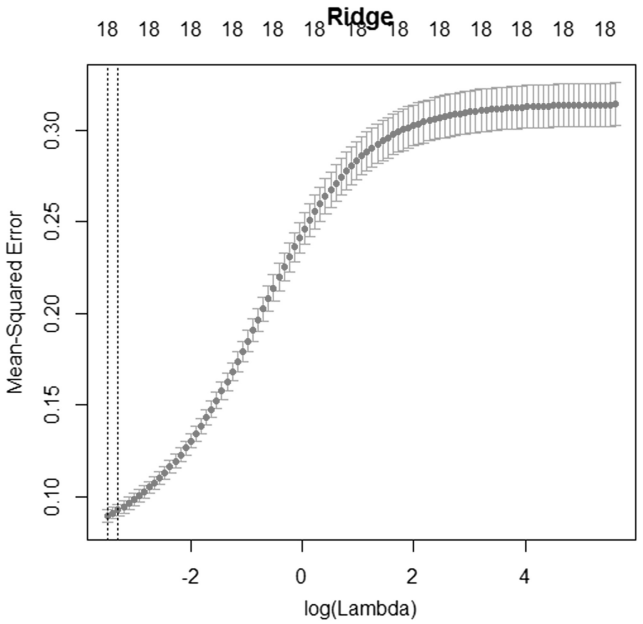

Fig. 2 Various 'λ' values depicting different mean squared errors for ridge regression on Chinese simulated data

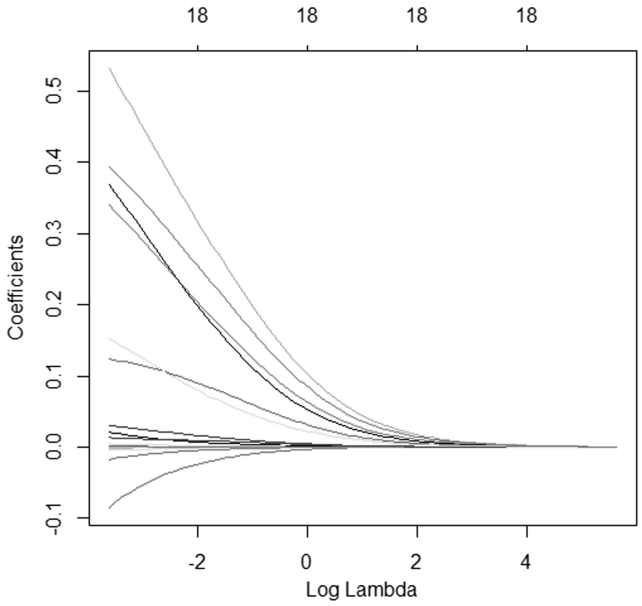

Fig. 3 Ridge regression pushes the coefficients of Chinese simulated data to zero as lambda increases

Applying the new ridge coefficients on Indian training set and determining probabilities by Eq. 3 resulted in a much more improved and varied spread out of predictions for DR. Risk scores are found by: risk score = $\beta_0 + X_1*\beta_1 + X_2*\beta_2 + \ldots + X_p*\beta_p$. Prevalence probability was then calculated from: p(y = 1) = exp(risk score)/1 + exp(risk score). Highest Youden's index (sensitivity + specificity − 1) [19] was exerted to select an optimal cut-off point from the probabilities. If a patients' risk falls above the cut-off point, there is a greater likelihood that he has developed DR compared with those that lie below the cut-off point.

Logistic regression produces probabilities for the current time at which the data was taken. Once the better fit between Chinese and Iranian model on Indian patients was decided, using these probabilities for 2 or 3 years prediction will be the next aim and will provide immense benefit in real application.

The achievement of this proposal was done using absorbing Markov chain [20]. Suppose we have a two-state model, with 'No DR' and 'Developed DR' as the two states. Consider 'Developed DR' as an absorbing state. Probabilities determined from the ridge coefficients portray the probabilities of going from No DR to Developed DR, that is, taking a step from a transient state to an absorbing state (denoted as P(DR)). Remaining probabilities can be observed from Supplementary Fig. 2, which are established on the characteristics of an absorbing state.

Next year's probabilities can be assessed from $x^{(n)} = x^{(0)}P^n$, where $x^{(0)}$ is the current known dichotomous situation of a patient, where the patient does not have DR and 'P' is the transition matrix. Defined in Eq. 5 are the two variables.

$$x^{(n)} = [1 \;\; 0] \begin{bmatrix} 1-P(DR) & P(DR) \\ 0 & 1 \end{bmatrix}^n \tag{5}$$

Substituting '$n = 1, 2, 3,\ldots$', we get the yearly models. For example, '$n = 1$' gives the probability of having DR at the current time the data was collected, '$n = 2$' shows the results for within 1 year, '$n = 3$' predicts within 2 years after first data collection and so on. A simpler method is formed below (Eq. 6), which can be used after determining the risk probabilities.

$$\left.\begin{array}{l} \text{I}^{\text{st}}\text{ Year DR Probability} = \text{Current P(No DR)} \times \text{Current P(DR)} + \text{ Current P(DR)} \\ \text{II}^{\text{nd}}\text{ Year DR Probability} = \text{Current P(No DR)} \times \text{I}^{\text{st}}\text{ Year P(DR)} + \text{Current P(DR)} \\ \text{III}^{\text{rd}}\text{ Year DR Probability} = \text{Current P(No DR)} \times \text{II}^{\text{nd}}\text{ Year P(DR)} + \text{Current P(DR); or in general,} \\ n^{\text{th}}\text{ Year DR Probability} = n^{\text{th}}\text{ Year P(No DR)} \times (n-1)^{\text{th}}\text{ Year P(DR)} + n^{\text{th}}\text{ Year P(DR)} \end{array}\right\} \tag{6}$$

The Indian test data set was used to validate results based on the ridge probabilities as well as the yearly probabilities obtained from the Markov model. Current prevalence of DR was tested by implementing Chinese ridge coefficients from Table 1, followed up by measuring logistic probabilities. Incidence of DR within year 1 and year 2 was determined

by estimating probabilities from the two-state Markov model. Diagnostic tests and area under ROC curve techniques were applied to justify our proposed method of predicting DR. Patients having any form of DR at the time of collection of data were used to estimate the current probabilities. These specific patients were then excluded from further calculations of first year and second year risk probabilities of developing DR.

Results

Clinical characteristics of study population

The collected 284 Indian training patients included 144 (50.7%) males and 140 (49.3%) females, average age being 51.18 ± 9.214 years (28–79 years). Two hundred thirty-one patients had hypertension out of which 225 were taking antihypertensive drugs. Average BMI was 25.51 ± 4.29 (15.63–40.63), leading towards 204 (71.8%) diabetics to be centrally obese. Duration of type 2 diabetes mellitus was on average 5.78 ± 5.15 (0.05–25.83) years. All clinical characteristics differentiated on the basis of with DR and without DR are given in Table 2.

Table 2 depicts that patients with developed DR had higher age, longer duration of diabetes, greater uncontrolled HbA1c, higher BMI, larger waist circumference, higher blood sugar fasting and larger systolic blood pressure. Being centrally obese, hypertensive, having positive history of taking antihypertensive medications and having some direct family member who is diabetic are all direct contributors towards DR. However, patients that do actually have high TC, TG and physical activeness are less likely to develop DR compared to those that have low values for such parameters. On the other hand, HDL-C, LDL-C, VLDL-C, diastolic blood pressure and diet control were similar for both patients, with or without DR.

Application of Iranian and Chinese DR models on Indian patients and predicting probabilities

Scores from the two studies were applied on our training sample and the total sum score was calculated. Upon analyzing these two models on our data, areas of computed diagnostic accuracies attained are 0.815 (95% CI 0.765–0.859) for Iranian and 0.776 (95% CI 0.734–0.832) for Chinese algorithms. Considering risk scores, Iranian score fits the Indian data more superiorly than the Chinese score, confirming the initial hypothesis. Optimal cut-off point for the Iranian score was assessed administering highest Youden's index and was evaluated as 65 with sensitivity and specificity as 78.26 and 74.33%. From the 23 patients diagnosed with diabetic retinopathy, 19 (82.6%) recorded a DR risk score ≥ 65, and from the 261 that did not develop DR, 163 (62.5%) patients noted a risk score < 65.

Table 2 Clinical characteristics of 388 Indian patients

Variables	With DR* ($n = 35$)	Without DR* ($n = 353$)
Age (years)	55.51 ± 10.34	51.57 ± 9.46
Gender: males	48.6% (17)	49.9% (176)
Duration of T2DM (years)	10.42 ± 5.38	5.51 ± 4.71
HbA1c % (mmol/mol)	9.38 (79) ± 1.78	7.37 (57) ± 1.16
BMI (kg/m^2)	26.17 ± 4.85	25.36 ± 4.35
TC (mg/dL)	173.57 ± 30.25	169.99 ± 29.86
TG(mg/dL)	151.29 ± 30.88	151.62 ± 37.12
BSF (mg/dL)	148.77 ± 46.48	109.58 ± 32.83
HDL-C (mg/dL)	40.80 ± 7.19	40.64 ± 7.13
LDL-C (mg/dL)	102.51 ± 18.22	99.05 ± 18.36
VLDL-C (mg/dL)	30.29 ± 6.19	30.33 ± 7.44
SBP (mmHg)	145.80 ± 12.55	132.64 ± 15.07
DBP (mmHg)	81.37 ± 4.94	81.55 ± 7.57
Waist circumference (cm)	96.14 ± 11.83	94.85 ± 12.53
Central obesity: yes	77.1% (27)	71.1% (251)
Hypertension: yes	91.4% (32)	80.2% (283)
History of antihypertensive drug treatment: yes	91.4% (32)	77.6% (274)
Diet control: yes	74.3% (26)	72.0% (254)
Family history of diabetes: yes	42.9% (15)	39.4% (139)
Physical activity: yes	68.6% (24)	64.0% (226)

Predicting the probabilities of DR by executing Eq. 3 was the next objective. Model 2 by Hosseini et al. resulted in an area under ROC curve of 0.62 (95% CI 0.561–0.677). On the other hand, model 2 by Wang et al. determined an area of 0.567 (95% CI 0.507–0.626). Both these models did not fit well and the probabilities were at the extreme boundaries of exact 0 or exact 1 (Fig. 1 and Supplementary Fig. 2). Iranian model fits extremely poorly, but Chinese model is a bit more spread out. However, majority of the points are forecasting probability 1.

To rectify this low accuracy criteria, two samples of size 3734 and 1869 were simulated to develop as close to the real Iranian and Chinese data as possible. Supplementary Table 1 provides coefficients obtained from logistic regression using enter method. Once again the probabilities were estimated and then accuracy was found by ROC curve. Firstly, application of simulated Iranian model produced an area under curve of 0.62 (95% CI 0.561–0.677). Secondly, the simulated Chinese model had an area of 0.748 (95% CI 0.693–0.797). Discriminatory analysis by Hosmer Lemeshow test for both results came out to be unacceptable. The areas clearly indicate that there is no improvement considering the Iranian fit. Although, Chinese fit amended quite a bit, reaching 0.748 from 0.567, the overall spread of prediction was reasonably poor.

To overcome the problem of overfitting, regularization technique was used. Ridge regression for binomial family was selected as the best method based on mean squared error. Optimal 'λ' came out to be 0.0235 and 0.0301 for Iranian and Chinese simulated ridge regression (Fig. 2). For these 'λ' values, optimal regression coefficients were constructed considering that ridge tries to push them close to zero to remove the errors (Table 1). Standard errors and confidence intervals are not written in the table because according to Goeman et al. [21], these results are not majorly useful and R software deliberately does not provide them. Reason being that standard errors are not explanatory for highly biased estimates as ridge regression reduces the variance but increases the bias of the penalized estimates. In most cases of penalized regression, it is difficult to calculate a precise estimate of bias. Bootstrapping can only produce an assessment of variance of the coefficients. It can be misleading to consider mean squared errors from this process as it ignores the inaccuracies caused by the bias. Therefore, affecting confidence intervals from bootstrapping as well, that depend on variance estimates.

One more time Eq. 3 was executed to determine the probabilities and its spread. Regularized Iranian simulated data formed an ROC curve with an area of 0.648 (95% CI 0.588–0.702) and regularized Chinese simulated data produced an area under ROC curve of 0.784 (95% CI 0.731–0.830). Both these models progressed regarding area under curve and the dissemination of data advanced as well (Supplementary Fig. 3 and Fig. 4). A cluster near the higher probabilities is seen in Supplementary Fig. 3, whereas Fig. 4 depicts a bigger cluster near low probabilities. Concerning probabilities, the coefficients obtained from ridge regression on simulated Chinese data is a much better fit on Indian patients than the Iranian data. Since, prevalence of DR is about 8.1% in the Indian data, probability of forecasting 1 by Iranian ridge model is quite over predictive.

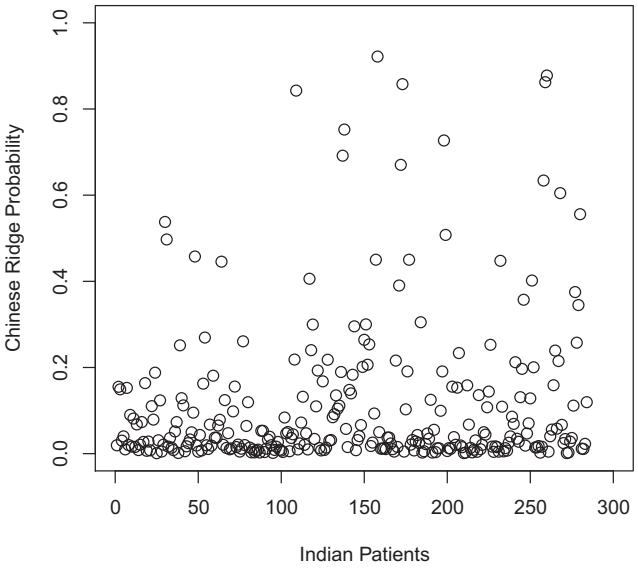

Fig. 4 Scatter plot of simulated Chinese ridge probabilities on Indian data

Since, Chinese model predicted applicable and correct probabilities for Indian patients, in this part only Chinese models' results will be discussed. It becomes vital to determine an optimal cut-off point which provides a level above which risk of having DR becomes greater. The optimal cut-off point for the Indian probabilities was assessed as 0.0934 with sensitivity and specificity as 78.26 and 71.26%. Justification was done by checking the number of patients with DR that actually got probability above 0.0934. Out of 23 patients diagnosed with DR, 18 (78.3%) noted a DR risk probability greater than 0.0934, and from the 261 that did not have DR, 185 (70.9%) patients observed a risk probability less than 0.0934. Ridge regression retains all variables; however, age, HbA1c, waist circumference, TC, HDL-C, LDL-C and BSF can be excluded if required due to the small impact of these coefficients.

Validation on Indian test sample

Out of a sample of 104 patients, 11 (10.5%) were currently having DR as a complication, 9 (9.6%) developed DR within a year and 12 (14.2%) developed DR within 2 years. Current ridge logistic probabilities were determined for each patient using Table 1 and the first year and second year probabilities were computed from Markov two-state model (Eq. 6). We know from the training sample that the cut-off point for current DR risk is 0.0934. Out of the 11 patients who were diagnosed with DR, 10 scored a risk probability of over 0.0934, and from the remaining 93 patients that did not have DR at

present, 58 scored less than this cut-off. Discriminatory analysis by ROC curve resulted in an area of 0.819 (0.732–0.888).

Since 11 patients had DR presently, these were then excluded from the total test sample to allow a better conclusion for 1-year risk probabilities. The cut-off point for 1-year risk is determined as 0.2071 with sensitivity and specificity as 88.89 and 76.19%. Out of the 9 patients who developed DR within a year, 8 had a risk probability above 0.2071, whereas 64 observed probability below 0.2071 in the 84 patients who did not develop DR. Area under ROC curve came out to be 0.811 (0.716–0.885).

Lastly, to work with the 2-year risk of DR, these nine patients were excluded as well. By highest Youden's index, the optimal cut-off value was decided as 0.15 with sensitivity and specificity as 91.67 and 45.83% (Table 3). For this part, the results were satisfying, as 11 DR patients within 2 years noted their risk above 0.15. ROC curve produced an area covering 0.686 (0.576–0.783). Although, 32 from 72 healthy subjects had their probability less than 0.15, overall the results were acceptable.

Table 3 Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and Youden's index to identify the optimal cut off point for 1- and 2-year diabetic retinopathy risk for two-state Markov model

Cut-off	Sensitivity	Specificity	NPV	PPV	Youden's index
One-year risk probability					
0.0203	100.00	26.19	100.0	10.7	0.2619
0.0831	100.00	36.90	100.0	12.3	0.369
0.1457	88.89	54.76	98.2	14.8	0.4365
0.1789	88.89	71.43	98.6	21.5	0.6032
0.2071	88.89	76.19	98.7	24.8	0.6508
0.2298	66.67	77.38	96.3	20.6	0.4405
0.3540	33.33	86.90	93.7	18.3	0.2023
0.4864	0.00	94.05	91.4	0.0	−0.0595
Two-year risk probability					
0.0183	100.00	23.61	100.0	10.3	0.2361
0.0323	100.00	31.94	100.0	11.5	0.3194
0.0603	91.67	36.11	98.0	11.2	0.2778
0.1221	91.67	41.67	98.3	12.2	0.3334
0.1500	91.67	45.83	98.4	13.0	0.375
0.1861	75.00	48.61	95.7	11.4	0.2361
0.2939	41.67	79.17	93.9	15.0	0.2084
0.5178	16.67	91.67	92.6	15.0	0.0834

Discussion

Diabetes registry conducted by ICMR reports that 26.4% of the patients have prevalence of at least one chronic complication, with retinopathy in type 2 diabetes mellitus being the most common [22]. We got 8.1% prevalence of DR for the current year and 14% incidence for a 2-year prediction. Since India has no risk score model for any complications caused by diabetes, it becomes essential to validate other countries' models on Indian patients and exerting statistical methods to alleviate the fit.

Iranian and Chinese DR risk scores, the only two models constructed on retinopathy, were applied on Indian patients. By one to one interaction with the patients and their recorded data files, clinical parameters were collected. The significant parameters required for verification of Iranian and Chinese models were sex, age, BMI, HbA1c, duration of diabetes, history of antihypertensive drug treatment and central obesity. Upon implementation of these scores on our collected data, diagnostic accuracies for the two models were 0.815 (95% CI 0.765–0.859) and 0.776 (95% CI 0.734–0.832) resulting in the better fit of Iranian score. Both the scores fitted the Indian data even better than their respective country data. Youden's index finalized 65 as the optimal cut-off point for the Iranian score.

Retinopathy is an important cause of visual impairment and therefore, risk scores predicting this are not adequate. Using regression coefficients for forecasting the likelihood of having DR was the next objective, permitting patients and doctors both to estimate a risk of DR based on probabilities. After several attempts to rectify and achieve the accurate probabilities from the two models, ridge regression on simulated data provided the precise probabilities. The ridge regression coefficients acquired from the Chinese simulated data satisfied the Indian data much better compared to the Iranian simulated data, producing an area under ROC curve of 0.784 (95% CI 0.731–0.830) and a much more distributed scatter plot of probabilities. For this measure, the optimal cut-off point selected was 0.0934.

Compared to other validation studies, such as the EuroSCORE on patients going through cardiac surgery, we got similar results. The EuroSCORE was validated on Indian and Australian patients and it got better results on Indian patients with the two areas under ROC curve being 0.8278 and 0.83 [23, 24]. We attempted this similar criterion and got the Iranian model to fit better with an area of 0.815. Our study went further and determined the current risk probabilities and afterwards estimated the following years' risk probabilities by Markov two-state model. To our knowledge this Markov method for forecasting has not been used in any risk score studies.

These results were validated on a test data and ROC curves for current, 1 year and 2 years prediction resulted in areas of 0.819, 0.811 and 0.686. Even though diabetes is a condition of high sugar level, both HbA1c and blood sugar fasting had a non-significant effect on DR based on

ridge regression for the test data. Considering average values, patients with DR had higher blood sugar fasting and HbA1c and the risk for developing DR grows considerably with the duration of diabetes.

Even though our findings are fitting well on the Indian data, one limitation is that we did not have the original data for the two countries to apply regularization techniques on. Data developed by simulation was attempted to be as near to the original data as imaginable and therefore, the deviation in the regression coefficients was observed. Nonetheless, the outcomes are extremely satisfying and can be applied by type 2 diabetes mellitus patients themselves. Construction of a DR risk model purely for India will require a bigger sample and thus was not approached in this study.

Consider an example of the Iranian score on the Indian data. Suppose a female patient of age 49 has had diabetes for 9.42 years, with HbA1c as 6.9% (52 mmol/mol) and BMI as 24.65. Such a patient gets a score of 55. Now an example of the regularized Chinese model would be to assume a male patient of age 60, who has had diabetes for 4.5 years, 7.6% (60 mmol/mol) HbA1c, 19.031 BMI, 81 cm waist circumference, 175 mg/dL triglyceride, 198 mg/dL total cholesterol, 47 mg/dL HDL-C, 116 mg/dL LDL-C, 134 mg/dL blood sugar fasting, no diet control, no family history of diabetes, no physical activity, hypertensive, 130 mmHg systolic blood pressure, 80 mmHg diastolic blood pressure and history of antihypertensive drugs. Such a patient gets a risk probability for prevalence of DR of 0.0725, within 1 year risk of 0.1397 and within 2-year risk of 0.2021.

Conclusions

In conclusion, the original Iranian score can be applied on Indian type 2 diabetes mellitus patients for current risk of having DR and ridge regression coefficients estimated from the Chinese model can be applied to calculate risk probabilities for prevalence of DR. Furthermore, Markov two-state model can be applied to forecast the yearly incidence of DR.

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Authors' contributions Faiz N.K. Yusufi carried out the collection of data, all data analysis and prepared the manuscript and takes responsibility as the guarantor.

Aquil Ahmed provided assistance in preparing the manuscript.

Jamal Ahmad supervised the collection of data and participated in manuscript preparation.

All authors have read and approved the content of the manuscript.

Compliance with ethical standards

Conflict of interest Faiz Noor Khan Yusufi, Aquil Ahmed and Jamal Ahmad declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval The patients' written consent was acquired in addition to an ethical approval through the Institutional Ethical Committee, Faculty of Medicine, J.N.M.C., A.M.U., Aligarh.

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ORIGINAL ARTICLE

Autoantibodies and HLA class II DR-DQ genotypes in Ugandan children and adolescents with type 1 diabetes mellitus

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Abstract

The aims were to determine the prevalence of autoantibodies in type 1 diabetes mellitus (T1DM) and further to investigate the human leukocyte antigen (HLA) class II DR-DQ genotypes associated with T1DM in Ugandan children and adolescents. Cross-sectional data were collected between January and December 2015 from 85 recently detected T1DM children and adolescents and 79 age-matched healthy controls. We measured serum concentrations of C-peptide, vitamin D, insulin autoantibodies (IAA), zinc transporter family member 8 antibodies (ZnT8-Ab), and glutamic acid decarboxylase autoantibodies (GADA). HLA-DBR1 and HLA-DQB1 typing was performed on EDTA-anticoagulated blood samples. The *t* test, chi-square test, and univariate logistic test were performed and multivariate logistic regression model fitted to identify associated factors of T1DM. Positive IAA and ZnT8-Ab were significantly higher in T1DM than in controls. GADA showed no difference between TIDM and controls. HLA-DQB1*02, unadjusted odds ratio (UOR) 4.2 (95% CI 1.4–12.7), and HLA-DBR1*04, adjusted odds ratio (AOR) 30.6 (95% CI 5.7–161.7), were significantly associated with T1DM. IAA and ZnT8-Ab are the likely significant positive antibodies in Ugandan children and adolescents with T1DM. The T1DM was associated with HLA-DQB1*02 and HLA-DBR1*04 (HLA-DR3 and HLA-DR4) genotypes.

Keywords Type 1 diabetes · Autoantibodies · HLA class II · Uganda

Introduction

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease characterized by the selective destruction of pancreatic β cells, with consequent absolute insulin deficiency, impaired glucose homeostasis, and physiological dependence on exogenous insulin [1]. The pathogenesis of the disease is determined by complex interactions between several genetic loci and environmental factors [2]. The incidence of T1DM is increasing worldwide and across all age groups in parallel with increased standard of living [3]. Global annual estimates by the DiaMond project were an increase of 2.4% for the period 1990–1994 and 3.4% for the period 1995–1999 [4]. This rise in T1DM incidence has continued unabated at an annual rate of approximately 3% throughout much of the world [5–8]. Few data are available from sub-Saharan Africa, but childhood-onset T1DM appears to have previously been rare [9], although this may have been due to high mortality of individuals with T1DM before diagnosis [6, 7, 10]. Recent reports on T1DM in sub-Saharan Africa

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suggest that childhood-onset T1DM is increasing [8, 11, 12] and cite genetic and environmental factors as the underlying drivers [3, 13, 14].

The major determinants of genetic susceptibility to T1DM reside in the human leukocyte antigen (HLA) class II region especially DR and DQ loci [15]. Individuals with the highest risk for T1DM express both predisposing haplotypes: DQA1*05:01-DQB1*02:01 [DQ2], frequently inherited with DRB1*03:01 [DR3]; and DQA1*03:01-DQB1*03:02 [DQ8], frequently inherited with DRB1*04:01 or DRB1*04:02 [DR4] [6, 16–20]. This high HLA susceptibility for T1DM is often simply referred to as DR3/DR4 and DQ2/DQ8 heterozygosity. An environmental triggering agent in individuals with high susceptibility leads to pancreatic β cell autoimmunity, with consequent pancreatic β cell destruction and insulin deficiency.

Beta cell destruction in T1DM may be assessed by measuring serum autoantibodies against incompletely identified islet cell cytoplasmic or molecularly defined antigens [21, 22] while insulin deficiency may be assessed by measuring C-peptide levels [23–26]. The most prevalent autoantibodies are directed at the 65 kDa isoform of glutamic acid decarboxylase (GADA) [21, 22, 27], the tyrosine phosphatase-like protein (IA)-2, insulin (IAA), and the zinc transporter family member 8 (ZnT8-Ab) [27]. Up to 90% of newly detected T1DM in Caucasians have one or more of these autoantigens and thus constitute the hallmark of immune-mediated T1DM in this population. Therefore, HLA allele typing and defining presence of autoantibodies against islet cell cytoplasmic antigens may assist in determining risk for T1DM and in understanding the pathogenesis of T1DM in various populations [28]. Moreover, the future trend is to incorporate some of these biological markers into the standard care of confirming the diagnosis of T1DM [27]. In sub-Saharan Africa, the impact of the immune markers in disease classification and diagnosis is still uncertain, with little information on the epidemiological, immunological, and genetics of childhood-onset T1DM. To address this gap, we investigated autoantibodies (GADA, ZnT8-Ab, IAA) and HLA class II haplotypes (HLA DRB1- DQB1 alleles) associated with T1DM in children and adolescents of Ugandan ethnicity.

Materials and methods

Study population

The study involved 19 health facilities with specialized T1DM clinics in the various regions of Uganda. The T1DM clinics are part of an ongoing World Diabetes Foundation (WDF)-sponsored project (WDF09-457)/Changing Diabetes in Children (CDiC®) program support by Novo Nordisk. The clinics were primarily started with the goal of improving care of children and adolescents with T1DM. The diagnosis of T1DM was made according to the established criteria [29–31]. All T1DM participants were on insulin therapy. Using a case-control study design, 85 individuals (43 females; 42 males; mean age ± standard deviation [SD] 15.9 ± 5.0 years) with recently (less than 12 months) detected T1DM and 79 healthy controls (53 females; 26 males; mean ± SD; 17.3 ± 3.7 years) were enrolled into the study between January 2015 and December 2015. In both the T1DM and controls, chronological age varied between 1 and 28 years. Six controls, all males and below the age of 10 years from different health facilities, declined participation, giving "fear of a blood sample being taken off" as reason for non-participation. The sample size calculation was based on a likelihood of at least a single positive autoantibody prevalence in T1DM of about 30 versus 10% in healthy individuals [32], non-response rate of 15%, and power of 80%. All the study participants had a complete medical history (including family history of diabetes and date of onset of diabetes, where applicable) followed by anthropometric measurements (age, weight, height, blood pressure) and venous blood samples drawn for blood chemistry and immunological studies. Participants with diabetes were enrolled after verification of the following criteria: (i) Ugandan ethnicity (both grandparents being black Ugandans) and having lived in Uganda at least for 6 months prior to diagnosis of diabetes, (ii) diagnosis of diabetes made within the past 12 months, (iii) chronological age of less than 30 years, and (iv) body mass index (BMI) not more than 30 kg/m^2. Controls were of Ugandan ethnicity, required to have lived in Uganda for at least the previous 6 months, were feeling healthy and had normal fasting plasma glucose (3.9–5.5 mmol/l). In addition, controls were matched for age (± 2 years), had no family history of diabetes, and no known immunological disease or clinically evident infection.

All laboratory procedures (except glycosylated hemoglobin [HbA1c] which was analyzed with Haemocue HbA1c 501® Analyser and plasma glucose which was analyzed with Haemocue RT201® Analyser, both procedures as point of care) were carried out at MBN Clinical Laboratory, Kampala, Uganda.

Fasting plasma glucose and glycosylated hemoglobin (HbA1c)

All participants were requested to fast from food and drinks from midnight and refrain from smoking and exercise before blood samples were taken in the morning between 07:00 and 09:00 am. Plain water could be taken ad lib.

A blood sample from a finger prick was taken for the measurement of fasting plasma glucose (Haemocue RT201® Analyser) and glycosylated hemoglobin (HbA1c) (Haemocue HbA1c 501® Analyser). The finger was first cleaned with cotton using plain water and then dried with

dry cotton. A single-use safety lancet (Unistik® 3, Comfort; Owen Mumford) was then used to prick the finger.

C-peptide, vitamin D, and antibody assays

A fasting venous blood sample for C-peptide, serum vitamin D, zinc transporter family member 8 antibodies (ZnT8-Ab), insulin autoantibody (IAA), and anti-glutamic acid decarboxylase antibody (GADA) was collected in a plain "red-top" venipuncture tube without additives. The blood was allowed to clot and then centrifuged to separate the serum from the cells. The serum was then immediately refrigerated to 2–8 °C and transported on a cold chain to St. Francis Hospital Central Laboratory and stored at 70 °C until analysis at MBN Clinical Laboratory. Freezing and thawing were especially avoided. C-peptide was analyzed using a microplate immunoenzymometric assay (TYPE 3) using kits purchased from Monobind Inc. Lake Forest, CA 92630, USA. Serum vitamin D concentrations were determined by Elecsys® vitamin D total test (Roche Diagnostics International Ltd. CH-6343 Rotkreuz, Switzerland). Anti-zinc transporter family member 8 antibodies (Zn8T-Ab) were analyzed by the ELISA enzyme immunoassay using Anti-ZnT8-Ab ELISA Assay Kits purchased from Eagle Biosciences, Inc. 20A NW Blvd, Suite 112, Nashua, NH 03063, USA. Antibodies to insulin in human serum (IAA) were determined by IAA ELISA ASSAY KIT purchased from Eagle Biosciences, Inc. 20A NW Blvd, Suite 112, Nashua, NH 03063, USA. A cut-off of less than 0.2 nmol/l for fasting C-peptide was used to define the categories of low and preserved C-peptide level [25]. In descriptive analysis, vitamin D levels were categorized insufficient (< 30 ng/ml) and sufficient (≥ 30 ng/ml) [33]. Antibody positivity was assigned according to the manufacturer's assay kit specifications.

Human leukocyte antigen (HLA) typing

Five milliliters of blood samples were collected in EDTA anticoagulant-containing venipuncture tubes and immediately transported to St. Francis Hospital, Kampala and stored at 2–8 °C until processed at MBN Clinical Laboratory, Kampala. HLA genotyping for DRB1 and DQB1 loci was performed using the DQ-DR Combi Tray Kit, Catalog number 101.704 (*Olerup* SSP AB Stockholm, Sweden; www.olerup-ssp.com). DNA was extracted from whole blood using the QIAmp DNA Mini Kit and spin columns (QIAGEN GmbH Hilden, Germany) and kept at minus 20 °C until used. Subsequent HLA typing was performed by polymerase chain reaction-sequence specific priming (PCR-SSP) method using micro-generic HLA DNA typing trays according to the manufacturer's protocol (GeneAmp PCR System 9700 Thermocyler, Applied Biosystems Inc. California; and Olerup SSP®, AB Stockholm, Sweden htttp://www.olerup.com/software/score). The software generated the HLA genotype including the common and rare alleles in the HLA-DRB1 and HLA-DQB1 loci. In the case of ambiguous or un-interpretable results, PCR tests were repeated and DNA bands re-analyzed. Nomenclature and serological equivalents were reported in accordance with the common and well-documented (CWD) catalog [34–36]. Because this typing system did not target all polymorphic sites and was of a low resolution, some alleles were not distinguished. That is, serologic specificity/broad specificity (two digit—allele group) was always obtained, but not always defining the accompanying encoding protein (four digits) resolution. To address this issue in the analysis, bivariate statistical analysis was done on the allele groups (which can be determined by serologic typing) and allele subtypes (which represent different amino acid sequences of encoded protein). For the logistic regression model, HLA-DRB1 and HLA-DQB1 alleles were collapsed into three categories each based on the premise that individuals with HLA-DRB1*03, *04; DQB1*02, and *03 genotypes have a high susceptibility of developing T1DM [27, 37]. The categories were designated HLA-DRB1*03, HLA-DRB1*04, and HLA-DRB1*XX; and HLA-DQB1*02, HLA-DQB1*03 HLA-DQB1*XX, where XX denoted the rest of the HLA alleles. The categories of *XX were taken as the references in logistic regression models.

Statistical analysis

The Shapiro-Francia test was used to assess if variables had a normal distribution [38]. Comparison of means between groups of cases and controls was performed by the Student's *t* test or Fisher-Pitman permutation test (nonparametric values) [39]. Categorical variables were analyzed with chi-square test or Fisher-Freeman-Halton test, as appropriate, to test for existence of associations. Adjusted odds ratios (AOR) and unadjusted odds ratios (UOR) with 95% confidence intervals (CI) were calculated under logistic regression to study the effect of the potential covariates on the T1DM. STATA version 14 (StataCorp LP, Texas, USA) statistical package was used for data analysis. For all tests, unless otherwise stated, the p value < 0.05 was considered to test the level of significance.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

The anthropometric, clinical, and immunological characteristics of the study participants are presented in Table 1.

Table 1 Anthropometric, clinical, genetic, and immunological characteristics of study participants

Characteristic Sample distribution (*n*)	Patients with T1DM 85	Control 79	*p* value 164
Age in years (mean ± SD) [range]	15.9 ± 5.0 [0.9–24.9]	17.3 ± 3.7 [7.3–27.9]	0.04
Sex ratio (F:M)	43:42	53:26	0.032
Weight [kg] (mean ± SD)	44.9 ± 13.9	48.4 ± 13.0	0.09
Height [cm] (mean ± SD)	152.3 ± 17.0	155.6 ± 13.0	0.17
BMI [kg/m^2] (mean ± SD)	18.9 ± 3.8	19.6 ± 3.6	0.24
SBP[a] [mmHg] (mean ± SD)	107 ± 14.9	111 ± 9.5	0.11
DBP[a] [mmHg] (mean ± SD)	69.9 ± 13.1	68.1 ± 9.2	0.39
PG [mmol/l] (mean ± SD)	13.2 ± 7.1	5.6 ± 2.3	< 0.001
HbA1c [%] (mean ± SD)	9.3 ± 2.9	4.8 ± 0.04	< 0.001
C-peptide[a] [ng/ml] (mean ± SD)	0.78 ± 1.26	1.49 ± 1.49	0.007
Vitamin D [ng/ml] (mean ± SD)	46.62 ± 25.50	39.92 ± 48.40	0.26
GADA[a], positive [%] (CI)	4.9 (1.8–12.4)	29.7 (19.7–42.1)	0.08
IAA, positive [%] (CI)	76.8 (66.3–84.8)	60.9 (48.4–72.2)	< 0.001
ZnT8-Ab, positive [%] (CI)	18.3 (11.2–28.3)	9.3 (4.2–19.5)	0.05

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PG* plasma glucose, *HbA1c* glycosylated hemoglobin, *GADA* glutamic acid decarboxylase, *IAA* insulin auto antibody, *ZnT8-Ab* zinc transporter 8 autoantibody, *CI* confidence interval

[a] Missing data for ≥ 1% of participants: SBP and DBP, 30 patients with T1DM and 24 in controls anti-GADA65, 6 patients with T1DM and 2 in controls; C-peptide, 37 patients with T1DM and 4 in controls

Differences were observed in the mean age, plasma glucose (PG) concentrations, level of glycated hemoglobin (HbA1c), C-peptide concentration, insulin autoantibodies, and zinc transporter family member 8 autoantibodies (ZnT8-Ab). The mean age was significantly lower in T1DM participants than in controls ($p = 0.04$); mean ± standard deviation (SD) was 15.9 ± 5.0 years (range 0.9–24.9) and 17.3 ± 3.7 (7.3–27.9). The PG concentration and HbA1c level were all significantly higher in participants with T1DM than in the controls ($p < 0.001$). The mean ± SD plasma glucose in T1DM, 13.2 ± 7.1 mmol/l, was significantly higher than in controls, 5.6 ± 2.3 mmol/l ($p < 0.001$). The mean ± SD HbA1c was significantly higher in T1DM, 85.1 mmol/mol, than in controls, 29.4 mmol/mol ($p < 0.001$). The mean ± SD C-peptide concentration was significantly lower in T1DM than in controls ($p = 0.007$), 0.78 ± 1.25 and 1.49 ± 1.48 nmol/l respectively. No significant difference was observed between T1DM and controls in the mean serum vitamin D concentrations; however, the proportion of participants with insufficient vitamin D levels (≤ 30 ng/ml) was significantly higher among controls than in T1DM participants.

The prevalence of positive IAA was higher in T1DM, 76.8% (95% CI 66.3–84.8) than in controls, 60.9% (95% CI 48.3–72.2) ($p < 0.001$). The prevalence of positive ZnT8-Ab was higher in T1DM, 18.3% (95% CI 11.3–28.3), than controls 9.3% (95% CI 4.2–19.5), ($p = 0.05$). The prevalence of positive GADA was 4.9% (95% CI 1.8–12.4) in T1DM and 29.7% (95% CI 19.7–42.1) in controls ($p = 0.08$). Table 2 summarizes the prevalence of autoantibodies, proportion of participants with preserved C-peptide, and insufficient vitamin D levels.

Table 3 displays the frequency of HLA-DRB1 and DQB1 alleles in T1DM and controls. DQB1*02 was significantly associated with T1DM, unadjusted odds ratio (UOR) 4.2 (95% CI 1.4–12.7) ($p = 0.01$). DQB1*02 allele had a frequency of 4:1 and was significantly associated with T1DM in comparison to the controls UOR 0.83 (95% CI 1.73–19.70) ($p = 0.005$).

In the logistic regression model, residing in the Central Region of Uganda [odds ratio (OR) 26.7 (95% CI 5.0–141.2)], having a normal level of vitamin D [OR 10.6 (95% CI 3.0–37.3)], being positive for IAA [OR 23.4 (95% CI 5.6–97.8)], and possessing HLA-DRB1*04 allele [OR 30.2 (95% CI 5.7–161.7)] were significantly associated with T1DM ($p < 0.01$).

Discussion

Data on T1DM in Uganda is scanty. This study involved children and adolescents with recently detected T1DM, who were consecutively enrolled into the study from 19 health units from several regions of Uganda. The T1DM participants consisted of 42 males and 43 females, mean age (mean ± SD) 15.9 ± 5.0, range 0.9–24.9 years. The observed mean age of the T1DM participants is lower than the commonly reported peak incidence of T1DM in the African population of 20–29 years [12], but within the range reported for the

Table 2 Prevalence of Autoantibodies; proportion of participants with preserved C-peptide; and sufficient Vitamin D

Characteristic	*n* (T1DM/controls)	T1DM (%)	Controls *n* (%)	*p* value
GADA [a], positive % (95% CI)	73/76	4.9 (1.8–12.4)	29.7 (19.7–42.1)	0.08
ZnT8-AbA, positive % (95% CI)	85/79	18.3 (11.3–28.3)	9.3 (4.2–19.5)	0.05
IAA, positive % (95% CI)	85/79	76.8 (66.3–84.8)	60.9 (48.3–72.2)	< 0.001
C-peptide[a], % ≥ 0.2 nmol/l (95% CI)	48/75	50.0 (35.9–64.0)	85.3 (75.2–91.7)	< 0.001
Vitamin D, % ≤ 30 ng/ml (95% CI)	85/79	29.4 (20.6–40.1)	62.0 (50.7–72.1)	< 0.001

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* glycosylated hemoglobin, *GADA* glutamic acid decarboxylase, *IAA* insulin auto antibody, *ZnT8-Ab* zinc transporter 8 auto-antibody, *n* positive units in a subgroup

[a] Missing data for ≥ 1% of participants: GADA65, 6 patients with T1DM and 2 controls; C-peptide, 37 patients with T1DM and 4 Controls

Western World [40]. Mean C-peptide concentration (mean ± SD; 0.78 ± 1.25 nmol/l) in T1DM group was low, a reflection of insulin deficiency characteristic of T1DM found in other studies [23, 24, 26, 32]. The degree of insulin deficiency was however modest, as only 50.0% (95% CI 35.9–64.0%) had C-peptide concentration < 0.2 nmol/l. C-peptide concentration < 0.2 nmol/l in the fasting state is associated with decreased endogenous insulin secretion and β cell dysfunction [23, 24]. Furthermore, fasting C-peptide concentration in T1DM declines with duration of diabetes and therefore moderately preserved C-peptide secretion is expected in the recently detected diabetes, as in these participants [26].It is however important to note that C-peptide > 0.2 nmol/l may also be attributed to some participants not disclosing a non-fasting state, which is associated with an elevation of C-peptide. C-peptide > 0.2 nmol/l is also observed in children and adolescents with maturity onset diabetes in the young (MODY) or type 2 diabetes in the young [25]. While we excluded obese children to minimize the likelihood of including type 2 diabetes in the study, the exclusion criteria were uncertain on MODY, although highly unlikely, as assigning the diagnosis of T1DM at diagnosis did take into consideration family history of diabetes and degree of metabolic dysregulation, features characteristic of MODY for its exclusion.

About 80% of T1DM participants had glycosylated hemoglobin (HbA1c) greater than recommended target of below 53.0 mmol/l. Participants with T1DM were enrolled at the first opportunity (after clinical stabilization) following the detection of diabetes. The HbA1c was therefore not expected to have substantially declined.

There were differences in the mean concentrations of vitamin D levels between T1DM and controls, although the proportion of individuals with insufficient vitamin D levels was higher among controls than individuals with T1DM. Similar findings have been observed in other studies [3, 41]. Some studies have, however, reported a protective effect of vitamin D against development of T1DM [42] while others have reported an inverse association of vitamin D and T1DM [43].

This study examined the prevalence IAA, ZnT8-Ab, and GADA autoantibodies between T1DM and healthy controls. IAA had the highest positive prevalence among T1DM: 76.8% (95% CI 66.3–84.8) versus 60.9% (95% CI 48.3–

Table 3 Frequency of HLA-DRB1 and DQB1 Alleles group (serological) in participants with T1DM and the healthy control

HLA-DRB1 allele	TID[a] (*n* = 74) AF	Controls[a] (*n* = 69) AF	UOR (95% CI)	*p* value	HLA-DQB1 allele	TID (*n* = 74) AF	Control (*n* = 69) AF	UOR (95% CI)	*p* value
DRB1*01	4	3	1.1 (0.2–7.5)	0.91	DQB1*02	16	4	4.2 (1.4–12.7)	0.01
DRB1*03	5	1	4.2 (0.4–48.4)	0.25	DQB1*03	9	11	0.952 (0.3–2.6)	0.92
DRB1*04	1	–	–	–	DQB1*04	8	4	1.4 (0.4–5.3)	0.62
DRB1*07	4	2	1.7 (0.2–13.2)	0.63	DQB1*05	17	15	1.3 (0.4–3.9)	0.64
DRB1*08	3	5	0.5 (0.1–3.2)	0.46	DQB1*06	24	35	1.00.	–
DRB1*09	7	3	1.9 (0.3–11.7)	0.47					
DRB1*10	2	–	–	–					
DRB1*11	11	18	0.5 (0.1–2.1)	0.35					
DRB1*12	1	1	0.8 (0.0–17.0)	0.91					
DRB1*13	23	15	1.3 (0.3–4.9)	0.72					
DRB1*15	7	16	0.4 (0.1–1.6)	0.18					
DRB1*16	6	5	–	–					

T1DM type 1 diabetes, *UOR* unadjusted odds ratio, *AF* allele frequency

[a] Missing data of > 1% of participants: 11 in T1DM and 10 healthy controls

72.2) in controls; followed by ZnT8-Ab: 17.6% (95% CI 10.8–27.4%) in T1DM versus 7.6% (95% CI 3.4–16.0%) in controls. There was no difference in the prevalence of GADA-positive antibodies between the T1DM and the control group: 44.3% (95% CI 33.6–55.5%) versus 58.4% (47.0–69.0%).

Insulin auto antibodies are known to peak early in the development of T1DM and decline following the onset of clinical symptoms of the disease [44]. An association between levels of IAA and risk for T1DM has been reported [45]. In this study, the proportion of T1DM with IAA-positive antibody titers above 4.0 U/ml (high titer) was 78.8% (95% CI 68.7–86.3) and 34.2% (95% CI 24.5–45.4) in the control group with UOR of 7.2 likelihood to have T1DM [($p < 0.001$); 95% CI 3.6–14.4]. In association with other autoantibodies, IAA may be a good predictor of T1DM [45]. However, all the T1DM participants were on insulin therapy, which is associated with the development of IAA and may therefore partly explain the high prevalence of positive IAA observed in T1DM. The relatively high prevalence of IAA in the control group could imply a significant number of healthy individuals are likely to develop T1DM in the future [46].

Overall, GADA positivity had a prevalence of 4.9% in T1DM and with no significant difference from controls (29.7%). This is in contrast to other studies especially in Caucasians [46]. Asanghanwa et al. [32] reported a prevalence of 24% in T1DM versus 7% in controls for GADA in Cameroon T1DM individuals. GADA positivity may be found in other forms of impaired β cell function [47], and together with the finding of increased IAA positivity among the control group in this study, it is tempting to speculate future β cell dysfunction in the non-diabetic control group [48].

The prevalence of ZnT8-Ab antibodies in this study was attenuated but significant: 18.3% in T1DM versus 9.3% in controls. Similar low rates of ZnT8-Ab positivity have been reported elsewhere in African populations [12, 32, 49, 50]. There are no clear reasons for low antibody positivity in some populations [12, 45]. In this study, the prevalence of at least one positive antibody in T1DM versus controls was 96.5% (95% CI 89.5–98.9%) and 81.0% (95% CI 70.7–88.3%); for at least two antibodies was 48.2% (95% CI 37.7–58.9%) and 32.9% (95% CI 23.4–44.1%); for at least three antibodies was 3.5% (95% CI 1.1–10.5%) versus 5.1% (1.9–12.9%). Asanghanwa and co-workers reported an overall prevalence of 29% in patients with T1DM having at least one diabetes-associated antibody versus 9% in healthy controls among Cameroonians [32]. The study of Cameroonian patients did not include IAA, which may be contributing to the high prevalence of the presence of at least one positive autoantibody being detected in T1DM in this study.

In this study, HLA-DQB1*02 and HLA-DRB1*04 were significantly associated with T1DM suggesting that high-risk genotypes HLA-DQB1*02 and HLA-DRB1*04 are involved in the pathogenesis of T1DM in the Ugandan population. DRB1*03-DQB1*02:01 (alleles often referred to as DR3) and DRB1*04-DQB1*03:02 (alleles often referred to as DR4) have been known to carry a high susceptibility for developing T1DM [37]. Type 1 diabetes is known to be strongly associated with HLA-DRB1*03-DQB1*02:01 (DR3) and HLA-DRB1*04-DQB1*03:02 (DR4) haplotypes, alone or in combination [51].

Residing in the central region of Uganda, having a normal range of vitamin D level and having HLA-DRB1*04 haplotype were independently associated with having T1DM. The incidence of T1DM has been shown to increase in parallel with the standard of living [3]. This may be the underlying factor of an independent association of T1DM and living in the central region. The diagnosis of T1DM is more likely to be made in central region than elsewhere in Uganda because of the access to healthcare facilities. Three of the four initial specialized type 1 diabetes clinics started in the central region of Uganda in 2009. Having a normal level of vitamin D was significantly associated with T1DM. It may be speculated that this is because in Uganda, when children fall ill, the first medications are given alongside multivitamins that contain vitamin D (personal observation).

Conclusion

Insulin autoantibodies (IAA) and of zinc transporter family member 8 antibodies (ZnT8-Ab) are the likely significant positive antibodies in Ugandan children and adolescents with T1DM. Type 1 diabetes in the Ugandan children and adolescents is associated with high-risk HLA-DQB1*02 and HLA-DRB1*04 genotypes.

Limitations

This study has some limitations. This was a cross-sectional study and therefore unable to draw conclusions on cause and effect. Although insulinoma-associated-2-autoantiobodies (IA-2A) and islet cell cytoplasmic autoantibodies (ICA) were not measured because of financial constraints yet their estimation would have shed more light on the antibody pattern of T1DM in the Ugandan population. Only a limited scope of HLA typing was carried out, leaving some haplotypes not typed to the gene-specific level. The gene-specific level (protein encoding) is the desired level at which the determination of the genes conferring risk or protection against development of type 1 diabetes is fully assessed. Although unlikely, there was a possibility that the type 1 diabetes group may have included patients with other forms of diabetes, notably MODY. Despite these limitations, this study is important as it is the first study to report the presence of factors for increased susceptibility of developing type 1 diabetes in Ugandan children and adolescents. Indeed, the study further

flags on the fears that the disease is not rare in this population, as previously suggested, but is fatally misdiagnosed. More studies are required in this field of T1DM.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study: children aged 8–17 years assented to participate, with parents of children below age of 18 years giving the informed consent. Participants above the age of 18 years were eligible and gave informed consent to participate.

Ethical approval Ethical approval was obtained from St. Francis Hospital, Nsambya and Mulago Hospital Institutional Review Boards/ Review Ethical Committees; both of which are accredited by the Uganda National Council of Science and Technology (UNCST). All procedures performed in this study were in accordance with the ethical standards of UNCST and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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ORIGINAL ARTICLE

Zinc transporter 8 autoantibody (ZnT8A) by ELISA for diagnosing type 1 diabetes among Chinese people

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Abstract
This study aimed to evaluate the utility of enzyme-linked immunosorbent assay (ELISA) to measure ZnT8A for diagnosing type 1 diabetes among Chinese people. We recruited a group of 95 patients with type 1 diabetes, 130 patients with type 2 diabetes, and 110 subjects without diabetes. We measured ZnT8A level by ELISA and glutamic acid decarboxylase antibody (GADA) level by radioimmunoassay. We collected data on their history-based variables, body mass index (BMI), fasting blood glucose, glycosylated hemoglobin, and lipid levels. 24.2% were positive for ZnT8A in type 1 diabetics, compared to 0.0% in type 2 diabetics and 0.9% in the participants without diabetes (both $p < 0.001$). And the type 1 diabetics had higher ZnT8A level compared with the latter two groups (both $p < 0.001$). The frequency of ZnT8A in the "classical" type 1 diabetics was higher than that in patients with latent autoimmune diabetes in adults (45.0 vs. 18.7%, $p < 0.05$). The frequency/level of ZnT8A was higher in the youngest group (all $p < 0.05$). The ROC curve area was 0.892. The combination of ZnT8A and GADA increased the diagnostic sensitivity. The ZnT8A level was correlated with the GADA level. ZnT8A-positive type 1 diabetics had younger age at diagnosis ($p = 0.022$), lower BMI scores ($p = 0.016$), and more frequent ketosis ($p = 0.034$) and needed more insulin ($p = 0.041$) than ZnT8A-negative type 1 diabetics. This study demonstrated the value of ZnT8A in addition to GADA for the diagnosis of type 1 diabetes.

Keywords Zinc transporter 8 autoantibody · Enzyme-linked immunosorbent assay · Glutamic acid decarboxylase antibody · Type 1 diabetes mellitus · Chinese

Introduction

Type 1 diabetes (T1D) is one kind of organ-specific autoimmune disease related to the autoimmune response against pancreatic β-cells. Measurement of the autoantibodies against pancreatic β-cell antigens, such as glutamic acid decarboxylase antibody (GADA), anti-insulin autoantibody (IAA), insulinoma-associated antigen-2 antibody (IA-2A), and islet cell autoantibody (ICA), is helpful to diagnose T1D. Zinc transporter 8 (ZnT8), an islet-specific gene product localized to the β-cell insulin granule, is recently identified as an autoantigen in T1D, and the autoantibodies to ZnT8 (ZnT8A) become an additional and independent diagnostic marker for T1D [1]. The introduction of ZnT8A in the routine diagnostic process of T1D may improve the overall autoantibody sensitivity [2]. However, the use of ZnT8A test is uncommon in Chinese medical field. Furthermore, ZnT8A is often measured by radioligand binding assay. Few studies have measured ZnT8A by enzyme-linked immunosorbent assay (ELISA), which is easy to implement and convenient to apply [3–5]. Therefore, we designed this study to evaluate the utility of ELISA to measure ZnT8A for diagnosing T1D among Chinese people.

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Subjects and methods

Subject selection

This cross-sectional association finding study was approved by the ethical committees of the local hospital and complied with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Ninety-five patients with T1D were enrolled from the Department of Endocrinology from January 2014 to April 2016. The median age was 33 (range 12–73) years with 58 males and 37 females. One hundred and thirty patients with type 2 diabetes mellitus (T2D) included 78 males and 52 females, and the median age was 52 (range 19–84) years. Patients with T1D and T2D were diagnosed according to the American Diabetes Association [6] and confirmed by reviewing laboratory data and medical records or by communicating with the participants' primary care physicians. Patients with T1D were classified as patients with latent autoimmune diabetes in adults (LADA) ($n = 75$) if their ages at onset were not less than 18 years and they had no requirement for insulin for at least 6 months after diagnosis, with GADA positivity [7]. Patients with T1D were classified as "classical" type 1 diabetics ($n = 20$) if they needed insulin therapy within 6 months after diagnosis [8]. Subjects ($n = 110$) without diabetes or other autoimmune diseases were recruited from healthy volunteers as a control group. All volunteers maintained normal oral glucose tolerance and had no family history of diabetes.

Data collection

We interviewed the subjects to collect data on their demographic, height, and weight. Body mass index (BMI = weight/height ^{2}) was computed. We noted the age at diagnosis, duration of diabetes, the insulin dose, and the appearance of ketosis at the 6 months after diagnosis. Ketosis was diagnosed by the elevation of urine and/or serum ketone bodies [9]. ZnT8A level was analyzed using ELISA kit (BioVendor-Laboratorni Medicina, Brno, Czech Republic) according to the manufacturer's specifications. GADA level was measured by a radioimmunoassay kit (CIS Bio-International, Gif sur Yvette, France) according to the manufacturer's instructions [10]. The cutoff value of ZnT8A and GADA positivity in our lab was 15 and 1 U/ml, separately. The concentrations of fasting blood glucose (FBG), plasma cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride were measured using an AU-2700 automated analyzer (Olympus, Mishima, Japan). Hemoglobin A1c (HbA1c) was measured using a DCA Vantage analyzer (Siemens Medical Solutions Diagnostics, NY, USA).

Statistical analysis

SPSS 13.0 software (IBM SPSS, Chicago, IL, USA) was used for data analysis. After testing for normality with Kolmogorov–Smirnov test, all normally distributed data were expressed as mean ± standard deviation, and non-normally distributed data were expressed as the median (interquartile range). Categorical data were expressed with numbers or percentage. Differences in parametric data were tested by t test. Differences in non-parametric data were analyzed by the Mann–Whitney U test or Kruskal–Wallis H test. Frequency differences were compared with chi-square test (with Yates continuity correction) or Fisher exact test when appropriate. Bivariate correlation was used to analyze the correlation between the concentration of ZnT8A and GADA. Receiver operator characteristic (ROC) curve analysis was used to evaluate the performance of testing ZnT8A level by ELISA for screening T1D. $p < 0.05$ was considered to be statistically significant, and the significance threshold became $p < 0.017$ when three comparisons for each test were involved [11].

Results

Positivity and level of ZnT8A

Twenty-three (24.2%) out of 95 were positive for ZnT8A in the patients with T1D, compared to 0/130 (0.0%, Pearson chi-square = 35.057, $p = 0.000$) in the patients with T2D and 1/110 (0.9%, Pearson chi-square = 26.776, $p = 0.000$) in the participants without diabetes. The type 1 diabetic patients had higher level of ZnT8A than the type 2 diabetic patients (median 13.59 vs. 6.67, $Z = -9.835$, $p = 0.000$) and the participants without diabetes (median 13.59 vs. 2.76, $Z = -9.877$, $p = 0.000$). The type 2 diabetic patients had higher concentration of ZnT8A compared with the participants without diabetes (median 6.67 vs. 2.76, $Z = -7.581$, $p = 0.000$).

The frequency of ZnT8A in the "classical" type 1 diabetics was higher than that in the LADA patients (45.0 vs. 18.7%, continuity correction = 4.618, $p = 0.032$). The ZnT8A level in the "classical" type 1 diabetics was higher than that in the LADA patients (median 14.00 vs. 13.42, $Z = -2.013$, $p = 0.044$). The frequency of ZnT8A in the type 1 diabetic patients aged < 18 years was higher than that in the 18- to 40-year-old type 1 diabetic patients (63.6 vs. 20.9%, continuity correction = 5.750, $p = 0.016$) and that in the type 1 diabetic patients aged > 40 years (63.6 vs. 17.1%, continuity correction = 7.338, $p = 0.007$). The ZnT8A level in the type 1 diabetic patients aged < 18 years was higher than that in the 18- to 40-year-old type 1 diabetic patients (median 23.62 vs. 13.23, $Z = -2.459$, $p = 0.014$) and that in the type 1 diabetic patients aged > 40 years (median 23.62 vs. 13.70, $Z = -2.521$, $p = 0.012$). The frequency of ZnT8A (20.9 vs. 17.1%, Pearson

chi-square = 0.202, $p = 0.653$) and the ZnT8A level (median 13.23 vs. 13.70, $Z = -0.855$, $p = 0.393$) in the latter two groups had no significant difference.

ROC curve for ZnT8A

ROC curve was shown in Fig. 1. An area under the curve (AUC) of 0.892 (95% CI 0.853 to 0.930) indicates that the test had relatively high accuracy. ZnT8A demonstrated low sensitivity (25.3%) and high specificity (99.6%) for diagnosing T1D at a cutoff of 15 U/ml.

Combinations and associations between GADA and ZnT8A

Thirty-eight patients with T1D were GADA-positive alone, and seven were ZnT8A-positive alone. Additionally, 16 patients with T1D were 2-antibodies-positive. Other 34 patients with T1D were 2-antibodies-negative. The proportion of GADA positive was 100% for "classical" type 1 diabetics and 45.3% for LADA patients. The level of ZnT8A was correlated with the level of GADA (correlation coefficient = 0.447, $p = 0.000$). The combination of ZnT8A and GADA increased the diagnostic sensitivity from 56.8% (the GADA positivity) to 64.2%.

Clinical difference between type 1 diabetics with ZnT8A+ and ZnT8A–

The history-based, anthropometric and metabolic characteristics of the patients with T1D were shown in Table 1. The ZnT8A-positive type 1 diabetics had younger age at diagnosis and lower BMI scores than the ZnT8A-negative type 1 diabetics. Compared with the ZnT8A-negative type 1 diabetic patients, the ZnT8A-positive type 1 diabetic patients had more frequent ketosis and needed more insulin. No significant difference was found in duration of diabetes, FBG, HbA1c, and the lipids (cholesterol, triglyceride, LDL-C, and HDL-C) levels between the two groups.

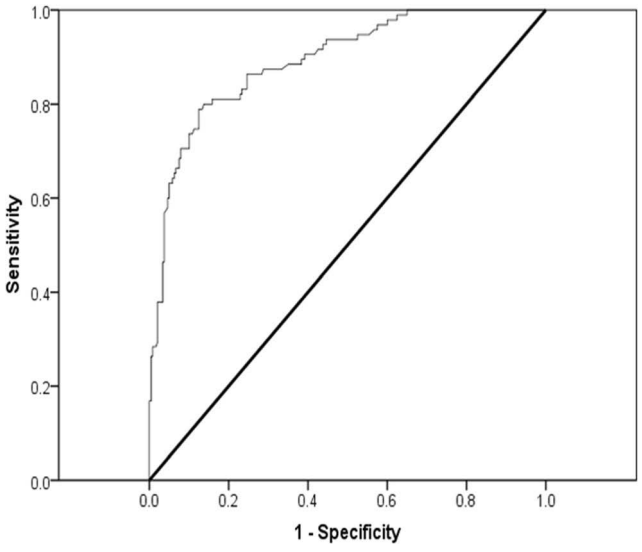

Fig. 1 ROC curve for ZnT8A as a screening test for type 1 diabetes

Discussion

As a novel β-cell-specific autoantibody, ZnT8A has widely clinical applications for screening T1D [12]. A study in UK reported that 54.3% patients with T1D were ZnT8A-positive [13]. Faccinetti et al. indicated that 65% Argentinian type 1 diabetic patients were ZnT8A-positive [14]. However, a much lower ZnT8A frequency (28%) was reported in Japanese patients with T1D [15]. In addition, the prevalence of ZnT8A was 31.8% in the Indian juveniles with T1D [16]. Our study found that the frequency of ZnT8A positivity in Chinese type 1 diabetics was 24.2%, which was in accordance with the frequency (24.1%) of ZnT8A measured by radioligand assay among our population with T1D [17]. Hence, the prevalence of ZnT8A in Asian patients with T1D may be lower, and the large difference of ZnT8A frequency worldwide warrants additional exploration.

The frequency of ZnT8A within LADA patients fluctuated from 6.2 to 32.4% [18, 19]. In current study, the ZnT8A frequency in LADA patients was 18.7%. The varying frequencies of ZnT8A in LADA patients might be due to geographical and ethnic differences. Besides, in terms of age, the diagnostic criteria are also different. In china, ≥ 18 years old at T1D onset would be diagnosed with LADA, whereas ≥ 30 years old at T1D onset would be considered so. The difference of age criteria may also result in the fluctuations of ZnT8A frequencies. Anyhow, the comparisons of ZnT8A positivity between "classical" type 1 diabetics and LADA patients were similar. It was previously published that the prevalence of ZnT8A positivity was higher in adult-onset "classical" type 1 diabetics as compared to that in LADA patients [19]. Collectively, our study showed the frequency of ZnT8A was 45.0% in "classical" type 1 diabetics vs. 18.7% in the LADA patients.

Lampasona et al. [20] reported that the prevalence of ZnT8A positivity in autoimmune patients with either GADA and/or IA-2A was higher in younger patients and declined with age: 12.2% in subjects aged 0–49 years; 6.6% in subjects aged 49.1–58.8 years; and 4.4% in subjects aged > 58.9 years. In our study, we found that the frequency of ZnT8A in the type 1 diabetic patients aged < 18 years was higher than that in the 18- to 40-year-old type 1 diabetic patients and that in the type 1 diabetic patients aged > 40 years. Moreover, the ZnT8A level in the type 1 diabetic patients aged < 18 years was also higher than that in the two latter groups. This was consistent with the reports previously published by Vaziri-Sani et al., who found ZnT8A titers were significantly higher in the 2–

Table 1 Clinical characteristics of the patients with type 1 diabetes

Variable	ZnT8A+ ($n = 23$)	ZnT8A− ($n = 72$)	$t/Z/\chi^2$	p
Age at diagnosis (years)	22 (24)	32 (31)	− 2.282[c]	0.022
Duration of diabetes (years)	2 (6)	3 (6)	− 1.011[c]	0.312
Ketosis	34.8%	12.5%	4.472[a]	0.034
Insulin dose (IU)	26.74 ± 11.32	21.15 ± 9.73	− 2.128[b]	0.041
BMI	21.72 (6.36)	24.89 (5.49)	− 2.415[c]	0.016
FBG (mmol/L)	9.34 (4.88)	9.23 (5.79)	− 0.547[c]	0.584
HbA1c (%)	8.80 (2.90)	8.65 (3.00)	− 0.578[c]	0.563
Cholesterol (mmol/L)	4.41 ± 1.07	4.55 ± 1.23	0.523[b]	0.604
Triglyceride (mmol/L)	1.85(0.98)	1.35(1.29)	− 1.772[c]	0.076
LDL-C (mmol/L)	3.05 ± 0.70	3.13 ± 0.92	0.435[b]	0.666
HDL-C (mmol/L)	1.05 ± 0.22	1.15 ± 0.31	1.639[b]	0.107

All normally distributed data were expressed as mean ± standard deviation, and non-normally distributed data were expressed as the median (interquartile range). Categorical data were expressed with percentage

BMI, body mass index; *FBG*, fasting blood glucose; *HbA1c*, hemoglobin A1c; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol

[a] Continuity correction value by chi-square test with Yates continuity correction

[b] t value

[c] Z value

17-year-old type 1 diabetic patients as compared to the 15–34-year-old cohort [21]. Nevertheless, another study, which was conducted in children and adolescents who were younger than 15 years and were newly diagnosed with diabetes, reported that the ZnT8A positivity was associated with older age [22]. As above, varying ages may lead to different effects on the ZnT8A frequency. Likewise, age of onset also has an effect on the ZnT8A frequency. It was reported that the prevalence of ZnT8A declined with the increasing age at diagnosis in Chinese and Belgian patients with T1D [17, 23]. Besides, the children aged 6–10 years had higher prevalence of ZnT8A than other older French within 6 months of T1D onset [5]. In this study, the age at diagnosis of ZnT8A-positive group was younger than that of ZnT8A-negative group. However, the ZnT8A prevalence increased with increasing age of onset to a plateau at 8–16 years and then fell [24]. Taken together, it is speculated that the age at diagnosis may play an important role in the ZnT8A level.

The Diabetes Antibody Standardization Program workshop identified that ZnT8A measured by radioligand assay showed a median ROC-AUC of 0.848 [25]. Dunseath et al. [13] showed an AUC of 0.80, 54% sensitivity, and 99% specificity by conducting ROC analysis of ZnT8A by ELISA in type 1 diabetics and healthy blood donors. In the present study, the ROC-AUC for ZnT8A by ELISA was 0.892. Besides, the sensitivity in our study was 25.3% and the specificity was 99.6%. Hence, we speculate that the ZnT8A test by ELISA may have high accuracy, not lower than the test by radioligand assay. Additionally, ZnT8A by ELISA, as a convenient and reproducible method, achieves a high degree of specificity and may be suitable for more widespread clinical application.

ZnT8A, as an additional and independent predictive marker for T1D, was found in 26% of type 1 diabetics previously classified as autoantibody-negative on the basis of GADA, IA-2A, IAA, and ICA [26]. In addition, it has been reported that the positive prevalence of ZnT8A in phenotypic T2D patients was 1.99%, and the ZnT8A assay could enhance the diagnostic prevalence of LADA based on GADA and IA-2A positivity [27]. As a consequence, the discriminatory value of a positive ZnT8A in differentiating LADA from youth onset T2D plays a role in the precise diagnosis and early insulin treatment for LADA patients. This study demonstrated that the combination of ZnT8A and GADA increased the diagnostic sensitivity. As the GADA test was common in China, the introduction of ZnT8A should be recommended as soon as possible. Moreover, it was previously reported that ZnT8A was associated with a high GADA titer [20]. We found that the ZnT8A level was correlated with the GADA level. Further analysis should be carried out in order to confirm the relationship between the GADA and ZnT8A.

Mbanya et al. [28] exhibited that the ZnT8A-positive patients with diabetes had significantly lower BMI scores than the ZnT8A-negative ones. It was also observed decreasing frequencies of ZnT8A accompanied by increasing BMI, among 120 type 2 diabetic patients positive for at least one of the following autoantibodies: GADA, IA-2_{IC}A, and IA-$2_{(256–760)}$ A [29]. In the present study, the ZnT8A-positive type 1 diabetics had lower BMI scores than the ZnT8A-negative type 1 diabetics. The above results support the idea that leaner patients with diabetes may have higher frequency of ZnT8A.

ZnT8 is highly β-cell-specific, and the autoimmune damage by the presence of ZnT8A may result in severe insulin

deficiency. A study in Finland discovered that ZnT8A-positive children had lower serum C-peptide concentrations and higher insulin doses and were more likely to have ketoacidosis than their ZnT8A-negative peers [22]. Similarly, it was found that the ZnT8A-positive type 1 diabetic patients had more frequent ketosis and needed more insulin, as contrast to the ZnT8A-negative type 1 diabetic patients in the present study. Therefore, the ZnT8A positivity might reflect a more aggressive disease for the type 1 diabetics.

Yang et al. [17] found no difference in ZnT8A prevalence in subjects stratified with duration of diabetes. Collectively, there was no difference between the duration of type 1 diabetic patients with ZnT8A+ and ZnT8A− in the present study. By contrast, it has been reported that 61% of patients with T1D onset were positive for ZnT8A, and the proportion was only 33% in patients with T1D for more than 6 months [5]. The correlation between duration of diabetes and ZnT8A prevalence warrants additional exploration. Meanwhile, some longitudinal studies are needed for dynamic monitoring of ZnT8A titer in Chinese patients with T1D. Moreover, we found that the level of lipid, FBG, and HbA1c exhibited no significant difference between type 1 diabetic patients with ZnT8A+ and ZnT8A−. This finding agreed with the study reported by Mbanya et al. [28], who demonstrated that lipid profiles were similar in the ZnT8A-positive and ZnT8A-negative groups. Additionally, another study in China reported that no difference was observed between the HbA1c levels in the ZnT8A-positive alone group and in the 3-antibodies-negative (GADA-negative, IA-2A-negative, and ZnT8A-negative) group [17].

The level of IA-2A was not studied, which is one of the major limitations of the current study. Seventy-five percent of the type 1 diabetics recruited in this study were LADA patients, and the proportion of "classical" type 1 diabetics was so small that it might influence some results, such as the prevalence of ZnT8A in type 1 diabetics. As ZnT8A titers decline exponentially from the clinical onset of T1D, the low prevalence of ZnT8A in this study could be due to the inclusion of patients with long duration of T1D. In our following study, we will recruit more patients with T1D onset and further study the ZnT8A prevalence.

Overall, the credibility of ZnT8A by ELISA is worth recommending as an easy and convenient method, especially when it is combined with GADA. As an easy and convenient method, ZnT8A by ELISA has a widely potential for clinical applications in the diagnosis of T1D. ZnT8A may be correlated with some clinical characteristics in the patients with T1D.

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Authors' contributions Xuan Qiu: literature search, design, clinical studies, statistical analysis, manuscript preparation; Cuili Ning: clinical studies, data acquisition, manuscript editing; Lin Xiao: data analysis, manuscript editing; Zhenyun Mu: data analysis, statistical analysis, manuscript editing; Kuanzhi Liu: concepts, design, definition of intellectual content, manuscript review. All authors have read and contributed to the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Identification of novel variants in neonatal diabetes mellitus genes in Egyptian patients with permanent NDM

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Abstract

Neonatal diabetes mellitus (NDM) is a monogenic form of diabetes resulting from mutations in more than 20 different genes encoding proteins playing a key role in the normal function of the pancreatic beta-cell. Mutations in the genes encoding the ATP-sensitive potassium channel, *ABCC8*, and *KCNJ11* and insulin (*INS*) gene are the most common causes of NDM; however, in consanguineous populations, *EIF2AK3* mutations are more common. Identification of the causative mutations by genetic testing is critical for appropriate management and to guide genetic counseling. To determine the genetic etiology of NDM in diabetic neonates and infants diagnosed before the age of 1 year and to describe their phenotype/genotype characteristics, DNA sequencing of coding regions and intronic boundaries of *ABCC8*, *KCNJ11*, *INS*, and *EIF2AK3* genes was undertaken in 20 patients. Further, targeted next-generation sequencing was performed for other genes known to cause NDM. *ABCC8* mutations were found in two patients (10%), with compound heterozygous mutations (p.N131 K/p.R598*) in one patient and a homozygous mutation (p.R1554Q) in the another patient. Heterozygous p.A174G and p.V59M mutations of *KCNJ11* were identified in two patients (10%), and homozygous *EIF2AK3* mutations were identified in two further patients (p.T905fs and p.R653T) (10%). No *INS* mutations were identified. Further testing identified a *SLC19A2* mutation (p.W387*) in one patient (5%) and the same homozygous *GCK* mutation in two siblings (p.A188T). *ABCC8*, *KCNJ11*, and *EIF2AK3* mutations were the main genetic causes of permanent NDM among Egyptian neonates.

Keywords NDM in Egypt · Genetic screening · ABBC8 · KCNJ11 · INS mutations

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Introduction

Neonatal diabetes mellitus (NDM) is defined as persistent hyperglycemia typically presenting before the age of 6 months of life and caused by a single gene mutation [1]. More than 22 known genetic causes of neonatal diabetes have been identified so far which define different clinical subtypes including isolated permanent neonatal diabetes (PNDM), transient neonatal diabetes (TNDM), and complex syndromes in which neonatal diabetes is often the presenting feature. Identifying the genetic cause has important implications for clinical management and treatment [2, 3].

Heterozygous mutations in the *KCNJ11*, *ABCC8*, and *INS* genes are the most common causes of PNDM accounting for about 60% of cases [4]. Rarer genetic causes of PNDM including homozygous glucokinase gene (*GCK*) mutations [5] or syndromic forms such as Roger's (TRMA) [6] and Wolcott Rallison syndromes [7] account for the majority of cases in populations with a high consanguinity rate [8]. It is clinically very important to identify mutations in *KCNJ11* and *ABCC8* in patients with NDM because the majority of these mutations result in diabetes responsive to sulfonylurea (SU) therapy, which dramatically improves diabetes control and decreases the risk of hypoglycemia [1, 9].

In this study, we aimed to determine the genetic etiology of NDM in a group of 20 diabetic neonates and infants diagnosed before the age of 1 year and to describe their phenotype/genotype characteristics.

Materials and methods

Subjects and methods

This cross-sectional study was conducted on 20 patients referred to the Diabetic Endocrine and Metabolic Pediatric Unit (DEMPU) at the Cairo University Children Hospital (CUCH). The study was started in the molecular biology unit of the Chemical pathology department, Faculty of Medicine, Cairo University, and continued at University College London, Institute of Child Health, Genetics and Genomics Medicine program (UCL-ICH-GGM).

The patients were diagnosed with diabetes mellitus according to the American Diabetes Association (ADA) criteria [10]. Inclusion criteria were insulin-treated diabetic infants diagnosed within the first year of life (17 cases were diagnosed with diabetes before/at 6 months of age and the rest were diagnosed at 6–12 months). Exclusion criteria included patients who were diagnosed as having type 1 diabetes mellitus having positive pancreatic autoantibodies (glutamic acid decarboxylase autoantibodies). Clinical data such as age at onset, family history of diabetes, history of consanguinity, birth weight, gestational age, and history of remission of DM were recorded. The signs and symptoms at presentation, extra-pancreatic features, blood glucose level, HbA1c %, and insulin dose were also obtained. Physical examination and neurological findings were also recorded. The study was approved by the Local Ethical Committee after informed verbal consents were obtained from parents of all patients. Whenever available, parent's blood samples were collected to check the segregation of the mutations identified.

Genetic analysis

All samples had undergone genomic DNA extraction from peripheral blood leukocytes using QiAamp DNA blood mini kit (Qiagen, Valencia, CA). In 18 patients, the coding regions and exon/intron boundaries of the *ABCC8*, *KCNJ11*, *INS*, and *EIF2AK3* genes were amplified by polymerase chain reaction (PCR) then sequenced by Sanger sequencing as previously described [3, 11–13].

Analysis of the sequencing data was performed using Mutation Surveyor v4.0.6 (Soft Genetics, State College, PA) where comparison with reference sequences (NM_000525.3, NM_000207.2, NM_001287174.1, AF110146.1) and sequence variations was checked against published polymorphisms and mutations and for conservation across species using ALAMUT (Interactive Biosoftware, Rouen, France) to predict the effect of novel unreported variants.

Targeted next-generation sequencing of other known neonatal diabetes genes including *FOXP3*, *GATA4*, *GATA6*, *GCK*, *GLIS3*, *HNF1B*, *IER3IP1*, *PDX1*, *PTF1A*, *NEUROD1*, *NEUROG3*, *NKX2-2*, *RFX6*, *SLC2A2*, *SLC19A2*, *STAT3*, *WFS1*, and *ZFP57* was subsequently performed as previously described [2] for patients in whom a mutation was not identified and sufficient DNA was available.

The phenotype for two patients was strongly suggestive of a specific neonatal diabetes subtype, and Sanger sequencing of the suspected gene was performed. In one case, sequencing of all coding regions and exon/intron boundaries of the *SLC19A2* gene (NM_006996.2) was undertaken. In the second case and their affected sibling, sequencing of all coding regions and exon/intron boundaries of the *GCK* gene (NM_000162.3) was performed. For these two siblings, homozygosity mapping was undertaken as they were the offspring of consanguineous parents using Infinium HD Ultra Assay protocol (Ilumina Inc., San Diego, USA) and analyzed using the Illumina Genome studio software by UCL genomics.

Sequence variants identified were looked for in parental DNA samples, whenever available to determine segregation. The Sanger sequencing and targeted next-generation sequencing testing were performed by the Exeter Molecular Genetics laboratory, University of Exeter.

Statistical analysis

Data were analyzed using IBM SSPS advanced statistics version 19.0 (SSPS Inc., Chicago, IL). Quantitative data were expressed as mean ± SD when normally distributed and as median and range when not normally distributed. *P* value < 0.05 is considered significant.

Results

Twenty PNDM neonates and infants were tested. The demographic and clinical characteristics of the studied group are presented in Table 1. Consanguinity was reported in 55% of the patients and four patients had syndromic PNDM with associated extra-pancreatic manifestations.

Pathogenic mutations were identified in 9 out of the 20 PNDM patients (Fig. 1) and their phenotypic and genotypic characteristics are listed in Table 2. Two patients (10%) had *ABCC8* mutations, two patients (10%) had *KCNJ11* mutations, and two patients (10%) had *EIF2AK3* mutations. Following screening of the above-listed genes, negative cases were screened for other genetic causes, two patients (10%) had *GCK* mutations and one patient (5%) had a *SLC19A2* mutation; in six patients, DNA was insufficient for tNGS and in five patients, no mutation could be detected in the 22 genes known to cause NDM.

The two cases with *ABCC8* mutations were born to consanguineous parents. The first was a male infant diagnosed with isolated PNDM at 2 months of age. He was found to be a compound heterozygote for two *ABCC8* mutations (c.393C > A, p.N131 K in exon 3 and c.1792C > T, p.R598* in exon 12). Mutation testing of his parents revealed that the father was heterozygous for the p.N131K mutation and the mother was heterozygous for the p.R598* mutation. The second patient was a female infant diagnosed with isolated PNDM at 1 month of age. She was found to be homozygous for the c.4661G > A, p.R1554Q mutation in exon 39. Her parents were both heterozygous for this mutation.

Table 1 Demographic and phenotypic features of the studied PNDM group

	PNDM ($n = 20$)
Age at presentations (days)	60 (7–330)[a]
Gestational age (weeks)	37.5 ± 1.2[b]
Birth weight (g)	2750 ± 808.6[b]
Male/female	11/9
DKA at presentation, *n* (%)	13/20 (65%)
Positive family history of DM, *n* (%)	12/20 (60%)
Consanguinity, *n* (%)	11/20 (55%)
Isolated PNDM/syndromic PNDM	16/4
Plasma glucose (mg/dl)	545 ± 165.7[b]
HbA1C %	8.5 ± 2.04[b]
C-peptide (ng/ml)	0.6 (0.2–3.2)[a]
Insulin dose (U/kg/day)	0.802 ± 0.6[b]

Qualitative data expressed as frequency and percentage

PNDM permanent neonatal diabetes mellitus, *DKA* diabetic ketoacidosis

[a] Median (range)

[b] Mean ± standard deviation (SD)

Two patients had *KCNJ11* mutations; both were born to non-consanguineous parents. The first was a male infant diagnosed with isolated PNDM at 4 months of age. He was heterozygous for the c.521C > G, p.A174G mutation. The second patient was also a male infant who presented with diabetic ketoacidosis (DKA) at the age of 45 days. He also had motor and mental developmental delay in the form of delayed walking and speech, as well as repeated epileptic seizures (DEND syndrome). He was heterozygous for the c.175G > A; p.V59 M missense mutation. Transfer to sulfonylurea was attempted in this patient upon mutation identification at the age of 27 months, and improvement in glycemic control was evident with sulfonylurea treatment. There was no improvement in his motor capabilities, but he started to show some improvement in his speech upon training.

EIF2AK3 gene mutations were identified in two patients born to consanguineous parents who presented with features suggestive of Wolcott Rallison Syndrome (WRS) in the form of acute episodes of liver dysfunction and skeletal dysplasia (in one patient). The first patient was homozygous for a frameshift mutation (c.2713dup, p.T905fs) in exon 12. The second patient was homozygous for a missense mutation (c.1958G > C; p.R653T) also located in exon 12. Both mutations have not been reported previously and are both predicted to be pathogenic.

SLC19A2 gene homozygous mutation (p.W387* in exon 4) was identified in one patient who presented features suggestive of a diagnosis of thiamine-responsive megaloblastic anemia syndrome (TRMA) (also called Roger's syndrome) which included pancytopenia, sensorineural hearing loss, and NDM. Management with thiamine attempted in this patient who responded well with complete withdrawal of insulin was achieved.

A *GCK* gene mutation was identified in two siblings born to consanguineous parents, who were diagnosed with isolated NDM during the early weeks of their life. This shared phenotype prompted the use of homozygosity mapping to detect regions of loss of heterozygosity (LOH) shared among the two siblings. This approach assumes the existence of a disease gene adjacent to a marker locus due to the observation of excessive homozygosity that is too significant to be attributed solely to chance. Comparison of the LOH regions identified the *GCK* gene among the shared LOH regions on chromosome 7. This finding strongly suggested *GCK* as a candidate gene in this family. Sanger sequencing of *GCK* gene in the

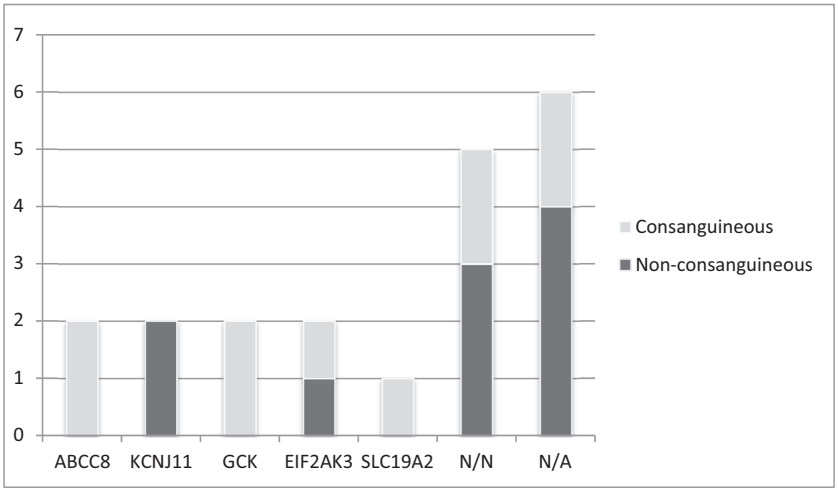

Fig. 1 Number of NDM gene mutations identified among the study group in relation to consanguinity. N/N no mutation identified, N/A no available sample for tNGS

siblings identified a previously reported homozygous missense mutation, p.Ala188Thr, in both individuals. Their mother, elder sister, and father had been diagnosed with diabetes at the age of 17, 4, and 27 years, respectively. Genetic analysis for the mother and elder sister showed that they were heterozygous for the same *GCK* mutation, thus having glucokinase-maturity onset diabetes of the young (*GCK*-MODY) and so is likely to be the father (his sample was not available) as predicted by the recessive inheritance.

Management of the PNDM cases: all the patients were treated with insulin since diagnosis, with an average dose of 0.802 ± 0.6 U/kg/day. Once the blood glucose levels were stable with insulin control, SU therapy (Glibenclamide) was attempted in only three patients (since switching treatment required hospitalization, such attempt was not feasible for most of the patients). One patient with a *KCNJ11* mutation (p.V59M mutation) showed a good response and transferred to SU therapy, while the other two patients who were later identified to have GCK-PNDM did not respond and shifted back to insulin.

Discussion

This study is to the best of our knowledge the largest undertaken to investigate various genetic causes of NDM in Egypt. The study included 20 neonates and infants admitted to a tertiary care unit from different regions in Egypt with a diagnosis of diabetes mellitus before the age of 1 year. Following ISPAD guidelines on monogenic diabetes, most of this study group were diagnosed below the age of 6 months; however, there were three patients in the age range 9–12 months with negative anti-GAD autoantibodies who were also included as NDM may present, although rarely, in this age range [14, 15].

The PNDM testing pipeline first analyzed *KCNJ11*, *ABCC8*, *INS*, and *EIF2AK3* genes, only four patients (20%) had mutations in *KCNJ11* and *ABCC8* (two mutations for each), and two patients had mutations in *EIF2AK3* causing Wolcott Rallison syndrome while no mutations were detected in the *INS* gene. Of the *ABCC8* mutations detected in this study, the p.N131K missense mutation has not been previously reported, but in silico pathogenicity analysis predicted the mutation to be likely affecting the protein's function. The p.R598* mutation was previously reported to cause hyperinsulinism (HI) [16]. Ellard et al. had previously described the mutational mechanism by which a heterozygous activating mutation resulted in PNDM when a second loss-of-function mutation was also present, probably because this loss of function mutation leads to a decrease in functional protein and hence the majority of pancreatic ATP channels will be homomeric for the gain of function mutation [11]. This is likely to be the underlying mechanism in our case where the p.N131K mutation is the gain-of-function mutation and p.R598* is the loss-of-function mutation. The second *ABCC8* mutation identified, p.R1554Q, had not been previously reported in sequence variant databases. It is likely pathogenic since the arginine residue at codon 1554 is highly conserved across species and two patients with transient neonatal diabetes who are heterozygous for this mutation have been previously identified by the Exeter laboratory (unpublished data).

The *KCNJ11* gene mutation p.A174G has been previously reported by Suzuki et al. to cause TNDM in a Japanese patient that recovered and exhibited normalized blood glucose at 307 days of age but relapse occurred at the age of 13 years [17]. In our study, till the age of 540 days, diabetes did not remit; however, further follow-up is required since remission in TNDM is reported to occur up to the age of 18 months [18]. The p.V59M missense mutation was previously reported as the most common mutation to cause PNDM associated with neurological features [3]. Gloyn et al. were the first to report this mutation and suggested that since Kir6.2 is the pore-forming subunit of KATP channels in skeletal muscle and neurons throughout the brain, altered activity of these

Table 2 Genotype-phenotype characteristics of mutation-positive PNDM patients

Patient no.	Gene	Gender	Age at diagnosis (weeks)	Gestational age (weeks)	Birth weight (grams)	Site of mutation	DNA changes	Zygosity/novelty	Family history of DM	Sulfonylurea		Associated disease and follow up
1	*ABCC8*	Male	8	39	2700	Exon 3 Exon 12	c.393C > A (p.N131 K) c.1792C > T (p.R598*)	Compound HT/novel	T2DM (father)	No	No	Patent foramen ovale
2	*ABCC8*	Female	4	37	2750	Exon 39	c.4661G > A (p.R1554Q)	HM/novel	No	No	No	No
3	*KCNJ11*	Male	6	37	2750	Exon 1	c.175G > A (p.V59 M)	HT/previously reported	T2DM 2 maternal uncles	Yes	Responsive	iDEN D
4	*KCNJ11*	Male	16	38	2700	Exon 1	c.521C > G (p.A174G)	HT/novel	T2DM paternal grandmother and aunts	No	No	No
5	*EIF2AK3*	Female	12	39	3100	Exon 12	c.2713dup (p.T905fs)	HM/Novel	No	No	No	Elevated liver enzymes
6	*EIF2AK3*	Female	4	38	3500	Exon 12	c.1958 G > C (p.R653T)	HM/Novel	No	No	No	Elevated liver enzymes, impaired kidney function, and skeletal defects
7	*SLC19A2*	Male	24	37	3100	Exon 4	c.1160G > A (p.W387*)	HM/Novel	deceased sister with DM and atrial septal defect	No	No	Pancytopenia, sensorineural hearing loss, atrial septal defect
8	*GCK*	Female	0.5	38	3200	Exon 5	c.562 G > A (p.A188T)	HM/previously reported	Yes, paternal and maternal grandmother	Yes	No response	No
9	*GCK*	Male	5	36	1600	Exon 5	c.562 G > A (p.A188T)	HM/previously reported	Yes	Yes	No response	No

PNDM permanent neonatal diabetes mellitus, *HT* heterozygous, *HM* homozygous, *SU* sulfonylurea

channels could cause developmental delay, muscle weakness, and epilepsy [19]. Also, Hattersley and Aschroft's study investigated a group of 12 patients being heterozygous for this mutation and reported that a few patients had presented with isolated PNDM but the majority presented with intermediate DEND (iDEND), a less severe clinical picture consisting of neonatal diabetes with developmental delay and/or muscle weakness but not epilepsy [20]. In the present study, transfer to sulfonylurea was initiated upon identification of the p.V59M mutation at the age of 27 months and improvement in glycemic control was evident with sulfonylurea treatment. There was no improvement in the patient's motor capabilities, but he started to show some improvement in his speech upon training. No other KCNJ11 mutations were detected in this study. In a previous study, we detected one patient with R201C mutation in KCNJ 11 gene who was shifted successfully from insulin to sulfonylurea treatment [21].

Mutations in *EIF2AK3* are the most common genetic cause of neonatal diabetes among patients born to consanguineous parents [3]. The *EIF2AK3* gene encodes for a transmembrane enzyme, the pancreatic eIF-2 alpha kinase (PERK), which localizes exclusively in the endoplasmic reticulum. Lack of PERK activity leads to cell death by apoptosis in different tissues. The high level of expression of *EIF2AK3* in both pancreatic beta-cells and bone tissue explains the development of early-onset diabetes mellitus and skeletal abnormalities in most patients with Wolcott Rallison syndrome, and hence, their association is considered a cornerstone for diagnosing this rare clinical entity [8].

In our study, one case showed clinical features of TRMA syndrome which is described in the literature by the triad of (i) megaloblastic anemia with ringed sideroblasts, (ii) non-autoimmune diabetes mellitus, and (iii) early-onset sensorineural deafness caused by *SLC19A2* mutations [22]. *SLC19A2* is the only known thiamine transporter in bone marrow, pancreatic beta-cells, and a subgroup of cochlear cells; therefore, anemia, diabetes, and deafness are the consequences of *SLC19A2* deficiency. Mikstiene et al. reported that most TRMA-causing mutations are nonsense or frameshift mutations in exon 2 [23]. These mutations lead to the absence of protein synthesis and thus a complete impairment of cellular thiamine transport. High doses of thiamine supplementation slow down the onset of diabetes and can decrease the requirement for insulin [24]. In our study, a nonsense mutation was identified but in exon 4 and the subsequent improvement in management with thiamine was a paradigm for the effectiveness of molecular testing in a better clinical outcome.

A crucial enzyme-regulating insulin secretion through ATP synthesis is glucokinase. Homozygous inactivating mutations in *GCK*, the gene encoding for glucokinase, causes complete glucokinase deficiency leading to PNDM and should be suspected in families of known consanguinity or family members having a *GCK*-MODY (MODY2) phenotype. Moreover, this molecular diagnosis is most likely when consanguineous probands present with diabetes within the first 3 weeks of life [5]. The two siblings with a *GCK* mutation in our study presented with diabetes at the age of 40 and 3 days and were born to consanguineous parents with *GCK*-MODY. Empirical SU treatment was tried with both siblings before attaining genetic diagnosis, but no response was achieved. Although one study reported that one NDM case with a *GCK* mutation had an improved glycemic control and decreased insulin dose upon SU introduction, all cases should be treated with insulin [25]. The p.A188T mutation was previously reported and is predicted to be pathogenic [26].

For five patients, no mutation was identified in the genes known to cause PNDM; three of them were in the group diagnosed 9–12 months. This may suggest that either there are mutations in other novel genes, so comprehensive genetic study and whole genome analysis is needed to detect these genes, or they may be T1DM with a late-onset appearance of autoantibodies. The other six patients who had insufficient DNA for tNGS may be positive for mutations in NDM genes other than *ABCC8*, *KCNJ11*, *INS*, and *EIF2AK3*.

Conclusion

Our study group comes from a single diabetes center which receives referrals from different regions over the country and thus can give a representative picture of the genetic causes of NDM in Egypt. Heterogeneous genetic causes were identified in our study group. *ABCC8*, *KCNJ11*, and *EIF2AK3* mutations were found to be the main genetic causes of PNDM among Egyptian neonates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from parents of all patients included in the study.

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ORIGINAL ARTICLE

Type 2 diabetes and FTO *rs9939609* gene polymorphism: a study among the two tribal population groups of Manipur, North East India

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Abstract

Diabetes is one of the most underrated epidemic worldwide, and its prevalence has increased rapidly in developing nations like India. It has increased not only in the general population per se, but even among the indigenous tribal populations also. Several candidate genes have been associated with type 2 diabetes, and the association of type 2 diabetes and FTO *rs9939609* gene polymorphism is a matter of debate. The present study aims to understand the prevalence of type-2 diabetes and its association with FTO *rs9939609* gene polymorphism, among the Naga and Mizo tribe of Manipur, North East India. Demographic, somatometric variables and blood samples from 521 individuals were collected and FTO *rs9939609* variant was screened. The prevalence of type 2 diabetes/impaired fasting glucose was found to be 10.1 and 43.73% among the Liangmai and Mizo tribe, respectively. The FTO variant showed an increased risk for impaired fasting glucose (OR 1.25; CI 0.38–4.1) among the Liangmai tribe, but among Mizo tribe, it showed an increased risk for type 2 diabetes (OR 1.34; CI 0.73–2.4), albeit with no statistical significances. This suggests that there seems to be diverse effect of FTO *rs9939609* A allele in the two tribes, i.e., disadvantageous effect among Liangmai tribe and an adaptive effect among Mizo tribe.

Keywords Type 2 diabetes · FTO gene · Genetic association variation · Tribal population · North East India

Abbreviations

T2D	Type 2 diabetes
BMI	Body mass index
CI	Confidence interval
DNA	Deoxyribonucleic acid
FTO	Fat mass and obesity associated
HC	Hip circumference
OB	Obese
OR	Odds ratio
OW	Overweight
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
WC	Waist circumference
WHR	Waist-hip ratio
WHtR	Waist-height ratio

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Introduction

The rising prevalence of type 2 diabetes in the last two decades is a concern of global public health. In developed countries, it was considered as "the Western killer", the developing countries are also not untouched by this biggest epidemic in human history [1]. In developing countries, its prevalence has

increased from 108 million in 1980 to 422 million in 2014, and International Federation of Diabetes estimated that in 2040, it will be around 642 million worldwide [2–4]. India is the second leading country in the world just after China, with 69 million people affected by diabetes [4]. According to the WHO report, in India, every 26 per 100,000 persons die due to diabetes, though this rate declined marginally but for males it has increased between 2000 and 2012 [5]. The primary causes of the epidemic of diabetes are the rapid epidemiological transition with changes in the lifestyle and dietary habits and also some sort of demographic transition and their exposure to rapid expanding urbanization and globalization [6]. The populations in India are non-tribal (caste populations) or tribal (indigenous) [2]. Even among the tribal areas of India, the prevalence of diabetes ranges from 1 to 10% and further increasing [7].

Diabetes is multifactorial in origin and among the diabetic individuals, type 2 diabetes (T2D) has contributed the maximum number because of the globally increasing trend of body weight and physical inactivity [8]. Not only the environmental factor, genetic factors have also taken an important role in the pathogenesis of type 2 diabetes [9]. Recently, several genome wide association studies (GWAS) on type 2 diabetes revealed an array of genes that are associated with type 2 diabetes [10–12]. Fat mass and obesity-associated (FTO) gene located in chromosome 16q12.2 is one of the novel genes that was identified by GWAS, and in initial stages, this gene was only found to be associated with obesity [13]. This *FTO* gene was first identified as type 2 diabetes susceptibility gene, but after adjustment of the BMI, the association disappeared [14]. Further BMI dependent role of FTO in T2D remains a matter of debate not only in Asians and but also in Europeans, hinting towards the role of ethnicity in the association [15–25].The effect of the FTO genetic variant on T2D varies among different ethnic populations, because of the differences in their environmental factors and genetic architecture [26]. Further, recent literature has focused on the gene and environment interactions in the causation of disease phenotype. India is a country with tremendous diversity at every level (dietary, climatic, geographic, socio-cultural) giving rise to simple mendelian gene pools that are stratified at the level of genetic architecture. The genetic architecture may not be compatible with the changing environment and may behave differently in different population groups. Further, the two selected tribal population groups are supposed to have different ancestries, Liangmai (East Asian Ancestry) and Mizo (European), but still this is debatable because morphologically they resemble people of East Asian Ancestry (Mongoloid feature) [27, 28]. Inspite of the fact that both the study tribes are from same geographical region, they reside at different altitudes, where Liangmai tribe resides in relatively higher altitude compared to the Mizo. Further, the two tribes are neither explored for the prevalence of T2D nor for the distribution of the FTO *rs9939609* and nor for the association between the FTO *rs9939609* and T2D. So the aim of the present study is to find out the prevalence of diabetes among the Liangmai and Mizo tribes and also examine whether the FTO *rs9939609* has the same effect in the two selected tribal populations with respect to type 2 diabetes, that have different environmental and genetic backgrounds.

Materials and methods

Study subjects

The present study was conducted among the two tribal populations of Manipur state, North East India, namely Liangmai Naga and Mizo. The Liangmai tribe was recruited from the Tamenglong and Senapati district and Mizo tribe from Churachandpur district, where they reside predominantly. Fieldwork for the present study was conducted between 2014 and 2016.

A total sample size of 521 individuals (258 Liangmai and 263 Mizo) of both sexes of the age between 18 and 60 years were recruited randomly from the two study population groups. The recruitment of the participant of the present study was done through household survey. As the study involves genetic variant, only individuals unrelated up to first cousin were recruited.

Demographic and somatometric measurements (anthropometry)

An interview schedule and standard techniques were used to collect the detailed data on demographic (name, age, sex, place of birth) and somatometric (height vertex, weight, waist circumference, hip circumference) variables. The Ethical committee, Department of Anthropology, University of Delhi, India approved the study. The written informed consents were obtained from all the participants before conducting the study.

Somatometric measurements including height, weight, waist, and hip circumferences were taken from all the participant individuals. Body weight (kg) and height (cm) were measured from all the subjects in lightweight clothing and without shoes, using weighing machine and anthropometer rod respectively. Body mass index (BMI) was calculated using the formula—weight in kilogram divided by height in meter square (kg/m^2). The participant subjects were classified as underweight (< 18.5 kg/m^2), normal weight (18.5–22.99 kg/m^2), overweight (23–25 kg/m^2), and obese (> 25 kg/m^2), according to their BMI value. (Asia Pacific population criteria) [29]. Waist circumference (cm) was measured at the least circumferences between the lower ribs and the iliac crest. Waist circumference < 90 cm for males and < 80 cm for female was taken as normal [30]. Hip circumference was measured at the buttock yielding the

Table 1 Distribution of somatometric indices and FTO *rs9939609* genotype, with respect to fasting blood glucose among the Liangmai and Mizo tribe of Manipur

Variables	Liangmai						Mizo				
	Normoglycemia		IFG	X^2 *p* value	T2D	X^2 *p* value	Normoglycemia	IFG	X^2 *p* value	T2D	X^2 *p* value
Age	40		39	0.71	50	< 0.003	43	46	0.25	49	< 0.001
Sex	M	56 (24)	2 (13.3)	0.5*	2 (18.2)	0.9*	56 (37.8)	10 (35.8)	0.8	40 (45.9)	0.2
	F	176 (76)	13 (86.7)		9 (81.8)		92 (62.8)	18 (64.2)		47 (54.1)	
BMI	N	89 (38.3)	3 (20)	0.2*	2 (18.1)	0.4*	46 (31.1)	6 (20.6)	0.3	27 (31.1)	0.1
	OW	47 (20.5)	7 (46.6)		2 (18.1)		34 (22.9)	5 (17.8)		12 (13.7)	
	OB	96 (41.3)	5 (33.4)		7 (63.6)		68 (45.9)	17 (60.7)		48 (55.2)	
WC	N	72 (33.1)	2 (13.3)	0.1*	1 (9)	0.1*	51 (34.4)	8 (28.5)	0.5	23 (26.4)	0.2
	AB	145 (66.8)	13 (86.7)		10 (91)		97 (65.6)	20 (71.5)		64 (73.5)	
WHR	N	21 (9.7)	0	0.4*	0	0.5*	24 (16.1)	4 (14.2)	0.9*	10 (11.4)	0.3
	AB	197 (90.3)	15 (100)		11 (100)		125 (83.9)	24 (85.8)		77 (88.5)	
Genotype FTO *rs9939609*											
TT		180 (77.6)	11 (73.3)	0.9*	10 (90.9)	0.5*	102 (68.9)	20 (71)	0.7	58 (66.6)	0.7
TA + AA		52 (22.4)	4 (26.7)		1 (9)		46 (31)	8 (28.5)		29 (33.4)	

Data are presented as *N* (%), *M* male, *F* female, *N* normal blood glucose, *OW* overweight, *OB* obese, *IFG* impaired fasting glucose, *T2D* type 2 diabetes, *BMI* body mass index, *WC* waist circumference, *WHR* waist-hip ratio

Yates corrected,*p value < 0.5 are significant

maximum circumference. Waist-hip ratio was calculated using the formula waist (cm) divided by hip (cm), i.e., (W/H) [31]. The circumferences were measured using a non-expendable steel tape. Normal WHR calculated, as was classified as < 0.90 (males), < 0.80 (females) [30].

Type 2 diabetes diagnosis

Intravenous blood samples (5 ml from each individual) were collected from the participant subjects, after overnight fasting by a trained technician. The collected blood samples were stored at 2–8 °C in ice box and transported to the molecular laboratory, Manipur University. The collected blood samples were centrifuged, and aliquots of plasma were separated for glucose analysis within 6 h after the blood collection. The plasma glucose levels were measured by using spectrophotometry using commercially available kits (Randox, USA). The World Health Organization standard was used for classification of glycemic status of the recruited subjects [32]. A participant is classified as having T2D if the individual has FPG ≥ 7.0 mmol/L (126 mg/dL) or 2-h OGTT ≥ 11.1 mmol/L (200 mg/dL), impaired fasting glucose (IFG) < 126–≥ 110 mg/dL or 2-h OGTT ≥ 140–< 200 mg/dL, and normal glucose level < 110 mg/dL or 2-h OGTT < 140 mg/dl. For the present study, the value of FPG was used for the classification of the recruited subjects to normal, impaired fasting glucose and type 2 diabetes.

Table 2 Distribution and prevalence of fasting blood glucose among the Liangmai and Mizo tribe of Manipur

Fasting blood glucose	Liangmai (*N* %)	Mizo (*N* %)	X^2 *p* value
Normoglycemia	232 (89.9)	148 (56.27)	< 0.001
Impaired fasting glucose	15 (5.8)	28 (10.65)	
Type 2 diabetes	11 (4.3)	87 (33.08)	
Total	258	263	

Data are presented as *N* (%)

p value < 0.5 are significant

PCR and genotyping

DNA was extracted from the whole blood using salting-out method [33]. Genotyping of *FTO rs9939609* gene polymorphism was carried out using forward primer sequence (AAC TGG CTC TTG AAT GAA ATA GGA TTC AGA) and reverse primer sequence (AGA GTA ACA GAG ACT ATC CAA GTG CAG TAC) [34]. The PCR was carried out using the Thermocycler (C-1000 Touch™, Bio-Rad, USA). The PCR reaction mixture of 10 ul, comprised of 50 ng DNA, 1× *Taq* Buffer, 2.5 mM MgCl2, 200 μM of dNTP, 10 pmol of each primer, and 1 U *Taq* DNA polymerase. The PCR conditions were maintained as initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min, and final extension at 72 °C for 10 min and then 4 °C forever. Amplified products were digested overnight at 37 °C with *ScaI* restriction enzyme to obtain fragment lengths of 182 bp (*TT* genotype), 154 bp (*TA* genotype) and 28 bp (*AA* genotype) [34].

Table 3 Odds ratio analysis of FTO *rs9939609* polymorphism in different categories of fasting blood glucose

Liangmai	Model 1		Model 2		Model 3	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
N vs IFG	1.25 (0.38–4.1)	0.7	1.23 (0.37–4)	0.7	1.22 (0.37–4)	0.7
N vs T2D	0.34 (0.4–2.7)	0.3	0.25 (0.2–2.2)	0.2	0.19 (0.1–2.2)	0.1
Mizo						
N vs IFG	0.88 (0.36–2.1)	0.8	0.86 (0.35–2.1)	0.7	0.86 (0.35–2.1)	0.7
N vs T2D	1.10 (0.63–1.9)	0.5	1.28 (0.71–2.3)	0.4	1.29 (0.71–2.3)	0.4

OR odds ratio, *CI* confidence interval, *N* normoglycemia, *IFG* impaired fasting glucose, *T2D* type 2 diabetes, *BMI* body mass index

Model 1 unadjusted odds ratio, *Model 2* age and sex adjusted and *Model 3* age, sex, and BMI adjusted

$p < 0.05$ are significant

Statistical analysis

The independent *t* test was performed to assess the differences in the continuous variables (age) between the two populations. Chi-square ($\chi 2$) test was used to observe the differences in the distribution of genotype and categorical variables (sex, BMI, WC, and WHR) in the studied population groups. Logistic regression was used to calculate odds ratio (OR) in three models, model 1 (unadjusted), model 2 (adjusted for age and sex), and model 3 (adjusted for age, sex, and BMI) at 95% confidence interval for FTO *rs9939609* variant and categorical somatometric variables. Adjustments were performed by including the covariates (age, sex, and BMI). All analyses were performed using the Statistical Package for the Social Sciences (SPSS, for Windows version 20.0). Statistical significance was taken at p value ≤ 0.05 for all the statistical tests.

Results

Table 1 shows the general characteristics and distribution of somatometric variables and FTO *rs9939609* genotype on the basis of fasting blood glucose levels (normoglycemia, IFG, and T2D) of the two study populations. No differences in the distribution of somatometric values, and FTO genotype were found in both the selected population groups. Although, T2D group is found to have a significant higher age as compared to the normoglycemia groups both in the Liangmai and Mizo tribes. Table 2 shows that individuals with IFG/T2D were found to be significantly higher among the Mizo tribe (43.73%) compared to Liangmai tribe (10.1%) ($p < 0.001$). Table 3 shows the association between the FTO *rs9939609* gene polymorphism with impaired fasting glucose and type 2 diabetes. The associations were examined in three models, viz., model 1 (unadjusted), model 2 (age and sex adjusted), and model 3 (adjusted for age, sex, and BMI). Among the Liangmai tribe, FTO variant showed an increased risk for impaired fasting glucose in all the three models, and the highest risk was found in the unadjusted model (odds ratio 1.25; 95% CI 0.38–4.1), albeit with no statistical significance. Among Mizo tribe, FTO *rs9939609* was seen to pose increased risk in all the selected models, but the highest risk was observed in model 2 (odds ratio 1.34; 95% CI 0.38–4.1), though there was no statistical significance.

Ten-year age-cohort wise distribution of FTO *rs9939609* gene polymorphism, with respect to three categories of fasting glucose levels (normoglycemia, IFG, and T2D), revealed a consistent presence of A allele among the T2D individuals in all the age groups among the Mizo tribe. Surprisingly, its

Table 4 Ten-year age-cohort wise distributions of FTO *rs9939609* A allele with respect to fasting blood glucose

Age-cohort	Liangmai			Mizo		
	Normoglycemia (TT/TA)	IFG (TT/TA)	T2D (TT/TA)	Normoglycemia (TT/TA)	IFG (TT/TA)	T2D (TT/TA)
≤ 29	40 (32/8)	3 (3/0)	0	25 (14/11)	3 (1/2)	6 (3/3)
30–39	71 (56/15)	4 (3/1)	0	37 (25/12)	10 (8/2)	14 (10/4)
40–49	73 (53/20)	5 (3/2)	4 (3/1)	27 (18/10)	7 (5/2)	17 (9/8)
50–59	43 (36/7)	3 (2/1)	7 (7/0)	41 (30/11)	5 (4/1)	26 (16/10)
≥ 60	5 (3/2)	0	0	18 (15/3)	3 (2/1)	24 (20/4)

[1] *N* normoglycemia, *IFG* impaired fasting glucose, *T2D* type 2 diabetes

presence was evident only in 40–49 age groups in the respective counterparts (T2D) of the Liangmai tribe (Table 4).

Discussion

The association between FTO *rs9939609* polymorphism and type 2 diabetes is a matter of debate [15–25], due to inconsistency in their association in various earlier studies attributable to the ethnicity, environmental factors, and genetic architecture of the populations [26]. In the present study for the first time, an attempt is made in order to understand the association of *rs9939609* A allele polymorphism with T2D in two North-Eastern tribal populations of India, occupying the same geographical area. However, these selected study populations have different ancestral origin [27, 35] and are also different with respect to the prevalence of obesity, where obesity among Mizo tribe is more compared to the Liangmai tribe [36]. The prevalence of FTO A allele, impaired fasting glucose, and type 2 diabetes are found to be significantly higher among the Mizo as compared to that of the Liangmai tribe. In the present study populations, A allele of FTO *rs9939609* polymorphism is not found to be significantly associated with type 2 diabetes. Similar findings have been reported from other East Asian populations from China and Japan [37, 38]. But, in all the analyzed models in the present study, A allele of FTO *rs9939609* polymorphism is found to pose more than onefold increase risk for IFG in Liangmai tribe and for type 2 diabetes in Mizo tribe. Whereas, a reverse trend, i.e., A allele to be posing a reduced risk for diabetes in Liangmai tribe and for IFG in Mizo tribe, though the observed risk is not found to be statistically significant. Absence of significance could possibly be because of lower sample size of the present study.

But the results hint towards population-specific association, where A allele of FTO *rs9939609* polymorphism is associated with high BMI [36]; the same allele may be deleterious in Liangmai tribe in combination with diabetes. Similar finding was reported in North Indian population where FTO A allele was significantly found to be significantly associated with BMI but not with the T2D [39]. In the present study, A allele of FTO *rs9939609* polymorphism seems to have disadvantageous effect in combination with T2D, as suggested by simultaneous existence of low frequency of T2D, FTO A allele, and BMI in the Liangmai tribe.

Further, among the Mizo tribe with high frequency of A allele, high frequency of obesity and T2D, the polymorphic allele seems to be adapted and so lower effect on FBG and very low risk but not significant for diabetes. The association studies of FTO *rs9939609* variant and T2D were done in different ethnic groups of India, representing Indo-European and Dravidian ethnicity; similar results of association with FTO rs9939609 A allele and T2D were obtained from all the studies reported [17, 19].

Conclusions

In the present study, the association between A allele of FTO *rs9939609* polymorphism and T2D was not found; however, a very low frequency of A carrying individuals among the T2D cases among Liangmai tribe hints towards the selective disadvantages of this allele with T2D. In contrast, higher number of individuals possessing A allele and T2D as compared to the respective control groups hint towards the selective advantage of A allele among the Mizo tribe. In term of biological research specifically including human subjects, the data need to be interpreted cautiously not just by going through the statistical significance. The clinical application of the present study is that individuals carrying A allele should be cautiously treated and managed especially among the Liangmai tribe.

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Authors' contributions Somorjit Singh Ningombam, Sunanda Rajkumari, Naorem Kiranmala Devi, and Kallur Nava Saraswathy analyzed the data and drafted the manuscript. Naorem Kiranmala Devi, Prakash Ranjan Mondal, and Kallur Nava Saraswathy designed the study and directed implementation and data collection. Somorjit Singh Ningombam, Varhlun Chhungi, Masan Kambo Newmei, and Sunanda Rajkumari collected the data and laboratory analysis, and Kallur Nava Saraswathy provided necessary logistic support. Somorjit Singh Ningombam, Naorem Kiranmala Devi, and Kallur Nava Saraswathy edited the manuscript for intellectual content and provided critical comment on the manuscript.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval The Ethical committee, Department of Anthropology, University of Delhi, India approved the study. The written informed consents were obtained from all the subject participants before conducting the study.

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ORIGINAL ARTICLE

Insulin resistance in relation to inflammatory gene expression and metabolic features in apparently healthy obese individuals

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Abstract

The present study aimed to investigate the association of insulin resistance (IR) with inflammatory gene expression levels, metabolic health, lipid profile, and body composition in the apparently healthy obese. In this cross-sectional study, 88 apparently healthy obese subjects were recruited and divided into insulin-resistant and non-insulin-resistant (NIR) groups. Fasting blood samples were taken to determine serum metabolic features. mRNA expression of inflammatory genes were assessed in freshly isolated peripheral blood mononuclear cells (PBMCs), using quantitative real-time PCR (qPCR). Bioelectrical impedance analysis (BIA) was used to describe body composition. Among inflammatory genes, toll-like receptor 4 (TLR4) mRNA revealed significant upregulation in PBMCs of IR group compared with NIR individuals ($p = 0.035$). High-density lipoprotein cholesterol (HDL-C, $p = 0.04$), low-density lipoprotein cholesterol (LDL-C, $p < 0.001$), waist circumference ($p = 0.025$), and waist to hip ratio ($p = 0.013$) were significantly different between the two groups. A significant but weak correlation of HDL-C was observed with TLR4 ($r = -0.305$; $p = 0.011$) and myeloid differentiation factor 88 (MyD88, $r = -0.27$; $p = 0.024$) expression level. Also, LDL-C was found to be correlated with TLR4 ($r = 0.302$; $p = 0.012$) and MyD88 ($r = 0.267$; $p = 0.027$) expression levels. There was also a significant correlation between HOMA-IR and HDL-C ($r = -0.25$; $p = 0.019$). The results of this study indicated the possible link between IR and TLR4. Also, there was a significant correlation between HDL-C and LDL-C as well as between TLR4 and MyD88. Some inflammatory genes and metabolic parameters were also significantly correlated.

Keywords Insulin resistance · Inflammatory genes · Metabolic health · Lipid profile · Healthy obese

Abbreviations

BP	Blood pressure
BMI	Body mass index
FBS	Fasting blood sugar
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model of insulin resistance
IR	Insulin resistance
LDL-C	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MHO	Metabolically healthy obese
MUO	Metabolically unhealthy obese
MyD88	Myeloid differentiation factor 88
NFκB	Nuclear factor kappa B
NIR	Non-insulin resistance
PBMCs	Peripheral blood mononuclear cells
QUICKI	Quantitative insulin sensitivity check index
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TRIF	TIR-domain containing adaptor-inducing interferon-β
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
WC	Waist circumference
WHR	Waist/hip ratio

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Introduction

Obesity as a serious concern to public health is reaching epidemic rates worldwide. The major health complications associated with the growing prevalence of obesity are type 2 diabetes mellitus (T2DM), fatty liver disease, cardiovascular disease, and obesity-related cancers [1]. Obesity is also associated with abnormal inflammatory responses that are a risk for obesity-related insulin resistance (IR) [2].

IR is linked to various chronic diseases such as hypertension, hyperlipidemia, and atherosclerosis, the hallmarks of metabolic syndrome (MetS) [3]. The dyslipidemia of IR seems to be triggered by features of IR than obesity. However, not all subjects with diminished insulin sensitivity develop dyslipidemia [4]. In fact, adipocyte size is proposed as a key factor for determining the contributing degree of adipose tissue to dyslipidemia. Enlargement of adipocytes, enhanced in lipolysis, can lead to more circulating free fatty acids and their delivery to the liver to increase triglyceride synthesis, exacerbate IR, and promote dyslipidemia [5]. The study of Veilleux et al. [6] reported the association between enlargement of visceral adipocytes and dyslipidemia independent of body composition in obese subjects (181). In another study [7], serum triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were directly correlated with IR and visceral fat. A research [8] on two groups with normal BMI showed that individuals were severely IR. Previous studies propose that the distribution of fat, particularly visceral obesity, may be a more important determinant of IR than overall obesity [9–11]. The key role of adipose tissue in the development of IR by releasing a wide range of proinflammatory cytokines and chemokines is clarified, but molecular basis for the link between obesity, IR, and metabolic state is not thoroughly identified.

There is considerable evidence that activation of membrane receptors such as toll-like receptors (TLRs) plays a significant role in the initiation and development of IR [2, 12, 13]. Glucose and saturated fatty acids can contribute to TLR expression and activation in human mononuclear cells and induce inflammatory cytokine production [14]. TLR4 recognizes free fatty acids which contribute to the pathogenesis of IR [15, 16]. Animal studies remarkably show that lack of TLR4 can protect mice from diet-induced obesity [13, 17, 18]. Furthermore, inhibition of TLR2 improved insulin sensitivity and signaling in muscles and white adipose tissue of mice [19]. Hardy et al. [20] reported increased TLR2 and TLR4 expression in adolescents with MetS compared to BMI-matched controls. However, studies between metabolically healthy and unhealthy obese individuals showed no significant differences in TLR2 and TLR4 gene expression levels [21, 22].

TLRs act via two downstream molecules, myeloid differentiation factor 88 (MyD88) and TIR domain-containing adaptor-inducing interferon-β (TRIF), which have connections with insulin homeostasis [2, 14]. An animal survey indicated that mice lacking MyD88 have decreased β cell mass compared to wild-type controls and they have normal glucose tolerance [23]. Also, another study revealed that TRIF deficiency induces decreased glucose tolerance and β-cell dysfunction [24].

In the study of Jialal et al. [25], there was a positive correlation between HOMA-IR and increased levels of TLR2 and TLR4 in patients with MetS. However, they did not report any downstream signaling proteins. While these important findings imply that TLR2 and TLR4 activation is important in the pathogenesis of IR, the association of IR and inflammatory genes is rarely studied. Therefore, the purpose of this study was to test the association of IR with TLR2 and TLR4 and downstream signaling in peripheral blood mononuclear cells (PBMCs) isolated from apparently healthy obese persons. Also, the association of IR with lipid profile, metabolic health, and body composition was studied.

Materials and methods

Study participants

In this cross-sectional study, 88 apparently healthy obese persons aged 29–43 years were recruited from numerous clinics in the northwest of Iran from June 15th to November 6th, 2015. All of the participants were classified as abdominally obese (waist circumference (WC) ≥ 95 cm) based on the Iranian National Committee of Obesity [26]. Informed consent was obtained from each participant and the study was approved by regional ethics committee of Tabriz University of Medical Sciences, Tabriz, Iran. The whole investigation was conducted according to the principles of the Declaration of Helsinki (ethical code: TBZMED.REC.1394.1191). Metabolic health status was defined using Meigs et al.'s [27] criteria in which the presence of less than three of the following components was considered as metabolically healthy state: high WC (≥ 95 cm), high serum triglyceride (TG) concentration (≥ 150 mg/dl), low serum high-density lipoprotein cholesterol (HDL-C) (< 40 mg/dl for men and < 50 mg/dl for women), elevated blood pressure (BP) (≥ 130/85 mmHg), and fasting blood sugar (FBS) (≥ 100 mg/dl).

The exclusion criteria were pregnancy and lactation, postmenopausal, recent change or misreport in energy intake (i.e., < 800 or ≥ 4200 kcal/d), chronic high-intensity exercise (> 100 min/week), smoking, alcohol consumption, individuals with serum TG (> 400 mg/dl), malabsorption, irritable bowel

syndrome, recent gastrointestinal surgery in the past 1 year, and diarrhea for 3 consecutive days in the past 3 months. Patients with diabetes mellitus, acute or chronic infectious or inflammatory disease, thyroid disease, cardiovascular disease, abnormal complete blood count, malignant disease, kidney disease, and mental disorders were excluded from the study. Individuals receiving medications/therapies including anticoagulant therapy, anti-obesity drugs, steroid therapy, anti-inflammatory drugs, antibiotics, beta blockers, corticosteroids, oral contraceptives, and dietary supplements in the past 2 months were also excluded. Demographic data, medical history, and physical history questionnaires were obtained. Anthropometric indices were measured according to standard measurement protocols as described in our previous study [28]. Bioelectrical impedance analysis (BIA: BC-418MA, Tanita, Japan) was used to define the fat percentage, fat mass (FM), and fat-free mass (FFM).

Laboratory assays

After a 12-h overnight fast, 5 cm^3 blood was obtained. Instantly, after centrifugation at 3000 rpm for 5 min, metabolic parameters were examined. FBS, total cholesterol (TC), TG, and HDL-C were assayed, using the standard enzymatic methods via Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands). Inter- and intra-assay coefficient of variation (CV) was < 5% for all analyses. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula: LDL-C (mg/dl) = [TC] − [HDL-C] − [TG] 5.0. Insulin was assayed using ELISA Monobind kit.

The homeostasis model of insulin resistance (HOMA-IR) was calculated as

$$[\text{fasting insulin}\,(\mu\text{IU/ml}) \times \text{fasting glucose}\,(\text{mg/dl})/405] \quad [29]$$

Quantitative insulin sensitivity check index (QUICKI), an indicator of insulin sensitivity, was calculated as

$$[1/(\log \text{fasting insulin} + \log \text{fasting glucose in mg/dl})] \quad [30]$$

The patients were divided into insulin-resistant (IR) and non-insulin-resistant (NIR) groups.

Peripheral blood mononuclear cell isolation

PBMCs were isolated using Ficoll-Hypaque gradient density centrifugation (Baharafshan, Tehran, Iran).

Real-time PCR

RNA was extracted from PBMCs, using Accusol (Bioneer Pacific, USA). cDNA from total RNA was synthesized, using the Revert Aid First Strand cDNA Synthesis kit (Fermentas, Thermo fisher Scientific, USA). Real-Time PCR was performed using primers specific for TLR2, TLR4, MyD88, TRIF, and NFκB (Invitro Gen), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as normalizer (Invitro Gen). Reactions were performed in triplicate, using a light cycler 96 real-time PCR instrument (Roche, Switzerland). Data were calculated via the $2^{-\Delta\Delta CT}$ method [31, 32].

Statistical analysis

All analyses were performed using SPSS software version 17.0 for Windows (PASW Statistics; SPSS Inc., Chicago, IL, USA). Normality of the data was checked, using Kolmogorov-Smirnov test. Mean ± standard deviation (SD) was used for parametric data and median (25th, 75th) for

Table 1 General characteristics and anthropometric measurements between IR and NIR groups of obese individuals

Variable	NIR group ($n = 27$)	IR group ($n = 61$)	$p^{\dagger}$
Age (years)	36.11 ± 7.95	36.83 ± 7.21	0.675
Weight (kg)	83.75 ± 11.80	85.68 ± 15.03	0.557
Height (cm)	165.66 ± 9.21	164.15 ± 12.89	0.584
Body mass index (kg/m^2)	30.66 ± 3.36	31.77 ± 4.22	0.232
Waist circumference (cm)	102.37 ± 6.32	106.022 ± 7.71	0.025
Hip circumference (cm)	110.14 ± 6.34	110.62 ± 8.30	0.792
Waist/hip ratio (cm)	0.92 ± 0.054	0.95 ± 0.056	0.013
Body fat (%)	32.25 ± 8.12	33.20 ± 7.70	0.603
Body fat mass (kg)	26.82 ± 6.33	28.32 ± 7.98	0.390
Fat-free mass (kg)	57.26 ± 11.86	57.40 ± 12.60	0.962
Systolic blood pressure (mmHg)	113.70 ± 19.24	112 ± 13.52	0.680
Diastolic blood pressure (mmHg)	73.33 ± 13.08	76.58 ± 11.36	0.243

Data are presented as mean ± SD

† Based on independent sample *t* test

Table 2 Metabolic parameters between IR and NIR groups of obese individuals

Variable	NIR group ($n = 27$)	IR group ($n = 61$)	$p^{\dagger}$
TG (mg/dl)	198.40 ± 92.54	170.37 ± 80.30	0.250
HDL-C (mg/dl)	46.00 ± 7.44	40.60 ± 8.14	0.040
LDL-C (mg/dl)	102.40 ± 29.17	121.36 ± 30.00	0.302
Cholesterol (mg/dl)	183.88 ± 34.87	196.54 ± 34.63	0.137
FBS (mg/dl)	90.88 ± 8.67	93.32 ± 9.08	0.242
Insulin (μU/ml)	6.14 ± 1.72	22.26 ± 8.28	< 0.001
HOMA-IR	1.38 ± 0.41	5.13 ± 1.99	< 0.001
QUICKI	0.57 ± 0.03	0.43 ± 0.03	< 0.001

Data are presented as mean ± SD

TG triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *FBS* fasting blood sugar, *HOMA-IR* homeostasis model of insulin resistance, *QUICKI* quantitative insulin sensitivity check index

† Based on independent sample *t* test

nonparametric variables. Standard statistical tests for comparison of the means of the two groups were independent sample *t* test for normal values and Mann-Whitney *U* test for abnormal data. Correlation between gene expression levels and other quantitative variables was assessed, using the Spearman correlation test. A *p* value less than 0.05 was considered significant. The sample size was estimated to be 67 persons, according to a previous study [33] based on serum HDL-C level, with 80% power and an α-error of 5%. Considering a drop-out rate of 30%, the total sample size required was 88.

Results

The general characteristics and anthropometric measurements of the participants are given in Table 1. Despite similar age (36.11 vs. 36.83 years) and BMI (30.66 vs. 31.77 kg/m^2), the waist

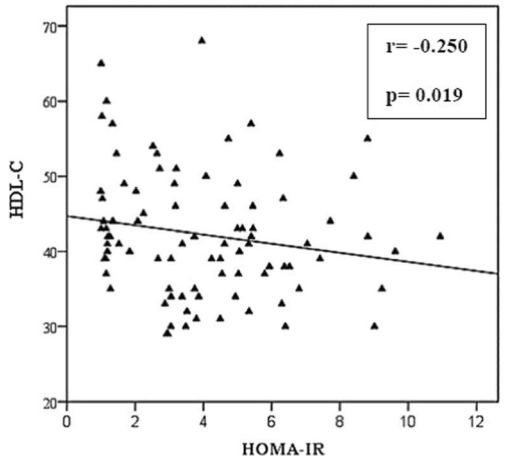

Fig. 1 Pearson correlation between HDL-C and HOMA-IR. HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model of insulin resistance

Table 3 Gene expression results between IR and NIR groups of obese individuals

Variable	NIR group ($n = 19$)	IR group ($n = 51$)	p^{+}
FC TLR2	1.84 (1.01–9.41)	0.611 (0.26–5.09)	0.074
FC Myd88	1.03 (0.27–3.78)	4.02 (0.48–15.56)	0.063
FC NFκB	1.21 (0.21–5.85)	2.05 (0.43–4.11)	0.383
FC TLR4	0.52 (0.01–12.80)	4.89 (0.46–16)	0.035
FC TRIF	0.702 (0.17–3.73)	0.92 (0.27–4.9)	0.409

Data are presented as median (25th, 75th)

FC fold change, *TLR2* toll-like receptor 2, *MyD88* myeloid differentiation factor 88, *NFκB* nuclear factor κB, *TLR4* toll-like receptor 4, *TRIF* TIR domain-containing adaptor-inducing interferon-β

+ Based on the Mann-Whitney *U* test

circumference (WC) ($p = 0.025$) and the waist/hip ratio (WHR) ($p = 0.013$) were higher in the IR group than the NIR group. There was no significant difference in body fat mass (FM) and fat-free mass (FFM) measured by bioelectric impedance between the two groups. The IR and NIR groups had no significant difference in terms of systolic and diastolic blood pressure.

The IR group had significantly lower HDL-C ($p = 0.04$) and higher low-density lipoprotein cholesterol (LDL-C) ($p = 0.02$) compared with the NIR group; however, there were no significant differences in other components of lipid profile (Table 2). Fasting insulin concentration and HOMA-IR were significantly higher ($p < 0.001$) and QUICKI was significantly lower ($p < 0.001$) in the IR compared to the NIR group (Table 2). There was a significant correlation between HOMA-IR and HDL-C ($r = -0.25$; $p = 0.019$) (Fig. 1).

TLR4 mRNA revealed significant upregulation in PBMCs of the IR group compared with the NIR individuals ($p = 0.035$). However, TLR2 mRNA expression revealed no significant difference between the two groups. There was also no significant difference in mRNA expression of downstream signaling proteins of TLRs including MyD88, TRIF, and NFκB between the two groups (Table 3). But there was a significant correlation between HDL-C and TLR4 ($r = -0.305$; $p = 0.011$) and MyD88 ($r = -0.27$; $p = 0.024$) expression level in all the persons (Fig. 2). A significant correlation of LDL-C was also observed with TLR4 ($r = 0.302$; $p = 0.012$) as well as with MyD88 ($r = 0.267$; $p = 0.027$) expression level (Fig. 3). Gene mRNA expression revealed no significant difference between the IR groups compared with the NIR individuals when analyzed based on gender (Table 4).

Discussion

The present study investigated the relationship of IR with inflammatory gene expression, lipid profile, metabolic health, and body composition in apparently healthy obese subjects. We found that TLR4 mRNA expression was significantly

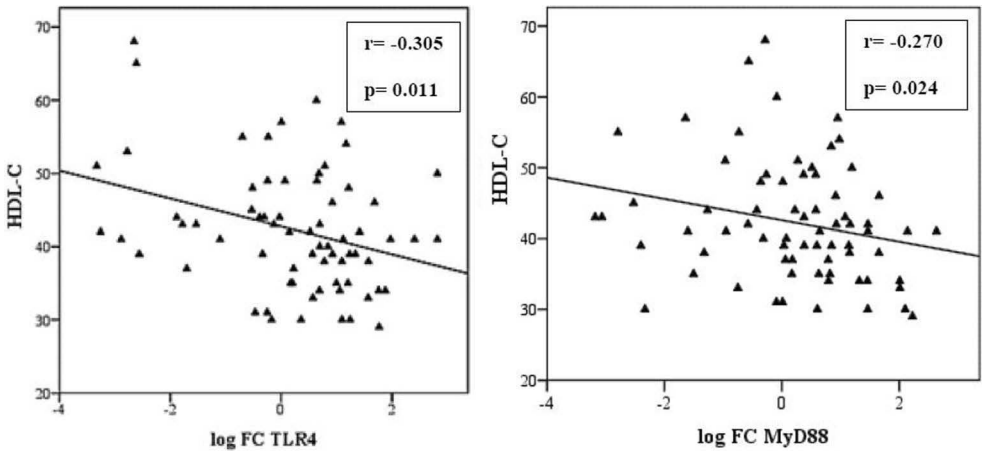

Fig. 2 Spearman correlations of HDL-C with log FC TLR4 as well as log FC MyD88. HDL-C: high-density lipoprotein cholesterol; FC: fold change, TLR4: toll-like receptor 4, Myd88: myeloid differentiation factor 88

different in PBMCs of the IR group compared with the NIR individuals. Though Myd88 and NFκB gene expression were increased in the IR group compared with the NIR group, it did not reach the significant level. However, there was a significant link of HDL-C and LDL-C with TLR4 and Myd88 expression. Approximately 70% of the participants were insulin-resistant. According to prior studies, high-risk abdominally obese patients are characterized by the cluster of metabolic abnormalities particularly high fasting insulin concentration [3]. Furthermore, it is proved that IR in population is related more to abdominal obesity than to general adiposity [8, 34]. Hence, there is a cause and effect relationship between them.

In the present study, we hypothesized that TLR4 signaling might be activated in obese condition which may mediate IR. We observed that mRNA expression of TLR4 was significantly increased in PBMCs of the IR group compared with the NIR group. In line with our study, the research conducted by Kim et al. [35] showed that TLR4 is a mediating key factor in the development of vascular inflammation and IR in diet-induced obesity via upregulation of transcriptional factors such as NFκB. Also, it was recently proved that free fatty acids (FFAs) can activate inflammatory pathways by activating TLR4 signaling in different cells like adipocytes and macrophages. In fact, in the absence of TLR4, initiation of inflammatory signaling pathways cannot occur [13].

The research conducted by Dasu et al. [36] on subjects with T2DM indicated that TLR4 expression was significantly increased compared to control subjects. It also had a positive correlation with HOMA-IR. Moreover, Reyna et al. [37] in a study on skeletal muscle of insulin-resistant subjects ($n = 22$) reported abnormal TLR4 expression with little information on TLR4-MyD88 signaling pathway and its correlation to IR. In addition, Creely et al. [38] showed increased TLR2 expression in the adipose tissue of T2DM patients with strong correlates to endotoxin levels and with no change in TLR4 expression.

We failed to find a significant difference in the mRNA expression of TLR2 and downstream signaling proteins of TLRs including MyD88, TRIF, and NFκB between the two groups, though significant correlations were observed between expression levels of inflammatory genes. Likewise, Telle-Hansen et al. [22] reported no differences in the expression level of TLR2 and TLR4 in the PBMCs of metabolically unhealthy obese (MUO) subjects compared with metabolically healthy obese (MHO) persons, although in MUO group, HOMA-IR was significantly higher than MHO group. In a study conducted by Gomez-Ambrosi et al. [21], no significant

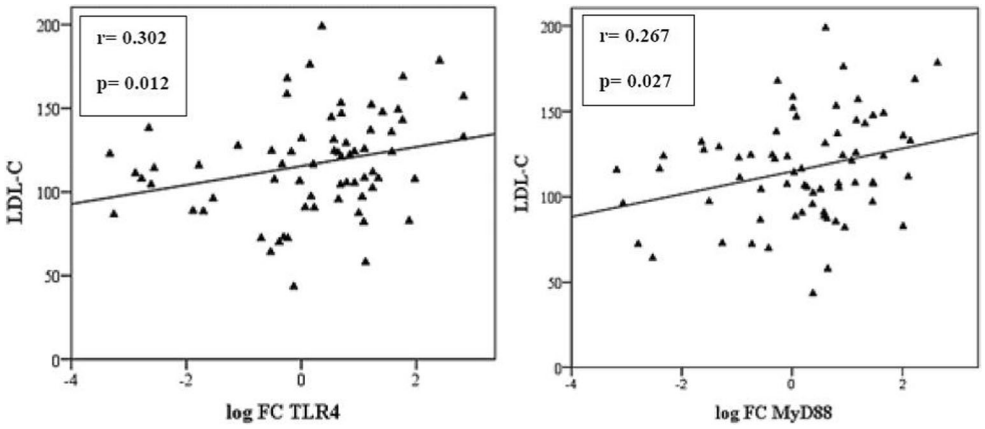

Fig. 3 Spearman correlations of LDL-C with log FC TLR4 as well as log FC MyD88. LDL-C: low-density lipoprotein cholesterol; FC: fold change, TLR4: toll-like receptor 4, Myd88: myeloid differentiation factor 88

Table 4 Gender differences in gene expression levels between IR and NIR groups of obese individuals

Variable	NIR group ($n = 19$)			IR group ($n = 51$)		
	Females ($n = 10$)	Males ($n = 9$)	p	Females ($n = 26$)	Males ($n = 25$)	p^+
FC TLR2	3.13 (1.01–8.56)	1.34 (0.65–11.08)	0.71	0.64 (0.30–8.23)	0.53 (0.23–4.99)	0.48
FC Myd88	1.67 (0.54–3.08)	0.48 (0.21–6.84)	0.46	4.25 (0.5–11.48)	3.98 (0.14–28.7)	0.79
FC NFκB	2.60 (0.23–5.83)	0.84 (0.05–6)	0.46	1.61 (0.42–3.20)	2.15 (0.05–6)	0.64
FC TLR4	0.93 (0.007–15.63)	0.4 (0.14–6.34)	0.65	3.31 (0.26–14.54)	4.92 (1.62–18.5)	0.32
FC TRIF	0.77 (0.3–5.2)	0.61 (0.062–4.81)	0.56	0.81 (0.25–4.45)	1.0 (0.26–6.68)	0.94

Data are presented as median (25th, 75th)

FC fold change, *TLR2* toll-like receptor 2, *MyD88* myeloid differentiation factor 88, *NFκB* nuclear factor κB, *TLR4* toll-like receptor 4, *TRIF* TIR domain-containing adaptor-inducing interferon-β

[+] Based on the Mann-Whitney *U* test

difference was noticed in the expression level of TLR4 and TNF-α between MHO and MUO groups with similar IR status. Although some studies [21, 22] showed that metabolic state of obese persons can be an important factor in determining inflammatory gene expression, our smaller sample size might have contributed to such results.

Our study also showed that among lipid profile, the levels of HDL-C and LDL-C were significantly different between the two groups, a finding which is consistent with prior reports [39–43]. Additionally, HDL-C levels inversely and LDL-C levels positively correlated with TLR4 and Myd88 expression levels. Although these correlations were significant, they were not strong, possibly due to lower sample size of our study. In line with our results, in a microarray study on obese subjects, TLR4 and Myd88 were inversely associated with plasma HDL-C levels [44]. Also, another study revealed that HDL-C causes MyD88-specific downregulation of TLR4 expression and signaling [45]. Moreover, several researchers [12, 46, 47] have shown that accumulation of LDL-C can trigger TLR2 and TLR4 signaling. Therefore, there is a strong link between HDL-C and LDL-C with TLR4 signaling and its relation with metabolic disorders like IR.

As the present work showed, there was a significant correlation of HOMA-IR with HDL-C and FBS. Similar to our finding, in a study [48] on 2283 patients with coronary heart disease (CHD), HDL-C was correlated with HOMA-IR levels. Another research on people with T2DM or MetS indicated significant positive correlations between the HOMA-IR and TC/HDL and between HOMA-IR and TG/HDL in MetS and T2DM patients [49]. Thus, low HDL-C may contribute to the high prevalence of IR; also, the combination of HOMA-IR and HDL-C as available and economic markers would help in identifying high-risk patients in clinical practice.

In this research, the mean WC and WHR were higher in the IR than those in the NIR patients. In line with this result, a study [50] from Quebec Heart Institute revealed that high WC is powerfully related to plasma insulin levels. Besides, Reaven et al. [51] showed that WC is a better predictor than BMI of insulin-mediated glucose uptake. Therefore, WC not only contributes to IR, but also can be used as a powerful predictor of clinical outcomes linked to IR.

Overall, to the best of our knowledge, this study is the first to be conducted on Tabriz population and to investigate the association of IR in relation to inflammatory gene expression levels, metabolic health, lipid profile, and body composition in apparently healthy obese individuals. However, some limitations of the study need to be considered, first, the small sample size and second, the case control nature of the study the cause of which could not be assessed through it.

Conclusion

The results of this study indicated the possible link between IR with TLR4. Also, there was significant correlation between HDL-C and LDL-C with TLR4 and MyD88. Some inflammatory genes and metabolic parameters were also significantly correlated.

Acknowledgments Thanks are due to all who participated in the study.

Authors' contribution MSA and PA wrote the study protocol and study design. BB and DS helped with qPCR. MSA and PA analyzed and interpreted the data. PA, MN, and SM helped with the sampling. PA and MSA were involved in drafting the manuscript or revising it critically for content. All authors have given final approval of the version to be published.

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Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Interrelationship between nuclear factor-erythroid-2-related factor 2, NADPH quinone oxidoreductase and lipoprotein-associated phospholipase A2 expression in young patients of metabolic syndrome

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Abstract
Metabolic syndrome (MS) is associated with inflammation and oxidative stress (OS). Keap1/Nrf2/ARE is a cytoprotective pathway induced by OS and inflammation. This study aims to evaluate the expression of nuclear factor-erythroid-2-related factor 2 (Nrf2) and its downstream target gene NADPH quinone oxidoreductase-1 (NQO-1) in MS. Since lipoprotein-associated phospholipase A2(LpPLA2) is an important inflammatory marker believed to have a role in complications of MS, the association of its expression with that of Nrf2 and NQO-1 was also studied. Medical students ($n = 26$) were categorised in two groups according to NCEP ATP III criteria with WHO criteria for obesity for South Asian region: patients of MS ($n = 13$) and controls ($n = 13$). mRNA expression of Nrf2, NQO-1 and LpPLA2 genes was evaluated by qPCR in blood using specific primers. Fold change was calculated by $2^{-\Delta\Delta cT}$ method keeping β-actin as internal control. Expression of NQO-1 and LpPLA2 was found to be higher in MS. However, Nrf2 expression was low in patients who had hypertriglyceridemia when compared with patients with normal triglyceride levels. A significant correlation was observed in expression of LpPLA2, with Nrf2 and NQO-1. Our data suggests that there may be compensatory activation of antioxidant defence mechanism in young patients of MS. Further evidence is provided by higher expression of LpPLA2 and its correlation with Nrf2 and NQO-1 in MS which suggests that inflammatory stress may induce expression of genes of cytoprotective pathways. Additionally, this study, for the first time, indicates that Nrf2 may have some role in regulating triglyceride (TG) concentration.

Keywords Metabolic syndrome · Lipoprotein-associated phospholipase A2 · Nuclear factor E2-related factor 2 · NAD(P)H quinone oxidoreductase 1

Introduction

Obesity, metabolic syndrome (MS) and type 2 diabetes are multifactorial diseases, characterised by insulin resistance (IR), chronic inflammation and oxidative stress (OS) and are of growing interest and concern due to their increasing prevalence among various populations of the world [1]. Prevalence of MS is rapidly increasing in Indian population, particularly with the adoption of a modernised sedentary lifestyle [2, 3]. Metabolic dysregulation, inflammation and OS are implicated in various complications, e.g. risk of cardiovascular events, cancer and other diseases [4, 5]. The knowledge on the pathophysiology of obesity, IR and diabetes is constantly expanding and many new molecular pathways are studied and proposed to have role in pathogenesis of these diseases and their complications. In the past decade, a lot of interest is generated in Keap1/Nrf2/ARE pathway. This pathway is believed to regulate cellular detoxification processes and redox status [6]. Under basal conditions, nuclear factor-erythroid-2-related factor 2(Nrf2) is associated with Kelch-like ECH-associated protein 1(Keap-1) which prevents its translocation to nucleus. In presence of stress, Nrf2 dissociates from Keap-1

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and translocates to nucleus where it binds to the promoter sequence of "antioxidant responsive element (ARE)". This leads to coordinated upregulation of ARE-driven detoxification and antioxidant genes. Products of Nrf2-induced genes are involved in conjugation/detoxification reactions (e.g. glutathione S-transferases-GSTs), antioxidative responses (e.g. NADPH quinone oxidoreductase –NQO-1) etc. [7]. Nrf2 is ubiquitously expressed and is considered to be a multi-organ protector [8]. Its role in obesity and metabolic dysregulation has already been established in animals [9, 10].

NADPH oxidase encoded by *NQO-1* gene is located on chromosome 16 and is an important downstream target for Nrf2. It is a widely distributed flavin adenine dinucleotide (FAD)-dependent inducible enzyme and acts as potent antioxidant [11]. Thus, the expression of these genes may affect the generation and disposal of reactive oxygen species (ROS). Although NQO-1 is shown to have an effect on MS in rodents, there is a scarcity of literature on human studies [12].

In addition to Keap1/Nrf2/ARE pathway, other circulating markers of inflammation have also been studied in MS. One of these is lipoprotein-associated phospholipase A2 (LpPLA2) which is secreted by macrophages and monocytes and is considered a risk marker for atherosclerosis and cardiovascular disease (CVD) [13]. LpPLA2 circulates with low-density lipoproteins (LDL) and high-density lipoproteins (HDL) and acts on the oxidised phospholipids producing lysophospholipids and oxidised fatty acids [14]. Independent studies have reported elevated mass and activity of LpPLA2 as well as high mRNA expression in MS [15–17].

Although numerous studies have proved that inflammation and OS are the key components of biochemical mechanism involved in MS and its complications, Nrf2, a master regulator of antioxidant and protective genes, has not been studied sufficiently in humans. In addition, not enough literature regarding the expression studies of Keap1/Nrf2/ARE pathway is available. Since the prevalence of MS has increased in the younger populations, the present study aimed to evaluate the expression of Nrf2 and its downstream target NQO-1 and their association with LpPLA2 expression in young patients of MS.

Material and methods

The study was a comparative cross-sectional study conducted in the Department of Biochemistry of University College of Medical Sciences, Delhi, as a pilot project. Ethical clearance was obtained from the Institutional Ethical Committee for human studies. Participation in this study was voluntary and those who were ≥ 18 years of age and gave informed written consent were recruited.

Study groups

A total of 26 undergraduate students of the college (those pursuing MBBS course) were recruited in the study. Thirteen patients of MS and equal number of controls were matched for age and gender and an informed consent was obtained from each participant. Each participant was interviewed and underwent anthropometric analysis. Exclusion criteria included overt CVD, past history of CVD, respiratory diseases, chronic allergic conditions, fever and infections, as assessed from history. Careful history regarding smoking status was obtained and smokers were excluded from the study. Individuals on any kind of medication were also excluded. Total of 7 ml venous blood was collected after an overnight fast in appropriate sample collection tubes (EDTA vials for mRNA levels, fluoride vial for plasma glucose estimation and plain vials for biochemical analysis) and was processed immediately.

Definition of the metabolic syndrome

Presence of MS was defined as per the criteria of the National Cholesterol Education Program Adult treatment Panel III (NCEP/ATPIII) [18]. Participants who had three or more of the following criteria were considered to have MS: hypertriglyceridemia (triglycerides level ≥ 150 mg/dl), low HDL (< 40 mg/dl for men and < 50 mg/dl for women), high blood pressure (≥ 130/85 mmHg), elevated fasting plasma glucose (≥ 110 mg/dl) and abdominal obesity. For defining abdominal obesity, cutoffs specific for South Asian region were considered (> 90 cm for men and > 80 cm for women) [19].

Anthropometric measurement

Waist circumference and hip circumference were measured according to WHO STEPS protocol [20]. Weight was measured using digital weighing machine and height using wall-mounted scale and these parameters were further used in deriving the body mass index (BMI) which was calculated as weight in kg divided by squared height (m^2). Systolic and diastolic blood pressure was obtained with a mercury sphygmomanometer using auscultatory method.

Expression analysis of Nrf2 and NQO-1 mRNA

Whole blood was collected in EDTA and used for gene expression analysis. Total ribonucleic acid (RNA) was isolated from whole blood using Tri-reagent BD from Sigma Chemicals, USA, following manufacturer's protocol. The quality of RNA was checked by taking the optical density ratio at 260/280; a ratio of 1.8–2.0 was considered adequate. First-strand cDNA synthesis was carried out using 200 U reverse transcriptase (Revert Aid from Thermo Scientific Inc., USA),

100pM random hexamer and oligo dT in the ratio 1:1 (Sigma Aldrich, India), 10 mM dNTPs, 40 U ribonuclease (RNase) inhibitor (Thermo Scientific Inc., USA) and 500 ng of RNA, 4 μl of 5× reaction buffer and the final volume made to 20 μl with diethyl pyrocarbonate (DEPC)-treated water. Incubation at 25 °C for 10 min was carried out, followed by 45 °C for 60 min, following which the reverse transcriptase was inactivated at 70 °C for 10 min in a thermocycler (Eppendorf Mastercycler Gradient-5331). This cDNA was stored at − 20 °C and used as template sample for qPCR.

Relative expression of the genes was analysed by real-time polymerase chain reaction (PCR) using specific primers. Ten microlitres of Hot-Start PCR master mix (Thermo Scientific Inc., USA), 0.5 μl of forward and reverse primers, 1 μl of syto 9 dye (diluted 1:100), 1 μl of diluted cDNA and volume made up to 20 μl with DEPC treated water was used for real time-PCR for Nrf2, NQO-1and LpPLA2 as well as housekeeping genes. The cycling conditions were same for all the genes except the annealing temperature (hold 95 °C for 4 mins, cycling for 35 cycles; 95 °C for 15 s, annealing at 54 °C temperature for 30 s and 72 °C for 30 s). The fluorescence was acquired at 72 °C. All the reactions were run in duplicates. The primer sequences for all the genes are given in Table 1. β-actin was used as internal reference gene. The relative expression of the gene was analysed by ΔΔcT and fold change was calculated using $2^{-\Delta\Delta cT}$ method.

Biochemical analysis

Blood samples were collected by venipuncture after an overnight fast for 12–14 h. Serum was separated by centrifugation at 3000 rpm for 5 mins and was used for routine biochemical parameters to rule out any liver and renal abnormalities. Plasma glucose, serum cholesterol and serum triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were estimated using system packs (Olympus AU 400, Japan), and low-density lipoprotein cholesterol (LDL-C) was calculated by Freidwald formula [21].

Table 1 Primer sequence

Name	Sequence 5′ to 3′
NRF2 (forward)	ACACGGTCCACAGCTCATC
(Reverse)	TGTCAATCAAATCCATGTCCTG
NQO1 (Forward)	GGCAGAAGAGCACTGATCGTA
(Reverse)	TGATGGGATTGAAGTTCATGC
LpPLA2 (forward)	CCACCCAAATTGCATGTGC
(Reverse)	GCCAGTCAAAAGGATAAACCACAG
β-actin (forward)	TCATGAAGTGTGACGTTGACATCCGT
(Reverse)	CCTAGAAGCATTTGCGGTGCACGATG

Statistical analysis

Data was analysed using SPSS software. The data in the two groups were compared using Mann-Whitney *U* test. To calculate fold change in expression, $2^{-\Delta\Delta cT}$ method was used. For this, ΔcT was calculated by subtracting cT value for target gene from the cT value for housekeeping gene for each participant. The average ΔcT values for cases and control were calculated. To calculate ΔΔcT average, ΔcT of cases was subtracted from average ΔcT of controls. Fold change for each patient was also calculated by the formula $2^{-\Delta\Delta cT}$. Correlation studies were done using the Pearson correlation coefficient analysis taking ΔcT as one of the variables. For this, Δ cT was calculated for each participant. A p value less than 0.05 was considered statistically significant.

Results

All participants in the study were aged 18–26 years and there was no significant difference in mean age between the two groups. There were three females in the control group and two females in the patient group. Table 2 depicts the anthropometric, clinical and biochemical parameters. Waist circumference, hip circumference, weight and BMI were significantly higher in patients of MS. However, two participants were obese and one had low HDL-C in control group also. While TG levels in all were in normal range (< 150 mg/dl) in the control group, five participants in the patient group were having hypertriglyceridemia (HTG). Fasting plasma glucose was within normal range in all participants (range 69–91 mg/dl). None of the participants were taking any sort of medicines.

Expression analysis

mRNA levels of NQO-1 were found to be higher in MS as compared to controls. Taking β-actin as reference gene, it was observed that the fold change in mRNA expression of NQO-1 in whole blood was 1.9. Expression of LpPLA2 was also 4.9 times higher in patients (MS). However, mRNA expression of Nrf2 was similar in both groups (fold change 1.1). Studies indicate that Nrf2 has role in lipid metabolism [10]; we therefore compared Nrf2 expression in patients of MS with normal TG levels (< 150 mg/dl) and HTG(≥ 150 mg/dl). HTG was found to be associated with 7.7 times lower Nrf2 expression.

Correlation studies

Correlation studies indicated that mRNA expression of Nrf2 correlated significantly with expression of NQO-1 ($r = 0.662$, $p = 0.001$) (Fig. 1). Expression of both genes (Nrf2 and NQO-1) also had significant positive correlation with waist circumference, hip circumference and weight (Table 3). However,

Table 2 Anthropometric, clinical and biochemical parameters

	Controls ($n = 13$)	Patients ($n = 13$)	p value
Age (years)	19.83 ± 0.9.3	20.67 ± 2.53	0.298
Hip circumference (cm)	95.33 ± 5.64	103.92 ± 8.02	0.006
Abdominal circumference (cm)	82.93 ± 9.90	105.92 ± 9.62	0.000
Waist hip ratio	0.82 ± 0.07	1.02 ± 0.09	0.000
Weight (kg)	62.33 ± 11.47	83.33 ± 14.69	0.001
BMI (kg/m^2)	21.98 ± 4.61	27.36 ± 4.07	0.006
Triglycerides (mg/dl)	80.25 ± 19.33	121.9 ± 40.52	0.004
HDL cholesterol(mg/dl)	50.5 ± 13.28	33.08 ± 11.58	0.002
LDL cholesterol(mg/dl)	107.03 ± 24.64	124.45 ± 27.38	0.116
Total cholesterol(mg/dl)	173.58 ± 24.08	181.92 ± 40.24	0.545
Systolic BP (mmHg)	121.42 ± 5.89	138.89 ± 3.94	0.000
Diastolic BP(mmHg)	76.25 ± 9.30	86.75 ± 2.92	0.001
Fasting glucose (mg/dl)	84.0 ± 7.17	81.92 ± 5.42	0.431

All values are expressed in mean ± SD, comparison of parameters in the two groups is carried out by unpaired student's t test, $p < 0.05$ is significant

Hip circumference, abdominal circumference, waist hip ratio, weight and BMI were significantly high in patients as were triglyceride levels and blood pressure. HDL cholesterol was significantly low in patients

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *BP* blood pressure

waist hip ratio correlated with expression of Nrf2 only. In addition, expression of NQO-1 correlated positively with BP and negatively with HDL-C in all participants ($n = 26$). A significant association of LpPLA2 mRNA expression was observed with expression of Nrf2 ($r = 0.653$, $p = 0.001$) and NQO-1 (0.748, $p = 0.000$) as shown in Fig. 2.

Discussion

Transcription of genes is a dynamic process. Any change in the biological/ metabolic processes is accompanied with regulatory modulation of the expression of various genes [22]. Patients with MS are subjected to cellular insult by ongoing chronic inflammation and metabolic dysregulation. In this study, we evaluated the expression of genes involved in cytoprotective pathways, i.e. Nrf2, its downstream target and NQO-1 in MS as well as LpPLA2, which is involved in inflammatory pathway. We observed that mRNA expression of NQO-1 is higher in young patients of MS. NAD(P)H:(quinone acceptor) oxidoreductase 1 (NQO-1) is an important antioxidant enzyme which has been assigned with multiple protective roles that include and extend beyond its antioxidant role [11]. Higher expression of NQO-1 gene in MS indicates activation of antioxidant defence mechanism in young patients.

NQO-1 is a downstream target for Nrf2, and it was expected that the induction of antioxidant enzyme would have been mediated through Nrf2. However, contrary to the expectation, the mRNA expression of Nrf2 was similar in both groups. According to previous studies, induction of Nrf2/ARE pathway

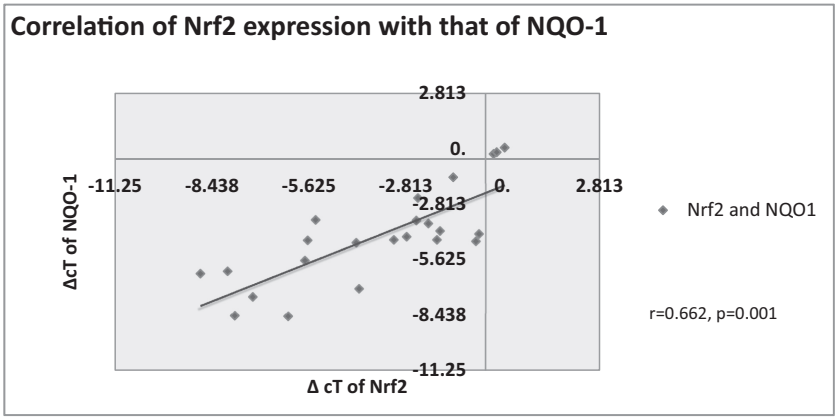

Fig. 1 Correlation of mRNA expression of Nrf2 with that of NQO-1. ΔcT of Nrf2 and NQO-1 are used as variables. r value is the Pearson correlation coefficient, $p < 0.05$ is significant. Significant correlation is observed between mRNA expression of Nrf2 and NQO-1

Table 3 Correlation of mRNA expression of Nrf2 and NQO-1 genes with anthropometric and biochemical parameters

Parameters ($n = 26$)	mRNA of Nrf2		mRNA of NQO-1	
	Correlation coefficient (r value)	Significance (p value)	Correlation coefficient (r value)	Significance (p value)
Hip circumference	0.458	0.024	0.488	0.018
Waist circumference	0.537	0.007	0.519	0.011
Waist hip ratio	0.480	0.018	0.399	0.06
Weight	0.521	0.009	0.523	0.010
Body mass index	0.349	0.095	0.335	0.118
Systolic blood pressure	0.299	0.155	0.498	0.016
Diastolic blood pressure	0.200	0.348	0.458	0.028
Total cholesterol	− 0.280	0.186	0.014	0.950
Triglycerides	− 0.021	0.923	0.291	0.178
High-density lipoprotein	− 0.385	0.063	− 0.421	0.045
Low-density lipoprotein	− 0.144	0.502	0.176	0.421
Fasting glucose	− 0.185	0.386	− 0.294	0.173

Correlation was evaluated by using the Pearson correlation coefficient. Dependent variables: ΔcT of Nrf2 and ΔcT of NQO-1

p value < 0.05 is significant

can be attributed to either increased expression of Nrf2 gene or by reduced ubiquitination and proteasomal degradation of Nrf2 [23, 24]. The results of our study indicate that presence of MS results in activation of Nrf2/ARE pathway, probably by stabilising Nrf2 rather than increasing the expression.

Nrf2 is ubiquitously expressed including adipose tissue. Nrf2 has been proposed to have role in obesity through its action in adipocyte differentiation [9]. Studies have reported that Nrf2 deficiency in human preadipocytes impairs adipogenesis [9]. This indicates that Nrf2 has direct association with obesity. These findings are reiterated in our present study also. Rather than in adipocytes, we studied the mRNA levels in blood and observed that a significant correlation of the expression exists with parameters of obesity, i.e. waist circumference, hip circumference, waist hip ratio and weight. The results of our study are similar to those reported by Das et al. [25]. They also demonstrated that Nrf2 expression correlates positively with obesity and Nrf 2-mediated oxidative stress is upregulated in obesity. However, our findings are contrary to those reported by Santillán et al. [26]. They reported that Nrf2 expression is reduced in overweight boys and negatively correlated with BMI and TG.

Our study also revealed that there is low mRNA expression of Nrf2 (7.7 times) in blood in HTG indicating that Nrf2 may

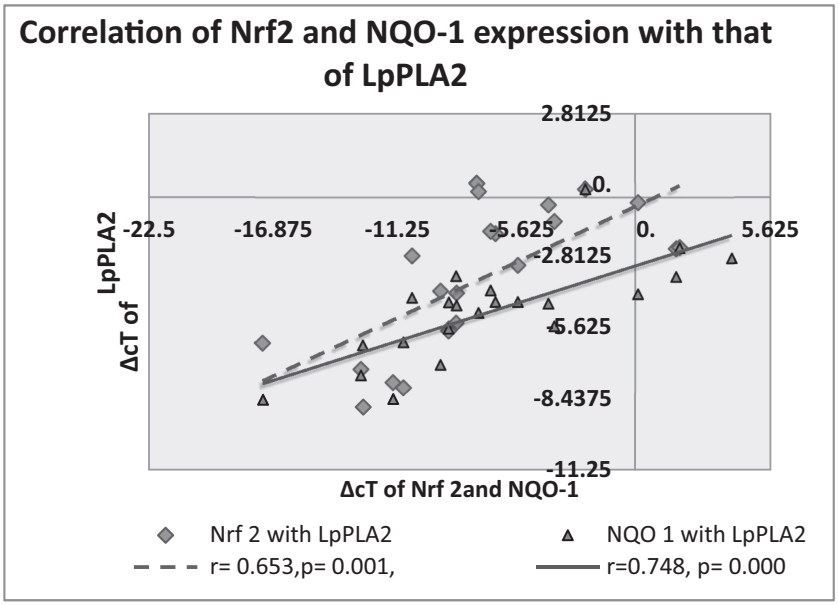

Fig. 2 Correlation of mRNA expression of Nrf2 and NQO-1 with that of LpPLA2. ΔcT of Nrf2, NQO-1 and LpPLA2 are used as variables. r value is the Pearson correlation coefficient, $p < 0.05$ is significant. Significant correlation of mRNA expression of Nrf2 and NQO-1 is observed with that of LpPLA2

have some role in TG homeostasis, although correlation was not observed between TG levels and Nrf2 expression. Many groups have carried out the studies on different animal models ranging from those on standard diet to long-term and short-term high-fat diet-induced insulin resistance and hepatic steatosis as well as methionine- and choline-deficient diet (MCD) as a model of fatty liver [27–30]. While Yates et al. [27] described that activation of Nrf2 signalling in murine liver may result in the lower liver lipid levels, Kitteringham et al. [28] observed that deletion of Nrf2 in mouse liver upregulates enzymes of lipogenesis in animals on standard diet. The use of MCD as a model of fatty liver has revealed that Nrf2 deletion accelerates the onset and progression to steatohepatitis by causing accumulation of TGs in liver [27]. However, none of these studies evaluated the expression of Nrf2 and/or NQO-1. While most studies which proposed a role of Nrf2 in lipid metabolism have been carried out in hepatic tissues in animal models with either Nrf2 deletion or activation, our study is carried out in the whole blood in humans. The dynamics of gene expression in peripheral blood mononuclear cells (PBMCs) may be different from those in hepatic tissue. While a high expression of NQO-1 in PBMCs in MS may be attributed to its antioxidant role, the same cannot be stated for Nrf2. Rather, it appears from this study, that Nrf2 expression in blood may be associated with circulating TG levels.

Since PBMCs are involved in inflammatory and oxidative pathways, we studied the expression of LpPLA2, an important circulating inflammatory marker in blood. The relevance of evaluation of this marker with Nrf2 and NQO-1 in MS is that obesity and dyslipidemia are associated with systemic inflammation and OS with Nrf2-ARE axis at the helm of cytoprotection in OS. In addition, anti-inflammatory role has also been attributed to Nrf2 [31].

Several studies have indicated that mRNA expression of LpPLA2 and its mass and/or activity are associated with obesity, dyslipemia and MS [15–17]. In this study also, the expression of LpPLA2 was higher in MS. We also observed a significant association of LpPLA2 expression with Nrf2 and NQO-1 expression. Previously, it has been reported that oxidative species like oxidised LDL(oxLDL) which is the substrate for the enzyme can also induce the expression of LpPLA2 [32]. In this light, it is important to note that LpPLA2 as well as Nrf2-NQO-1 axis can be induced by oxidative or electrophilic stress. Action of LpPLA2 enzyme on oxidised phospholipids generates other oxidant/inflammatory mediators like lysophophatidylcoline, ox-fatty acids and isopronates [14]. The association of mRNA levels of LpPLA2 with those of Nrf2 and NQO-1 thus again brings our focus on induction of Nrf2-ARE axis in conditions of OS and inflammatory stress. However, it will be worthwhile to establish the molecular pathway for the same.

Apart from being the first study evaluating mRNA expression of Nrf2 and NQO-1in MS in Indian population, strength of our study is involvement of young individuals who are not under any medication. Since various drugs are known to be potent inducers of Keap1-Nrf2-ARE axis [33], and aspirin and cholesterol lowering-drugs affect the LpPLA2 activity [34], it seems pertinent that the expression profile should be studied in young individuals who are not taking any drugs. Our study thus evaluated the expression of these genes without effect of confounding variables like age and drug intake.

Although the data from our study reveals what is happening at the level of expression of the genes in whole blood in MS, information about the cause and effect relationship between various metabolites and products of these genes needs to be evaluated further. Moreover, association of Nrf2 expression with TG homeostasis needs to be confirmed in a larger sample, for which the research is already underway.

Conclusions

The results of our study indicate higher expression of NQO-1in blood in MS. A significant association of LpPLA2 expression with both Nrf2 and NQO-1 expression indicates that inflammatory stress is associated with compensatory induction of cytoprotective pathway, i.e. Nrf2-ARE axis. This study also reveals that Nrf2 may have some role in maintaining TG concentration although this finding needs to be confirmed in a larger sample size.

Implications Our study gives an insight into the probable pathophysiology and molecular mechanisms pertaining to OS and inflammation in young patients of MS. This is the first study which highlights the link between expression profile of Nrf2 in blood and concentration of TGs. Therefore, it paves the way for further studies on the role of Nrf2 in TG metabolism. Detailed studies may be conducted regarding the expression of mRNA and protein levels in blood as well as organs involved in TG metabolism, i.e. liver and adipose tissues. This may help to develop Nrf2 as a target for the therapeutic intervention in treating HTG.

Limitations Our study has certain limitations. For the purpose of evaluation of correlation between variables, sample size is relatively small. So potential for selection bias may raise some concerns on the statistical precisions of the estimates. Therefore, a large-scale study is required to validate our findings. In addition, this study has not evaluated the mRNA and expression in tissues which are involved in lipid metabolism. Since different genes are expressed differently in various tissues, it will be worthwhile to evaluate the same in different tissues to ascertain their role in MS.

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Authors' contributions Dr. Seema Garg and Dr. Mohit Mehndiratta conceptualised and designed the study. Pranav Malik and Dr. Seema Garg acquired the data with help from Dr. Rajarshi and Dr. Mohit Mehndiratta. Dr. Seema Garg statistically analysed and interpreted the data. Manuscript was drafted by Dr. Seema Garg, Dr. Rajarshi Kar and Dr. Mohit Mehndiratta. Critical review of manuscript was done by all authors.

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Compliance with ethical standards

Conflict of interest.

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Glossary

Nrf2	Nuclear factor-erythroid-2-related factor 2 (Nrf2)
NQO-1	NAD(P)H quinone oxidoreductase-1
TG	Triglycerides
HTG	Hypertriglyceridemia
LpPLA2	Lipoprotein-associated phospholipase A2
MS	Metabolic syndrome
OS	Oxidative stress
MCD	Methionine- and choline-deficient diet

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ORIGINAL ARTICLE

Correlates of ghrelin and visfatin in metabolic syndrome patients with and without prediabetes

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Abstract
Ghrelin is deregulated in obesity-associated insulin resistance (IR) while visfatin features a role in glucose uptake regulation, inflammation and IR. This study aimed to conduct comparisons and correlations of ghrelin and visfatin plasma levels in nondiabetic metabolic syndrome (MetS) and MetS-prediabetic/type 2 diabetes mellitus (T2DM) patients. In a cross-sectional study of 30 normoglycemic lean subjects (control), 31 MetS subjects, and 30 MetS-prediabetic/T2DM, plasma ghrelin and visfatin were measured by colorimetric-enzymatic assays. The comparison of both biomarkers between study groups and the correlation between them as well as with participants' adiposity, hematologic, and atherogenicity indices were conducted. Ghrelin levels (pg/mL) lacked any statistically significant difference between each of nondiabetic MetS (618.10 ± 93.22, p = 0.103) or MetS-pre/T2DM (498.17 ± 103.21, p = 0.454) vs. the normoglycemic lean control (369.38 ± 111.76). Visfatin level (ng/mL) in MetS patients with pre/T2DM (19.24 ± 2.05, p = 0.003) or without pre/T2DM (18.43 ± 1.83, p = 0.002) was significantly higher as compared to healthy controls' (8.62 ± 2.23). There was a direct ghrelin-visfatin correlation in the whole study population as well as in both MetS and MetS-pre/T2DM arms (p < 0.001). In nondiabetic MetS patients, ghrelin and visfatin proportionally correlated with waist/hip ratio (WHR; p = 0.032 vs. p = 0.008, respectively) while ghrelin correlated directly with BMI (p = 0.034). In MetS-pre/T2DM, visfatin correlated directly with body adiposity index (p = 0.039) but inversely with WHR (p = 0.011), while ghrelin and visfatin directly correlated with mean platelet volume (p = 0.025 vs. p = 0.030, respectively) and ghrelin proportionally correlated with platelet/lymphocyte ratio (p = 0.034). In effect, ghrelin and visfatin molecular interplays with adiposity and blood indices in the MetS derangements may present potential pharmacotherapeutic targets in metabolism and prediabetes anomalies.

Keywords Visfatin · Ghrelin · Prediabetes · Metabolic syndrome

Introduction

Type 2 diabetes mellitus (T2DM) previously known as "noninsulin-dependent diabetes" is the most common type of diabetes [1]. Jordan has high prevalence of DM; it constitutes 13–17% [2–4] and 15–20% for prediabetes (FBS 100–125 mg/100 mL) [5]. Overweight or obesity is a major condition that can lead to insulin resistance (IR) developing, which increases the risk of DM [6]. Furthermore, metabolic syndrome (MetS) increases diabetic risk approximately by fivefold [7, 8]. Excess adiposity, which predisposes to excessive fatty acids and various adipokine releases, increasing the risk of both DM and cardiovascular disease (CVD) [9]. Adiponectin serum levels are decreased in people with excessive adipose tissue leading to decrease grade of systemic inflammation [10]. Ghrelin is 28-amino acid peptide produced by A/X-cells in stomach [11]. Ghrelin is acylated by ghrelin O-acyl transferase (GOAT) [12]; the acylated form is the biologically active form [13]. Decreased circulating ghrelin levels have been demonstrated in people who have DM and IR [14], while anorexia and cachexia elevate the plasma ghrelin levels [15]. Ghrelin is involved in energy balance, cardiovascular, and gastrointestinal functions [16, 17].Visfatin is a protein produced by visceral adipose tissue (VAT); hence, it gains "visfatin" [18]. It regulates insulin sensitivity and hypoglycemia by interacting directly with insulin receptors [19]. Importantly, visfatin levels have been reported to be elevated

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in fatty liver disease, T2DM, obesity, and polycystic ovary syndrome [20]. Consequently, not surprisingly, visfatin antiinflammatory effects are via modulating cytokine secretion [21].

Few previous studies investigated the correlation between ghrelin and visfatin in diabetic and nondiabetic patients with metabolic syndrome [14–22]. Thus, our aim was to further explore such correlation as well as correlations with clinical and laboratory parameters such as hematologic, adiposity, and atherogenic indices. In addition, we made the comparison of above parameters between the three study groups.

Subjects, materials, and methods

Study subjects

Ninety-one Jordanian individuals were recruited from the Jordan University Hospital (JUH) between March 2016 and October 2016. Study participants were divided into the following: MetS group of 31 normoglycemic patients who met the International Diabetic Federation (IDF) criteria of MetS [23] (Table 1), MetS-pre/T2DM group that included 30 prediabetic or newly diagnosed antihyperglycemic treatment-naïve T2DM patients [6] with MetS [23], and 30 healthy participants who were normoglycemic (HbA1C < 5.7%, FBS < 100 mg/dL) and lean (BMI < 25 kg/m^2). All individuals were subjected to complete full history and clinical examination. Exclusion criteria were the presence of autoimmune or inflammatory diseases, any life-threatening disease, endocrine disorder other than MetS or DM, previous treatment with an antihyperglycemic agent, and pregnancy.

An informed consent was obtained from all included subjects. The study started after obtaining approval from the Scientific Research Committee at the School of Pharmacy, the Deanship of Academic Research, the University of Jordan and approval from the JUH Institutional Review Board (IRB) committee.

Anthropometric measurements

Anthropometric measurements [height, weight (Wt), waist circumference (WC), hip circumference (HC)] were taken for each participant. In details, WC was measured using flexible measuring tape that was placed around the individual in the horizontal plane, at the midpoint between the lowest rib and the upper part of the anterosuperior iliac crest, and HC was measured at the most prominent point of the gluteus maximus.

The conicity index was calculated as follows [24]:

$$\text{C-index} = \text{WC (m)}/[0.109 \times \text{square root of weight (kg)/height (m)}].$$

The body adiposity index was calculated as follows [25]:

$$\text{Body adiposity index (BAI)} = \text{hip circumferemce}_{(\text{cm})}\left[\text{Height}_{(\text{m})}1.5{-}18\right]$$

Atherogenic index of plasma (AIP) was calculated according to the following formula [26]:

$$\log (\text{TG (mg/dL)/HDL-C (mg/dL)}).$$

Blood sample collection

Blood samples were collected in lithium heparin tubes and centrifuged to obtain plasma samples, which were stored at − 80 °C until biochemical analysis.

Biochemical analyses

The biochemical tests of HbA1c, fasting plasma glucose, and fasting lipid profile [triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)] and the hematological tests of mean platelet volume (MPV), red blood cell distribution width (RDW), monocyte-to-lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) were performed for each consented patient. Visfatin (My BioSource Inc., USA; sensitivity = 1.0 ng/mL) and ghrelin

Table 1 Diagnostic criteria of metabolic syndrome

Criteria	Diagnosis
Central obesity plus any two of the following four factors:	Waist circumference ≥ 94 cm in men or ≥ 80 cm in women.[a]
Elevated triglycerides	≥ 150 mg/dL, or specific treatment for this lipid abnormality
Low HDL-C	< 40 mg/dL in men or < 50 mg/dL in women or specific treatment for this lipid abnormality.
Elevated blood pressure	Systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg or treatment of previously diagnosed hypertension.
Elevated fasting plasma glucose	≥ 100 mg/dL or previously diagnosed T2DM

Source: [23]

[a] For Eastern Mediterranean and Middle East (Arab) populations; IDF uses European data until more specific data are available

(My BioSource Inc., USA; sensitivity = 10 pg/mL) were determined using strip plate sandwich (Quantitative) ELISA (enzyme-linked immunosorbent assay) kits.

Statistical analysis

All data were encoded, entered, and analyzed using SPSS© 22 (SPSS, Inc., USA). Chi-square test was used to compare categorical variables among the groups. For continuous variables, comparison between study groups was conducted by ANCOVA test using age as a covariate. Spearman correlation was used to assess relationship between continuous variables. All differences were considered to be significant if p value was < 0.05.

Results

Patients' demographic data and clinical characteristics

All participants were Caucasians; they were almost equally distributed between the two genders in all three study groups (Table 2). The systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP, mmHg) were significantly higher in MetS and MetS-pre/T2DM vs. control ($p < 0.01$) (Table 2).

MetS-pre/T2DM fasting plasma glucose (mg/dL) and glycosylated hemoglobin (%) were significantly higher than those of the normoglycemic controls and those of MetS patients ($p < 0.001$). Triglycerides (TG) level (mg/dL), total cholesterol (TC, mg/dL), and body mass index (BMI) were significantly higher in both MetS and MetS-pre/T2DM when compared to the controls ($p < 0.001$). Also, LDL-C, mg/dL was significantly higher in both MetS ($p < 0.05$) and MetS-pre/T2DM ($p < 0.01$) when compared to the lean control (Table 2).

Furthermore, WC (cm), HC (cm), WHtR, body adiposity index (BAI) ($p < 0.001$), and C-index ($p < 0.01$) were significantly higher in both MetS groups when compared to the control group. Moreover, WHR was markedly higher when comparing MetS vs. MetS-pre/T2DM and both vs. controls ($p < 0.05$) (Table 3). MPV (fL) in both MetS ($p < 0.001$) and MetS-pre/T2DM ($P2 < 0.01$) groups was greater pronouncedly than in the controls. Notably, platelet count was higher markedly ($p < 0.05$) in nondiabetic MetS patients as compared to controls. On the other hand, substantial intergroup difference was observed in PLR between MetS vs. MetS-pre/T2DM) ($p < 0.05$). Nevertheless, there were neither any appreciable intergroup variations in RDW, MLR, or NLR nor were there any obvious intergroup discrepancies in leukocyte differential counts (Table 3). Table 3 also demonstrates the paired group-comparisons of atherogenic indices expressed as AIP, TC/HDL-C, and LDL-C/HDL-C ratios. Obviously, AIP in both MetS and MetS-pre/T2DM were identified with significant intergroup differences ($p < 0.001$ for both comparisons) when compared to healthy control participants. On the other hand, TC/HDL-C and LDL-C/HDL-C ratios were significantly higher in MetS-pre/T2DM only (but not in MetS) vs. healthy lean controls ($p < 0.05$).

Ghrelin and visfatin level comparisons and correlation studies

Ghrelin levels lacked any statistically significant variations in nondiabetic MetS or MetS-pre/T2DM groups ($p > 0.05$ for

Table 2 Gender distribution and comparison of clinical parameters between study arms

Parameters		Control group ($N = 30$) (mean ± SE)[a]	MetS group ($N = 31$) (mean ± SE)[a]	MetS-pre/T2DM group ($N = 30$) (mean ± SE)[a]	P1[b]	P2[b]	P3[b]	P[a]
Gender, N (%)	Male	13 (43.3%)	16 (51.6%)	14 (46.7%)				0.808
	Female	17 (56.7%)	15 (48.4%)	16 (53.3%)				
SBP (mmHg)		112.29 ± 3.15	131. 87 ± 2.57	131. 34 ± 2.92	*< 0.001*	*< 0.001*	0.888	
DBP (mmHg)		70.09 ± 2.12	80.00 ± 1.73	81.18 ± 1.97	*0.001*	*0.001*	0.643	
FPG (mg/dL)		85.69 ± 5	91.34 ± 4.08	122.33 ± 4.64	0.404	*< 0.001*	*< 0.001*	
HbA1c (%)		5.12 ± 0.15	5.19 ± 0.12	6.37 ± 0.14	0.724	*< 0.001*	*< 0.001*	
TG (mg/dL)		63.67 ± 19.42	186.79 ± 15.85	211.04 ± 18.02	*< 0.001*	*< 0.001*	0.296	
LDL-C (mg/dL)		97.58 ± 7.34	121.81 ± 6	134.13 ± 6.81	*0.016*	*0.002*	0.161	
HDL-C (mg/dL)		47.63 ± 2.89	42.62 ± 2.36	39.52 ± 2.68	0.203	0.071	0.368	
TC (mg/dL)		158.10 ± 8.23	201.81 ± 6.72	215.91 ± 7.64	*< 0.001*	*< 0.001*	0.153	
BMI (kg/m^2)		21.37 ± 0.97	33.47 ± 0.79	34.47 ± .90	*< 0.001*	*< 0.001*	0.390	

BMI body mass index, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycosylated hemoglobin, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides

[a] Covariates appearing in the model are evaluated at the following values: age = 42.47; by chi-square

[b] Data obtained by ANCOVA. P1: MetS vs. control, P2: MetS-pre/T2DM vs. control; P3: MetS-pre/T2DM vs. MetS

Table 3 Comparison of adiposity, hematologic, atherogenic indices, and molecular metabolic risk biomarkers between study arms

Parameters	Control group ($N = 30$) (mean ± SE)[a]	MetS group ($N = 30$) (mean ± SE)[a]	MetS-pre/T2DM group ($N = 30$) (mean ± SE)[a]	P1[b]	P2[b]	P3[b]
Adiposity indices						
WC (cm)	78.41 ± 1.9	103.27 ± 1.55	105.51 ± 1.76	< 0.001	< 0.001	0.324
HC (cm)	93.65 ± 2.12	115.77 ± 1.73	119.42 ± 1.97	< 0.001	< 0.001	0.150
WHR	0.84 ± 0.02	0.90 ± .01	0.89 ± .01	0.020	0.023	0.018
WHtR	0.48 ± 0.01	0.62 ± .01	0.63 ± .01	< 0.001	< 0.001	0.396
C-index	1.21 ± .01	1.27 ± .01	1.28 ± .01	0.003	0.003	0.600
BAI	26.34 ± 1.34	35.39 ± 1.1	36.83 ± 1.24	< 0.001	< 0.001	0.365
Hematologic indices						
RDW-CV%	13.62 ± 0.33	13.15 ± 0.27	13.70 ± 0.30	0.287	0.871	0.157
MPV (fL)	6.94 ± 0.41	9.08 ± 0.34	8.65 ± 0.38	< 0.001	0.008	0.381
PLT count ($\times 10^3/\mu L$)	234.44 ± 12.89	276.09 ± 10.52	261.91 ± 11.96	0.019	0.169	0.357
Monocytes ($\times 10^3/\mu L$)	0.82 ± 0.14	0.58 ± 0.12	0.58 ± 0.13	0.209	0.272	0.996
Neutrophils ($\times 10^3/\mu L$)	5.93 ± 1.19	4.38 ± 1.13	5.81 ± 1.29	0.409	0.957	0.386
Lymphocytes ($\times 10^3/\mu L$)	2.61 ± 0.19	2.46 ± 0.15	2.85 ± 0.18	0.569	0.407	0.088
MLR	0.27 ± .04	0.25 ± 0.03	0.20 ± 0.04	0.650	0.281	0.377
NLR	2.36 ± 0.40	1.87 ± 0.33	0.25 ± 0.03	0.369	0.607	0.722
PLR	104.03 ± 9.11	121.58 ± 7.44	97.02 ± 8.45	0.157	0.618	0.026
Atherogenic indices						
AIP	0.20 ± 0.05	0.64 ± 0.04	0.69 ± 0.05	< 0.001	< 0.001	0.455
TC/HDL-C	3.39 ± 0.82	5.04 ± 0.67	6.63 ± 0.76	0.139	0.012	0.106
LDL-C/HDL-C	2.15 ± 0.68	3.03 ± 0.55	4.29 ± 0.63	0.335	0.042	0.122
TG/HDL-C	1.22 ± 0.64	5.05 ± 0.76	6.69 ± 0.89	0.003	< 0.001	0.143
Molecular metabolic risk biomarkers						
Ghrelin (pg/mL)	369.78 ± 111.76	618.10 ± 93.22	498.17 ± 103.21	0.103	0.454	0.376
Visfatin (ng/mL)	8.62 ± 2.23	18.43 ± 1.83	19.24 ± 2.05	0.002	0.003	0.762

Data obtained by ANCOVA

AIP atherogenic index of plasma, *BAI* body adiposity index, *C-index* conicity index, *HC* hip circumference, *MLR* monocyte-to-lymphocyte ratio, *MPV* mean platelet volume, *NLR* neutrophil-to-lymphocyte ratio, *PLR* platelet-to-lymphocyte ratio, *PLT count* platelet count, *RDW* red blood cell distribution width, *TC/HDL-C* total cholesterol/high-density lipoprotein-cholesterol ratio, *TG/HDL-C* triglycerides/high-density lipoprotein-cholesterol ratio, *WC* waist circumference, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio

[a] Covariates appearing in the model are evaluated at the following values: age = 42.47

[b] Data obtained by ANCOVA. P1: MetS vs. control; P2: MetS-pre/T2DM vs. control; P3: MetS-pre/T2DM vs. MetS

both comparisons vs. control). Meanwhile, visfatin level was significantly higher in both nondiabetic MetS and MetS-pre/T2DM groups in comparison to healthy controls ($p < 0.01$) (Table 3; Fig. 1).

There was a direct ghrelin-visfatin correlation in the whole study population as well as in both MetS and MetS-pre/T2DM arms ($p < 0.001$). In the whole study population, both biomarkers correlated directly with BMI, SBP, TC, and LDL-C ($p < 0.05$) (Table 4). Both ghrelin and visfatin lacked any marked intra-group associations with other clinical parameters ($p \geq 0.05$) except direct BMI correlation with ghrelin in both control and MetS groups ($p < 0.05$).

Particularly, in MetS group, ghrelin correlated proportionally with BMI and WHR ($p < 0.05$) while visfatin correlated

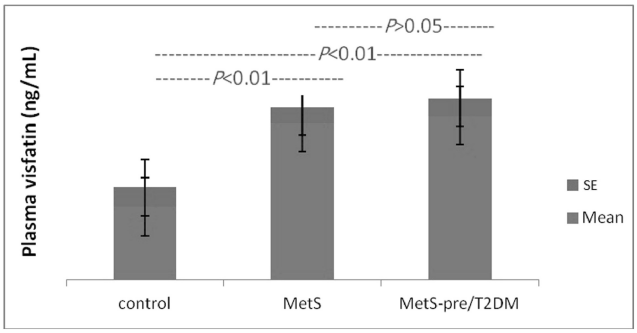

Fig. 1 Visfatin intergroup significant differences (with age as a covariate; results are mean ± SE)

Table 4 Correlation of clinical parameters with molecular metabolic risk biomarkers in the total study population and each study arm

Group			Ghrelin (pg/mL)	Visfatin (ng/mL)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	FPG (mg/dL)	A1C (%)	TG (mg/dL)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Total	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.635**	0.271*	0.215*	0.191	0.132	0.065	0.188	0.270*	0.247*	−0.190
		Sig. (2-tailed)		< 0.001	0.011	0.044	0.075	0.219	0.549	0.080	0.011	0.021	0.077
	Visfatin (ng/mL)	Correlation coefficient	0.635**	1.000	0.414**	0.382**	0.243*	0.200	0.181	0.336**	0.372**	0.300**	−0.168
		Sig. (2-tailed)	< 0.001		< 0.001	< 0.001	0.022	0.061	0.090	0.001	< 0.001	0.004	0.115
Control	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.229	0.439*	0.053	0.267	0.345	−0.052	0.273	0.342	0.335	−0.173
		Sig. (2-tailed)		0.232	0.017	0.785	0.162	0.067	0.789	0.151	0.070	0.075	0.368
	Visfatin (ng/mL)	Correlation coefficient	0.229	1.000	−0.073	0.325	0.349	0.145	−0.142	0.221	0.329	0.238	0.021
		Sig. (2-tailed)	0.232		0.705	0.085	0.064	0.454	0.462	0.248	0.081	0.213	0.915
MetS	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.917**	0.395*	0.297	0.170	0.080	0.027	0.134	0.144	0.143	−0.145
		Sig. (2-tailed)		< 0.001	0.034	0.117	0.378	0.679	0.889	0.487	0.457	0.458	0.453
	Visfatin (ng/mL)	Correlation coefficient	0.917**	1.000	0.202	0.344	0.256	0.090	0.058	0.097	0.039	0.059	−0.267
		Sig. (2-tailed)	< 0.001		0.285	0.063	0.173	0.636	0.759	0.609	0.838	0.759	0.154
MetS-pre/T2DM	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.477**	0.077	−0.019	−0.058	−0.069	0.060	0.192	0.276	0.265	−0.223
		Sig. (2-tailed)		0.008	0.685	0.919	0.761	0.719	0.752	0.309	0.140	0.157	0.237
	Visfatin (ng/mL)	Correlation coefficient	0.477**	1.000	0.153	−0.023	−0.098	−0.003	−0.049	0.034	0.188	0.148	0.069
		Sig. (2-tailed)	0.008		0.419	0.903	0.606	0.986	0.796	0.860	0.319	0.435	0.716

Data obtained by Spearman test

BMI body mass index, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycosylated hemoglobin, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed)

proportionally with WHR ($p < 0.01$). Exceptionally, visfatin in MetS pre/T2DM group correlated with both WHR (negatively) and BAI (positively) ($p < 0.05$) but none of these correlations were evident in case of ghrelin (Table 5).

Collectively of total population's atherogenic indices, ghrelin and visfatin directly correlated with both TC/HDL-C and LDL-C/HDL-C ratios ($p < 0.01$). Strikingly, visfatin also correlated with AIP proportionally ($p < 0.01$) with no significant intra-group associations for either biomarkers (Table 5).

Collectively of total population's hematologic indices, both biomarkers significantly and proportionally correlated with platelet count ($p < 0.01$). Furthermore, visfatin positively correlated with monocyte counts ($p < 0.05$) and MPV ($p < 0.01$). In MetS-pre/T2DM group, both biomarkers directly correlated with MPV ($p < 0.05$) and ghrelin correlated positively with PLR (Table 6).

Discussion and conclusion

In a recent study conducted by Sharifi et al. [27], TG concentration in diabetic group was higher compared to the two other groups ($p < 0.001$). Although HDL-C concentrations were lower in diabetic and prediabetic subjects compared with normal individuals ($p < 0.001$), the mean levels of LDL-C had no significant differences among the three groups. In contrast, in our study, not only TG but also TC level (mg/dL) ($p < 0.01$) and LDL-C (mg/dL) ($p < 0.05$) levels were significantly higher in both MetS and MetS-pre/T2DM when compared to the controls (Table 7).

Acylated ghrelin concentrations in all three groups of Sharifi et al. [27] study were lower than normal values ($p = 0.006$). In contrast with the data of the above study, ghrelin levels in our study lacked any statistically significant variations in both MetS and MetS-pre/T2DM groups when compared to control ($p > 0.05$). The differences in ghrelin levels can be due to measurement of total ghrelin vs. specifically acylated entities measured in the study by Sharifi et al. [27].

Importantly and unlike our findings where ghrelin was directly correlated to BMI, a significant inverse relationship was found between serum ghrelin concentration and BMI by Sharifi et al. [27]. Notably, in parallel to our results, no significant discrepancy was demonstrated between MetS-diabetic and MetS-prediabetic patients for their age, in comparison to significantly younger normoglycemic lean subjects ($p < 0.001$); additionally, there were also no significant intergroup variations in gender distribution in Sharifi et al. [27] study.

More recently, Chen et al. [28] characterized the type 2 diabetic study group to have a higher BMI, WHR and LDL ($p < 0.001$) as compared with control, being in agreement with our study results (Table 8). In contrast to our findings of lack of differences in plasma ghrelin levels ($p = 0.454$) between the three study groups, the above study demonstrated significantly lower plasma ghrelin level in the type 2 diabetic group as compared to control ($p < 0.001$).

It is necessary to emphasize that adiposity and blood indices, such as TG/HDL-C, CI, RDW, and MPV, were not examined in either Sharifi et al. [27] or Chen et al. [28] studies. Interestingly TG/HDL-C, CI, and MPV were significantly higher in both MetS and MetS-pre/T2DM when compared to the controls ($p < 0.01$), while there were not any appreciable intergroup variations in RDW.

Interestingly and similar to our findings, Akdoğan et al. [29] indicated that MPV, TG, and AIP were higher in T2DM patients compared to control. Akdoğan et al. [29] also reported that HbA1c levels correlated very weakly with MPV, PDW, and PLR and weakly with the RDW, while the atherogenic index correlated very weakly with the PLR ratio and RDW.

This study provided several ideas regarding relation of visfatin with obesity, MetS, T2DM, atherogenic, adiposity, and hematologic indices. Our results indicate that plasma concentration of visfatin was significantly increased in MetS as compared with lean healthy control. There have been contradictory findings on the association between visfatin and MetS. A cross-sectional study by Baltacı et al. [30] that enrolled a total of 169 subjects divided into two groups, obese and nonobese patients, could find that serum visfatin levels increase along with rising insulin resistance and body mass index, but without substantial associations with metabolic syndrome or impaired glucose metabolism.

Haider et al. [31] also reported that visfatin levels were substantially increased in morbidly obese individuals and its level was reduced after gastric banding surgery. Conversely, Pagano et al. [32] found that plasma levels of visfatin were significantly lower in obese subjects. On the other hand, the negative correlation we found in obese patients between visfatin and adiposity is not in agreement with data previously reported by Berndt et al. [33]. Mainly, visfatin plasma concentrations and visceral visfatin mRNA expression correlated with measures of obesity but not with visceral fat mass or WHR, and unlike our study, clinical parameters were not evaluated. Correspondingly, Kamińska et al. [34] found elevated levels of visfatin in obese subjects along with negative correlation with WHR, but, surprisingly, visfatin did not correlate with the majority of anthropometric parameters. In our study, plasma visfatin correlated with obesity and WHR.

El-Shafey et al. [35] recruited a sample of 74 subjects that were divided in to three groups (20 subject in each). They reported appositive correlation between MetS and the elevation of both TC and serum TG levels. They also reported that HDL-C and LDL-C levels significantly increased in obese diabetic patients compared with obese nondiabetic patients [35]; these results are in contrast with our study where we did not have significant differences in lipid profile between the two MetS groups, normoglycemic and pre/DM. El-Shafey

Table 5 Correlation of adiposity and atherogenic indices with molecular metabolic risk biomarkers in the total study population and each study arm

Group			Ghrelin (pg/mL)	Visfatin (ng/mL)	WC (cm)	HC (cm)	WHR	WHtR	C-Index	BAI	AIP	TC/HDL-C	LDL-C/HDL-C
Total	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.635**	0.204	0.206	0.147	0.205	0.039	0.171	0.230*	0.309**	0.281**
		Sig. (2-tailed)	0.	< 0.001	0.057	0.054	0.171	0.055	0.721	0.112	0.031	0.003	0.008
	Visfatin (ng/mL)	Correlation coefficient	0.635**	1.000	0.353**	0.326**	0.193	0.344**	0.117	0.295**	0.358**	0.392**	0.328**
		Sig. (2-tailed)	< 0.001	0.	0.001	0.002	0.070	0.001	0.273	0.005	0.001	< 0.001	0.002
Control	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.229	0.504**	0.375*	0.116	0.540**	0.230	0.109	0.273	0.366	0.339
		Sig. (2-tailed)	0.	0.232	0.005	0.045	0.550	0.002	0.230	0.574	0.152	0.051	0.072
	Visfatin (ng/mL)	Correlation coefficient	0.229	1.000	0.127	0.018	0.181	0.191	0.226	− 0.045	0.201	0.117	0.087
		Sig. (2-tailed)	0.232	0.	0.511	0.928	0.348	0.322	0.238	0.818	0.295	0.545	0.652
MetS	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.917**	0.248	− 0.055	0.398*	0.147	− 0.179	− 0.062	0.174	0.285	0.196
		Sig. (2-tailed)	0.	< 0.001	0.194	0.778	0.032	0.446	0.352	0.748	0.367	0.134	0.309
	Visfatin (ng/mL)	Correlation coefficient	0.917**	1.000	0.147	− 0.236	0.475**	− 0.010	− 0.129	− 0.250	0.224	0.289	0.190
		Sig. (2-tailed)	< 0.001	0.	0.438	0.209	0.008	0.956	0.498	0.183	0.235	0.121	0.314
MetS-pre/T2DM	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.477**	− 0.128	0.171	− 0.272	− 0.040	− 0.234	0.244	0.227	0.324	0.325
		Sig. (2-tailed)	0.	0.008	0.499	0.367	0.145	0.833	0.213	0.194	0.228	0.081	0.080
	Visfatin (ng/mL)	Correlation coefficient	0.477**	1.000	− 0.075	0.299	− 0.459*	− 0.024	− 0.276	0.379*	− 0.064	0.076	0.064
		Sig. (2-tailed)	0.008	0.	0.694	0.109	0.011	0.902	0.140	0.039	0.736	0.689	0.736

Data obtained by Spearman test

AIP atherogenic index of plasma, *BAI* body adiposity index, *C-index* conicity index, *HC* hip circumference, *TC/HDL-C* total cholesterol/high-density lipoprotein-cholesterol ratio, *TG/HDL-C* triglycerides/high-density lipoprotein-cholesterol ratio, *WC* waist circumference, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed)

Table 6 Correlation of hematological indices with molecular metabolic risk biomarkers in the total study population and each study arm

Group			Ghrelin (pg/mL)	Visfatin (ng/mL)	Monocytes ($\times 10^3/\mu L$)	Neutrophils ($\times 10^3/\mu L$))	Lymphocytes ($\times 10^3/\mu L$)	MLR	NLR	PLR	RDW (%)	MPV (fL)	Plt count ($\times 10^3/\mu L$)
Total	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.635**	0.113	0.066	−0.042	0.061	0.073	0.192	−0.178	0.073	0.309**
		Sig. (2-tailed)		< 0.001	0.295	0.543	0.698	0.570	0.499	0.074	0.097	0.501	0.003
	Visfatin (ng/mL)	Correlation coefficient	0.635**	1.000	0.234*	−0.031	0.033	0.144	−0.069	0.117	−0.149	0.330**	0.291**
		Sig. (2-tailed)	< 0.001		0.027	0.773	0.759	0.179	0.523	0.274	0.163	0.002	0.006
Control	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.229	0.072	0.456*	−0.131	0.004	0.455*	0.007	−0.413*	−0.487**	−0.041
		Sig. (2-tailed)		0.232	0.711	0.013	0.499	0.983	0.013	0.973	0.026	0.007	0.834
	Visfatin (ng/mL)	Correlation coefficient	0.229	1.000	0.174	0.080	0.175	0.076	−0.064	−0.257	−0.183	−0.073	−0.136
		Sig. (2-tailed)	0.232		0.368	0.682	0.364	0.696	0.742	0.178	0.341	0.707	0.482
MetS	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.917**	0.139	0.018	0.260	−0.043	−0.257	0.069	−0.206	−0.218	0.303
		Sig. (2-tailed)		< 0.001	0.473	0.925	0.174	0.823	0.179	0.722	0.284	0.256	0.110
	Visfatin (ng/mL)	Correlation coefficient	0.917**	1.000	0.221	0.067	0.355	−0.001	−0.306	−0.033	−0.267	−0.141	0.251
		Sig. (2-tailed)	< 0.001		0.240	0.723	0.054	0.995	0.100	0.864	0.154	0.456	0.181
MetS-pre/T2DM	Ghrelin (pg/mL)	Correlation coefficient	1.000	0.477**	0.269	−0.141	−0.191	0.285	0.035	0.388*	0.044	0.407*	0.457*
		Sig. (2-tailed)		0.008	0.150	0.456	0.313	0.128	0.853	0.034	0.816	0.025	0.011
	Visfatin (ng/mL)	Correlation coefficient	0.477**	10.000	0.203	−0.068	−0.280	0.174	0.107	0.316	−0.243	0.398*	0.267
		Sig. (2-tailed)	0.008		0.283	0.720	0.134	0.357	0.572	0.089	0.196	0.030	0.154

Data obtained by Spearman test

MLR monocyte to lymphocyte ratio, *MPV* mean platelet volume, *NLR* neutrophil to lymphocyte ratio, *PLR* platelet-to lymphocyte ratio, *PLT* count platelet count, *RDW* red blood cell distribution width

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed)

Table 7 Comparison between our study findings of ghrelin and clinical parameters vs. those of Sharifi et al. [27]

Parameters	Findings by Sharifi et al. [27]				Our findings					
	Normoglycemic ($N = 29$)	Pre-DM ($N = 29$)	DM ($N = 29$)	P	Control group ($n = 30$)	MetS nondiabetic group ($n = 30$)	MetS Pre/T2DM group ($n = 29$)	P1	P2	P3
Age (years)	[35.4 ± 9.2]	53.5 ± 13.2	47.8 ± 11.6	< 0.001	42.45 (20–70)	30.17 (20–53)	45.29 (20–70)	< 0.001	< 0.001	< 0.001
BMI (kg/m^2)	28.8 ± 4.3	27.8 ± 4.5	29.2 ± 5.4	0.548	21.37 ± 0.97	33.47 ± 0.79	34.47 ± .90	< 0.001	< 0.001	0.390
WHR	0.83 ± 0.05	0.86 ± 0.06	0.88 ± 0.08	0.009	0.84 ± 0.02	0.90 ± 0.01	0.89 ± 0.01	0.020	0.023	0.018
SBP (mmHg)	120.1 ± 13.3	130 ± 17.3	129.6 ± 18.6	0.04	112. 29 ± 3.15	131. 87 ± 2.57	131. 34 ± 2.92	< 0.001	< 0.001	0.888
DBP (mmHg)	73.2 ± 9.2	76.4 ± 9.2	78.5 ± 11.5	0.138	70.09 ± 2.12	80.00 ± 1.73	81.18 ± 1.97	0.001	0.001	0.643
TC (mg/dL)	172.8 ± 20.1	190 ± 45.6	188.3 ± 31.4	0.1	158.10 ± 8.23	201.81 ± 6.72	215.91 ± 7.64	< 0.001	< 0.001	0.153
TG (mg/dL)	104 ± 44.5	169 ± 82	190.9 ± 91.7	< 0.001	63.67 ± 19.42	186.79 ± 15.85	211.04 ± 18.02	< 0.001	< 0.001	0.296
HDL-C (mg/dL)	52.2 ± 3.9	43.3 ± 7.9	46.5 ± 6.4	< 0.001	47.63 ± 2.89	42.62 ± 2.36	39.52 ± 2.68	0.203	0.071	0.368
LDL-C (mg/dL)	99.7 ± 17.2	113.2 ± 38.7	103 ± 35.5	0.2	97.58 ± 7.34	121.81 ± 6	134.13 ± 6.81	0.016	0.002	0.161
Ghrelin[c] (pg/mL)	51.5 ± 40.2	45.5 ± 10.5	45.3 ± 33.2	0.1	369.78 ± 111.76	618.10 ± 93.22	498.17 ± 103.21	0.103	0.454	0.376

Data obtained in our study by ANCOVA

BMI body mass index, *DBP* diastolic blood pressure, *FBG* fasting plasma glucose, *HDL-C*, high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *WHR* waist-to-hip ratio

[a] Covariates appearing in the model are evaluated at the following value: age = 42.47

[b] Data obtained by ANCOVA. P1: MetS vs. control; P2: MetS-pre/T2DM vs. control; P3: MetS-pre/T2DM vs. MetS

[c] Acylated ghrelin in a study by Sharifi et al. [27]

Table 8 Comparison of our study results of ghrelin vs. those of Chen et al. [28]

Parameters	Chen et al. [28] findings			Parameters	Our findings					
	Control group ($n = 95$)	T2DM group ($n = 75$)	P		Control group ($n = 30$)	MetS nondiabetic group ($n = 30$)	MetS Pre/T2DM group ($n = 29$)	P1	P2	P3
BMI (kg/m^2)	23.01 ± 1.12	25.23 ± 3.01	< 0.001	BMI (kg/m^2)	21.37 ± 0.97	33.47 ± 0.79	34.47 ± 0.90	< 0.001	< 0.001	0.390
WHR	0.84 ± 0.03	0.87 ± 0.04	< 0.001	WHR	0.84 ± 0.02	0.90 ± 0.01	0.89 ± 0.01	0.020	0.023	0.018
FPG (mmol/L)	5.06 ± 0.41	9.27 ± 1.11	< 0.001	FPG (mg/dL)	85.69 ± 5	91.34 ± 4.08	122.33 ± 4.64	0.404	< 0.001	< 0.001
HbA1c (%)	5.13 ± 0.32	8.04 ± 1.63	< 0.001	HbA1c (%)	5.12 ± 0.15	5.19 ± 0.12	6.37 ± 0.14	0.724	< 0.001	< 0.001
TG (mmol/L)	1.42 ± 0.81	1.55 ± 0.73	0.12	TG (mg/dL)	63.67 ± 19.42	186.79 ± 15.85	211.04 ± 18.02	< 0.001	< 0.001	0.296
TC (mmol/L)	4.58 ± 0.86	4.65 ± 1.03	0.46	TC (mg/dL)	158.10 ± 8.23	201.81 ± 6.72	215.91 ± 7.64	< 0.001	< 0.001	0.153
LDL-C (mmol/L)	2.33 ± 0.44	2.77 ± 0.86	0.206	LDL (mg/dL)	97.58 ± 7.34	121.81 ± 6	134.13 ± 6.81	0.016	0.002	0.161
HDL-C (mmol/L)	1.61 ± 0.46	1.27 ± 0.35	< 0.001	HDL (mg/dL)	47.63 ± 2.89	42.62 ± 2.36	39.52 ± 2.68	0.203	0.071	0.368
Ghrelin (μg/L)	6.74 ± 2.13	2.85 ± 1.59	< 0.001	Ghrelin (pg/mL)	369.78 ± 111.76	618.10 ± 93.22	498.17 ± 103.21	0.103	0.454	0.376

Data obtained in our study by ANCOVA

BMI body mass index, *FPG* fasting plasma glucose, *HbA1c* glycosylated hemoglobin, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *TC* total cholesterol, *TG* triglyceride, *WHR* waist-to-hip ratio

[a] Covariates appearing in the model are evaluated at the following value: age = 42.47

[b] Data obtained by ANCOVA. P1: MetS vs. control; P2: MetS-pre/T2DM vs. control; P3: MetS-pre/T2DM vs. MetS

et al. [35] could also demonstrate that visfatin levels were significantly elevated in obese patients and that visfatin was associated with abdominal obesity which is similar to our findings. Unlike our findings, El-Shafey et al. [35] could evidence a significant proportional correlation between visfatin concentration and BMI in obese diabetic patients. On the other hand, Filippatos et al. [36] revealed that plasma visfatin levels were increased in overweight and obese subjects with MetS compared with those individuals without MetS. In accordance with our finding, Olszanecka-Glinianowicz et al. [37] reported that comparable visfatin levels were observed in MetS group vs. non-MetS group. Impressively, our study confirms the findings of the above two studies.

In a study by Akdoğan et al. [29], HbA1c, which is a marker of long-term glycemic control, weakly correlated with MPV, PDW, RDW, PLR, and NLR ratios and the AIP.

The novelty in our study was the analysis of the comparisons and correlations between plasma levels of ghrelin and visfatin in Jordanian patients with metabolic syndrome. Previous studies did not show correlation between ghrelin and visfatin with hematologic indices, atherogenic indices, or adiposity indices. Based on a cross-sectional study, we report that patients with either MetS or MetS-pre/T2DM have elevated visfatin levels, while ghrelin levels do not differ from the apparently healthy controls. Correlations exist between the two biomarkers and between adiposity, hematologic, and atherogenic indices; however, these correlations differ between the whole sample and each of the study group as well as between the individual groups.

Study strength The novelty in our study was that we analyzed the comparisons and correlations between plasma levels of ghrelin and visfatin in Jordanian patients with metabolic syndrome.

Study limitation Time and financial constrains impacted and limited our research capacity to accommodate more participants.

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Compliance with ethical standards

An informed consent was obtained from all included subjects. The study started after obtaining approval from the Scientific Research Committee at the School of Pharmacy, the Deanship of Academic Research, the University of Jordan and approval from the JUH Institutional Review Board (IRB) committee.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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ORIGINAL ARTICLE

The clinical and biochemical profiles of patients with IFG

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Abstract
To study the clinical and biochemical profiles across the different ranges of impaired fasting glucose (IFG) based on American Diabetes Association (ADA) and World Health Organization (WHO) criteria. A cross-sectional study was conducted on 149 subjects, of which 63 belonged to group 1 (IFG = 100–110 mg/dl) and 86 to group 2 (IFG = 111–125 mg/dl). Basic anthropometric and clinical examinations were done for all subjects. Data was collected from patient by a questionnaire, which included the history of hypertension and diabetes and other comorbidities and complications. Biochemical profiles including Fasting Plasma Glucose (FPG), Oral Glucose Tolerance Test (OGTT), HbA1c, Fasting insulin levels and Fasting Lipid Profile were measured. Assessment of insulin resistance and beta cell function was done by Homeostasis Model Assessment (HOMA). Data were analysed using SPSS software version 15 and $p < 0.05$ considered as statistically significant. Family history of diabetes, prevalence of hypertension and higher BMI were noted to be significant higher in group 2 compared to group 1. Clustering of cardiovascular risk factors suggesting metabolic syndrome was also much higher in group 2 (60.5 vs 39.7% p value = 0.012). Impaired glucose tolerance was significantly higher in group 2 (73.3 vs 28.6 $p < 0.001$) denoting more glycemia. Insulin resistance (HOMA-IR) was significantly higher in group 2 ($p = 0.001$). Beta cell function (HOMA-β) was also higher in group 2 but not statistically significant ($p = 110$). In IFG, the higher range of blood sugar 111 to 125 mg/dl is associated with more glycemia, cardiovascular risk factors and insulin resistance. Beta cell function though higher in this group is inadequate to compensate for higher insulin resistance.

Keywords Impaired fasting glucose · Insulin resistance · Beta cell function · Homeostasis model

Introduction

Most complications of diabetes mellitus have been found to have a direct relationship with the level of blood glucose. It is now known that blood glucose levels below the defined diabetic range have also been found to have these complications, at incidence rates that cannot be ignored.

Because of the tendency to cause complications at the so-called normoglycemic range, the concept of prediabetes came into inception. In 1979, impaired glucose tolerance (IGT) was introduced to categorize those blood glucose levels which were above normal postprandial sugars but not in the diabetic range [1]. In 1997, a similar concept of impaired fasting glucose (IFG) was introduced for those fasting sugars which were above normal but not in the diabetic range [2]. Both these entities, in further studies, were found to cause diabetes related complications. Together they came to be known as prediabetes. This is the "submerged part of the iceberg" of glycemia which clinicians had long ignored.

IFG and IGT were found to be pathologically distinct entities, with IFG due to hepatic insulin resistance and IGT due to muscle insulin resistance. IFG was initially defined within the range of 110–125 mg/dl. In 2003, the American Diabetes Association (ADA) extended the range of IFG to include 100–110 mg/dl; thus now, IFG as per ADA criteria is 100–125 mg/dl, while that in the World Health Organization (WHO) criteria is still 110–125 mg/dl. The ADA lowered the cut-off based on the premise that glycaemic complications do begin above 100 mg/dl blood sugar itself. However, it is

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not delineated if the deleterious glycemic consequences and comorbidities are the same, across this wide range.

We undertook this study to see the clinical and biochemical profiles across the ADA range of IFG, and whether based on this the newly added group, i.e. 100–110 mg/dl, is at the same risk of complications as the traditional 110–125 mg/dl range. We also tried to analyse the HOMA-IR (homeostasis model assessment-insulin resistance) and HOMA-β (homeostasis model assessment-beta cell function) in IFG subjects across the different levels of FPG and whether IFG's association with IGT was influenced by insulin resistance and beta cell function.

Materials and methods

This was a cross-sectional time bound study on out patients who came to hospital for routine health checkup and in-patients recruited from Sept 2012 to June 2014, in a tertiary care hospital in southern Karnataka, who were aged ≥ 18 years with FPG 100-125 mg/dl. The subjects who were known diabetics; subjects on steroids, contraceptives or on lipid lowering medication; subjects with active infections, chronic kidney or liver dysfunction; any endocrine causes of raised sugars; and gestational diabetes were excluded from the study. The study protocol was approved by the institutional ethics committee (IEC 415/2012).

Data was collected interviewing the subject by a specific questionnaire, which included the history of hypertension in the subject and family history of diabetes (in first-degree relatives).

Height and weight of each study participant were measured, and body mass index (BMI) was calculated using the formula BMI = weight (kg)/[height (m^2)]. Waist circumference was measured midway between inferior margin of ribs and the superior border of iliac crest at the end of normal expiration (WHO STEPS protocol). Waist circumference (WC) of ≥ 90 cm for males and ≥ 80 cm for females was considered as abnormal. Hip circumference (HC) was measured at the widest portion of the buttocks with the tape parallel to the floor. The waist-hip ratio was then calculated (IDF criteria) [3].

Fasting plasma glucose (FPG) was measured using hexokinase method by Roche Cobas E601 analyser machine. All subjects underwent a 2-h oral glucose tolerance test (OGTT) (with 82.5 g glucose-monohydrate equivalent to 75 g anhydrous glucose) to document IGT. Fasting insulin levels were measured by electrochemiluminescence immunoassay (ECLIA) method using Roche Cobas E601 analyser machine [4]. Assessment of insulin resistance and beta cell function was done by using homeostasis model assessment 2 (HOMA 2) computerized method [5], which has been shown to correlate well with a euglycemic clamp for use in cross-sectional studies [6]. IR was expressed as HOMA-IR. Beta cell secretary capacity was expressed as HOMA-β.

HOMA-R and HOMA-β were calculated by the following formula

$$\text{HOMA-IR} = \frac{\text{Fasting insulin(FIL)} \times \text{FPG}}{405} \quad \text{HOMA-}\beta = \frac{360 \times \text{FIL}}{\text{FPG-63}}$$

where FPG is in milligrams per decilitre, and FIL is in microunits per litre.

HbA1c was measured using NGSP-certified high-performance liquid chromatography (HPLC) using Bio-Rad Variant Hemoglobin analyser.

Fasting Lipid Profile was measured including triglycerides (TGs) (GPO grinder method using Roche Cobas E601 analyser machine) and HDL (direct homogenous method using Roche Cobas E601 analyser machine). LDL was then calculated by using Friedewald formula (LDL-c (mg/dl) = TC (mg/dl) − HDL-c (mg/dl) − TG (mg/dl)/5) [7].

In the present study, subjects were divided into two groups based on FPG value. Group 1 consists of individuals with IFG in the range of 100–110 mg/dl which is only in the ADA criteria, and for group 2, the IFG was in the range of 110–125 mg /dl which is part of ADA and WHO definitions.

Statistical analysis

Pearson's chi-square test was done to study the relation between FPG and other variables like hypertension, family history of type 2 DM, HbA1c, IGT, metabolic syndrome, dyslipidemia and TG/HDL ratio. Correlation between FPG and HbA1c was assessed by calculating Pearson's correlation coefficient. Mann-Whitney *U* test, a non-parametric test, was used to compare HOMA-IR, HOMA-β and waist-hip ratio between groups 1 and 2. Independent samples *t* test was used to compare the anthropometric measurements between male and female in each group. p value < 0.05 was taken as significant. Data were analysed using the statistical package for social sciences (SPSS version 15, Chicago, IL, USA).

Results

Clinical profile

After obtaining informed consent, a total of 149 subjects were recruited in the study from Sept 2012 to June 2014. Of which, 63 subjects belonged to group 1 and 86 subjects belonged to group 2. The gender distribution in two groups is shown in Table 1.

The anthropometric measurements between male and female in each group are shown in Table 1. In each group,

Table 1 Anthropometric measurements between male and female in each group

Anthropometric Measurement	Group 1		*p* value	Group 2		*p* value
	Male (40)	Female (23)		Male (49)	Female (37)	
Age (years)	55.32 ± 13.23	57.30 ± 10.51	0.559	54.98 ± 12.33	56.16 ± 13.17	0.834
Waist circumference (cm)	88.23 ± 6.50	91.26 ± 7.73	0.347	92.45 ± 15.10	92.44 ± 9.96	0.786
Waist/hip ratio	0.98 ± 0.03	0.96 ± 0.05	0.02*	0.99 ± 0.03	0.95 ± 0.06	0.06
BMI (kg/m^2)	23.79 ± 1.79	25.64 ± 3.64	0.002*	25.89 ± 2.87	26.23 ± 5.44	0.005*

SD standard deviation

*Statistically significant

females had a statistically significant higher mean BMI than males. In each group, males had higher waist-hip ratio (WHR) compared to females, and in group 1, it was statistically significant. Other anthropometric measurements such as age and waist circumference did not differ markedly between two groups. The details of anthropometric measurements are shown in Table 1.

About 45% subjects of group 2 had hypertension, 52.3% subjects of group 2 had a family history of type 2 DM, and this prevalence was statistically significant in comparison with group 1 (p value 0.002 and p value < 0.001 respectively). These comorbidities are detailed in Table 2.

Biochemical profile

We analysed the biochemical parameters, namely the HbA1c, the insulin resistance and beta cell function (HOMA-IR and HOMA-β respectively) and the lipid profile in all subjects (Table 3).

In the > 6.4% HbA1c range, group 2 had a higher prevalence 20.9% (vs 15.9% in group 1, p value = 0.04). A higher percentage of subjects in group 1 had HbA1c in the proposed 5.7–6.4% range (69.8 vs 50%, p value = 0.04). But because this did not define the correlation between HbA1c levels and the levels of FPG in IFG subjects, we looked at FPG and HbA1c as continuous variables. On looking at FPG and HbA1c as continues variables, we found that they had an extremely poor correlation (Pearson's correlation coefficient = 0.06), as seen in the scatter diagram in Fig. 1.

The prevalence of IGT was much higher in group 2 than in group 1 (73.3 vs 28.6%) with a significant p value of < 0.001 denoting more glycemia. To further study if this glycaemia was due to insulin resistance or beta cell dysfunction, we calculated the HOMA-IR and the HOMA-β.

We studied the relation of insulin resistance (HOMA-IR) with the level of IFG and is given in Table 3. A Mann-Whitney *U* test was performed, showing that group 2 had a worse profile than group 1, with median HOMA-IR higher in group 2 than group 1, and this difference was statistically significant with $p < 0.001$. On studying the beta cell function (HOMA-β) in these subjects of IFG, we found that though median HOMA-β was higher in group 2 than in group 1, this difference was not significant (p value = 0.110).

Metabolic syndrome prevalence in the two groups followed the same trend as insulin resistance HOMA-IR. Group 2 had a prevalence of 60.5% while group 1 had 39.7%, and this difference was significant (p value = 0.012).

We studied the lipid profile between these two groups, based on three categories: subjects with increased triglycerides or decreased HDL (suggestive of diabetic atherogenic profile), those with increased LDL and subjects with no dyslipidemia. We found no difference in the dyslipidemia pattern of the two groups (p value = 0.761).

The TG/HDL ratio (ratio ≥ 3 which is well established as a marker of insulin resistance) was also compared between the two groups. Even though there was a statistical difference HOMA-IR and prevalence of metabolic syndrome between the two groups, there was no statistical difference in prevalence of significant TG/HDL.

Discussion

In this study, we focussed on comparing the clinical and biochemical differences between the two groups of IFG, between the 100–110 mg/dl range which is the ADA criteria (group 1) and the 110–125 mg/dl range which is part of ADA and WHO definitions (group 2).

Table 2 Comparison of clinical profile of study participants between the two IFG groups

Clinical profile	Group 1 $n = 63$	Group 2 $n = 86$	*p* value
Hypertension	13 (20.6%)	39 (45.3%)	0.002*
Family history of type 2 DM	10 (15.9%)	45 (52.3%)	< 0.001*

*Statistically significant

Table 3 Comparison of biochemical profile of study participants between the two IFG groups

Biochemical profile	Group 1, $n = 63$		Group 2, $n = 86$		p value
IGT	18 (28.6%)		63 (73.3%)		< 0.001*
HOMA-IR	2.3 (2.1, 3.1)[a]		4.19 (3.2, 4.8)[a]		< 0.001*
HOMA-β	79.69 (66.3, 99.92)[a]		90.59 (73.7, 106.62)[a]		0.110
Dyslipidemia	↑TG/↓HDL ↑LDL	27 (42.9%) 4 (6.3%)	↑TG/↓HDL ↑LDL	41 (47.7%) 6 (7%)	0.659
	↑TG/↓HDL + ↑ LDL	10 (15.9%)	↑TG/↓HDL + ↑LDL	16 (18.6%)	
TG/HDL	≥ 3	32 (50.8%)	≥ 3	50 (58.1%)	0.373
	< 3	31 (49.2%)	< 3	36 (41.9%)	
Metabolic syndrome	25 (39.7%)		52 (60.5%)		0.012*

SD standard deviation

*Statistically significant

[a] Median (first quartile Q1, third quartile Q3)

In our study, family history of diabetes was also higher in group 2 than group 1 with a p value of < 0.001. This may suggest that family history does add to the higher glycemia prevalent in group 2. We found that IGT was significantly associated with group 2 as compared to group 1. Robert Wagner et al. also found that family history of type 2 diabetes mellitus (FHD) in first-degree relatives was associated with the risk of IFG and IFG with IGT (OR of 1.37 and 1.64 respectively). However, this association was found to be insignificant with isolated IGT [8].

Considering comorbidities across the two groups, we found that hypertension was significantly higher in group 2. Morio et al. [9] studied 2943 Japanese middle-aged subjects and concluded that IFG is an independent risk factor for the development of hypertension in normotensives with the odds ratio of 1.66 for men and 2.62 for women after a follow-up of 5.6 years. In India, Mohan et al. in the CUPS-19 (Chennai Urban Population Study) [10] study also found that the blood pressure was higher in IFG subjects than in normoglycemic. The higher levels of hypertension in group 2 may be due to higher insulin to compensate for the increased insulin resistance which leads to increased sympathetic activity and to a lesser extent due to insulin-mediated sodium and water retention [11].

Anthropometry impacts FPG in a major way. BMI was shown to be positively associated with IFG, by Thompson et al. [12]. Lee et al. in a prospective cohort study on 14,006 men showed that BMI > 30 kg/m^2 and a waist > 102 were both associated with higher risk of development of IFG and type 2 DM [13]. Shweta Sahai et al. compared

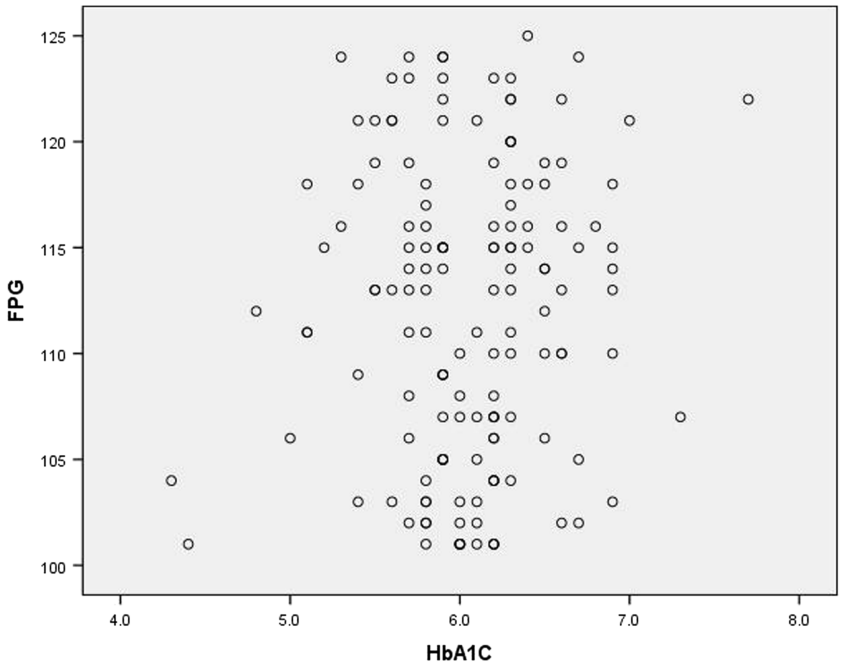

Fig. 1 Scatter plot of FPG and HbA1c shows extremely poor correlation (Pearson's correlation coefficient = 0.06)

BMI, waist, and waist/hip ratio between prediabetics and non-diabetics and found strong correlation of IFG with waist/hip ratio (p value = 0.0234) but a poor correlation with waist circumference and BMI (p value—0.1296 and 0.1482 respectively) [14].

In our study, in each group, females had a statistically significant higher mean BMI than male (p value = 0.002 in group 1 and 0.005 in group 2). In each group, males had higher WHR compared to females and in group 1, it was statistically significant (p value = 0.02). Other anthropometric measurements such as age and waist circumference did not differ markedly between two groups.

As per the ADA, HbA1c of 5.7 to 6.4% represents the prediabetic range. They opined that HbA1c was less sensitive but highly specific to identify not only subjects with IFG and/or IGT. We studied the trend of HbA1c across the different levels of IFG and found that there was an extremely poor correlation between the levels of HbA1c and IFG (Pearson's correlation coefficient $r = 0.06$). Lorenzo et al. [15], in the IRAS (Insulin Resistance Atherosclerosis Study), tried to study the efficacy of HbA1c of 5.7 to 6.4%. HbA1c had a sensitivity of 23.6% in identifying prediabetics, FPG had a sensitivity of 69.1% and OGTT had a sensitivity of 59.5%. The combined sensitivity of FPG and OGTT was 95.8% but that of FPG and HbA1c was only 75.6%. In the Indian context, Mohan et al. [16] showed that the HbA1c performed better with the sensitivity of 60% for the WHO criteria of IFG and 65.1% for the ADA criteria of IFG. Thus, there might be an inter-racial difference in the efficacy of HbA1c to identify prediabetics and probably a combination of HbA1c, FPG and OGTT might perform better in identifying prediabetics.

In our study, we found that 73.3% in group 2 had associated IGT as opposed to 28.6% in group 1 and this difference was highly significant (p value < 0.001). This could be because of a "spillage" in the insulin resistance from the liver to the muscles resulting in an additive effect in the insulin resistance and a higher prevalence of IFG plus IGT in group 2. Thus, this group of IFG subjects which had both IFG and IGT is at a higher risk of developing overt diabetes, which too earlier [17] placing them at a higher risk of macrovascular complications.

HOMA-IR has been compared in various studies amongst subjects of IFG and IGT. Meyer et al., showed that there was no statistical difference in the HOMA-IR between IFG and IGT subjects (p value > 0.4) [18]. This was in contrast to results shown by Festa et al. [19], who showed that insulin resistance was higher in IFG subjects than the IGT subjects ($p = 0.036$).

In our study, we found that group 2 (FPG—111–125 mg/dl) has a significantly higher HOMA-IR (4.19 vs 2.3 in group 1) (p value < 0.001). The question that whether the higher HOMA-IR in group 2 was merely the additive effect of the peripheral and hepatic insulin resistance or a greater hepatic insulin resistance in group 2 as compared to group 1 would probably require a larger number or subjects to see the trend of HOMA-IR across the FPG data.

Similarly, a number of studies compared HOMA-β between subjects of IFG and IGT. Wasada et al. opined that insulin resistance was higher in the isolated IGT and IFG + IGT groups than in the isolated IFG group. Beta cell function was higher in the isolated IGT and IFG + IGT than in the normoglycaemic and IFG subjects [20]. Snehalatha et al. showed similar results in South Asian Indians [21].

In our study, there was no significant difference in the B cell function (group 1 = 79.69% and group 2–90.59%, p value = 0.110). Beta cell function of group 2 could not compensate adequately with the increase in insulin resistance; thus, the fasting sugars increased. This partial compensation is seen as an insignificant increase in beta cell function, thus preventing its "drift" into overt diabetes until the beta cells begin to fail [22].

After assessing the above parameters, metabolic syndrome was assessed in IFG subjects. Meigs et al. [23] in the 2803 Framingham offspring study showed that metabolic syndrome was found to be a predictor of future risk of type 2 diabetes, independent of the insulin resistance. Gupta et al. [24] compared metabolic syndrome to IFG in 14,120 hypertensives as a predictor of Type 2 DM, concluding that metabolic syndrome was a better predictor of type 2 DM than IFG (p value = < 0.001).

In the current study too, we found that the prevalence metabolic syndrome was significantly higher in group 2 (p value < 0.012), a clear indication of the higher insulin resistance. This again may point to the clustering of cardiovascular risk factors in group 2 which may translate into at a higher risk of macrovascular complications.

This study highlights the differences between the newly added group with FPG 100–110 mg/dl based on ADA criteria and the WHO-based IFG. Although the newly added lower spectrum of the ADA group may be at a realistic risk of diabetes related complications, comparing their profile to the WHO range of 111–125 mg/dl shows that, metabolically, the WHO range shows more dysglycaemia with more IGT. Insulin resistance rises, and beta cell compensation does fail in group 2. HbA1C may not identify this high-risk group with adequate sensitivity. Comorbidities such as hypertension and the metabolic syndrome cluster are also much higher in group 2 which translate into cardiovascular risks.

The strengths of this study are that all patients with IFG were assessed for anthropometric and biochemical parameters, including calculation of HOMA-IR and HOMA-β. The study has certain limitations. It is cross-sectional, and there is no follow-up on their future conversion to diabetes. Further studies with follow-up may have implications for clinical practice. However, since patients in group 2 did have more comorbidities, these subjects may need more frequent follow-

up and screening for complications given their greater clustering of risk factors. In short, the WHO criteria do help to identify more dysglycemic subjects, with higher cardiovascular risk. In resource poor countries, this group may need more focussed counselling and follow-up to prevent glycemia and its complications.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Leisure but not household physical activities associates with metabolic syndrome in middle-aged and older individuals: a cross-sectional study

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Abstract
Metabolic syndrome (MS) increases risk of diabetes. Physical activity (PA) is acknowledged to prevent MS, but a few studies in developing countries investigated the relationship between spontaneous PA and MS in older populations. To investigate the association between household and leisure physical activities vs. MS in a cohort of individuals older than 50 years, dwelling in the metropolitan area of Rio de Janeiro (RJ), southeastern Brazil. In 225 individuals aged 51–91 years (62 ± 9 years), MS and related risk factors were assessed through anthropometric measurements, blood pressure, and biochemical analyses, while habitual PA was classified using the Modified Baecke Questionnaire. MS was diagnosed in 64% of the participants. Multivariate logistic regression showed that low levels of household activities discriminated patients with higher blood glucose, obesity, and diabetes mellitus, but were not associated with the risk of having MS. Conversely, leisure PA not only discriminated individual risk factors (total cholesterol, triglycerides, and abdominal circumference), but also associated with MS. Patients declaring not having performed leisure PA within the last year had 2.5 higher risk of exhibiting MS [95% CI (1.22–5.34)], after adjustments for age, body mass index, VLDL, and household PA. Leisure but not household PA is associated with the risk of having MS in an urban population of Brazilian middle-aged and elder individuals. These findings suggest that tasks usually performed at home would not be of sufficient intensity/volume to prevent MS in this population.

Keywords Logistic models · Cardiovascular diseases · Aging · Adult health · Baecke Questionnaire

Introduction

The prevalence of metabolic syndrome (MS) is exponentially greater in middle-aged/older than that in young groups [1, 2]. On the other hand, physical activity is known as an effective strategy to prevent metabolic disorders, such as obesity and hyperlipidemia [3]. However, the amount of recommended physical activity to prevent MS remains controversial. While it has been recognized that cardiometabolic effects of exercise would be proportional to its intensity and volume [4, 5], some research indicated that even low levels of physical activity vs. inactivity would be capable of lowering mortality among adults with MS [6].

Despite this, sedentary behavior remains predominant in the population [7]. This is not different in developing countries such as Brazil—previous surveys suggest that proportion of active vs. inactive citizens would be around 15–17% vs. 80–85%, respectively [8, 9]. Assuming that exercise intensity is not primordial in physical activity programs aiming to promote health, adequate amounts of unstructured physical activity might help preventing MS. Hence, research is needed on how spontaneous physical activity performed on daily basis reduces MS occurrence. However, most prior studies focused on structured and controlled aerobic exercise routines [10–13].

In Brazil, studies about the relationship between physical activity and MS are scarce, particularly among middle-aged and older groups. A national survey coordinated by the Brazilian Society of Cardiology [8] detailed the prevalence of several risk factors for cardiovascular disease in all regions of the country, including physical activity, but data concerning

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MS were not provided. The few studies investigating whether levels of physical activity in Brazilian senior citizens would differ among those with and without MS observed very restricted and peculiar groups [14–17].

In brief, information about the relationship between MS and spontaneous physical activity would be important to ascertain its role in cardiovascular disease prevention, particularly in middle-aged and older groups. There is a lack of data on this matter in developing countries, and Brazil is no exception. Hence, this study investigated the association between household and leisure physical activities vs. MS, in individuals older than 50 years dwelling at Rio de Janeiro (RJ), southeastern Brazil.

Methods

Subjects

A sample of 225 consecutive patients aged 51 to 91 years (62 ± 9 years) from the geriatric outpatient clinic of Antonio Pedro University Hospital (Fluminense Federal University, Niteroi, RJ, Brazil) enrolled in this cross-sectional study. This clinic attends to people that live in the urban area of the cities of Rio de Janeiro and Niteroi.

Compliance with ethical standards

Informed consent was obtained from all individual participants included in the study, which gained approval from the Salgado de Oliveira University Institutional Ethics Committee (process no. 033/2012). The authors declare they have no conflict of interest.

Procedures

Diagnosis of MS followed recommendations of the National Cholesterol Education Program - Adult Treatment Panel III (NCEP-ATP III) [18]. MS was diagnosed based on three of five criteria: (1) abdominal circumference (AC) > 88 cm (women) or 102 cm (men); (2) systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg; (3) fasting plasma glucose ≥ 100 mg/dL; (4) triglycerides (TG) ≥ 150 mg/dL; (5) high-density lipoprotein (HDL) < 50 mg/dL (women) or < 40 mg/dL (men).

Body mass and height were measured with a 100-g precision scale and 1-cm stadiometer (Filizola™, São Paulo, SP, Brazil), respectively. Body mass index (BMI) was calculated. Abdominal circumference (AC) was measured as the distance between the lowest costal margin and iliac crest in the standing position. A single experienced researcher performed all evaluations.

Biochemical analyses [glucose, total cholesterol (TC), low-density lipoprotein (LDL), HDL, very low-density lipoprotein (VLDL), and TG], SBP, and DBP were extracted from medical recordings. Patients fasted for 12 h prior to biochemical examinations, and procedures for data collecting/processing were performed at the University Hospital facilities. Serum glucose levels were measured using the bichromatic GLU method (Dimension Clinical Chemistry System, Siemens™, Lapa de Baixo, SP, Brazil), adapted from the hexokinase-glucose-6-phosphate dehydrogenase method [19]. Serum was collected from centrifuged samples after which HDL, TC, and triglyceride levels were determined fluorometrically using an automatic analyzer (Dimension™, Clinical Chemistry System, Siemens, Erlangen, Germany) and enzymatic kits (Roche Diagnostics™, Mannheim, Germany). LDL was calculated through Friedewald formula (LDL = TC − HDL − TG/5), which is valid for TG levels below 400 mg/dL [20].

Physical activity levels were assessed through the Modified Baecke Questionnaire (MBQ), which quantifies in older persons the amount of physical activity performed within the last year [21, 22]. The instrument includes questions on household, sports, and leisure time domains. Questions regarding household activities have four to five possible answers, from inactive to very active. Questions about sports and leisure time include type of activity, frequency, and months of practice. A continuous dimensionless activity score is calculated by summing scores pertaining to each domain.

Statistical analysis

Statistical power of the sample was calculated considering a medium effect size (0.50) and 225 subjects included in the study. Assuming $\alpha = 0.05$, the sample achieved a statistical power of 0.97. Univariate analysis confirmed the normality of continuous variables. Therefore, data are presented as mean ± standard deviation, whereas categorical variables are presented as percentage. The Student *t* test was applied to compare continuous variables between groups with and without MS. The chi-square was used to compare categorical variables. Differences between groups for continuous variables were tested by *t* tests for independent samples. Potential differences among groups defined according tertiles of physical activity in regard to risk markers were tested by ANOVA followed by Tukey post hoc verifications. Association between physical activity and MS was tested by univariate and multivariate logistic regression as appropriate, adjusted for age, clinical conditions, and smoking. Physical activity scores were included in statistical models as categorical variables. In all cases, significance level was set at $p \leq 0.05$ and calculations were performed using the NCSS software (NCSS™, Kayesville, UT, USA).

Results

Table 1 exhibits overall characteristics of patients. MS was present in 64% of patients, with a prevalence of 66.9% in

women and 53.2% in men. Groups did not differ for age or smoking occurrence, but weight, BMI, and AC were higher in subjects with vs. without MS. HDL was lower in patients with than without MS, while triglycerides, glucose, SBP, and DBP were higher in those with MS. As expected, obesity was more prevalent among patients with MS. In regard to physical activity, household scores were similar across groups, but leisure and total physical activity were higher in those without MS. Comparisons within genders showed no difference between scores in men, regardless the type of activity. In women, leisure (1.32 vs. 0.47; $p = 0.002$) and total physical activity (3.2 vs. 2.3; $p = 0.001$) were higher in patients without MS.

Table 2 presents data of clinical history and risk factors in groups stratified according to physical activity scores. Household activity contribution for the overall score was of 72%, while 28% corresponded to leisure activities. No participant declared to have been involved in sports activities within the last year. HDL ($p = 0.007$) and prevalence of obesity ($p = 0.04$) were lower in subjects classified in first vs. third tertiles of household activities, while blood glucose ($p = 0.02$) and prevalence of diabetes ($p = 0.05$) were higher in first vs. second tertiles. Patients who practiced any leisure time physical activity exhibited lower total cholesterol ($p = 0.02$), triglycerides ($p = 0.01$), AC ($p = 0.04$), and overall risk for cardiometabolic disease ($p < 0.001$), as well as lower obesity ($p = 0.047$). As for total MBQ score, patients in second tertile exhibited higher triglycerides ($p = 0.02$) and sum of risk factors ($p = 0.05$) vs. third tertile, while prevalence of heart disease was greater in first vs. third tertiles ($p = 0.05$).

Table 3 depicts results of univariate and multivariate logistic regressions, with MS as dependent variable. All variables not used to define MS entered in the univariate model. In this model, only BMI [OR (IC 95%)] [1.11 (1.05–1.18)], VLDL [1.12 (1.08–1.16)], and leisure physical activity [3.41 (1.85–6.27)] were retained as significant predictors of MS. Patients that did not practice any leisure physical activity had 3.4-fold higher chance of exhibiting MS than those who did so. As for multivariate logistic regression, age and all significant variables included in univariate analyses were retained, except total physical activity due to collinearity with leisure physical activity. In the multivariate model, BMI and VLDL were significant predictors of MS, albeit leisure physical activity remained as the strongest marker. Actually, risk of MS was nearly 2.5-fold (1.22–5.34) higher among patients who declared to be inactive vs. active during free time.

Discussion

The present study investigated the association between physical activity and MS in a cohort of 225 patients aged 51 to 91 years old (mean 62 ± 9 years), dwelling in the urban area of Rio de Janeiro, in the southeast of Brazil. The major finding was that physical activity during leisure time was a strong predictor of MS and related risk factors, while household activities did not relate to risk of developing MS. Since MS risk factors interact synergistically to increase the chance of developing cardiovascular and metabolic diseases, our findings are aligned with the premise that sedentary lifestyle increases obesity and cardiovascular risk [23–27]. Patients with MS had lower physical activity scores and, as expected, worse profiles in regard to anthropometric variables (weight, BMI, AC), blood analysis (HDL, triglycerides, glucose), clinical history (obesity), and arterial blood pressure [14, 15, 23–25, 27].

Prior research in developed and developing countries indicates that regular physical activity may reduce risk of MS. Kim et al. [28] showed that moderate to vigorous physical activity reduced the occurrence of MS among middle-aged Japanese of both sexes. In Norway, a prospective study demonstrated that even low levels of physical activity were capable to reduce mortality rates due to cardiovascular diseases among adults with MS [6]. A study developed in Iran [29] ratified the association between MS and poor physical activity, by demonstrating an inverse relationship between this syndrome and the amount of daily walking.

As for studies with Brazilian populations, effects of physical activity to prevent MS have been confirmed by some [16, 27], but not all studies [14, 15, 17]. Moreira et al. [27] evaluated MS in 667 men and 702 women over 18 year old in the city of São José do Rio Preto, southeastern Brazil, as well as its association with age, gender, clinical variables, socioeconomic status, and physical activity. MS was present in 22.7% of the population, increasing with age, higher BMI, and sedentary lifestyle. Prevalence of MS was of 16.7 and 26.1% in active/very active vs. minimally active/inactive participants. Overall, inactive individuals were 56% more likely to have MS vs. active/very active. Turi et al. [16] tested associations between MS and physical inactivity among 963 users (aged > 50 years) of public healthcare system in Bauru, southeastern Brazil. Overall prevalence of MS was of 33.6% (range 30.7 to 36.6). High rates of hypertension, diabetes, hypercholesterolemia, abdominal obesity, and MS related to lower levels of total physical activity. However, after adjustment for potential confounders (basic health care units, age, sex, smoking habit, economic condition, waist circumference, overweight, SBP, and DBP), the multivariate model showed that physical activity in leisure time, but not sports practice or occupational domains, significantly associated with higher prevalence of diabetes (OR = 1.79 [1.17–2.72]), hypercholesterolemia (OR = 1.85 [1.24–2.76]), and MS (OR = 1.48 [1.08–2.05]).

On the other hand, Rocha et al. [17] investigated the relationship between MS and health variables among 348 elderly persons (67.8% women) aged 60 to 104 years (mean 72 ± 9 years), at the northeastern city of Campina Grande. Participants were considered as "active" if answered positively to a question regarding whether they performed physical

Table 1 Characteristics of individuals with and without metabolic syndrome

Variable	Total $n = 225$	Metabolic syndrome		p value
		Present $n = 144$	Absent $n = 81$	
Demographic characteristic				
Female (%)	79.1	66.9	33.1	0.461
Men (%)	20.9	53.2	46.8	0.844
Age (years)	62 ± 9	64 ± 8	62 ± 9	0.148
Weight (kg)	75.0 ± 14.6	77.3 ± 14.5	70.5 ± 13.8	< 0.001
BMI (kg/m^2)	30.7 ± 5.4	31.6 ± 5.4	28.9 ± 4.8	< 0.001
Risk factor				
Total cholesterol (mg/dL)	207 ± 49	211 ± 52	198 ± 42	0.069
Abdominal circumference (cm)	98.2 ± 13	101 ± 11	95.0 ± 11	< 0.001
LDL (mg/dL)	125 ± 41	126 ± 43	122 ± 37	0.456
HDL (mg/dL)	49 ± 13	45 ± 12	56 ± 13	< 0.001
Triglycerides (mg/dL)	167 ± 117	200 ± 129	101 ± 42	< 0.001
Glycemia (mg/dL)	125 ± 48	132 ± 51	110 ± 37	0.005
SBP (mmHg)	139 ± 26	144 ± 22	134 ± 18	0.003
DBP (mmHg)	86 ± 12	87 ± 13	83 ± 9	0.006
Clinical history (%)				
Smoking (%)	9.3	9.4	7.9	0.708
Diabetes mellitus	54.2	57.7	47.4	0.141
Hypertension	90.7	90.6	90.8	0.964
Obesity (BMI ≥ 30 kg/m^2)	56.4	65.1	39.5	< 0.001
Dyslipidemia	86.2	87.9	82.9	0.301
Heart disease (AMI, coronary disease)	7.1	7.4	7.9	0.891
Habitual physical activity				
HPA	1.7 ± 0.6	1.7 ± 0.5	1.7 ± 0.5	0.712
LPA	1.0 ± 2.7	0.8 ± 2.2	1.4 ± 2.7	0.050
TPA	2.7 ± 2.4	2.5 ± 2.2	3.2 ± 2.8	0.049

p values refer to differences between groups with and without metabolic syndrome

BMI body mass index, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *AMI* acute myocardial infarction, *HPA* household physical activity, *LPA* leisure physical activity, *TPA* total physical activity

activity for at least 30 min, three times a week or more ("yes" vs. "no"). MS was more prevalent in women (67.4%) than that in men (38.4%) and increased in those suffering from osteoarthritis or heart problems. However, no significant association was found between MS and regular exercise. Doro et al. [15] investigated the relationship between physical activity and MS adjusted for a number of variables in 1.330 Japanese-Brazilians (46.1% men) aged ≥ 30 years living in Bauru. An expressive majority of subjects referred to perform light or moderate physical activities in leisure time (81.2% of men and 86.6% of women). However, only 384 men and 280 women referred to be working; of these, 87.8% of men and 96.1% of women classified their effort as light or moderate. Prevalence of MS was higher in older individuals. Age, BMI, HOMA-IR, and C-reactive protein were independently associated with MS, but not work or leisure physical activity. Dalacorte et al. [14] investigated physical activity in 362 citizens aged 60–79 years with and without MS from Novo Hamburgo, southern Brazil. MS was present in 64% of women and 44% of men. No difference in regard to physical activity was found between MS and non-MS groups. Consequently, odds of having MS as calculated by logistic regression were the same irrespective of physical activity.

Altogether, those studies might suggest that physical activity would not prevent MS in specific Brazilian older populations. However, methodological limitations have been acknowledged. Dalacorte et al. [14] and Doro et al. [15] warned that the excessive inactivity in their samples has possibly precluded the detection of protective effects of physical activity. Moreover, physical activity is often assessed by instruments too imprecise to classify individuals into correct physical activity levels, since low levels of activity are difficult to

Table 2 Risk factors [mean ± SD] and prevalence of diseases [*n*(%)] related to metabolic syndrome according to household and total activity scores (tertiles) and of leisure activity score ("yes" or "no")

	Household activity score			Leisure activity score		Total activity score		
	1° *N* = 71	2° *N* = 70	3° *N* = 84	Yes *N* = 64	No *N* = 161	1° *N* = 78	2° *N* = 79	3° *N* = 68
Total cholesterol (mg/dL)	197.2 ± 44.0	212.0 ± 49.8	210.1 ± 52.3	195.3 ± 42.7	211.1 ± 50.5	202.8 ± 50.8	217.2 ± 48.9	198.8 ± 44.8
HDL (mg/dL)	46.1 ± 10.9	47.9 ± 11.6	52.3 ± 14.8#	49.6 ± 13.4	48.7 ± 12.8	47.6 ± 11.2	49.3 ± 14.1	50.2 ± 13.4
LDL (mg/dL)	118.5 ± 38.1	130.9 ± 43.2	126.1 ± 40.8	119.3 ± 39.3	127.5 ± 41.3	122.0 ± 41.9	131.4 ± 39.9	121.6 ± 40.5
Triglycerides (mg/dL)	166.7 ± 88.5	168.9 ± 112.3	165.5 ± 141.9	135.2 ± 73.9	179.5 ± 128.8*	168.9 ± 97.7	188.8 ± 155.8	139.2 ± 74.4$
Glycemia (mg/dL)	136.9 ± 48.7	115.0 ± 40.7*	123.6 ± 50.3	116.1 ± 43.5	128.7 ± 48.8	134.2 ± 48.6	121.5 ± 45.7	119.0 ± 48.8
SBP (mmHg)	142.7 ± 20.8	139.5 ± 21.6	139.5 ± 21.6	137.0 ± 17.3	141.9 ± 22.2	142.8 ± 22.6	139.6 ± 19.7	139.0 ± 20.6
DBP (mmHg)	85.5 ± 11.9	84.5 ± 10.3	87.0 ± 13.0	85.4 ± 9.5	85.9 ± 12.7	85.1 ± 12.0	86.3 ± 13.0	85.9 ± 10.4
Abdominal circ. (cm)	99.9 ± 12.8	97.2 ± 11.4	98.8 ± 9.6	96.3 ± 9.4	99.6 ± 11.8*	100.8 ± 13.5	97.8 ± 10.0	97.0 ± 9.5
Risk factors	3.1 ± 1.1	2.9 ± 1.1	3.0 ± 1.2	2.5 ± 1.2	3.2 ± 1.0*	3.1 ± 1.1	3.2 ± 1.0	2.7 ± 1.3$
Clinical history								
Diabetes mellitus	45 (63.4)	31 (44.3)*	46 (54.8)	33 (51.6)	89 (55.3)	48 (61.5)	38 (48.1)	36 (52.9)
Hypertension	63 (88.7)	66 (94.3)	75 (89.3)	59 (92.2)	145 (90.1)	71 (91.0)	72 (91.1)	61 (89.7)
Obesity	33 (46.5)	38 (54.3)	56 (66.7)#	30 (46.1)	97 (60.6)*	41 (52.6)	50 (63.3)	36 (52.9)
Dyslipidemia	62 (87.3)	61 (87.1)	71 (84.5)	55 (85.9)	139 (86.3)	67 (85.9)	69 (87.3)	58 (85.3)
Heart disease	7 (9.8)	2 (2.8)	8 (9.5)	2 (3.1)	15 (9.3)	9 (11.5)	6 (7.6)	2 (2.9)

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *circ.* circumference, *HAS* household activity score, *LAS* leisure activity score, *TAS* total activity score

*$p < 0.05$ between 1° and 2° tertiles or yes and no; #$p < 0.05$ between 1° and 3° tertiles; $$p < 0.05$ between 2° and 3° tertiles

Table 3 Univariate and multivariate logistic regressions having metabolic syndrome as dependent variable ($n = 225$)

Variable	Univariate odds ratio (CI 95%)	Multivariate odds ratio (CI 95%)
Age (years)	1.00 (0.97–1.03)	1.02 (0.98–1.07)
BMI (kg/m^2)	1.11 (1.05–1.18)	1.12 (1.04–1.21)
Total cholesterol (mg/dL)	1.00 (0.97–1.03)	
LDL (mg/dL)	1.00 (0.99–1.00)	
VLDL (mg/dL)	1.12 (1.08–1.16)	1.13 (1.08–1.17)
Smoking (present)	1.04 (0.40–2.75)	
Leisure physical activity (absent)	3.41 (1.85–6.27)	2.55 (1.22–5.34)
Household physical activity		
1° tertile	0.98 (0.49–1.96)	0.56 (0.22–1.38)
2° tertile	0.84 (0.43–1.64)	0.62 (0.26–1.46)
3° tertile	1 (reference)	
Total physical activity		
1° tertile	1.86 (0.94–3.65)	
2° tertile	2.02 (1.02–4.01)	
3° tertile	1 (reference)	

BMI body mass index, *LDL* low-density lipoprotein, *VLDL* very low-density lipoprotein

measure by questionnaires. This is the case of the International Physical Activity Questionnaire applied by Rocha et al. [17].

The present study advanced the knowledge by demonstrating that household activities might not compensate for the lack of physical activity in free time. Univariate and multivariate analysis showed that BMI, VLDL, and physical activity were significant predictors of MS, which concurs with prior studies with young [30, 31] and older adults [32, 33]. However, the most relevant finding was that physical activity during leisure time was the strongest marker of MS, in both univariate and multivariate models. The risk of MS was about two times smaller in subjects classified in the third tertile of total physical activity vs. those into first/second tertiles. Considering only leisure physical activity, in both models, risk of MS was about three times greater among patients having not practiced any physical activity during leisure within the last year. Turi et al. [16] reached equivalent conclusions when applying the original Baecke Questionnaire to older people also living in southeastern Brazil. One could speculate that those findings could result from geographic bias, or resulting of sample artifacts, since there is no rationale for why household and leisure physical activities would have different effects upon MS. However, a recent meta-analysis showed that individuals with high involvement in physical activities during leisure time would be 20% less likely to develop MS than those with little engagement, regardless of their house or work occupations [34].

It is important to note that the present results do not exclude the benefits of some household activities for general health. Current guidelines recommend 150 min of moderate physical activity per week or 600 metabolic equivalents minutes per week (METs min/week) [35]. It is therefore possible to speculate that household activities as sweeping, window cleaning, and lawn mowing are vigorous enough [36] to induce health benefits if regularly performed. Unfortunately, this is frequently not the case and this helps to explain why our results do not ratify data from studies suggesting that increased involvement in household activities might help to prevent MS [37, 38], being in line with research questioning this possibility [5, 39]. It might be supposed that volume or intensity of household activities would not be enough to elicit adaptations in isolate components of MS. Our findings partially concur with this premise, since higher scores for household domain did not significantly reduce odds ratio of MS; on the other hand, patients classified into the highest tertile of this domain exhibited better risk profile (as blood glucose and HDL) and lower diabetes occurrence. This is particularly relevant when acknowledging that older individuals, particularly women, are more likely to occupy their time with household tasks and rarely have regular involvement in leisure physical activities with adequate intensity/volume to prevent cardiometabolic disease [40, 41]. In senior populations, household activities are more often performed by women than by men [42]. Accordingly, in our sample, men were more active in the free hours, while women majorly performed household tasks.

The main limitation of this study was the cross-sectional design, which does not allow cause-effect conclusions and does not exclude the possibility of reverse causality. Additionally, the prevalence of inactivity in our sample, particularly during leisure time, might have introduced bias when addressing risk of MS, even after adjustment for other variables. Finally, physical activity was indirectly assessed by questionnaire, which is always problematic.

In conclusion, poor engagement in physical activity during leisure time was shown to be a strong predictor of MS in 225 individuals over 50 year old, dwelling at Rio de Janeiro, southeastern Brazil. Higher scores of total and, particularly,

leisure time physical activity are related to better lipid profile and lower prevalence of obesity, diabetes, and heart disease. On the other hand, isolate household physical activities had poor relationship with overall risk of MS. Physical activities with greater intensity and volume practiced during leisure time should be encouraged to prevent cardiometabolic disease among senior citizens. Additional research is necessary to ratify those data in populations with distinct demographic, cultural, and social characteristics.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Physical activity correlates among persons with type 2 diabetes in Jamaica

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Abstract
Despite the importance of physical activity in the management of diabetes, many persons with diabetes do not meet the recommended levels. This may be influenced by lack of knowledge of the benefits of exercise and common barriers such as lack of time. The purpose of this study was to determine the factors associated with physical activity level in Jamaicans with type 2 diabetes. One hundred and ninety-four persons participated. A demographic questionnaire, a questionnaire to assess knowledge of exercise benefits, the barriers section of the Exercise Benefits and Barriers Questionnaire and the International Physical Activity Questionnaire were administered. The mean age of the sample was 57.5 years. Thirty-eight point seven percent (38.7%) was low active, 33.5% was moderately active and 26% was highly active. Common barriers were the following: perception of exercise as hard work, exercise as tiring and exercise as fatiguing. A greater percentage of those who were low active perceived exercise as tiring compared to those who were highly active ($p = 0.004$). Younger persons were more active than older persons ($p = 0.005$), and employed persons were more active than the unemployed ($p = 0.000$). Knowledge was not significantly related to physical activity level. Physical activity level is low in persons with diabetes in Jamaica. Perception of exercise as hard work, tiring and fatiguing is a common barrier. Older persons and the unemployed are more likely to be inactive. Education and individualized physical activity counselling are necessary to assist with overcoming barriers and increasing physical activity in this population.

Keywords Type 2 diabetes · Physical activity · Barriers · Knowledge · Jamaica

Introduction

The worldwide prevalence of diabetes among adults over 18 years of age was reported to be 8.5% in 2014. It accounts for approximately 3.7 million deaths each year [1]. In Jamaica, diabetes affects 7.9% of the population and is the second leading cause of death in persons under age 70 [2, 3]. Type 2 is more prevalent affecting 90% of persons with diabetes. It is projected that by 2030, approximately 439,000 million adults will be affected by the disease [4]. The rising prevalence, particularly in low- and middle-income countries, is due in part to ageing of populations, unhealthy dietary practices and physical inactivity [1].

Physical activity is defined as bodily movement that is produced by contraction of skeletal muscle which substantially increases energy expenditure. Exercise, which is a type of physical activity, is defined as planned, structured, repetitive bodily movement done to improve or maintain physical fitness [5]. An important aspect of the management of diabetes is regular physical activity which has been shown to produce positive effects such as increase in glucose tolerance [6], increase in insulin sensitivity, lower fasting blood glucose levels [7], improved HbA1c [8], lower blood pressure and lower blood lipid levels [7].

Despite its importance, many persons with diabetes do not meet the recommended level of physical activity (150 min per week of moderate intensity or 75 min of vigorous physical activity or equivalent combination) [9]. Reported physical inactivity levels in persons with diabetes vary across different populations ranging from 31 to 91% [10, 11].

In Jamaica, 29.5% of persons aged 15–74 are inactive and 15% report low physical activity level [3]. To the best of our knowledge, physical activity level of Jamaicans with diabetes has not been reported. Against the background of general

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physical inactivity in the Jamaican population, low physical activity level among persons with diabetes would not be surprising and would suggest that there may be a deficit in the management of these individuals. Numerous barriers to physical activity have been identified and include lack of time, lack of social support, lack of proper equipment, other health problems, the perception that exercise is physically painful and environmental factors such as unsafe neighborhoods [12–14]. Barriers can affect the adoption of and adherence to physical activity recommendations. Hays and Clarke [15] found that, in a sample of 270 residents of the USA, persons with lower barriers to exercise were more likely to be active. Further, inactive individuals are more likely to report lack of time as a barrier compared to those who are active [16]. Other psychosocial variables associated with low physical activity levels include low self-efficacy, poor body image and lack of social support [17–19].

The influence of personal and demographic characteristics on physical activity, in persons with diabetes, has also been examined with inconsistent findings. For example, older persons and women with diabetes were less active in a sample of Canadians [17] while age and gender did not influence physical activity level in a study involving 274 residents, in rural communities in the USA [20].

Knowledge of the benefits of physical activity may also influence physical activity level. This is relatively unexplored among persons with diabetes. In a study involving 238 participants from India with type 2 diabetes, only 51% indicated that exercise participation can control diabetes, while 84% believed that exercise should only be done by persons with diabetes who are obese [21]. Among 217 persons with type 2 diabetes in Pretoria, South Africa, 92% had poor knowledge regarding the benefits of exercise [11].

Increasing physical activity among persons with diabetes in the Jamaican population would improve the management of the condition. The development of appropriate strategies to do this requires first assessing the physical activity level, determining the knowledge of the benefits of exercise as well as identifying barriers to exercise. The purpose of this study was, therefore, to determine the physical activity level of persons with diabetes living in urban Jamaica and how this is influenced by perceived barriers to exercise and knowledge of the benefits of exercise.

Methods

Study design

A cross-sectional design was used to meet the study objectives. The target population comprised Jamaicans aged 18 years old and over, who were living with diabetes. A sample of convenience was used. Outpatients who attended the Diabetes Association of Jamaica (DAJ), the University Hospital of the West Indies (UHWI) Diabetic Clinic and the University of the West Indies (UWI) Community Health Centre were asked to participate.

The study was approved by the Ethics Committee of the UWI, Mona Campus, and permission was received from the relevant authorities at the DAJ, the UHWI Diabetic Clinic and the UWI Community Health Centre to recruit persons from the facilities. Data collection was done, between February 2012 and March 2014, while patients were waiting to be seen by the doctor. Informed consent was obtained from all participants included in the study. Once signed informed consent was received, the questionnaires were interview-administered and this took approximately 20 min. Several visits were made to the facilities to recruit the required number of persons. The minimum sample size required to provide 80% power to detect a medium effect size of 0.25 for differences in barrier scores across physical activity level, at an alpha level of 0.05, was 156 [22].

Measures

Four questionnaires were utilized: a demographic questionnaire, knowledge of exercise benefits questionnaire, the barriers section of the Exercise Benefits and Barriers Questionnaire and the International Physical Activity Questionnaire.

Knowledge of exercise benefits questionnaire

An author-developed questionnaire was used to ascertain knowledge about the benefits of exercise for persons with diabetes and how they should exercise. Individuals were tested on their knowledge of benefits of exercise such as reduction in blood sugar level, reduction in HbA1c, weight loss, lower blood pressure and cholesterol levels. Questions on how persons with diabetes should exercise included the following: when they should and should not exercise, mode of exercise, frequency, duration and intensity. The questionnaire had a total of 13 questions, and each correct answer was given a score of 1 and each incorrect answer given a score of 0. The scores were expressed as a percentage.

Exercise Benefits/Barriers Scale—Exercise Benefits and Barriers Questionnaire

The Exercise Benefits/Barriers Scale [23] consists of 43 items and two components: the benefits scale and the barriers scale. The instrument may be scored and used in its entirety or as two separate scales. For the purpose of this research, only the "barrier" portion was used. It contains 14 items and assesses the perceived barriers to participation in regular exercise. Some of the barriers included are as follows: "takes too much

of my time", "exercise is tiring", "exercise is fatiguing", "exercise is hard work", "spouse not encouraging", "family not encouraging", "places to exercise too far away" and "costs too much to exercise". The instrument uses a Likert-type format with responses as follows: 4 for "strongly agree", 3 for "agree", 2 for "disagree" and 1 for "strongly disagree". When used alone, the barriers score ranges from 14 to 56; therefore, the higher the score on the Barriers Scale, the greater the perception of barriers to exercise. The Exercise Benefits/Barriers Scale (EBBS) has been tested for internal consistency showing a Cronbach's alpha of 0.86 for the barriers scale. Test-retest reliability was found to be 0.77 [23].

International Physical Activity Questionnaire

The International Physical Activity Questionnaire (IPAQ) short version [24, 25] was used to assess physical activity level. This self-administered questionnaire comprises four generic items which assess the last 7 days of total physical activity. From the IPAQ scores, physical activity level was classified as low active, moderately active and highly active.

The IPAQ has acceptable measurement properties for monitoring population levels of physical activity among 18–65-year-old adults in diverse settings [26].

Data analysis

Descriptive statistics were calculated for all variables as appropriate. To determine the relationship between knowledge of exercise (%) and level of physical activity (low, moderate, high), a one-way ANOVA was used. The relationships between barriers to exercise and physical activity level and between age and physical activity level were determined using a one-way ANOVA. The associations between gender, education, employment and physical activity level were analysed using a chi-square test. An alpha level of 0.05 was used for statistical significance.

Results

Characteristics of sample

Questionnaires were distributed to and collected from 194 persons. The IPAQ and the EBBS data were missing for two participants. For 14 participants, data were missing for the EBBS question on spousal encouragement. The mean age of the sample was 57.53 (13.15) years ranging from 18 to 90 years. Fifty-six (29%) were men, and 138 (71%) were women. Most (47%) had achieved secondary education, were employed (43.3%) and had been diagnosed for over 10 years (44.8%) (Table 1).

Table 1 Clinical and demographic characteristics of participants

	Number	Percent
Gender		
Male	56	28.9
Female	138	71.1
Parish: St. Andrew	33	17.0
Kingston	116	59.8
Other	45	23.2
Education		
Primary/secondary	151	77.8
Tertiary	43	77.2
Occupation: retired	52	26.8
Employed	84	43.3
Unemployed	26	13.4
Self-employed	32	16.5
Diagnosis time of < 1 year	21	10.8
1–10 years	86	44.3
> 10 years	87	44.8
Age, mean (SD)	57.53 (13.15)	

Physical activity levels of participants

As shown in Table 2, approximately 39% (75) of participants were low active, 33.5% (65) were moderately active and 26% (52) were highly active. Physical activity data were missing for two persons.

Knowledge of exercise and barriers to exercise

Table 2 shows details of the knowledge and barriers scores. One hundred and ninety-four persons completed the knowledge questionnaire. The mean percentage score was 64.4%. The mean score on the Exercise Barrier Scale was 26.9. There were five main barriers to exercise participation as indicated by "strongly agree" and "agree" responses combined: (1) "exercise is tiring" (50%), (2) "exercise is hard work" (28.9%), (3) "exercise is fatiguing" (26.3%), (4) "exercise takes too much time" (19.6%) and (5) "my spouse does not encourage exercising" (18.6%). See Table 3.

Table 2 Knowledge of exercise benefits, exercise barriers and physical activity

	N	Range	Mean (SD)
Knowledge (%)	194	30.7–92.3	64.49 (13.15)
Exercise barriers score	193	5–52	26.9 (5.9)
Physical activity level, no. (%)			
Low active	75 (38.7)		
Moderately active	65 (33.5)		
Highly active	52 (26.1)		

Table 3 Main exercise barriers among participants

Barrier	Number	Percent
Exercising takes too much of my time		
Strongly disagree	35	18.0
Disagree	119	61.3
Agree	32	16.5
Strongly agree	6	3.1
Missing	2	1.0
	Total 194	
Exercise tires me		
Strongly disagree	22	11.3
Disagree	74	38.1
Agree	86	44.3
Strongly agree	10	5.1
Missing	2	1.0
	Total 194	
My spouse does not encourage exercising		
Strongly disagree	47	24.2
Disagree	97	50.0
Agree	23	11.9
Strongly agree	13	6.7
Missing	14	7.2
	Total 194	
I am fatigued by exercise		
Strongly disagree	37	19.1
Disagree	104	53.6
Agree	43	22.2
Strongly agree	8	4.1
Missing	2	1.0
	Total 194	
Exercise is hard work for me		
Strongly disagree	36	18.6
Disagree	100	51.5
Agree	48	24.7
Strongly agree	8	4.1
Missing	2	1.0
	Total 194	

Correlates of physical activity

As shown in Table 4, the mean scores for knowledge of the benefits of exercise did not differ significantly by physical activity level (62.2% in those who were low active, 66.2% in those moderately active and 65.8% in the highly active). Similarly, mean scores for perceived barriers were not significantly different across different physical activity levels. When the specific barriers were examined in relation to physical activity, a significantly greater percentage of those who agreed that exercise was tiring were inactive compared to those who were very active (46.5 vs 16.3%; $p = 0.004$). Physical activity was also associated with perception of exercise facilities being far away, with a greater level of inactivity among those who agreed that facilities were too far away ($p = 0.028$). Physical activity level was not significantly related to any other specific barrier.

A greater percentage of men were highly active compared to women (36.4% vs 30.9), and more women were low active than men (42.3% vs 30.9 respectively; $p = 0.150$). The mean age (60.9 years) of those who were low active was significantly higher than that of those who were highly active (53.5 years; $p = 0.005$) [95% CI for mean difference, 1.78, 13.08]. Employment status was significantly related to physical activity level. More of those who were employed (42.7%) was physically active compared to those unemployed (19.2%; $p = 0.000$). Educational level achieved was not associated with physical activity level.

Discussion

The study investigated physical activity level and its correlates among persons with type 2 diabetes in Jamaica. Almost 39% of the sample was categorized as low active. This level of physical inactivity among persons with diabetes in Jamaica is similar to what has been observed in the general population [3]. Inactivity levels of 67% [16], 37% [20] and 63.7% [17] have been reported in groups from Scotland, the Midwestern United States and Canada, respectively. The differences may be due to variations in the measures of physical activity used.

Common barriers to exercise were the following: the perception that exercise was hard, exercise was tiring and exercise was fatiguing. The feeling of tiredness has been identified in previous studies as a barrier to exercise [16, 27]. In the study by Thomas et al. [16], feeling of tiredness was identified by older persons while lack of time was more of a problem for younger persons. Lack of time was not among the top three barriers in our study and may be explained by the large percentage of persons that were unemployed and retired and who may have had more leisure time. Proper consultation and individualized prescription, provided and monitored by appropriately trained health care professionals, could help persons to transition and progress gradually in an exercise programme to overcome feelings of excessive tiredness. A multidisciplinary diabetes care team could facilitate this.

Lack of spousal support was a barrier for 19% of the sample. Lack of social support such as provided by significant others has previously been observed as a barrier to exercise in other populations [28]. Approaching exercise counselling as a family affair, where all members of the family are educated about the benefits of exercise for improving/maintaining health and preventing illness, should be encouraged. In this

Table 4 Correlates of physical activity

	Physical activity level			*p* value
	Low active	Moderately active	Highly active	
	no. (%)			
Gender				
Male	17(30.9)	18 (32.7)	20 (36.4)	.150
Female	58 (42.3)	47 (34.3)	32 (23.4)	
Education				
Tertiary	17 (31.5)	19 (35.2)	18 (33.3)	.791
Primary/secondary	60 (40.3)	50 (33.6)	39 (26.2)	
Employment				
Retired	28 (53.8)	20 (38.5)	4 (7.7)	.000
Employed	19 (23.2)	28 (34.1)	35 (42.7)	
Unemployed	13 (50.0)	8 (30.8)	5 (19.2)	
Self-employed	15 (46.9)	9 (28.1)	8 (25.0)	
Barriers score				
Mean (SD)	27.7 (5.9)	26.9 (5.4)	26.5 (5.5)	.441
Knowledge (%)				
Mean (SD)	62.2 (12.8)	66.2 (13.7)	65.8 (14.3)	.159
Age				
Mean (SD)	60.9 (14.1)	57.0 (13.1)	53.5 (10.6)	.007

way, family members could support each other and all would benefit, including those with diabetes.

The perception of barriers to exercise, indicated by the questionnaire score, did not influence physical activity level. This is in contrast to the findings of others [15, 29]. The fact that overall perception of less barriers did not result in higher physical activity may be suggesting that other factors not assessed in this study, such as self-efficacy, may have influenced exercise participation [30]. Two specific barriers were significantly associated with physical activity level: perception of exercise as tiring and places for exercising being too far away. Persons who perceived exercise as tiring were less active. Feelings of tiredness could cause persons to avoid physical activity. Emphasizing the benefits and advising them to break up exercise time into two or three shorter periods (10–15 min) for the day could help minimize the sensation of effort. Additionally, advice on incorporating physical activity into daily routine and gradually increasing the amount and intensity could be of help, for example encouraging walking to shops and other facilities in the neighborhood instead of driving and stair climbing instead of elevator use. Those who considered exercise facilities as too far away were also less active. Counselling on using facilities and space within community, neighborhood and home environment could minimize this barrier.

The mean percentage score on the knowledge of the benefits of exercise among persons with diabetes was 64%, suggesting some deficit in this area and the need for more education. While knowledge of diabetes has been explored in some studies, only a few have examined specifically knowledge of the benefits of exercise. Knuth and colleagues [31] reported that 63% of their sample of Brazilian adults believed that exercise was useful in the management of diabetes. Similarly, in the study by Padma et al. [32], almost 62% of the sample agreed that exercise was beneficial for control of diabetes. In both studies, however, assessment of knowledge was based on response to a single question. Using a six-item instrument, Okonta and colleagues [11] found 92% of their sample with poor knowledge of the benefits of exercise and weight loss in persons with diabetes.

Knowledge of the benefits of exercise does not necessarily translate to practice. In a South African sample of persons with type 2 diabetes [11], global knowledge of the benefits of exercise and a healthy diet showed no significant association with the practice of exercise. Similarly, in our study, knowledge of the benefits of exercise did not influence physical activity level. Knowledge scores for those who were moderately and very active were not significantly higher than scores for those who were low active, suggesting that knowledge did not influence behaviour.

Activity level did not differ significantly by gender though a higher percentage of women were inactive compared to men. This lack of significant association contrasts the findings of others [17, 30] and may be due to the much smaller number of men than women participating.

Physical activity level was positively related to employment status. This complements reports from population-based studies which have shown higher levels of objectively

measured physical activity in employed versus unemployed persons [33, 34]. This association between employment and self-reported physical activity was also observed in a sample of Japanese with type 2 diabetes [35]. The increased activity level observed in employed persons may be a consequence of specific job tasks involving physical activity. On the other hand, some jobs can be categorized as sedentary because specific associated tasks do not involve much physical activity. However, the positive association has been observed even in persons engaged in typically sedentary jobs. In these cases, the enhanced activity would be more than likely related to the daily routines of full-time employment and active transport to and from places of work [34].

Older persons had significantly lower activity level than younger persons. This is consistent with the findings of Thomas et al. [16], who also observed that younger persons in their sample were more likely to be active. Older persons with diabetes may be more inactive due to other age-related health problems. Increasing age is oftentimes associated with comorbidities such as painful arthritis involving lower limbs joints, which can be helped by exercise [36], but may make exercise initiation difficult. Persons may not be willing to persevere beyond the initial difficulty to experience the positive benefits [30]. Exercise recommendations which minimize impact on the joints may be necessary to help with overcoming this problem and increasing physical activity level [37]. The relative inactivity of older persons may also be attributed to their perception or belief that as people age, they should exercise less [15].

Some limitations of the study must be noted. Self-report on physical activity was utilized as opposed to more objective measures such as accelerometry. The possibility of misinformation or recall bias, therefore, cannot be ruled out. Additionally, the cross-sectional nature of the study does not allow for any determination of causality of the identified factors with physical activity level.

A standardized tool for assessing barriers to exercise was used. The tool, however, does not capture all possible barriers to exercise that could be faced by persons with diabetes. The results of the study should therefore be interpreted with this in mind.

Despite the limitations, this study, to our knowledge, is the first to provide data on the correlates of physical activity in persons with diabetes in the Jamaica and therefore fills an important gap.

Conclusion

Physical activity is an important adjunct to drug therapy in the management of diabetes. The findings of the study indicate that physical activity level is low in persons with diabetes and, more so, in older persons and the unemployed. Diabetes care is clearly less than optimal. Feelings of tiredness, fatigue and the perception that exercise is hard work are common barriers. Having knowledge of the benefits of exercise does not seem to enable or empower persons to sufficiently overcome the perceived barriers. Education, individualized exercise counselling and encouraging spousal/family support are necessary to assist with overcoming some of the barriers and increasing physical activity participation in persons with diabetes. In developing strategies for increasing physical activity in persons with diabetes, the unemployed and elderly should be targeted.

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Author contribution C.G.—conception and design, literature search, data analysis and interpretation, manuscript preparation, manuscript editing and review, and final approval

G.N.—acquisition of the data, interpretation of the data, manuscript preparation, manuscript editing and review, and final approval

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the study were in accordance with the ethical standards of the University of the West Indies Ethics Committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Predicting relationship of eating behavior, physical activity and smoking with type II diabetes and related comorbidities among Saudi citizens: cross-sectional observational study

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Abstract

The aim of the study was to investigate the correlation pattern of social habits and cardiovascular risk assessment with disease history (e.g., obesity, T2DM and HPT) among free-living citizens of Saudi Arabia. Cross-sectional observational study design was used to collect the data among the citizens of Saudi Arabia. Convenient sampling technique was used to contact over 1163 free-living individuals. A self-administered questionnaire containing 49 items divided into following three sections was used to measure the correlation. Logistic regression analysis was used to predict the response. Sixty-seven percent ($n = 725$) of the respondents (male $n = 419$ (57.79%) vs. female $n = 306$ (42.21%), respectively; $p > 0.01$) perceived unhealthy nutritional habits. Also, a total of 785 (72.55%) participants (436 (55.54%) vs 349 (44.56%); males vs. females; $p > 0.05$) responded insufficient daily physical activity. Leisure time and uncontrolled eating were independently associated with BMI (S.E − 0.13, 95% CI − 1.81 to − 0.11, $p = 0.035$; and S.E 0.31, 95% CI 0.23 to 0.45, $p < 0.001$, respectively). T2DM showed significant independent association with leisure time, uncontrolled eating, and the Framingham risk (S.E − 0.25, 95% CI − 1.27 to − 0.14, $p = 0.022$; S.E 0.49, 95% CI 0.21 to 0.97, $p < 0.001$, and S.E − 0.17, 95% CI − 0.87 to − 0.13, $p < 0.001$, respectively). Study concluded a strong inverse correlation pattern between the level of physical activity (leisure time) with BMI, T2DM, obesity, and HPT. Uncontrolled eating behavior showed significant effect on BMI, obesity, and T2DM. However, HPT was significantly associated with work time (PA) and the Framingham risk assessment score.

Keywords Physical inability · Eating behavior · Type 2 diabetes mellitus · Obesity · Cross-section observational study

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Introduction

Diabetes mellitus (DM) is a world-wide concern. Among Saudi prevalence of the disease is reported to be 23.9% in the year 2013 [1] and is expected to be increasing. DM is associated with improper life style including lack of physical activity, unbalanced diet, smoking, and obesity [2]. All of these risk factors are noted in Saudi due to variety of reasons [2]. DM prevalence is reported to be more in Saudi men in comparison to women [3, 4]. Moreover, prevalence of DM is reported to be higher in Saudi living in urban areas compared to that living in rural areas [3].

Significant portion of adolescent males in Saudi (37%) are smokers; however, figures are comparatively less in females (6%). Saudi start smoking at a very early age which cannot be ruled as one major cause for diseases in later stages of life. Due to the advancements on the telecommunications, majority of the Saudi adolescent men (87%) are reported to be spending about 2 h of inactive periods a day. Moreover, their dietary habits are reported to be poor as well [5]. Across all the age groups, obesity and overweight among Saudi men and women is reported to be up to 45.60 and 39%, respectively [6].

Poor eating behavior, lack of physical activity, and smoking are the common risk factors for DM, obesity, and hypertension [4–6]. Till date, no epidemiological study has established a link between physical inactivity, eating behavior, and cardiovascular risk with obesity, type 2 diabetes mellitus, and hypertension. With such high prevalence of T2DM and related cardiovascular mortality in Saudi Arabia, it is necessary to determine the relationship of social habits on health status of free living. Thus, the objective of the study is to investigate the correlation pattern of social habits and cardiovascular risk assessment with disease history (e.g., obesity, T2DM, and HPT) among free-living citizens of Saudi Arabia.

Methodology

Ethics approval

The study protocol and instruments were approved by Taibah University Research Ethics committee and the Health Commission of Saudi Arabia.

Participant consent

Participants interested to participate in this study were required to sign a research informed consent form. Participants, who were illiterate, acquired an impartial witness to explain the study protocol before participation.

Study design and participant characteristics

Cross-sectional observational study design was used to collect the data between January 2016 and May 2017 among the citizens of Saudi Arabia (no expatriates). A multistage stratified cluster random sampling design was used to obtain a representative sample of Saudi Arabian population. Stratification was based on the number of regional health authorities in the country (i.e., Makkah, Madinah, Jeddah, and Riyadh), age, gender, and Saudi nationals only. Each region was assigned a sample proportionate in size to its population. A simple random sampling was used to select participants from primary healthcare center coverage area (PHCC). Kish method was used to select one participant within the identified area. Participants with age more than 18 years were included in the study. Convenient sampling technique was used to contact over 1163 free-living individuals from various places of four major cities of Saudi Arabia. One thousand and eighty-two of them (93.03% response rate) agreed to participate in the study and filled the initial questionnaire containing 10 items of general information. Selected participants were then given an appointment at a local primary healthcare clinic for biochemical and other study measurements including physical activity, eating behavior, and estimating risk for cardiovascular diseases. If any participant by chance missed the appointment schedule, the principal investigator managed to arrange another with the convenience of individual. All the biochemical and other measurements were undertaken under the supervision of research team to avoid any procedural or ethics bias.

Study instruments

General information

This section comprised of 10 items concerning gender, age, height, body weight, social history, and own health history of type 2 diabetes mellitus (T2DM), obesity, hypertension (HPT), high lipid profile (HLP), and cardiovascular disease (CVD).

Biochemical analysis

Taking into consideration that many, if not all of the participants, may be unable to recall their actual values of blood pressure, plasma glucose level or cholesterol level, the primary healthcare clinic to confirm the values and add the recent and precise values to the respective participant file.

Participant self-perception to nutritional habits (healthy/unhealthy) and physical activity (sufficient/insufficient) were reported. Individual body mass index (BMI) was calculated using height and body weight.

A self-administered questionnaire containing 49 items divided into following three sections were used to measure the correlation;

a. Baecke Physical Activity Questionnaire (BQ) [7]
b. Three-Factor Eating Questionnaire—TFEQ-R18 [8, 9]
c. The Framingham scale; The Framingham risk assessment for estimating patients' 10-year risk of developing cardiovascular disease [10].

Baecke Physical Activity Quesitonnaire

Physical activity level was measured by using BQ, developed for assessment of physical activity levels in large-population studies. Sixteen-item questionnaire was classified into three groups of physical activity, work, sports, and non-sport/leisure time. Five likert scale responses (never, seldom, sometimes, often, and very often) were used to measure the components. A score ranging from one (minimum) to five (maximum) was used to test the questionnaire as described by Baecke et al. [7].

The original BQ questionnaire was translated in Arabic using translation/retranslation procedure by two different independent professional translators. Face and content validation was performed by research experts from department of clinical and hospital pharmacy, Taibah University.

Eating behavior

Stunkard and Messick's [8] revised version of TEFQ-R18 [9] was used to evaluate the eating behavior among the study population. Every question was measured from 1 to 4 and the scores were summed up to obtain the following three classifications and has good validity and reproducibility in the general population.

This questionnaire measures three different aspects of eating behavior;

cognitive restraint—6 items (conscious control of food intake targeting weight loss or weight control), total score range (6–24)
uncontrolled eating—9 items (excessive food intake due to constant feeling of hunger or lack of self-control), total score range (9–36)
emotional eating—3 items (overeating in periods of negative mood), total score range (3–12) [9].

Higher score indicated high cognitive restraint, uncontrolled eating, and/or emotional eating. The original TEFQ-R18 questionnaire was translated in Arabic using translation/retranslation procedure by two different independent professional translators. Face and content validation was performed by research experts from department of clinical and hospital pharmacy, Taibah University.

The Framingham risk score

This scale was used to assess the 10-year risk for development of cardiovascular disorders. Individual score was calculated as per point system. Criteria were as follows: low risk $\leq 10\%$ (0.6%/year), moderate risk 11–19% (0.6–2%/year), and high-risk $\geq 20\%$ ($> 2\%$/year). Biochemical analyses required to calculate the score were total cholesterol (mmol/L), high density lipoproteins (mmol/L), and systolic blood pressure (mm of Hg). Other general values were available in the general questionnaire filled by each participant. The principle investigator was the only responsible person to calculate individual risk score to avoid multi-hand bias.

Statistical analysis

The measured data are presented as number (n), percentage (%), or mean ± SD as indicated. Gender comparison was tested with two-sided Student's t tests and chi-square. Pearson and Spearman correlation coefficients were used to evaluate correlation patterns. Point-biserial correlation method was used for categorical variables against continuous variables. One-way ANNOVA was used for the comparison between tertile groups of physical activity, eating behavior, and the Framingham scale with BMI, however chi-square test with T2DM, smoking, and other disease conditions. Logistic regression model was used to predict the response. Statistical package for social sciences (SPSS 20 ®) was used to execute all the analysis.

Results and findings

A total of 1082 participants were recruited in the study with a response rate of 93.03%; the detail response rate as per location is provided in Fig. 1. Similar rates were reported all over the recruitment site, i.e., Makkah—92.06%; Madinah—92.25%; Jeddah—95.08%; and Riyadh—92.78%). Sixty-seven percent ($n = 725$) of the respondents (male $n = 419$ (57.79%) vs. female $n = 306$ (42.21%), respectively; $p > 0.01$) perceived unhealthy nutritional habits. Also a total of 785 (72.55%) participants (436 (55.54%) vs 349 (44.56%); males vs. females; $p > 0.05$) responded insufficient daily physical activity.

The characteristics of study population ($n = 1082$) are presented in Table 1. Male to female ratio 1:0.8, mean ± SD age was 35.7 ± 14.4 years for males and 36.1 ± 15.1 years for females ($p = 0.169$). Male participants had significantly high BMI compared to female (28.3 ± 5.9 vs. 24.1 ± 4.8; $p > 0.001$). Total of $n = 506$ (46.76%) participants within

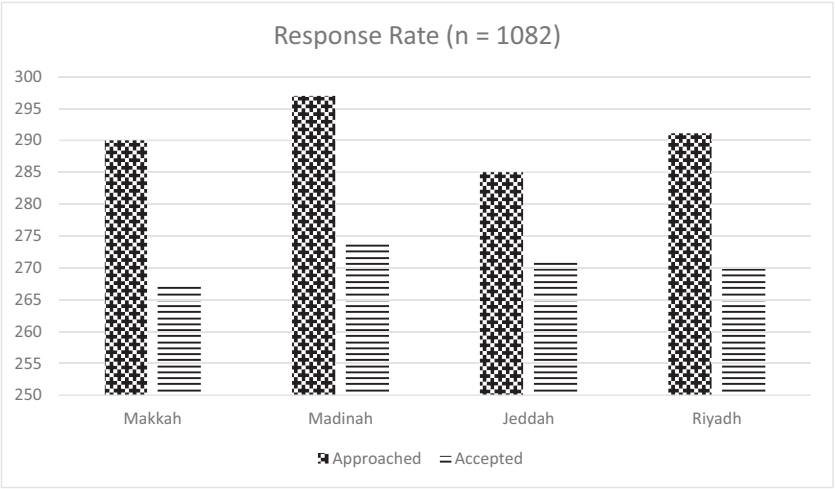

Fig. 1 Response rate distribution pattern over the site of recruitments

normal BMI range rest $n = 576$ (53.23%) had high BMI (either overweight or obese). Disease history showed no significant difference among gender, except HLP ($p = 0.001$) and obesity ($p = 0.041$). About 65 % of the study population had a history of T2DM.

Physical activity-leisure time showed significant inverse correlation pattern with BMI, smoking, and disease history (except HLP, $p = 0.813$). Sport time physical activity also correlated significantly with BMI, smoking, and disease history (except T2DM ($p = 0.652$). All the aspects of eating behavior, uncontrolled eating, and emotional eating showed significant direct and inverse correlation with BMI, smoking, and disease history (except HLP). The Framingham risk assessment identified significant direct correlation with low-risk tertile to BMI, smoking, HPT, and HLP; however, high-risk score tertile was found to be significantly inversely correlated to all the observed parameters (Table 2).

Further analysis focused to compare BMI and prevalence of T2DM in the tertile groups of leisure time physical activity,

Table 1 Characteristics of the study participants ($n = 1082$)

Characteristics	Male	Female	Total	*p*-gender diff.
Number (%)	596 (55.08)	486 (44.92)	1082 (100.00)	
Age (years) mean ± SD	35.7 ± 14.4	36.1 ± 15.1	36.6 ± 12.3	0.169
BMI (kg/m^2) mean ± SD	28.3 ± 5.9	24.1 ± 4.8	26.3 ± 6.1	0.001*
Overweight, *n* (%)	215 (36.07)	152 (31.27)	367 (33.92)	0.023*
Obesity, *n* (%)	117 (19.63)	92 (18.93)	209 (19.3)	0.041*
Smoker, *n* (%)				
Ever	424 (71.14)	185 (38.06)	609 (56.28)	0.001*
Never	179 (28.86)	301 (61.94)	473 (43.72)	
Type II diabetes mellitus, *n* (%)				
Yes	417 (69.97)	284 (58.44)	701 (64.79)	0.435
No	179 (30.03)	202 (41.56)	381 (35.21)	
Cardiovascular disease, *n* (%)				
Yes	218 (36.58)	141 (29.01)	359 (33.18)	0.886
No	378 (63.42)	345 (70.99)	723 (66.82)	
Hypertension (HPT), *n* (%)				
Yes	193 (32.38)	178 (36.63)	371 (34.29)	0.421
No	403 (67.62)	308 (63.37)	711 (65.71)	
High lipid profile (HLP), *n* (%)				
Yes	351 (58.89)	201 (41.36)	552 (51.02)	0.001*
No	245 (41.11)	285 (58.64)	530 (48.98)	

Data are presented as n, mean ± SD, and %. Overweight = BMI ≥ 25.0 ≤ 29.9 kg/m^2 ; Obesity = BMI ≥ 30 kg/m^2

*$p < 0.05$ between genders (two-sided Student's *t* test and chi-square)

Table 2 Correlation of physical activity, eating behavior, and the Framingham risk with BMI, smoking, and disease history

Characteristics		BMI	Smoking	T2DM	HPT	HLP	Obesity
Baecke (PAQ)	Leisure time - PA	− 0.281†	− 0.213†	− 0.187*	− 0.235*	NS	− 0.171*
	Sport - PA	− 0.211*	− 0.281†	NS	− 0.188*	− 0.271†	− 0.144*
	Work - PA	NS	NS	− 0.141*	− 0.317†	NS	− 0.195*
TFEQ – R18	Cognitive restraint	NS	NS	NS	NS	NS	NS
	Uncontrolled eating	0.266*	− 0.177*	0.161*	− 0.162*	NS	0.159*
	Emotional eating	− 0.267†	− 0.214*	0.241†	− 0.145*	NS	0.140*
Framingham	Low risk	0.126*	0.183*	NS	0.267†	0.231†	NS
	Moderate risk	NS	NS	NS	− 0.192*	− 0.147*	NS
	High risk	− 0.291†	− 0.245†	− 0.287†	− 0.261†	− 0.297†	− 0.273†

Low risk = < 10% CAD (0.6%/year), moderate risk = 10–20% (0.6–2%/year), high risk = > 20% (> 2% per year). Point-biserial correlation method

PA physical activity

*$p < 0.05$;† $p < 0.001$

sport time, and uncontrolled and emotional eating behavior. The BMI was significantly higher among the participants of the lowest vs middle (27.1 ± 9.6 vs 24.4 ± 5.1 kg/m^2, p = 0.017) and in the highest tertile of leisure time (28.3 ± 8.3 vs 23.9 ± 4.7 kg/m^2, $p = 0.023$). Similar pattern was observed with sport time to BMI and HPT. However, leisure time also showed significant difference with T2DM, obesity, and HPT ($p > 0.01$) among the lowest to the highest tertile participants (Table 3).

In addition, participants of the highest tertile of both uncontrolled and emotional eating behavior had significantly high BMI as compared to the middle and lowest tertiles ($p > 0.01$). Smoking cohort showed no significant difference among tertiles of both physical activity and eating behavior, hence considered to remove from further analysis. Similar associations found when the tertile groups were replicated separately among male and female (data no shown).

Logistic regression analysis was made to investigate the impact of physical activity, eating behavior, and the Framingham risk on BMI, T2DM, obesity, and HPT (gender and age were adjusted for potential confounders). As shown in Table 4, leisure time and uncontrolled eating were independently associated with BMI (S.E − 0.13, 95% CI − 1.81 to − 0.11, $p = 0.035$; and S.E 0.31, 95% CI 0.23 to 0.45, $p < 0.001$, respectively). T2DM showed significant independent association with leisure time, uncontrolled eating, and the Framingham risk (S.E − 0.25, 95% CI − 1.27 to − 0.14, $p = 0.022$; S.E 0.49, 95% CI

Table 3 Inter-tertile association with BMI, smoking, T2DM, obesity, and HPT

Characteristics		Tertile	BMI	Smoking	T2DM	Obesity	HPT
Baecke - PAQ	Leisure time -PA	Highest	0.023	NS	0.001†	0.001†	0.001†
		Middle	0.017	NS	0.010	0.022	NS
		Lowest	NS	NS	NS	NS	NS
	Sport -PA	Highest	0.001†	0.033	0.027	0.001†	0.018
		Middle	0.014	NS	NS	NS	0.011
		Lowest	NS	NS	NS	NS	NS
TFEQ	Uncontrolled eating	Highest	0.001†	0.026	0.001†	0.001†	0.023
		Middle	0.001†	NS	0.001†	NS	NS
		Lowest	NS	NS	NS	NS	NS
	Emotional eating	Highest	0.001†	0.014	0.037	0.001†	0.019
		Middle	NS	NS	NS	0.041	NS
		Lowest	NS	NS	NS	NS	NS

$p < 0.05$; one-way ANNOVA and chi-square

† $p < 0.001$

Mid middle, *Mod* moderate, *low* lowest

Table 4 Results of logistic regression analysis with BMI, T2DM, obesity, and HPT

Dependent	Independent	B	S.E	95% CI	*p*
BMI	Leisure time-PA	− 0.96	− 0.13	− 1.81 to − 0.11	0.035
	Sport – PA	− 0.55	− 0.08	− 0.83 to 0.21	NS
	Uncontrolled eating	0.34	0.31	0.23 to 0.45	0.001
	Emotional eating	0.29	0.28	0.21–0.37	NS
T2DM	Leisure time-PA	− 0.81	− 0.25	− 1.27 to − 0.14	0.022
	Sport – PA	− 0.37	− 0.07	− 1.20 to 0.17	NS
	Uncontrolled eating	0.49	0.27	0.21 to 0.97	0.001
	Emotional eating	− 0.45	− 0.05	− 1.51 to 0.51	NS
	Framingham	− 0.38	− 0.17	− 0.87 to − 0.13	0.001
Obesity	Leisure time-PA	− 0.91	− 0.11	− 1.63 to − 0.10	0.017
	Sport – PA	− 0.67	− 0.21	− 1.40 to − 0.07	0.001
	Uncontrolled eating	0.39	0.22	0.19 to 0.59	0.037
	Emotional eating	0.44	0.28	0.23 to 0.61	0.041
	Framingham	− 0.51	− 0.31	− 0.89 to − 0.21	0.001
HPT	Leisure time-PA	− 0.81	− 0.23	− 1.64 to − 0.19	0.034
	Sport -PA	− 0.59	− 0.09	− 1.40 to − 0.21	NS
	Work – PA	− 0.77	− 0.36	− 1.21 to − 0.64	0.021
	Uncontrolled eating	− 0.41	0.05	− 1.01 to 0.81	NS
	Emotional eating	− 0.21	0.09	− 1.49 to 0.43	NS
	Framingham	− 0.51	− 0.91	− 1.83 to − 0.46	0.001

The model included all 1082 participants and was adjusted for age and sex as potential confounders. Nagelkerke's $R^2 = 0.87$

0.21 to 0.97, $p < 0.001$ and S.E − 0.17, 95% CI − 0.87 to − 0.13, $p < 0.001$, respectively). HPT showed similar pattern with leisure time and work time physical activity and also with the Framingham risk assessment. However, obesity showed significant independent association with leisure time, sport time, uncontrolled eating, emotional eating, and also the Framingham risk assessment.

Discussion

The current study contributed to establish the relationship of physical activity, eating behavior, and the Framingham cardiovascular risk with obesity, T2DM, and HPT in a country population known to have the highest T2DM-related cardiovascular mortality prevalence in Middle-east. The following highlighted the major findings of the study: (a) participants with low physical activities (leisure time and sport) exhibit significantly higher BMI values, (b) T2DM independently associated with the leisure time, uncontrolled eating behavior, and the Framingham risk score, (c) individuals with high uncontrolled and emotion eating behavior showed inverse effect on HPT, (d) T2DM has no significant association with either sport time (PA) or emotional eating behavior among the participants, and (e) except BMI, all three aspects (T2DM, obesity, and HPT) were significantly associated with the Framingham risk assessment.

It has considered that obesity is directly concern with the imbalance in the positive energy imbalance; decreased energy outlay (e.g., physical inactivity); and increased intake (e.g., excessive eating) or combination of both phenomena. Somehow scientific evidence has already unequivocally determined the relationship between reduced activity and obesity [11]. Abdulmohsen and colleagues [12] investigated the level of physical activity among Saudi population ($n = 4758$) by using the Gobal Physical Activity Questionnaire (GPAQ) and reported high level of physical inactivity, but unfortunately did not establish the association between the inactivity and eating behavior to certain community diseases and risk factor for cardiovascular disease development. The present study also identified no significant correlation of work (PA) with BMI, smoking, and lipid profile. These findings are in concordance with Packer and colleagues [13] systemic review on the physical inability effect on quality of life and disease symptoms.

In addition, BMI, smoking, T2DM, and HLP in the present study were significantly higher, and the prevalence of overweight was significantly greater in males than in females [12]. Typically, males have higher BMI, smoking, T2DM, and HLP than females (except HPT) and we have not previously observed this pattern in any of regional studies. Also in the current study, males are at lower age, which makes the difference in BMI and HPT between genders (data no presented due to adjustment in regression modeling).

A significant trend towards decrease in HLP, HPT, and obesity was found with increased sport (PA). However, statistical difference was only observed between the lowest and highest tertile group. Previous Saudi literature suggested the inactivity among Saudi adults and adolescents is a major public health concern that requires urgent intervention [5, 14]. Our findings in support of the impression of reduced energy expenditure during leisure time and reduced sport (PA) are the core determinants of obesity epidemic in Saudi Arabia.

The importance of unhealthy lifestyle (e.g., physical inactivity and excessive eating) was considered as major determinant of T2DM and HPT; literature is well established [15, 16]. In the present study, BMI, T2DM, HPT, and HLP were found to be significantly different among genders. However, after it was examined in tertile groups of leisure time PA, sport PA, and uncontrolled and emotional eating behavior separately among gender, respectively, the associations observed in the whole studied population were replicated. This indicates that the relationship between BMI, T2DM, HPT, and obesity with physical activity and nutrition habits is not only valid in general, but also among genders [17–19].

A relatively large portion of study population was aware about their unhealthy nutritional habits and insufficient daily PA. This finding should, however, be carefully interpreted, since it does not necessarily mean that participants will take part in lifestyle modification programs, if such are offered.

Like all the studies, this study also comes across with some potential limitations which limit any inference about the direction of causality and the utilization of questionnaire for assessment of nutritional habits instead of real-time situation, since under or over reporting is often observed with such type of data collection method. This will restrict us from drawing definitive conclusions. However, this is the first kind of study that reported the interaction between different aspects of physical activity, eating behavior, and the Framingham risk assessment among the citizens of Saudi Arabia. Hence, this study not only extends the findings of previous literature [12, 14] of physical inability but develops a link with cardiovascular-related mortality [20] and also provide the understanding of current epidemics of obesity, T2DM, HPT, HLP [16–19] and risk assessment among the Saudi nationals.

Limitations of the study

a. Cross-sectional observational study design has its own limitations but in this study, we tried to reduce bias by sorting patients to type II diabetes mellitus.
b. Data collected from multiple cities cannot generalize for Arab population. Yet, future studies are recommended to address this limitation.
c. Different assessment tools might reveal different findings, so it is highly recommended to compare and validate various tools in Saudi population and also contrast the results; to provide better information to healthcare professionals involved in health care setting.

Conclusion

Study concluded a strong inverse correlation pattern between the level of physical activity (leisure time) with BMI, T2DM, obesity, and HPT. Uncontrolled eating behavior showed significant effect on BMI, obesity, and T2DM. However, HPT was significantly associated with work time (PA) and the Framingham risk assessment score.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study protocol and instruments were approved by Taibah University Research Ethics committee and the Health Commission of Saudi Arabia. All procedures performed in this study were in accordance with the ethical standards of the Taibah University research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Participants who were interested to participate in this study were required to sign a research informed consent form. Participants, who were illiterate, acquired an impartial witness to explain the study protocol before participation.

Abbreviations T2DM, type II diabetes mellitus; HPT, hypertension; HLP, high lipid profile; PA, physical activity; BMI, body mass index; BQ, Baecke Physical Activity Questionnaire; TFEQ-R18, Three-Factor Eating Questionnaire

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ORIGINAL ARTICLE

Effectiveness of a cluster-randomized controlled trial community-based lifestyle intervention program to control prehypertension and/or prediabetes in Thailand

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Abstract
Lifestyle intervention is recommended as the primary management for prehypertension and prediabetes. The aim of the study was to examine the effect of a community-based lifestyle intervention on glucose and blood pressure levels in Nakhon Pathom Province, Thailand. We conducted a cluster-randomized community trial in 12 Buddhist temples, enrolling 443 members, aged 35–65 years, with prediabetes and/or prehypertension. The temple (and their members) was randomized to six 2-h group session of lifestyle intervention (physical activity and nutrition) over 6 months guided by the Health Action Process Approach model or receiving a healthy lifestyle pamphlet. Physical outcomes (glucose, blood pressure, lipid profiles, and anthropometric measures) were assessed at 6 and/or 12 months following baseline assessments. The effects of the lifestyle intervention were assessed with generalized linear mixed models. The study recruited 443 participants in 12 temples (220 in the intervention and 223 in the control group), and 89% completed the 12-month evaluation. At 12-month follow-up, mixed modeling found significant interaction effects on fasting plasma glucose ($p = 0.017$) and diastolic blood pressure ($p = 0.002$). Improvements were found for systolic blood pressure, but no significant interaction effect was detected. In conclusion, the lifestyle intervention was only partially effective. More research is needed to investigate lifestyle interventions in this community-based setting. Trial registration number: TCTR20170721001

Keywords Community-based intervention · Lifestyle intervention · Prediabetes · Prehypertension · Thailand

Introduction

The prevalence of diabetes and impaired fasting glucose was 7.5 and 10.6%, respectively among adults aged 20 years and older in 2009 in Thailand [1]. The prevalence of hypertension and prehypertension weighted to the Thai national 2009 population 15 years and older was 21.5 and 38.3% respectively [2]. Levels of awareness of hypertension and diabetes have improved but were still low across the country [1, 2]. Prediabetes and prehypertension are major risk factors of preventable chronic cardiovascular disease [3].

Lifestyle interventions have been found to be effective to prevent the progression of prehypertension to hypertension and prediabetes to diabetes [4–9]. Such nonpharmacological primary prevention approaches can have enormous public health effects [4]. In order for these proven lifestyle interventions to have a public health impact, they need to be implemented in community settings [10, 11]. Several studies in a church-based, mainly American, community setting found promising results of lifestyle interventions in lowering diabetes and hypertension risk [12–15]. In Thailand, the Buddhist temple has been recognized as an important community health promotion intervention setting [16–20]. There is a lack of studies in Asia on community (Buddhist temple) lifestyle interventions to reduce hypertension and diabetes risk. Therefore, the aim of this study was to study the effectiveness

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of a community (Buddhist temple)-based lifestyle intervention program to control prehypertension and/or prediabetes in temple members in a cluster-randomized controlled trial in Nakhon Pathom Province, Thailand. It was hypothesized that participants in the study that receive a lifestyle intervention will have a greater reduction in blood pressure and blood glucose levels, compared with those who only received a health education leaflet.

Materials and methods

Study design

The study is a cluster-randomized controlled evaluation of a group-based program in accordance with the requirements of the "Consolidated Standards of Reporting Trials (CONSORT) statement" [21] and its extension to cluster-randomized trials [12]. From community settings (Buddhist temples) in Nakhon Pathom Province, 12 temples were randomly selected for inclusion in the study (six temples were intervention and six temples were control sites) (see Fig. 1).

Principles for recruitment

Inclusion criteria

Temples Twelve temples having a temple building and having at least 100 temple members in the locations of Nakhon Pathom Province were included in the study.

Temple members Males and females, aged 35 to 65 years, who visit the temple, and having been diagnosed with prediabetes and/or prehypertension (having FPG ≥ 100 and < 126 mg/dl or SBP/DBP ≥ 120–139/80–89 mmHg), were included in the study.

Exclusion criteria

Temple members Temple members under the age of 35 and above 65 years, with previous diagnosis of heart diseases; with previous diagnosis of hypertension and/or type 2 diabetes; on antihypertensive and/or glucose-influencing medication; with unstable pulmonary disease, substance abuse, severe psychiatric problems, or orthopedic or rheumatologic disease; and having kidney disease, were excluded.

Randomization

A list of the names of Buddhist temples in Nakhon Pathom Province was obtained from the National Office of Buddhism. Randomization was performed using a secure remote randomization agent, divided into six intervention and six control temple sites. At the temple level, all consecutive participants in screening sessions were recruited into the study over a period of 2 months from June to July 2016.

Blinding

Temple leaders were informed that their assignment to different study conditions was random, and that different temples would get different interventions. Random assignment occurred prior to baseline measurement in order to ascertain that research nurses conducting recruitment and assessment remained blind to the condition assignments.

Data collection

Blood pressure (BP) was assessed based on Thai guidelines [22] with a validated automated digital BP monitor (BpTRU) three times at two different sessions, with 1 week apart. Prediabetes was screened with questions of the Thai Diabetes Risk Score Tool [23]. Clients who scored 7 or more on the diabetes risk screen were considered to have elevated diabetes risk [23]. In confirmatory testing, a professional nurse collected fasting venous blood samples from the temple members for fasting plasma glucose and lipid tests. These tests were performed with a uniform glucose oxidase-peroxidase method and a cholesterol oxidase-phenol aminophenazone (CHOD-PAP) method. Participants with prehypertension (with a seating resting systolic blood pressure between 120 and 139 mmHg and/or a diastolic blood pressure of 80–89 mmHg) and/or prediabetes (fasting glucose level from 100 to 125 mg/dl or 5.6 to 7.0 mmol/l) and not taking any hypertension and/or glucose-influencing drugs were included in the study.

Anthropometric measurements were recorded by trained research nurses using standardized protocols [24]. A questionnaire on mental disorders, substance use, and sociodemographic information was interview-administered by trained researchers. They included the following: the *Patient Health Questionnare-9* (*PHQ-9*) was used to screen participants suffering from depression [25]. It has demonstrated high sensitivity (0.84) and specificity (0.77) in a validation study in Thailand, using a cutoff score of 9 or more as indicative for major depression [26]. Cronbach's alpha is 0.81.

Tobacco smoking was assessed with two questions: (1) "Have you ever smoked tobacco?" and (2) "Do you currently smoke tobacco?" (Response options were 1 = yes, daily; 2 = yes, less than daily; and 3 = no, not at all) [27]. Responses were grouped into never smoked, former smoker, and current smoker (daily or less than daily).

Problem drinking was assessed with the Alcohol Use Disorders Identification Test (AUDIT)-C [28]. The AUDIT has been validated in a study in Thailand [29]. Cronbach's alpha was 0.88 in this study.

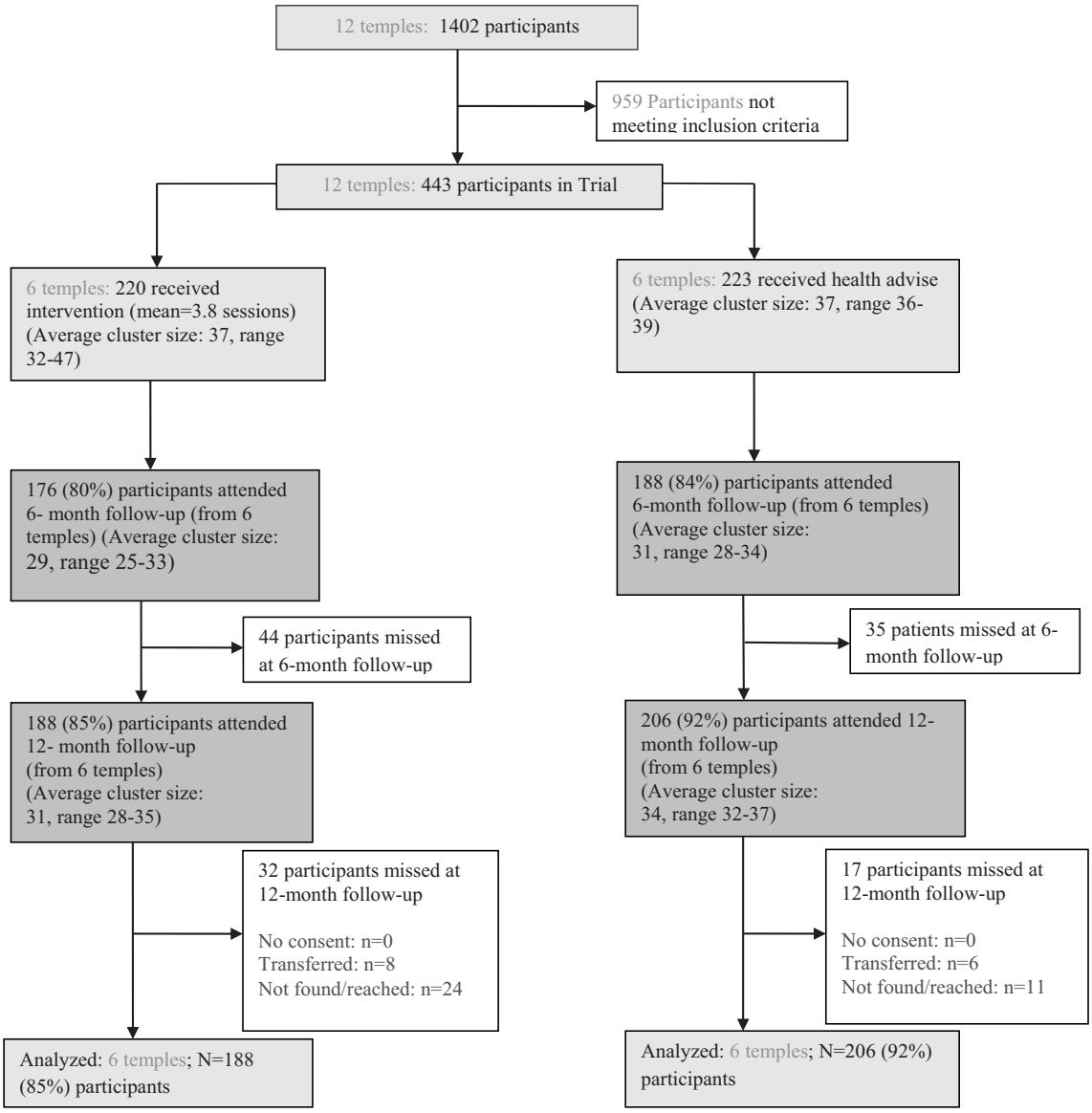

Fig. 1 Flow-chart of temples and participants in the trial

Measurements occurred at baseline and 12 months for biomarkers and at baseline, 6 months, and 12 months for the other measures.

The primary outcomes included the reduction in systolic blood pressure and blood glucose levels after the intervention.

Interventions

Lifestyle intervention

The intervention consisted of six group lifestyle (diet and physical activity) counseling sessions (on an average 60–90 min) over a period of 6 months which was conducted by trained study nurses in the temple premises and/or the adjacent health facility (Table 1). Specific diet and physical activity intervention program goals [30, 31] included the following: total energy from fat (< 30%) and from saturated fats (< 10%), ≥ 15 g per 1000 kcal fiber, ≥ 30 min per day of moderate-intensity physical activity, reduction of current body weight (≥ 5%), sodium intake ≤ 2400 mg/day, and alcohol intake (≤ 2 drinks/day for men and ≤ 1 drink/day for women) [30, 31]. The Health Action Process Approach (HAPA) model [32] and self-regulation theory [33] were utilized to determine individual goals of participants and help them in motivating the progression from intended goals to specific behavior change in order to achieve the diet and physical activity program goals [34–36].

Lifestyle education leaflet All the temple members randomized and allocated to the control group received standard care

Table 1 Lifestyle intervention contents

Session	Major intervention content
1	- Lifestyle influence health, diabetes, hypertension, risk factors and development, effects, prevention
	- Goals, planning, homework and other exercises, including sensible alcohol use
	- Homework assignments: monitoring own behavior with food diary (including identifying sodium content of foods) and physical activity schedule
2	- Returning of food diaries
	- Comparison of own habits with the diet and physical activity goals sufficient for prevention, role modeling, analysis, and re-attribution
	- Homework assignments: preparation for goal setting, monitoring physical activity and eating habits
3	- Feedback from the physical activity schedule
	- Goal planning
	- Homework assignments: feedback and re-enforcement, monitoring physical activity and eating habits
4	- Feedback based on findings from food diaries
	- Education on how to eat healthy
	- Goal planning, goal setting
	- Homework assignments: positive feedback in getting social support, monitoring physical activity and eating habits
5	- Evaluating and refining the goals
	- Routines, changed; intermediate goals
	- Exercise: how to overcome barriers, how to use resources in maintaining the behavior changes
	- Homework assignments: monitoring physical activity and eating habits
6	- Evaluating the goals
	- Routines, changed; analysis and re-attribution of success and failure
	- Future goals and evaluation

(as per routine health care provision) and a pamphlet on healthy diet and exercise benefits.

Quality assurance of assessment and intervention procedures

Quality assurance procedures were employed to ensure that assessment and intervention procedures were standardized across the temple study sites and across all participants. In order to accomplish this, standardized procedures and training materials were developed, and the research nurses were systematically trained in their application. Moreover, the activities of the interviews, physical assessment procedures and intervention program implementation were routinely supervised and monitored by senior researchers. Intervention sessions were audio-recorded, observed and reviewed for fidelity by senior research staff. Feedback was provided to the field work staff and protocol deviations or other problems addressed and corrected in a timely manner.

Sample size calculation

As reviewed from previous intervention studies [37], systolic blood pressure reduction between 5.3 and 5.9 mmHg could provide 90% power [34], while a blood glucose level reduction of 0.1 to 0.3 mmol is considered large enough to prevent the development of type 2 diabetes [30]. Utilizing the Trial Protocol Tool for RCTS software (2004), the sample size was calculated (at 90% power, an average cluster size of 20, a median intracluster correlation of 0.005) [38] and assuming an 80% success rate in reaching suitable recordings, the minimum unadjusted sample size was 96 and the clusters per group were 6. Based on similar previous research studies, a dropout rate of 25% was expected and we increased the sample size to 160 subjects in six clusters per treatment arm.

Data analysis

The initial comparability of control and intervention groups was assessed using nonparametric Mann-Whitney *U* tests for continuous and Pearson chi-square tests for categorical variables. The results were analyzed by the use of the intention-to-treat principle for participants who had completed the required assessments both at the baseline and at a 12-month follow-up. Of the 443 participants, 394 (89%) stayed in the intervention for the 1-year intervention and follow-up period and were included in the analysis. The intervention effects on continuous 12-month outcomes [i.e., fasting plasma glucose, systolic and diastolic blood pressure, triglyceride, low-density

Table 2 Baseline demographic and lifestyle characteristics

Variable	Intervention group ($n = 220$)	Control group ($n = 223$)	p value
Age in years; median (IQR)	51.0 (11)	52.0 (11)	
Gender; *n* (%)			
Male	56 (25.5)	58 (26.0)	0.894
Female	164 (74.5)	165 (74.0)	
Education; *n* (%)			
Primary or less	129 (58.6)	125 (57.1)	0.346
Secondary	54 (24.5)	46 (21.0)	
Post-secondary	37 (16.8)	48 (21.9)	
Marital status; *n* (%)			
Married/cohabiting	152 (69.1)	162 (74.0)	0.257
Unmarried/widowed/divorced	68 (30.9)	57 (26.0)	
Smoking status; *n* (%)			
Never	197 (89.0)	202 (90.9)	0.514
Ever	24 (10.9)	20 (9.0)	
Current	16 (7.3)	12 (5.4)	
Problem drinking; *n* (%)			
No	198 (89.4)	198 (89.0)	0.890
Yes	23 (10.6)	24 (11.0)	
Depression (PHQ-9)			
No	183 (85.3)	195 (89.0)	0.237
Yes	32 (14.7)	24 (11.0)	

lipoprotein (LDL), high-density lipoprotein (HDL), BMI and waist circumference] and diabetes and hypertension incidence outcomes were analyzed using linear, gamma, and logistic mixed-effect models adjusted for clustering (i.e., a random effect of temple community) and baseline covariates, namely age, gender, education, smoking, alcohol use and depression. Fixed effects in these analyses consisted of time, condition, a time-by-condition interaction (i.e., between-group changes) and baseline covariates. The data were analyzed using IBM-SPSS for Windows, version 24 (Chicago, IL, USA).

Table 3 Comparison of physical outcome measures between intervention and control groups

Variable	Intervention group ($n = 220$)	Control group ($n = 223$)	p value
Fasting plasma glucose (mg/dl), mean (SD)	97.5 (13.0)	96.0 (12.0)	0.235
Systolic blood pressure (mmHg), mean (SD)	126.7 (11.6)	125.7 (11.0)	0.849
Diastolic blood pressure (mmHg), mean (SD)	80.0 (8.3)	79.7 (8.7)	0.163
Prediabetes	89 (40.5)	82 (36.8)	0.426
Prehypertension	204 (92.7)	209 (93.7)	0.677
Prediabetes only	16 (7.3)	14 (6.4)	0.677
Prehypertension only	131 (59.5)	141 (63.2)	0.426
Prediabetes and prehypertension	73 (33.2)	68 (30.5)	0.544
Total cholesterol (mg/dl), mean (SD)	203 (42)	209 (57)	0.104
Triglyceride (mg/dl), mean (SD)	110.5 (50.5)	121 (64.1)	0.003
HDL cholesterol (mg/dl), median (IQR)	55.4 (17.3)	48.4 (6.8)	< 0.001
LDL cholesterol (mg/dl), median (OQR)	124 (44)	129.0 (46)	0.009
Body mass index (BMI, kg/m^2), mean (SD)	25.0 (3.9)	25.3 (4.1)	0.501
Waist circumference (male, cm), mean (SD)	87.9 (10.3)	87.6 (10.2)	0.845
Waist circumference (female, cm), mean (SD)	84.2 (9.8)	86.4 (8.7)	0.029

Results

A total of 1402 participants were screened for prediabetes and/or prehypertension, and 443 were cluster-randomized into intervention and control groups. The overall attrition rate at 12-month follow-up was 11%. Figure 1 shows the study flow of the trial.

Attrition analyses found that dropout was significantly related to intervention condition ($p = 0.020$) and depression symptom prevalence ($p = 0.010$).

The mean age of all participants was 51.4 years (SD = 7.3), with a range of 35–65 years. The majority (74.3%) of the participants were female, 71.5% were married or cohabiting, and 57.9% had primary education. In all, 6.3% were current

Table 4 Study outcomes of the intervention and control groups at time 1 (baseline), time 2 (6 months), and time 3 (12 months)

Variable	Intervention group	Control group	Group effects		Time effects		Group × time effects[3]	
			COE (SE)	*p* value	COE (SE)	*p* value	COE (SE)	*p* value
Fasting plasma glucose (mg/dl), mean (SD)[1]								
T1	97.5 (9.9)	96.3 (10.3)	− 1.03 (2.62)	0.701	2.36 (1.10)	0.032	3.66 (1.53)	0.017
T3	100.1 (14.0)	102.2 (11.0)						
Systolic blood pressure (mmHg), mean (SD)[1]								
T1	126.7 (7.1)	126.6 (7.1)						
T2	126.9 (9.8)	126.4 (7.8)	0.032 (1.43)	0.989	− 3.39 (0.91)	< 0.001	2.13 (1.26)	0.090
T3	123.4 (9.6)	124.7 (9.2)						
Diastolic blood pressure (mmHg), mean (SD)[1]								
T1	79.8 (6.5)	79.0 (6.5)						
T2	78.3 (6.6)	79.1 (6.5)	− 1.01 (1.06)	0.359	− 3.57 (0.72)	< 0.001	3.07 (0.99)	0.002
T3	76.5 (7.7)	78.3 (6.6)						
Total cholesterol (mg/dl), mean (SD)[1]								
T1	206.1 (35.4)	211.8 (37.6)	4.99 (7.27)	0.505	9.01 (3.72)	0.016	− 21.48 (5.18)	< 0.001
T3	212.1 (33.9)	199.4 (40.2)						
Triglyceride (mg/dl), median (IQR)[2]								
T1	110.5 (50.5)	121 (64.1)	0.14 (0.07)	0.077	0.06 (0.05)	0.244	− 0.14 (0.07)	0.036
T3	118.0 (58.0)	115.0 (58.5)						
HDL cholesterol (mg/dl), median (IQR)[2]								
T1	55.4 (17.3)	48.4 (6.8)	− 0.14 (0.05)	0.011	0.02 (0.02)	0.194	0.05 (0.03)	0.060
T3	59.3 (12.4)	53.6 (5.2)						
LDL cholesterol (mg/dl), mean (SD)[1]								
T1	124.7 (36.8)	133.3 (32.6)	8.24 (7.16)	0.273	5.61 (3.44)	0.103	− 19.12 (4.77)	< 0.001
T3	127.3 (30.2)	119.4 (35.8)						
Body mass index (BMI, kg/m^2), mean (SD)[1]								
T1	25.0 (3.9)	25.3 (4.1)						
T2	25.2 (3.8)	25.8 (3.8)	0.25 (0.47)	0.604	0.49 (0.48)	0.313	− 0.08 (0.07)	0.911
T3	25.8 (5.4)	25.6 (4.0)						
Waist circumference (male, cm), mean (SD)[1]								
T1	87.6 (10.2)	87.6 (10.2)						
T2	87.3 (10.6)	89.0 (10.3)	− 0.46 (2.05)	0.825	− 0.44 (2.45)	0.859	0.854 (3.33)	0.798
T3	87.4 (9.5)	87.9 (11.7)						
Waist circumference (female, cm), mean (SD)[1]								
T1	84.2 (9.8)	86.4 (8.7)	2.25 (1.26)	0.088	− 0.52 (1.13)	0.643	0.95 (1.55)	0.541
T2	82.4 (10.2)	86.4 (9.0)						
T3	83.7 (10.0)	86.9 (8.6)						

COE coefficient, *SE* standard error

[1] Linear mixed regression model

[2] Gamma mixed regression model

[3] Adjusted for cluster effects and baseline characteristics (demographic and lifestyle variables)

smokers, 10.8% engaged in problem drinking, and 12.8% had depression symptoms. No significant differences were found in these demographic and lifestyle variables between intervention and control groups (see Table 2).

Table 3 shows the physical outcome measures of the participants at baseline. Except for triglyceride, HDL, and LDL cholesterol, no significant group differences were found (see Table 3).

Table 4 presents the physical study outcomes in each data assessed and results of multilevel mixed modeling. At 12-month follow-up, mixed modeling found significant interaction effects on fasting plasma glucose ($p = 0.017$) and diastolic blood pressure ($p = 0.002$), while negative effects were found for total cholesterol ($p < 0.001$), triglyceride ($p = 0.036$), and LDL cholesterol ($p < 0.001$). Improvements were found for systolic blood pressure, but no significant interaction effect was detected. Further, there were no significant changes in terms of BMI and waist circumference (Table 4). Finally, Table 5 shows the development of diabetes and hypertension at 1 year. There was a significant time effect in developing diabetes and the incidence hypertension was higher in the control than in the intervention group, but this was only marginally significant (Table 4). On the other hand, among the prehypertensive groups at baseline, the intervention group showed a higher reduction of blood pressure to normotensive status compared to the control group, but it was not reaching significance ($p = 0.149$). Current smoking reduced by 81.3% from 7.3% at time 1 to 1.4% at time 3 in the intervention group and by 33.3% from 5.4% at time 1 to 3.6% at time 3 in the control group.

Discussion

To our knowledge, this is the first Buddhist temple cluster-randomized controlled trial evaluating the effects of a lifestyle intervention program to control prehypertension and/or prediabetes in temple members. Although the lifestyle intervention demonstrated positive effects on fasting glucose and blood pressure levels, no effects were found on BMI levels and negative effects on total cholesterol, triglyceride and LDL cholesterol. Increases in glucose levels in both intervention and control groups after a lifestyle intervention were also found in previous studies [40]. It should be noted that in this study, the average BMI levels (25.1 kg/m^2 at baseline) were low compared with a study in an Asian population in Hong Kong [39] but similar to a study in Vietnam [40]. Results from the current study cannot confirm, as in several previous studies [4–9, 12–15], that significant changes can be obtained in a 1-year study on temple-based intervention in blood pressure, glucose, lipids, BMI and waist circumference. One possible explanation for the limited intervention effect was the suboptimal session attendance of participants, on an average 3.8 sessions, with a target of six sessions, and more sessions are needed, as found in other studies [15, 41], to affect significant changes. In addition, participants in the intervention group with depression symptoms were more likely to drop out of the study compared to their control group counterparts. It is possible that being confronted with enormous lifestyle change efforts need in the intervention group participants made them develop more depressive symptoms, leading to the dropout of the study. Further, intervention fidelity evaluation found a lack of motivation and compliance of some of participants to conduct their food diaries, which may have led to an increase in the levels of total cholesterol and LDL. Similarly, in a lifestyle advice intervention for prehypertension with other cardiovascular risk factors by community pharmacists in Southern Thailand, it was found that a number of participants "remained in their previous stage of readiness to adopt life-style modification" [42].

Table 5 Changes to type 2 diabetes and/or hypertension

Variable	Intervention group	Control group	Group effects		Time effects		Group × time effects[1]	
	n (%)	*n* (%)	COE (SE)	*p* value	COE (SE)	*p* value	COE (SE)	*p* value
Type 2 diabetes[2]								
T1	0	0	− 0.00 (0.001)	1.000	− 3.99 (0.26)	< 0.001	0.58 (0.38)	0.131
T3	6 (7.1)	5 (4.1)						
Hypertension[2]								
T1	0	0	− 0.00 (0.10)	1.000	− 0.23 (0.15)	0.112	− 0.34 (0.19)	0.070
T3	2 (1.1)	7 (3.4)						

COE coefficient, *SE* standard error

[1] Adjusted for cluster effects and baseline characteristics (demographic and lifestyle variables)

[2] Logistic mixed regression model

Study limitations

The study found mixed results, and it is unclear which components of the lifestyle intervention program led to this finding. Several indicators such as substance use and depression symptoms were assessed by self-reporting, which has its limitations. The study only assessed short-term effects (6 months post intervention) and longer term of assessments (18 or 24 months) would be needed.

Conclusion

The lifestyle intervention was only partially effective. Session attendance was sub-optimal, and clients should be motivated to attend more sessions to improve effectiveness. More research is needed to investigate lifestyle interventions in this community-based setting.

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Author contribution Supa Pengpid and Karl Peltzer contributed to the design of the experiment, data analysis, and writing of the manuscript.

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Compliance with ethical standards

Ethical considerations The study was performed according to the principles of the Declaration of Helsinki. It was reviewed and approved by the Committee for Research Ethics (Social Sciences), Mahidol University (MU-SSIRB: 2016/053-B1). Written informed consent was obtained from all participants.

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ORIGINAL ARTICLE

Prevalence of gestational diabetes and contributing factors among pregnant Jordanian women attending Jordan University Hospital

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Abstract
To determine the prevalence of gestational diabetes mellitus (GDM) and associated risk factors among pregnant Jordanian women attending Jordan University hospital. A cross-sectional study conducted on 644 singleton pregnancies screened for GDM with 75-g, 2-h oral glucose tolerance test at 24–28 weeks of gestation between January 2015 and January 2016 in Jordan. The diagnosis of GDM was reached through WHO criteria. Maternal characteristics and demographic information, and obstetrics' histories, were collected. The prevalence of GDM with it's risk factors was then determined. The prevalence of GDM was 13.5%. A statistically significant increase in prevalence was observed among pregnant women with increase in the following variables: maternal age, gravidity, parity, maternal pre-pregnancy BMI, maternal BMI at the time of the tests and with the presence of acanthosis nigricans, past history of gestational diabetes, and family history of diabetes mellitus type II with a p `0.001, p 0.005, p 0.013, p 0.000, p 0.000, p 0.001, p 0.016, and p 0.001 respectively. The impact of GDM on maternal and infant health is of great clinical and public health importance and imposes a significant economic burden. The prevalence of GDM seems to be quite high in Jordan. Given that women with diabetes are unaware of their condition, all pregnant women should be screened for oral glucose tolerance test and encouraged to do it at the proper time.

Keywords GDM · Pregnancy

Introduction

Diabetes is one of the most common metabolic disorders that may cause pathological pregnancy [1]. Gestational diabetes mellitus (GDM) is defined as a carbohydrate intolerance resulting in hyperglycemia or any degree of glucose intolerance with onset or first recognition during pregnancy from 24 weeks' gestation onwards and which resolves following the birth of the baby [2]. GDM is associated with adverse perinatal outcome, and increased long-term risks of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disorders for both mother and offspring [3, 4]. About 50% of the women who suffered from gestational diabetes will suffer from type 2 diabetes during 5 years after pregnancy [5]. And those women with GDM have three to four times higher risk of metabolic syndrome later in life [6].

Pregnancies complicated by maternal diabetes in addition are associated with increased rate of Cesarean section delivery, macrosomia, admission to neonatal intensive care unit (NICU), and perinatal mortality [7]. Children born from pregnancies complicated with GDM also seem to have an increased risk of obesity, altered carbohydrate metabolism, and abdominal adiposity during childhood and adolescence [8]. Hence, the impact of GDM on maternal and infant health is of great clinical and public health importance [9] and imposes a significant economic burden [10]. Treating diabetes recognized during pregnancy results in lowering maternal and fetal complications [1]. GDM affects approximately 6–7% of pregnant women [11]. However, the prevalence of GDM has been

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reported to be increasing worldwide within the last 20 years [12] especially in developing countries [9].

GDM is most often asymptomatic and must be diagnosed by testing with oral glucose tolerance test (OGTT) [12]. Protocols for screening/diagnosing and management for GDM are controversial with several guidelines available [11]. Practices range from universal testing of all pregnant women to testing on a case-by-case basis according to clinician or patient decisions [12, 13]. However the publication of WHO criteria for the diagnosis of GDM guided us world wide [14].

The diagnosed prevalence of diabetes among female Jordanian adults aged from 18 to 34 and 35 to 49 years increased from 0.7–6.2% respectively in 2002 [15] to 5.3–10.4% in 2007 [16].

In our hospital, we adopted the recommendation of WHO, and since 2014, is in use. Our aim in this study is to determine GDM prevalence with its relation to maternal characteristics and demographic variants among women who are registered to deliver at Jordan University Hospital in order to direct health resource to improve the outcomes for these high-risk pregnancies.

Method

A cross-sectional study conducted on singleton pregnancies screened for GDM with 75-g, 2-h oral glucose tolerance test at 24–28 weeks of gestational age in a tertiary teaching hospital (Jordan University Hospital), between January 2015 and January 2016. Gestational age was determined by last menstrual period (LMP) and according to early ultrasound examination. The diagnosis of GDM was reached when one of the following plasma glucose values was met or exceeded the following values : fasting blood sugar, 92 g/dl; 1 hour blood sugar level, 180 g/dl; and the hour level, 153 g/dl [17].

Women with a singleton gestation and had no congenital anomalies in the current pregnancy were eligible for the study, while women with multiple gestations and those who were registered to deliver after 28 weeks of gestational age, and those who refused to have the test, were excluded from the study. In addition and those who have fasting level exceeds 126 g/dl. The following risk factors and obstetric history were determined: pre-pregnancy weight and height maternal weight measured when the OGTT was tested, the presence of medical diseases, previous pregnancy complicated with GDM, the presence of acanthosis nigricans and polycystic ovarian syndrome, birth weight ≥ 4000 g, stillbirth with no clear obstetric cause or major cardiovascular/CNS malformation, maternal educational level, and working status with the average family income. In addition, the presence for family history of diabetes type 2 and hypertension were all studied.

Maternal body mass index (BMI) was calculated on the subject's self-reported weight and height before pregnancy. The participants were weighed at the time of OGTT. The following BMI definitions were used in this study: underweight (< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥ 30 kg/m^2) [18].

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA). Frequency and percentage were calculated for the categorical data, and Pearson's chi-square test or Fisher's exact test were applied to determine potential factors associated with GDM and to determine whether there are any statistical differences between groups. The level of significance was set at a p 0.05 to test the hypothesis of no association. Fisher's exact test replaces chi-square test when the minimum expected count is less than five.

Results

A total of 644 pregnant women who are registered to deliver at Jordan University Hospital were tested for OGTT at 24–28 weeks of gestation. Women registered to deliver after 24–28 weeks of gestational age and refused to have the test were excluded from the study and those who have fasting level that exceeds 126 g/dl. Descriptive and medical information was collected by interview women and record abstraction.

In our study, 87 pregnant women were found to have gestational diabetes, with overall prevalence 13.5%. Of the 87 women with gestational diabetes, 56 (64.4%) had fasting blood glucose ≥ 92 g/dl, 17 (19.5%) had two abnormal values, and 14 (16.1%) had three abnormal values. The demographic characteristics of women with gestational diabetes and non-gestational diabetes are shown in Table 1.

From all the participants, 146 (23%) were primiparas. The mean age of l was 30 ± 5.6 years. The gestational age ranges from 24 to 28 weeks gestation with mean of 26.1 ± 1.4 weeks.

We found that the risk for GDM increased significantly with an increase of maternal age, increase of the gravidity, and the increase of the parity with p 0.001, p 0.005, and p 0.013 respectively. About the gravidity, we found that out of a total number of 127 nulliparous, 20 (16%) of them had GDM, and from a total of 29 grand multipara, 10 (34%) of them had GDM, p (0.01). Increased pre-pregnancy BMI and BMI at the time of the test were both significant, where we found GDM in 25 (28.7%) and 49 (56.3%) of morbid obese women with p 0.000 and p 0.01 respectively. History of previous pregnancy with GDM was also statistically significant, p 0.016. The

Table 1 Shows the demographic characteristics of women with gestational diabetes and non-gestational diabetes

Characteristics	Non- Diabetic Number % N=557 86.5%		GDM Number % N=87, 13.5%		Total women Number % 644, 100%		*p* - value
Age years (Mean ±SD)	29.9 ± 5.5		32.3 ± 5.8		30.2 ± 5.6		**0.001**
Age group (years)							
15-19	4	0.7%	0	0.0%	4	0.6%	**0.000**
20-29	273	49.0%	24	27.6%	297	46.1%	
30-39	252	45.2%	50	57.5%	302	46.9%	
40-49	28	5.0%	13	14.9%	41	6.4%	
Gestational age (Mean ±SD)	26.1±1.4		26.0 ± 1.3		26.1± 1.4		0.573
Gravidity (Mean ±SD)	3.1±1.9		3.9±2.6		3.2± 2.0		**0.005**
Parity (Mean ±SD)	1.6±1.5		2.0±1.7		1.7± 1.5		**0.013**
Nullipara	154	27.6%	20	23.0%	174	27.0%	**0.007**
Para 1-2	261	46.9%	36	41.4%	297	46.1%	
Para 3-4	123	22.1%	21	24.1%	144	22.4%	
Grand multipara	19	3.4%	10	11.5%	29	4.5%	
Neonatal weight of Previous deliveries							
<4000	392	49.9%	61	91%	453	94.4%	0.324
4001-4249	14	3.4%	4	6.0%	18	3.8%	
4250-4499	3	0.7%	0	0.0. %	3	0.6%	
4500-4999	4	1.0%	2	3.0%	6	1.2%	
Total	413	100%	67	100%	480	100%	
Abortus (Mean ±SD)	0.5± 0.9		0.8± 1.3		0.5± 1.0		0.117
No abortion	386	69.3%	48	55.2%	434	67.4%	**0.009**
1-3 abortion	166	29.8%	36	41.4%	202	31.4%	
> 3 abortions	5	0.9%	3	3.4%	8	1.2%	
Stillbirths							
Yes	5	0.9%	0	0.0%	5	0.8%	0.483
No	552	99.1%	87	100%	639	99.2%	
BMI (kg/m^2) before pregnancy							
< 18.4	25	4.5%	0.0	0.0%	25	3.9%	**0.000**
18.5-24.9	291	52.2%	30	34.5%	321	49.8%	
25 -29.9	168	30.2%	32	36.8%	200	31.1%	
>30	73	13.1%	25	28.7%	98	15.2%	
BMI (kg/m^2) of the mother at the time of the test							
18.5-24.9	142	25.5%	7	8.0 %	149	23.1%	**0.000**
25 -29.9	226	40.6%	31	35.6 %	257	39.9%	
>30	189	33.9%	49	56.3 %	238	37.0%	
Personal History Acanthosis Nigricans							
Yes	56	10.1%	20	23.0%	76	11.8%	**0.001**
No	501	89.9%	67	77.0%	568	88.2%	
Personal history of GDM							
Yes	16	2.9%	7	8%	23	3.6%	**0.016**
No	541	97.1%	80	92%	621	96.4%	
Personal History PCOs							
Yes	53	9.5%	12	13.8%	65	10.1%	0.218
No	504	90.5%	75	86.2%	579	89.9%	
Personal history of GHTN							
Yes	17	3.4%	3	3.1%	20	3.1%	0.843
No	540	96.6%	84	96.9%	624	96.9%	
Personal History of Thyroid							
Yes	7	1.3%	3	3.1%	10	1.6%	0.124
No	550	98.7%	84	96.9%	634	98.4%	

Table 1 (continued)

Characteristics	Non- Diabetic Number % N=557 86.5%		GDM Number % N=87, 13.5%		Total women Number % 644, 100%		*p* - value
Family History of DM Type 2							
Yes	288	51.7%	62	71.3%	350	54.3%	**0.001**
No	269	48.3%	25	28.7%	294	45.7%	
Family History of HTN							
Yes	278	49.9%	46	52.9%	324	50.3%	0.607
No	279	50.1%	41	47.1%	320	49.7%	
Working Status							
House wife	303	54.4%	53	60.9%	356	55.3%	0.255
Employed	253	45.6%	34	39.1%	288	44.7%	
Family income							
<500JD/month	260	46.7%	37	42.5%	297	46.1%	0.384
500-1000JD/month	280	50.3%	45	51.7%	325	50.5%	
> 1000JD/ month	17	3.1%	5	5.7%	22	3.4%	
Educational Level							
Secondary school or less	144	25.9%	25	28.7%	169	26.2%	0.34
Bachelor of Diploma	358	64.3%	54	62.1%	412	64.0%	
Masters	47	8.4%	5	5.8%	52	8.1%	
PhD	8	1.4%	3	3.45%	11	1.7%	

presence of first-degree family history of diabetes mellitus type 2 and the presence of acanthosis nigricans in the current pregnancy were also statistically significant with *p* 0.001 for both.

In our study, there was no statistically significant GDM with personal history of polycystic ovary syndrome, history of thyroid disease, personal history of hypertensions, history of stillbirths' birth weight of previous deliveries, family income, level of education, family history of high blood pressure, or working status of the mother with *p* 0.22, *p* 0.12, *p* 0.84, *p* 0.48, *p* 0.32, *p* 0.38, *p* 0.43, *p* 0.61, and *p* 0.26 respectively.

Discussion

It is well known that the prevalence of GDM varies widely worldwide [19]. The rates can be as high as 25% depending on the population and diagnostic criteria used [2]. Prevalence seems to depend on factors such as ethnic origin and varies from region to region [12, 20].

GDM prevalence worldwide differs from country to country. In European countries, GDM prevalence is most often reported as 2–6% of all pregnancies [12]. Lower prevalence of GDM was observed in Northern or Atlantic seaboard parts of Europe, with estimates mostly less than 4% in that region [12]; however, in another study, the prevalence was 7.4% in Nordic Caucasian women [21]. In the South Europe region, the prevalence estimate is around 6%; in some other parts of Europe such as Ireland it is 10%; in Finland, it reaches 10–11% [12, 22]. In the UK, one report by the National Institute for Health and Care Excellence (NICE) suggests that the prevalence of GDM in England and Wales is approximately 3.5% of all pregnancies [23]. In a study from United States (US), GDM prevalence in an ethnically diverse California population varied from approximately 5% in non-Hispanic White women to 8.5% in Asian women, with Black and Hispanic women at intermediate risk [24]. In another study from US, a higher prevalence of 9.2% was observed [25].

In Australia, the pooled GDM prevalence varies from 4.78 to 6.71% in Aboriginal and Torres Strait Islander women [26]. However, in a very recent publication, it was as high as 29.6% and valence was observed in all ethnic groups [27].

The prevalence of GDM in studies published from Asian developing countries showed that in Bangladesh, it is 9.6%; in Malaysia, 11.4% [9, 28, 29]; and in India, reaches 23.3% [30]. Prevalence figures ranged from 0% Tanzania to 13.9% Nigeria from Africa [31]. In studies from the Mediterranean region, the prevalence in Turkish people was 11.1% [32], and in Saudi Arabia 24.2% had GDM [33].

There was no published research performed concerning the prevalence of GDM among pregnant women in Jordan. There is only one unpublished Master Dissertation conducted in 2009 studying 300 pregnant women; their prevalence was 17.3% [34]. We studied 644 pregnant women and found prevalence of 13.5%; therefore, we are a high-risk population.

Maternal age is strongly associated with GDM as the incidence of GDM increased markedly with increasing maternal age [35], which coincides with our results where no women

under the age of 19 had GDM in comparison to 13 (14.9%) between the age of 40–49 years.

In previous studies [20, 21, 36], multigravida and multiparous women are more likely to develop GDM compared to primigravida and nulliparous which is similar to our results where p was 0.005 for multigravida and p 0.013 for multiparous women.

We also noticed that there is a statistically significant association between onset of GDM and previous GDM with p 0.016; this data is consistent with the observations published in some other studies [36–38].

Higher pre-pregnancy BMI as well excessive weight gain in early pregnancy was an important risk factor for the development of GDM in some other previous studies [35, 37, 39]. In our study, we noted that pre-pregnancy BMI had a high statistically significant association with GDM as there was no pregnant women with GDM when BMI was less than 18.4 kg/m^2 which increased to 25 (28.7%) in pregnant women when the BMI reaches more than 30 kg/m^2, p 0.000. On the other hand, 7 (8.0%) women had GDM when BMI was between 18.5–24.9 kg/m^2 at the time of the test compared to 49 (56.3%) when the BMI was above 30 kg/m^2 with p 0.000.

Acanthosis nigricans was noticed in one study to be associated with GDM [40], and we notice this also in our study, with p 0.001.

Family history of diabetes type 2, as risk factors, was more frequent in the GDM group compared to the group of pregnancies with normal glucose tolerance [38, 39, 41]. This was proved in our study, where there was a positive relationship between family history (first-degree relatives) of DM and family history of GDM with p 0.001.

Most of the previous studies that reported significant associations between GDM and some other maternal characteristics as history of polycystic ovary syndrome, birth weight of the previous child ≥ 4 kg, previous stillbirth [38, 41, 42], and lower socioeconomic background including low level of education and working status [43, 44] had higher number of participants and thus increased statistical power; however, those were not significant in our study.

Finally, we conclude that the prevalence of GDM is increased with increased maternal age, gravidity, and parity. The increase of BMI before pregnancy together with increase weight gain during pregnancy, previous history of GDM, and family history of type II DM are important risk factors that need more attention during antenatal care.

Conclusion

The impact of GDM on maternal and infant health is of great clinical and public health importance and imposes a significant economic burden. The prevalence of GDM seems quite high in Jordan, which is a trend observed in the majority of countries worldwide, as mothers are getting older with the rising incidence of obesity and other risk factors. Given that women with diabetes are unaware of their condition, all pregnant women should be offered OGTT and encouraged to do it at the proper time.

Compliance with ethical standards

The study was approved by The Ethics Committee for Medical Research at the Jordan University Hospital and the University of Jordan. The participants were pregnant women with gestational age which ranged from 24 to 28 weeks who gave a consent to participate during their usual antenatal clinic visits.

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

A comparison of serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in the first, second, and third trimester in obese and non-obese pregnant women with and without gestational diabetes mellitus

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Abstract

Previous study demonstrated that obesity and associated gestational diabetes mellitus (GDM) are increasing. However, the combination of obesity and GDM contribute to the pregnancy complication have yet to be elucidated. The aim of this study was to compare serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in the first, second, and third trimester in obese and non-obese pregnant women with and without gestational diabetes mellitus. We performed a prospective, case-control study in 30 obese women diagnosed with GDM, 30 non-obese pregnant women with GDM, and 30 non-obese and without GDM, age-matched pregnant women who were enrolled in the first, second, and third trimester and followed-up until delivery. Blood samples were collected once from each participant in the first trimester of pregnancy, during the fetal viability scan, once in the second trimester of gestation during screening for gestational diabetes mellitus, and once in the third trimester of gestation. Serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels were measured. In the third trimester, fructosamine level (1.8 ± 0.08) was slightly higher in obese women who were diagnosed with GDM than in the second and first trimester (1.70 ± 0.12, 1.70 ± 0.09), respectively. But, in the third trimester, phosphorus levels were slightly lower in GDM women who were GDM women than in the first trimester. In the first, second, and third trimester, in these subjects, the mean levels of vitamin D and calcium were slightly different. Our data tend to support the concept that fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus level assays can provide a good index of health control especially in obese. This study has increased our knowledge of biochemical changes in GDM among obese pregnant women with GDM.

Keywords Fructosamine · Gestational diabetes mellitus · 25-Hydroxyvitamin D · Calcium and phosphorus · Trimester

Abbreviations

GDM Gestational diabetes mellitus
DM Diabetes mellitus
BMI Body mass index
Glu Glucose

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Introduction

Gestational diabetes is defined as carbohydrate intolerance during pregnancy [1]. Also, researchers have reported hormone intolerance occurs such as alteration of leptin contents in pregnant women with type 1 diabetes mellitus [2]. Knowledge of the changes of biochemical marker such as HbA1 is important in pregnant diabetics [3]. Evidences have revealed that women may respond different from men to vitamin D supplementation regarding cardio-metabolic biomarkers [4]. Some biochemical markers such as irisin may be a useful biochemical marker in early pregnancy to predict

the development of gestational diabetes mellitus [5]. Limited data are available assessing the effects of vitamin D administration on markers of metabolism intolerance in gestational diabetes mellitus [6]. It is well known that nutrition plays a crucial role for both the mother and the fetus [7]. In this regard, omega-3 fatty acid supplementation in gestational diabetes mellitus (GDM) women had beneficial effects [8]. Researchers suggested that most of the analytes including plasma alkaline phosphatase, calcium lactate dehydrogenase, and phosphate change during normal pregnancy [9].Serum 25-hydroxyvitamin D (25(OH)D) has been shown to be inversely associated with gestational diabetes risk [10]. Also, researchers reported that dietary interventions can improve pregnancy outcomes in women with gestational diabetes mellitus [11].

Fructosamine is a marker of glucose control reflecting the average glycemic level over the preceding 2–3 weeks [12]. Researchers have investigated the role of maternal serum fructosamine in the management of gestational diabetes [13]. Researchers reported that GDM in obese patients is characterized by lower weight gain and higher baseline glucose [14]. Researchers investigated the validity of using fasting plasma glucose levels in conjunction with HbA1c or fructosamine for the screening of diabetes in high-risk individuals [15]. Emerging evidence suggests that during pregnancy, the mother is potentially subjected to gluco-toxicity as well as oxidative stress to help the fetus absorb more nutrients [16]. Previous studies established that micronutrient intake during pregnancy affects fetal organ development and mother's health [17]. Also, pharmacological interventions in obese pregnant women are important in preventing GDM [18].

The present study was designed firstly to measure the level of serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese pregnant women diagnosed with GDM to compare non-obese pregnant women with GDM and non-obese pregnant women without GDM and secondly to determine the pattern changes of the mentioned biochemical markers including serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese pregnant women with GDM in different time points. Results of this study may be useful to improve the follow-up and to evaluate the efficacy of nutrient and pharmacological treatment for obese pregnant women diagnosed with GDM.

Materials and methods

Methods

We performed a prospective, case-control study on 30 obese women diagnosed with GDM, 30 pregnant women with GDM, and 30 age-matched non-obese pregnant women without GDM enrolled in the first, second, and third trimester visit and were followed up until delivery. Subjects were between the age group of 18–35 years.

Medical history of gestational diabetic and control subjects was obtained through a structured questionnaire. All procedures performed in current study involving human participants were in accordance with the ethical standards of the Damghan University Committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained for all individual participants included in the study. The inclusion criteria include subjects with body mass index (BMI) of at least 30 kg/m^2 and history of gestational diabetes. Obesity was determined based on BMI. Subjects excluded from the study were women of age below 18 years and above 35 years with type 1 and type 2 diabetes mellitus and werre diagnosed with diabetes before pregnancy.

Blood samples were collected once from each participant in the first trimester of gestation and during the fetal viability scan, in the second trimester of gestation during screening for gestational diabetes mellitus, and in the third trimester of gestation. Blood samples were analyzed for serum calcium levels by colorimetric method [19]. Glucose level was measured by glucose oxidase method (Pars Azmoon, Tehran, IR Iran) [20, 21]. Phosphorus concentration was determined by colorimetric method [19]. Fructosamine level was measured by spectrophotometric method [22]. Vitamin D level was measured by chemiluminescence immune assay method [23].

Statistical analysis

Results were expressed as mean ± standard deviation (SD) in the study. The data were statistically analyzed. Descriptive statistics and analysis were performed in SPSS16 for Windows.

Results

The study evaluated the role of the pattern changes in serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels for obese women with GDM. The mean fructosamine levels in non-obese pregnant women without GDM, non-obese pregnant women with GDM, and obese pregnant women with GDM were found to be 1.40 ± 0.03, 2.10 ± 0.10, and 2.00 ± 0.08 mg/dl, respectively (Table 1). The serum vitamin D, fructosamine, and calcium concentrations were found to be high in groups II and III when compared to group I (Table 1). However, the serum phosphorus concentrations were found to be low in groups II and III when compared to group I (Table 1).

From the first to the third trimester of pregnancy, an increase in fructosamine occurs, from 1.70 ± 0.09 to 1.80 ± 0.08. In fact, there is an increase toward the third trimester. Nevertheless, from

Table 1 Fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in non-obese pregnant women without GDM, non-obese women with GDM, and obese women with GDM (mg/dl)

Variables	Group I: non-obese pregnant women without GDM	Group II: non-obese pregnant women with GDM	Group III: obese pregnant women with GDM	PV
Vitamin D	11.2 ± 0.7	11.7 ± 1.7	10.6 ± 1.6	0.85
Calcium	8.80 ± 0.22	9.2 ± 0.14	9.00 ± 0.09	0.38
Phosphorus	3.70 ± 0.08	3.70 ± 0.14	3.30 ± 0.10	0.02
Fructosamine	1.40 ± 0.03	2.10 ± 0.10	2.00 ± 0.08	0.001

The results shown are the means of triplicate experiments ± SD

the first to the third trimester of pregnancy, drop in vitamin D, calcium, and phosphorus levels occurs (Table 2); likewise, there is drop toward the third trimester. The correlation plots between blood levels of phosphorus, calcium, vitamin D, fructosamine, glucose, body mass index (BMI) levels, and age in groups I, II, and III are shown in Fig. 1.

Discussion

The key finding of this study is that the mean of fructosamine level has an increasing trend in non-obese pregnant women with GDM and obese pregnant women with GDM. Our results demonstrated that the combination of GDM and obesity aggravates the adverse pregnancy outcomes caused by either GDM or obesity alone, whereas 25-hydroxyvitamin D, calcium, and phosphorus levels were uniformed throughout the gestation period. These findings are consistent with prior studies [15]. A possible mechanism of our findings could be explained by the increasing glucose levels due to insulin resistance in obese women with GDM. In obese women with GDM, beta cells of pancreas tissue work overtime to produce sufficient insulin, but the insulin does not lower their blood sugar contents. Although insulin does not cross the placenta, sugar and other nutrients do. So extra blood glucose goes through the placenta. Nonetheless, the exact mechanism remains unclear. Fructosamine level demonstrated an increasing trend from the first trimester to the third trimester, whereas 25-hydroxyvitamin D, calcium, and phosphorus levels drop. The present data are in close agreement with the conclusion of other studies [12, 13, 17, 24].

Table 2 Serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in the first, second, and third trimester in obese pregnant women diagnosed with gestational diabetes mellitus

Variables	First trimester	Second trimester	Third trimester	PV
Vitamin D	11.9 ± 1.4	11.7 ± 1.2	9.90 ± 0.88	0.40
Calcium	9.20 ± 0.08	8.90 ± 0.10	8.70 ± 0.31	0.17
Phosphorus	3.70 ± 0.12	3.50 ± 0.11	3.50 ± 0.91	0.34
Fructosamine	1.70 ± 0.09	1.70 ± 0.12	1.80 ± 0.08	0.76

The results shown are the means of triplicate experiments ± SD

It was previously indicated that calcium fulfills a variety of roles in human physiology. Several factors are involved in regulating serum calcium. A number of recent studies have suggested that low serum calcium values can be seen in cases of vitamin D deficiency. It has been suggested that hypercalcemia is also seen in a variety of abnormalities. Hypophosphatemia, or decreased serum level of phosphate, may be seen in vitamin D deficiency. But increased serum level of phosphate is caused by chronic renal failure and excessive ingestion of vitamin D. When calcium and phosphorus levels in the body are within normal limits, the predominant dihydroxylated form of vitamin D synthesized is the less active metabolite. Recent evidences have suggested that intestinal calcium absorption decreases with age, and in postmenopausal women, decreased estrogen production is accompanied with plasma level decline of vitamin D3. Changes in fructosamine, calcium, phosphorus, and vitamin D levels may also be seen in complications of pregnancy. For comprehension of role of these biochemical factors, further studies are needed to elucidate whether these biomarkers can be useful in the management of human pregnancy.

We suggest that a combination of routinely measured clinical factors and biochemical marker such as fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels measured in the first, second, and third trimester in obese women diagnosed with GDM may provide a useful approach to the prediction of GDM outcome. Our data tend to support the concept that fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus level assays can provide a good index of health control especially in obese pregnant women with GDM. Implying the level of fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels can be used to screen obese women with GDM. Despite minor differences, 25-hydroxyvitamin D, calcium, and phosphorus levels were almost the same as in obese women diagnosed with GDM compared with GDM women without obesity.

Our results demonstrated that there is negative correlation between BMI and age ($r = -0.059$). There is positive correlation between BMI and glucose level ($r = 0.101$). Also, there is positive correlation between fructosamine and glucose level ($r = 0.0.209$). This study found that the levels of fructosamine levels were closely related to age, BMI, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese women with GDM. However, the molecular mechanisms underlying the relationship between the fructosamine levels and BMI, Glu, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese women with

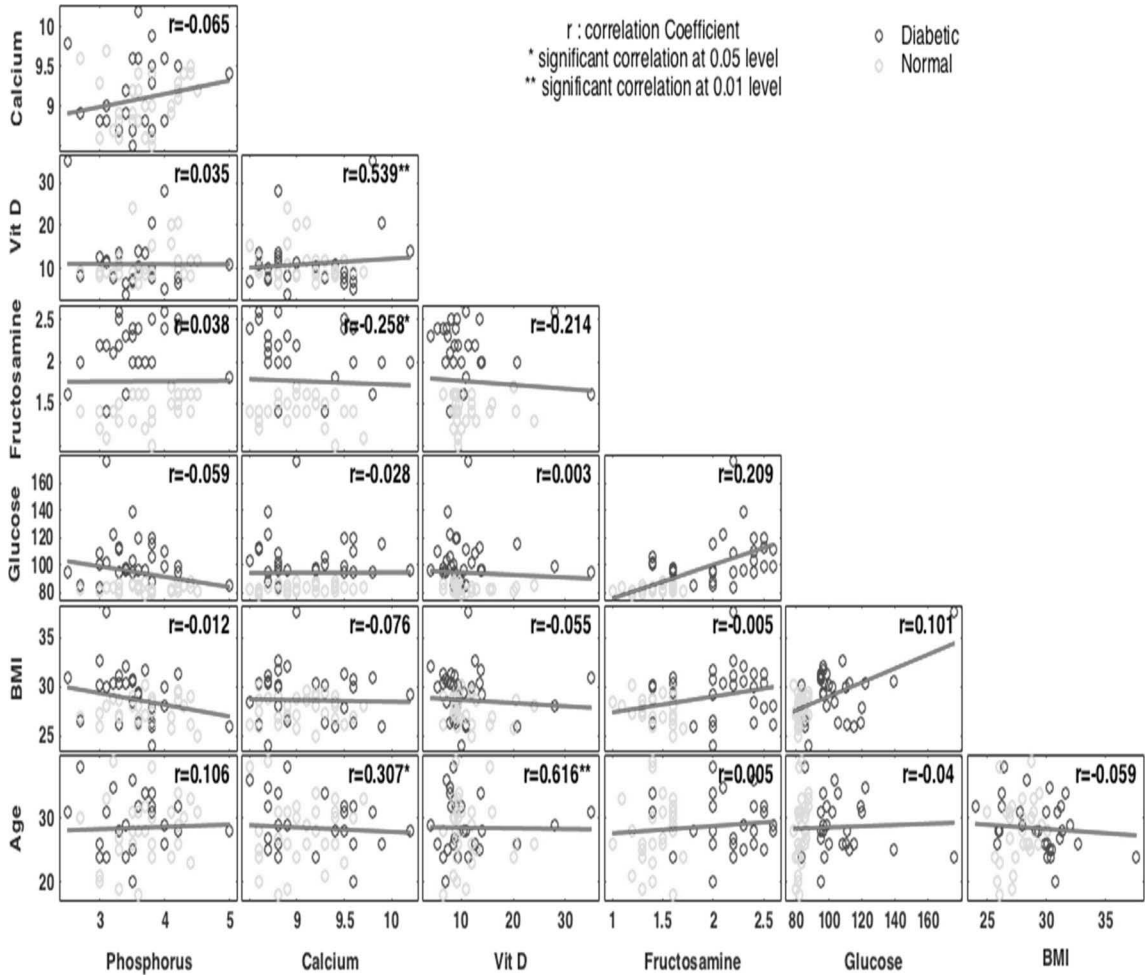

Fig. 1 The correlation plot between serum levels of phosphorus, calcium, vitamin D, fructosamine, glucose, body mass index (BMI), and age in obese pregnant women with GDM and non-obese pregnant women without GDM

GDM are not clear. These data may provide further information in elucidating the correlation between fructosamine levels and BMI, Glu, 25-hydroxyvitamin D, calcium, and phosphorus levels in GDM obese women.

This study has several strengths or strong points. To our knowledge, this is the first study to assess a biochemical marker associated with GDM in obese pregnant women in the first, second, and third trimester of pregnancy. The study population is representative of the general population. We were able to examine the association between biochemical markers including fructosamine, glucose, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese pregnant women with GDM. The weak points of our study are as follows: We had limited information on obese and GDM risk factors, such as family history. We cannot determine the usefulness of these results in clinical practice. The novelty of our work we found was that measuring the concentration of fructosamine in serum is easier and less costly than using other biochemical factors including insulin and adiponectin as a routine screening test to detect pregnancy complication.

Conclusions

In conclusion, this prospective study revealed the determination of serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels in obese pregnant women with GDM might prevent maternal complications. Our results indicate that the combination of GDM and obesity aggravates the adverse pregnancy outcomes caused by either GDM or obesity status alone.

This study provides new insight into the biochemical changes associated with GDM in obese women. Validation in a large prospective study is required to determine the usefulness of these results in clinical practice. Therefore, to reduce confounding factors and increase the accuracy of results, these studies must be carried out on a larger size of population.

Research agenda

To assess the serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels for gestational diabetes mellitus

Practice point

Serum fructosamine, 25-hydroxyvitamin D, calcium, and phosphorus levels for gestational diabetes mellitus

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Author contributions All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; DQ designed the experiments and preparation of the manuscript; MN conducted the experiments and performed analysis; BR was involved in conception and design, data analysis, and interpretation.

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Compliance with ethical standards

All procedures performed in current study involving human participants were in accordance with the ethical standards of the Damghan University Committee and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained for all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Bacteriological profile of sepsis and its correlation with procalcitonin in patients with diabetes mellitus

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Abstract
Bloodstream infections can lead to life-threatening sepsis and require rapid antimicrobial treatment. It is an accepted opinion that diabetes worsens prognosis of infection, particularly sepsis, although there is not much data published on this subject. The aim was to study the bacteriological profile of sepsis in patients of diabetes mellitus (DM) and the correlation of procalcitonin as biomarker of sepsis and blood culture positivity. Study was conducted at University College of Medical Sciences (UCMS) and Guru Teg Bahadur (GTB) Hospital from December 2013 to November 2014. Thirty known diabetic patients with signs and symptoms of sepsis were enrolled for the study irrespective of age, sex, and type of diabetes. Blood samples were taken for blood culture, estimation of procalcitonin and blood glucose levels, fasting and postprandial and glycosylated hemoglobin, and other relevant biochemical tests. Out of the 30 samples, 9 (30%) patients yielded growth; among them, 66.6% were found to be *Staphylococcus aureus* and 33.3% were *Klebsiella pneumoniae*. Median procalcitonin (PCT) levels in positive blood culture subjects were significantly higher than those with negative blood culture. We observed blood culture positivity in 30% of the patients of DM with sepsis, predominance of Gram-positive bacteria, and significantly higher PCT levels in blood culture-positive patients.

Keywords Diabetes mellitus · Sepsis · Bacteriological profile · Procalcitonin

Introduction

Diabetes mellitus is associated with an increased susceptibility to infection and sepsis. There is a close association between hyperglycemia and infections. Patients with DM are at higher risk for bacterial infections, such as urinary tract infections (UTIs), lower respiratory tract infections (LRTIs), as well as cellulitis, osteomyelitis, peritonitis, and sepsis [1]. Diabetics contract pneumonia, wound infections, and urinary tract infections more frequent than non-diabetics [2]. Sepsis can complicate bacterial infections in diabetic patients, which represent 20.1–22.7% of all patients with sepsis [3]. The main reason for which diabetes mellitus predisposes to infection appears to be abnormalities of the host response, particularly in neutrophil chemotaxis, adhesion, and intracellular killing; these defects have been attributed to the effect of hyperglycemia. Hyperglycemia of ≥ 11.1 mmol/l (≥ 200 mg/dl) has been associated with reduced neutrophil activity [4]. Due to frequent infections, diabetic patients have more exposure to antibacterial agents, which can lead to increased antibiotic resistance rates. Early diagnosis is crucial in case of life-threatening infections. Blood culture is the current "gold standard" for diagnosis of septicemic conditions and is based on the detection of viable microorganisms present in the blood. However, on some occasions, blood cultures have intrinsic limitations in terms of sensitivity and rapidity. The limitations of blood cultures have also fostered interest in the development of sensitive and rapid

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laboratory tests aimed at detecting nonspecific biomarkers of sepsis [5]. There are several biomarkers of sepsis, like C-reactive protein, serum procalcitonin (PCT), IL-6, IL-8, and lactate, of which PCT has been found to be the most effective. PCT has been proposed as an indicator of infection and as well as useful marker of the severity of sepsis [6]. There is an evidence suggesting that the use of PCT in monitoring patients with sepsis leads to reduce morbidity and mortality [7]. Many severe infections in patients with diabetes mellitus require early surgical and antibiotic treatments. Therapy by narrow-spectrum antibiotics can prevent the increase in antibiotic resistance and also emergence of multidrug resistance in diabetic patients.

This study was conducted to know the bacteriological profile in diabetic patients with sepsis and correlation of positive blood culture with procalcitonin levels.

Material and methods

A total of 30 subjects suffering from diabetes mellitus with signs and symptoms of sepsis including severe sepsis and septic shock irrespective of age and sex, admitted in the Endocrinology I.C.U and medical wards at UCMS and GTB Hospital, over the period from December 2013 to November 2014, were included in the study.

Subjects with other systemic complications like chronic kidney disease, congestive heart failure, and chronic respiratory infections or immune disorders were excluded from the study.

Approval from the institutional ethics committee was taken before the commencement of the study. Relevant details of the patients were recorded in a predesigned proforma.

The blood was collected before the administration of any antibiotics for routine hemogram, blood culture, procalcitonin levels, and blood glucose levels (fasting and postprandial), as well as glycosylated hemoglobin.

The blood samples were collected for blood culture in the BacTec blood culture bottles. Each vial contained 40-ml enriched soybean—casein digest broth, 0.02% SPS resin, CO_2, O_2, and a sensor for the detection of fluorescence. The vials were placed in BacTec 9120 system. A positive result was indicated by an audible alarm and yellow illumination of the positive indicator lamp at the site of positive vial. The bottles were incubated for 5 days before being reported as negative.

Samples for PCT were centrifuged to separate the serum and kept at − 70 °C for further analysis.

Bacteriological processing

Positive vials showing presumptive presence of viable organisms were subjected to subculture on blood agar, MacConkey agar and chocolate agar. The growth obtained was identified by colony morphology, Gram-stain of the isolated colonies, and conventional biochemical identification tests as per the standard protocol followed in our laboratory [8].

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the isolated organisms was performed by Kirby-Bauer's disk diffusion method on Mueller-Hinton agar plates, and the results were recorded as per the Clinical and Laboratory Standards Institute 2015 guidelines [9].

Antibiotics with disk strength used in identification were ampicillin 10 μg, amikacin 30 μg, erythromycin 15 μg, clindamycin 2 μg, gentamycin 10 μg, cefoxitin 30 μg, vancomycin 30 μg, chloremphenicol 30 μg, imipenam 10 μg, ciprofloxacin 5 μg, ceftazidime 30 μg, aztreonam 30 μg, teicoplanin 30 μg, tigecycline 15 μg, lineozolid 30 μg, colistin 10 μg, and piperacillin-tazobactam 100/10 μg.

Estimation of procalcitonin

Human Procalcitonin ELISA Kit was used for estimation of PCT. Assay range of kit is 9.3 to 300 ng/ml. The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assess the level of procalcitonin (PCT) in samples. Standard, test sample, and HRP-labeled PCT antibodies were added to enzyme wells which were pre-coated with PCT antibody, and then the plates were incubated and washed to remove the uncombined enzyme. Upon adding chromogen solutions A and B, the color of the liquid changed into blue, and the reaction with the acid caused the color to become yellow. The depth of color and the concentration of the PCT sample were positively correlated.

Results

A total of 30 blood samples were taken during the study period of 1 year. Patients were from age group 16 to 67 years (median age in years 45.7), out of whom 13 were male patients and 17 were female patients. Eighty-three percent (25/30) of the patients had type 2 diabetes mellitus.

Blood samples were processed for aerobic culture, and nine (30%) of these samples yielded growth. Out of the total, nine (30%) were bacterial isolates: six were from Endocrinology I.C.U. and three from medical wards. Maximum positivity was found from Endocrinology I.C.U. Gram-positive and Gram-negative organisms contributed to 66.6% (6/9) and 33.3% (3/9), respectively. Table 1 describes the distribution of the total bacterial isolates obtained from positive blood cultures along with the antibiogram and PCT levels. *Staphylococcus aureus* was the predominant organism isolated (66.6%) followed by *Klebsiella pneumoniae* (33.3%).

Table 1 Blood culture positivity with antimicrobial susceptibility results and PCT levels

S. no.	Subject number	Organisms isolated	Antimicrobial susceptibility testing results	PCT (ng/ml)
1	3	*Klebsiella pneumoniae*	S: TGC, CL, IPM, CH, AK, CAZ, PIT R: CIP, CTX, COT, AZT	10.95
2	6	*Staphylococcus aureus*	S: LZ, TEI, VA, FOX, AK R: E, CD, GEN, AMP, CIP	83.15
3	10	*Staphylococcus aureus*	S: FOX, TEI, LZ, GEN, VAN R: AK, AMP, CIP, E, CD	58.80
4	14	*Klebsiella pneumoniae*	S: TGC, COL, IPM, CH R: CIP, AK, PIT, CAZ, CTX, COT, AZT	120.60
5	15	*Staphylococcus aureus*	S: TEI, FOX, GEN, AMK, CD, AMP, VA, LZ R: E, CIP	9.11
6	16	*Klebsiella pneumoniae*	S: TGC, COL, IPM, CH, AK, CAZ R: CIP, PIT, CTX, COT, AZT	56.10
7	17	*Staphylococcus aureus*	S: E, CD, VA, TEI, FOX, LZ R: GEN, CIP, AK, AMP	10.12
8	19	*Staphylococcus aureus*	S: LZ, TEI, FOX, VA R: GEN, CIP, AK, AMP, E, CD	10.55
9	26	*Staphylococcus aureus*	S: LZ, TEI, VA, FOX, AK R: E, CD, GEN, AMP, CIP	115.60

S sensitive, *R* resistant, *AK* amikacin, *AMP* ampicillin, *AZT* aztreonam, *CAZ*, ceftazidime, *CIP* ciprofloxacin, *CH* chloramphenicol, *CD* clindamycin, *CTX* cefotaxime, *COL* colistin, *COT* co-trimoxazole, *E* erythromycin, *FOX* cefoxitin, *GEN* gentamicin, *IPM* imipenem, *LZ* linezolid, *PIT* piperacillin + tazobactam, *TEI* teicoplanin, *TGC* tigecycline, *VA* vancomycin

Median procalcitonin levels were higher in patients with positive blood culture ($p = 0.022$) as shown in Table 2.

Discussion

There is a lack of consensus regarding a bacteriological profile of sepsis and the correlation with level of procalcitonin in patients of diabetes mellitus. This study provides information on the bacteriological profile of isolates causing bloodstream infections along with their antibiotic susceptibility pattern and the correlation with PCT, which plays an important role in the effective management of sepsis in diabetic patients. In this study of 30 patients with signs and symptoms of sepsis, blood culture was positive among 9 (30%) and of whom 2 died (22.2%). In this study, sepsis contributed to 6.6% of all deaths, which is comparable to another study where it contributed to 10.8% deaths [10]. Another study has reported a prevalence of infection in diabetic patients of 30.4% with an average of 1.65 organisms per case in contrast to non-diabetic population having infection rates of 21.2% with an average 1.2 organisms per case [10]. Though there is a paucity of literature on the bacterial etiology of diabetic sepsis, *S. aureus* has been reported to be the single most frequent pathogen, followed by *E. coli* in diabetic foot. Other studies have also found the same [11–13].

Table 2 Comparison of PCT level with blood culture (PVSN)

Variable	Category	Median (IQR)	Significance[a]
Blood culture	P	56.10 (10.34–99.37)	0.022 (S)
	N	10.23 (5.92–17.26)	

IQR interquartile range (25th to 75th percentile), *P* positive, *N* negative

[a] Mann-Whitney *U* test

Despite strict glycemic control, diabetic patients have a 1.7-fold probability of developing an I.C.U.-acquired blood stream infection compared to non-diabetic subjects [14].

It is also true that diabetic patients have greater problems with healing of infections because of reduced blood supply, which affects the body's ability to fight infection [15]. Known risk factors which contribute to development or perpetuation of sepsis include the use of immunosuppressive agents (31 patients; 77.5%), recent hospitalization (10; 25.0%), diabetes mellitus (5; 12.5%), and smoking (5; 12.5%). The body surface area (BSA) involvement was also extensive (mean 53.6% ± 34.1%) in sepsis cases [16].

In our study, there is significant correlation between blood culture positivity and level of procalcitonin. Median PCT levels in positive blood culture subjects were significantly higher than negative blood culture subjects ($p = 0.022$). In a study, it is seen that there is a significant rise of PCT level in patient of positive blood culture and in urinary tract infection where diabetes was one of the comorbid conditions[14] which is correlating with this study also [17]. One study has demonstrated the superiority of procalcitonin to other laboratory values as a predictor of bacteremia [18]. Studies have also investigated how well PCT correlates with types of pathogens [19]. Different PCT cutoff levels suggest different bacterial species, with higher concentrations for Gram-negative bacteria (AUC 0.81 at cutoff 6.47 ng/ml) [20]. In this study also, PCT level is seen higher for Gram-negative bacteria.

Although unique in its own, our study did not have controls and was limited to an Endocrinology I.C.U. in a tertiary care hospital. The sample size being small, the results from this study should be carefully interpreted keeping individual population, related demographic, and microbiological variations in mind.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

High prevalence of disabling hearing loss in young to middle-aged adults with diabetes

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Abstract
South Africa has one of the highest diabetes prevalence numbers in Sub-Saharan Africa with more than 2 million diagnosed. There is an increase in evidence linking diabetes with hearing loss. This study aimed to determine prevalence and characterize the nature of hearing loss in patients with diabetes. An observational matched groups design was utilized with a total of 192 participants, 110 patients with diabetes (cohort) and 82 patients without diabetes (control). Pure tone audiometry findings showed a significantly higher prevalence of hearing loss in those with diabetes (55%) when compared to those without (20%) diabetes ($p < .001$). Further, in patients with diabetes (and diagnosed with hearing loss), the majority (74%) presented with sensorineural hearing loss. There was a higher number of participants with disabling hearing loss (pure tone average (PTA) 0.5, 1, 2 and 4 kHz > 41-dB hearing level (HL) in the better ear) in those with diabetes ($n = 48$) than those without ($n = 10$). Distortion product otoacoustic emission assessments showed significantly higher percentages of abnormalities ($p < 0.01$) in those with diabetes compared to those without diabetes. Findings of this study showed that participants who were diagnosed with diabetes had a higher proportion of disabling hearing loss when compared to those without diabetes. The findings of this study further strengthen the suggestion that hearing loss should be considered as a comorbidity associated with diabetes.

Keywords Hearing loss · Diabetes · Disabled persons · Prevalence

Introduction

The prevalence of non-communicable diseases (NCDs) such as diabetes is expected to surpass that of the current predominant communicable (infectious) diseases in the next 20 years [1]. African countries are estimated to have the world's largest increase in NCD morbidity and mortality over the next decade [2–5]. Diabetes is one of the most prevalent non-communicable diseases with approximately 422 million diagnosed worldwide with projections showing an expected increase to 642 million in the next two decades. Global mortality rates show that 5 million people between 20 and 79 years of age died from diabetes and diabetes-related complications, compared to only 3.6 million that died from human immunodeficiency virus (HIV), tuberculosis (TB), and malaria combined in 2015 [1–5].

The African continent has up to 25 million people living with diabetes along with the highest proportion of undiagnosed adults under 60 years of age [2]. The existence of diabetes in Africa was once a rare occurrence; however, the prevalence is rapidly rising with the increase in urbanization and lifestyle diseases affecting more in the population [5]. South Africa has one of the highest and fastest growing diabetes prevalence numbers in Sub-Saharan Africa with more than 2 million people living with diabetes [2–5]. The projected epidemiological trends of diabetes suggest that it is likely to be a significant contributor to the burden of disease globally, especially in developing countries.

A concern for hearing health care professionals is the emerging body of research evidence that associates diabetes with the risk of developing hearing loss [4, 6–12]. At present,

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research evidence linking diabetes with hearing loss is not extensive; however, the reported association is plausible based on pathological changes observed post-mortem in patients with diabetes revealing damage to the vasculature and neural system of the inner ear [10, 11]. Hearing loss is already a major public health issue globally with adult-onset hearing loss ranked second highest contributor to years lost to disease [12, 13]. Therefore, a potential increase in the prevalence of hearing loss among patients with diabetes (among other already-known risk factors) is a concern for health professionals, the public health system, and the economy, especially in developing countries [5].

Research investigating the impact of diabetes on hearing health in developing countries, Africa in particular, has been sparse; however, a study from Nigeria highlighted diabetes as one of the risk factors associated with hearing loss [12]. In terms of prevalence estimates, there are considerable regional differences in prevalence reports of hearing loss among patients with diabetes, with prevalence ranging between 13 and 78% [14–16]. In the USA, a study reported 13.1% hearing loss prevalence; in contrast, in Iran, a study reported a higher prevalence of 45% [14–16]. More recently, the highest prevalence was reported in a study from India with 78.2% prevalence of sensorineural hearing loss in their study focusing on participants with uncontrolled diabetes [17]. There are several factors that may help explain this variation in the prevalence of hearing loss reported in previous studies. These include study of methodological differences and limitations of study designs, sample sizes, and hearing loss assessment protocols carried out to determine prevalence.

Specific to the assessment protocols, varying prevalence reports may have been affected by three key factors. These included the classification of hearing loss in terms of hearing level (in dB, HL) norms, pure tone average (PTA) calculations, and the type of assessments used. First, the classification of hearing loss in terms of the cutoff threshold used to distinguish between presence and absence of hearing loss may have influenced prevalence reports. Presence of hearing loss is determined using pure tone hearing thresholds (measured in decibels, dB; HL) with 25-dB HL as the typical cutoff used to differentiate between presence and absence of hearing loss (in adults) [18]. However, there are other cutoff thresholds that can be used to classify presence and degree of hearing loss, e.g., 15-dB HL cutoff. Thus, prevalence of hearing loss reported will therefore vary depending on which one of these two cutoffs is used.

Second, the frequencies used to determine the PTA can also influence the prevalence of hearing loss reported. In most clinical settings, a PTA is derived using three frequencies that cover the low to mid ranges (0.5, 1, and 2 kHz); however, diabetes-related hearing loss first affects the high frequencies between 2 and 8 kHz [8, 16]. In order to observe possible diabetes-related hearing loss with PTA calculations that are inclusive of higher frequencies, some studies have added modified PTA calculations.

Third, the type of assessments used to establish diabetes-related auditory dysfunction can also impact on the prevalence findings reported. Most studies that have investigated the presence of auditory abnormalities in diabetes patients used pure tone audiometry assessments in the standard frequency range (250–8000 Hz) [7, 8]. Pure tone audiometry may not be sufficiently sensitive to detect diabetes-related damage; therefore, the addition of otoacoustic emissions (OAEs) as part of the test battery was recommended to cross-check of findings with an objective and sensitive assessment of the diabetes-related damage to the auditory pathway [11]. While OAEs do not test hearing acuity as such, the assessments give valuable information about the site of lesion by differentiating between sensory abnormalities that may be due to diabetic microvascular damage to the cochlea and a neural abnormality [11].

Therefore, considering the variations in the current literature as identified above, the current study design was conducted to document hearing loss in patients with diabetes in South Africa as a country with one of the highest diabetes prevalence findings in Africa, while avoiding the methodological shortfalls of previous studies [5].

Materials and methods

Study design

This study employed an observational cross-sectional matched groups design. The two groups used in this study comprised participants diagnosed with diabetes (cohort) and another group consisting of participants without diagnosis of diabetes (control). Participants in both groups were matched for age and gender to allow for comparison between the two groups. Participants included in this study if they met the following criteria: clinically confirmed diagnosis of diabetes of either type (cohort group only), age range between 18 and 55 years of age, and did not report any of the following: prolonged exposure to loud recreational or occupational noise, prior use of ototoxic drugs, history of head injury, radiotherapy to the head or ear surgery, and no clinical diagnoses or reports of neurological impairments.

Data collection

Ethics clearance was obtained from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC/Ref:134/2015) prior to the commencement of the study.

Data collection commenced after informed consent was obtained and included the following assessments: case history

Table 1 Participant demographics

	Cohort [*n* (%)]	Control [*n* (%)]	Statistical significance ($\alpha = 0.05$) based on independent *t* tests
Gender (%)			
Males	53 (48)	41 (50)	$t(175) = -0.58$, $p = 0.56$
Females	57 (52)	41 (50)	
Age (years)			
20–30 years	13 (12)	4 (5)	$t(6) = -0.75$, $p = 0.48$
31–40 years	25 (23)	13 (16)	$t(34) = 1.51$, $p = 0.14$
41–49 years	27 (24)	25 (30)	$t(37) = -0.80$, $p = 0.42$
> 49 years	45 (41)	40 (49)	$t(60) = 1.37$, $p = 0.17$

interview, otoscopy, tympanometry, pure tone audiometry, distortion product otoacoustic emmissions (DPOAE), and a medical folder review to obtain information related to participants' diabetes type and status.

Participants

A total of 232 individuals consented to participate in this study, 123 in the cohort and 109 in the control group. A total of 40 of the potential participants, 13 in the cohort group and 27 in the control group, were later excluded from this study because they did not meet the inclusion criteria for the study. Most of the exclusions were either due to age or prior exposure to occupational noise. The resultant study sample was a total of 192 participants, 110 in the cohort and 82 in the control arms of the study respectively. Participant description is detailed in Tables 1, 2, and 3 below.

Specific to the cohort group, see below on table a description of the diabetes characteristics and comorbidities

Table 2 Diabetes characteristics and comorbidities

Diabetes characteristics	Number of participants *n* (%)
Type	
Type I	9 (8)
Type II	101 (92)
Diabetes duration	
0 to 5 years	65 (59)
6–10 years	27 (24)
11–20 years	15 (14)
Over 20 years	3 (3)
Diabetes control	
Controlled, < 7 g mol	29 (26)
Uncontrolled, > 7 g mol	81 (74)
Comorbidities	Number of participants *n* (%)
Hypertension	62 (56)
Positive for diabetic neuropathy	56 (51)
Failed the vision screener	62 (56)

Comorbidities only investigated in the cohort (diabetic) group

Results

Hearing loss prevalence, type, and severity

Pure tone audiometry assessments showed a higher prevalence of hearing loss in the cohort (55%) when compared to the control (20%) group with both the conventional pure tone average (cPTA) and high-frequency pure tone average (HF-PTA). Most participants had symmetrical hearing regardless of hearing loss presence, cohort (80%) and control (71%). Utilizing the HF-PTA to describe the type of hearing loss, sensorineural hearing loss was the most prevalent type of hearing loss observed. Majority of the hearing losses observed were of a slight degree (16–25-dB HL). There were relatively

Table 3 Hearing loss information

Description	Cohort	Control
Total sample size	110	82
Total number of ears in sample	220	164
Number of ears with normal hearing	[*n* (%)]	[*n* (%)]
cPTA (conventional)	138 (63)	124 (76)
HF-PTA (high frequency)	98 (45)	132 (80)
Number of ears with hearing loss	[*n* (%)]	[*n* (%)]
cPTA (conventional)	82 (37)	40 (24)
HF-PTA (high frequency)	122 (55)	32 (20)
Type of hearing loss	[*n* (%)]	[*n* (%)]
Conductive	7 (6)	4 (12)
Sensorineural	91 (74)	22 (67)
Mixed	24 (20)	7 (21)
Severity of hearing loss	[*n* (%)]	[*n* (%)]
Slight (16–25-dB HL)	42 (35)	14 (44)
Mild (26–40-dB HL)	32 (26)	8 (25)
Moderate (41–70-dB HL)	39 (32)	7 (22)
Severe (71–90-dB HL)	9 (7)	3 (9)
Profound (> 90-dB HL)	0 (0)	0 (0)

cPTA = average of 0.5, 1, and 2 kHz and HF-PTA = average of 2, 4, and 8 kHz. Hearing loss severity classification according to [19]

Table 4 Predisposing risk factors and hearing loss

Predictor variables	Odds ratio	p value ($\alpha = 0.05$)	95% confidence interval	
			Lower	Upper
Diabetes duration (years)	1.120859	*0.013*	1.024482	1.226303
Diabetes control (g mol)	0.9920032	0.786	0.9362205	1.05111
Age (years)	2.904431	*0.019*	1.191974	7.0771
Gender[a]	0.2661657	*0.005*	0.1045702	0.6774794
Presence of hypertension	1.847933	0.215	0.7005446	4.874572

Regression calculated with $n = 110$, $R^2 = 0.181$, and log likelihood = − 62.149578

[a] Reference variable = female

The italic values indicate the statistically significant variables ($p < 0.05$) according to the logistic regression analysis. These values indicate that the variables were found to be statistically significant predisposing factors to hearing loss in the study sample investigated.

more ears (up to 3 times more) with disabling hearing loss (> 41-dB HL) in the cohort (48) than the control (10) group.

Odds of hearing loss presence

A logistic regression analysis was done to investigate the likelihood of hearing loss presence with the following (independent) variables: gender, age, and diabetes characteristics (duration and control) (see Table 4).

Otoacoustic emissions

There was a significantly higher proportion of ears with abnormal findings in the cohort than control group with both DPOAE signal-to-noise ratio and level findings (see Table 5).

Discussion

The prevalence of hearing loss in patients with diabetes was significantly higher compared to those without diabetes. Furthermore, the current study report of hearing loss prevalence was found to be slightly higher (55%) than previous study reports of 13.1% [15], 43.6% [8], and 45% [16]. Several factors may be plausible explanations for the variation in prevalence percentages of hearing loss reported in different studies, such as the use of different classifications of hearing loss in terms of the normative cutoff (in dB HL) of the range of frequencies used for PTA calculations.

A low cutoff hearing level norm (15-dB HL) was used in this study to include a slight hearing loss (16–25-dB HL). Slight hearing loss is not typically acknowledged by many researchers in their findings [20, 21] even though it is known to impact speech discrimination and comprehension even in adults [22]. Also, the current study made use of a HF-PTA in line with literature evidence indicating that diabetes-related hearing loss mainly affects the high-frequency hearing thresholds (2–8 kHz) which may be missed by the conventional PTA calculation (0.5–2 kHz) [8, 16]. With the use of a HF-PTA and a low cutoff norm to classify presence of hearing loss in the current study, there was an additional 42 ears (35%) with slight high-frequency hearing loss identified, which may have otherwise been missed.

In accordance with previous studies, the current study found sensorineural hearing loss to be the most common

Table 5 Abnormal diagnostic DPOAE findings

	Low frequencies	High frequencies
Signal-to-noise ratio (dB) (n [%])		
Cohort ($n = 220$)	112 (51)	186 (85)
Control ($n = 164$)	39 (24)	107 (65)
Significance of differences	$t(290) = -1.24$, $p = 0.21$	$t(381) = -6.65$, $p < 0.01$
DPOAE level (dB SPL) (n [%])		
Cohort ($n = 220$)	89 (40)	213 (97)
Control ($n = 164$)	65 (40)	139 (85)
Significance of differences	$t(358) = -8.06$, $p < 0.01$	$t(323) = -3.99$, $p < 0.01$

n = number of ears. Norms: signal to noise ratio > 6 dB, DPOAE level > 0 dB (Botelho et al. 2014)

type of hearing loss established in 74% of the participants with diabetes [7, 8, 11, 16]. While sensorineural hearing loss may be the most prevalent type of hearing loss in patients with diabetes, other hearing loss types are worth noting and discussing. In this study, conductive and mixed hearing losses were found in 15 and 26% respectively among patients with diabetes. Similar findings were reported by Thimmasettaiah and Shankar (2012) with mixed hearing loss in 16% of their participants with diabetes [9]. Hearing losses attributed to outer and middle ear pathologies such as otitis externa and otitis media should not be overlooked in patients with diabetes because their ability to heal and recover from wounds and infections may be compromised. Furthermore, unlike sensorineural hearing loss, losses attributed to outer and middle ear pathologies can be treated and reversed if detected early.

With respect to severity of hearing loss, moderate or worse hearing loss (i.e., thresholds > 40-dB HL) was established in a higher proportion in the cohort group (82%) than that in the control (18%). Moderate or worse hearing loss can be disabling as it can negatively impact audibility, discrimination, and comprehension of sound and speech to the listener [20] and often requires intervention in the form hearing amplification. Reports of a high proportion of ears diagnosed with disabling hearing loss in patients with diabetes were consistent with [8] study which also reported more than 20% of their participants had moderate to severe hearing loss. However, despite this strong evidence of a high proportion of disabling hearing loss in patients with diabetes reported in literature, it is a concern that disabling hearing loss is still not recognized as a comorbidity of diabetes.

Diabetes-related hearing loss has also been associated with diabetes duration [22–24]. The current study also found that each year, increase in diabetes duration was associated with about 12% (OR 1.12) increase in odds of hearing loss. These findings are in line with the hypothesis that an increased diabetes duration implies increased patient age, which is, apart from diabetes presence, a risk factor for hearing loss [3]. The current study findings also highlighted that, in adults younger than 49 years, age was a predictor variable for hearing loss although this age group may not be identified to be at risk for age-related impact to their hearing. This is important to note as it indicates that even in a younger population, diabetes may contribute to hearing deterioration [25, 26].

With respect to participant characteristics, males with diabetes had significantly higher odds of hearing loss presence (OR 0.74, $p < 0.01$), in comparison to females with diabetes. Previous studies have reported that hearing loss is generally more common in men than women [26]. The difference in risk to acquire hearing loss between men and women has been attributed to occupational differences, reaction time to symptoms, and/or frequency of doctor's visits [26]. Also, women have been shown to take better care in issues of health than men [26].

Conclusion

The current study has provided a broad description of hearing loss in patients with diabetes in a South African population. Overall, the findings of this study showed that participants diagnosed with diabetes had a higher proportion of hearing loss when compared to those in the control group. Also, the likelihood of hearing loss presence was associated with diabetes duration and participant factors (age and gender). The findings of the current study indicate that healthcare providers should include, as part of diabetes patient management, a referral for hearing screening for early detection of hearing loss. The current study highlights the need for an audiologist within the multidiscipliary team involved in diabetes patient care.

Specific to audiological testing protocols, clinical implications of the current study include the use of the high-frequency pure tone average calculation (PTA 2, 4 and 8 kHz), more stringent hearing loss classification norms (> 15-dB HL), and the inclusion of DPOAE assessments in combination with pure tone audiometry. Overall, the findings of the current study suggest that hearing loss should be recognized as a comorbidity of/with diabetes. Recognition in instruments such as the annual diabetes fact sheets of key organizations like the International Diabetes Federation and World Health Organization will increase awareness of the prevalence of hearing loss in patients with diabetes.

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Author contribution V. Hlayisi: concepts, design, definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review.

L. Petersen: definition of intellectual content, literature search, manuscript preparation, manuscript editing, and manuscript review.

L. Ramma: definition of intellectual content, literature search, data analysis manuscript preparation, manuscript editing, and manuscript review.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants All procedures performed in the study were in accordance with the 1964 Helsinki declaration (and its later amendments or comparable ethical standards) as well as the ethical standards of the institutional committee. Ethics clearance was obtained from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC/Ref:134/2015) prior to the commencement of the study.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

A risk scores for predicting prevalence of diabetes in the LAO population

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Abstract
To develop risk scores for predicting the prevalence of diabetes in the Lao population. This was a cross-sectional study of both men and women (age 30 to 70 years) living in rural villages of the Vientiane municipality in the Lao PDR. Multiple logistic regressions with backward stepwise selection were used; the diabetes risk score was derived from the β-coefficient. Performance of the score was determined by the area under the receiver operating characteristic curve (AUC), the sensitivity, the specificity, and the positive predictive value for the specified cut-off value. The prevalence of undiagnosed diabetes was 7%. The factors included in the predictive in model were 17 (40 to 70 years of age) + 14 (high waist circumference) + 11 (hypertension) + 7 (family history of diabetes). A cut-off point of risk scores of 29.5 out of 49 produced the optimal sum, leading to a sensitivity of 0.75, a specificity of 0.55, a positive predictive value of 17.8%, and an AUC of 0.70. Data suggested that the combination of age, waist circumference, hypertension, and family history of diabetes could be utilized to identify Lao individuals at high risk of undiagnosed diabetes. The generalizability for other Lao population needs further investigation.

Keywords Risk assessment model · Diabetes prevalence · Risk score · Undiagnosed diabetes · Lao diabetes prevalence

Introduction

Diabetes is a chronic disease that results in long-term damage and socio-economic problems. It is linear in its course with increasing morbidity, and it accounts for a high amount of health care services worldwide. The Lao PDR has no clear data sources examining the prevalence of diabetes. Based on the estimation by the International Diabetes Federation (IDF) in 2013, the national prevalence of diabetes was 4.44% among the population aged 20–79 years [1].

Individuals are diagnosed with pre-diabetes if they have the following characteristics, which are higher than standard values but not enough to qualify for a diagnosis of diabetes: impaired fasting glucose or IFG (100–125 mg/dl), impaired glucose tolerance or IGT (2 h of oral glucose tolerance test or OGTT 140–199 mg/dl), and/or glycated hemoglobin or A1C (5.7–6.4%). Pre-diabetes indicates a risk of type 2 diabetes progression in the order of 30 and 70% over 4 years and 30 years, respectively [2]. The common risk factors used in the prediction of the prevalence of diabetes consist of age, sex, family history of diabetes, body mass index (BMI), waist circumference (WC), waist-to hip ratio (WHR), hypertension, anti-hypertensive drugs, physical inactivity, smoking, history of dyslipidemia (low-density lipoproteins, high-density lipoproteins, and triglycerides), intake of anti-dyslipidemia drugs, history of gestational diabetes, and history of having a baby weighing > 4 kg. These factors play a role in the onset of type 2 diabetes [3]. The important step to preventing and/or delaying the onset of type 2 diabetes is to identify people with undiagnosed diabetes. Several studies have convincingly indicated that early interventions involving health education and providing appropriate care may address this problem [4, 5]. Undiagnosed diabetes was predicted by the screening of risk factors. A diabetes risk assessment model that is simple, less

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expensive, more convenient, and noninvasive has been developed by several investigators.

Most Lao people, who live in rural areas, are not very interested in assessing their health risk or having annual medical check-ups. In addition, the geographical area or location makes it difficult for them to access health care services. In Lao PDR, compared to other countries, the development risk score for predicting the prevalence of diabetes has not been studied extensively. To our knowledge, a risk assessment model might provide an acceptable prediction in the prevalence of diabetes, particularly as undiagnosed diabetes. Therefore, the current study aimed to develop risk scores for predicting the prevalence of diabetes in the Lao population. We believe that an appropriate screening risk score model for the early identification of undiagnosed diabetes could provide an important tool to prevent and delay the onset of diabetes mellitus. In addition, it is also necessary to evaluate the validation of risk scores in high-risk populations.

Materials and methods

Study design

This was a cross-sectional investigation conducted from December 2015 to July 2017 in 15 selected rural villages of two districts of the Vientiane Municipality in the Lao PDR. Participants were both men and women within the age range of 30 to 70 years living in the selected community. Participants diagnosed with diabetes and/or using any antidiabetic drugs were excluded.

The preferred sample size was estimated from the results of a previous study with an identical population. Its findings showed a 7.4% prevalence of diabetes, with four variables being significant factors [6]. A rule of thumb is that models should be developed with 10 to 20 events per variable (EPV) [7]. Accordingly, an acceptable sample size needed to assess the population prevalence with good precision can be estimated according to the following calculation: sample size needed for precision = 20 EPV × 4 variables = 80 participants as the sample size. Then, the sample size is needed to estimate the population: $n = 100\% \times 80 / 7.4\% = 1082$ participants.

The study protocol began with an interview on the demographic and behavioral information of each participant, followed by a physical exam and blood pressure measurement. After that, antecubital vein blood samples of the patients were collected in the morning 6.30-9.00,after undergoing the day before. The diabetes prevalence was first identified by an FPG level equivalent to or more than 126 mg/dl; then, a repeated testing was needed in order to affirm the presence of undiagnosed type 2 diabetes. In the risk assessment, all participants were divided into two subgroups of three-fourths (823 subjects) of the sample and one fourth (275 subjects) of the sample, which were utilized in developing the diabetes risk scores and validating the risk scores, respectively.

The demographic data were comprised of age; gender; family history of diabetes, including parents and siblings; history of having baby weighing more than 4 kg; gestational diabetes; and history of or current dyslipidemia (triglycerides > 150 mg/dl, LDL-C ≥ 100 mg/dl, HDL-C < 35 mg/dl). Behavioral data, including smoking habits and physical inactivity (less than 150 min/week or 3 day/week), were also obtained from each participant. After that, anthropometric measurements,blood pressure and venous blood collection were taken.

BMI was computed by dividing body weight (kg) by body height (m^2) using a weight and height scale with precision to the nearest 0.1 kg and 0.1 cm, respectively. The BMI categories, designated by the WHO as normal, overweight, and obesity for Asian people, are 18.5–22.9, 23.00–24.9, and ≥ 25 kg/m^2, respectively [8]. Waist circumference (WC) was measured using measuring tape at the midpoint between the superior border of iliac crest and the lowest rib, with the subject in the position of standing relaxed and in underclothes [9]. As recommended by IDF, the WC for healthy Asian people is classified as < 80 cm for females and < 90 cm for males. Hip circumference is measured at the level of maximal gluteal protrusion for a healthy waist-to-hip ratio (WHR), which is < 0.85 for females and < 0.9 for males [10]. WHR is the ratio of WC (cm) to hip circumference (cm).

Blood pressure (BP) was measured for the participants. After relaxing for 5 min, participants sat upright with their upper arm positioned at the heart level and were measured by an Omron blood pressure monitor. The assessment of blood pressure is determined according to the guidelines of the European Society of BP (ESH) and of the European Society of Cardiology (ESC) 2013 [11].

In the present study, fasting plasma glucose (FPG) was utilized to identify an individual who has either pre-diabetes or undiagnosed diabetes. Venous blood samples were collected 5 ml from the antecubital vein into the test tube and stored at − 20 °C. The blood glucose level was analyzed by a glucose oxidase method in the laboratory of Vientiane Mahosot Hospital using the automatic analyzer Huma Star 600-Human. Following the American Diabetes Association (ADA) standard, plasma glucose levels of < 100, 100–125, ≥ 126 mg/dl specify the health condition as normal, pre-diabetes, and type 2 diabetes, respectively [12].

The study was approved by the National Institute of Public Health National Ethics Committee for Health Research (NECHR), the Lao People's Democratic Republic. Written informed consent was obtained from all participants. The clinical trial number is NCT03311802 (ClinicalTrials.gov).

Statistical analysis

Development of the risk score Multiple logistic regressions with backward stepwise selection were computed in the statistical model for the development of the risk score, utilizing data from 823 participants, or three-fourths of the total number of participants. Variables with p values < 0.2 were acceptable for addition into the modeling procedure, and a p value < 0.05 was the cut-off for the level of statistical significance. The diabetes risk score values originated from the β-coefficient by multiplying its β-coefficient in the regression model by 10 for the simplified equation [13] to form an original equation:

$$\beta_1\ (x_1) + \beta_2\ (x_2) + \beta_3\ (x_3) + \beta_4\ (x_4) + \ldots\text{etc.}$$

Meanwhile, the probability value of having diabetes was calculated by this equation: $p = 1 / (1 + \exp.(-x))$ [13, 14].

Validating the risk score Receiver operating characteristic (ROC) curve analysis was utilized to verify the performance of the risk scores using data from one fourth of the total number of participants (275 samples). The area under the curve (AUC) demonstrated the accuracy of prediction. The cut-off point of risk score, sensitivity, specificity, and positive predictive value (PPV) were examined. The PPV was calculated as follows: (sensitivity × specificity) / [sensitivity × prevalence + (1 − specificity) × (1 − prevalence)].

Results

The characteristics of all participants and the prevalence of undiagnosed diabetes

Among 1098 subjects, there were more women (74.9%) than men (25.1%). A large proportion of the sample is comprised of the age group of 30–59 years. A family history of diabetes was present in 24.8% of the sample, while 0.5 and 2% of female participants previously had gestational diabetes and a history of delivering a baby with a birth weight of more than 4 kg, respectively. The prevalence of a history of taking antihypertensive drug(s), hypertension, high systolic blood pressure (SBP), and high diastolic blood pressure (DBP) was 20.1, 37.2, 26.5, and 31.4%, respectively, while the history of currently taking lipid-lowering drugs and dyslipidemia was 8.7 and 10.7%, respectively. Regarding health behaviors, 84.9% of the sample was physically inactive, and 11% of the sample smoked cigarettes. The percentages of those with high BMI, WC, and WHR were 50.5, 59.9, and 72.5%, respectively. By means of 77 participants having an FPG ≥ 126 mg/dl, the overall prevalence of undiagnosed diabetes was 7.0%.

Developing the risk score

According to the participants' characteristics, the crude and adjusted odds ratios (ORs) of undiagnosed diabetes are shown in Table 1. Of all of the risk factors, four risk factors played a significant role in the increased prevalence of undiagnosed diabetes in the final model: hypertension (OR = 3.09, p = 0.0003), waist circumference ≥ 80 cm for females and ≥ 90 cm for males (OR = 4.13, p = 0.001), age ≥ 40 years (OR = 5.55, p = 0.005), and family history of diabetes (OR = 2.08, p = 0.020). The risk equation of the diabetes risk score value was derived from the β-coefficient as follows: 1.7 (age ≥ 40) + 1.4 (WC) + 1.1 (hypertension or HTN) + 0.7 (family history of diabetes or FDM) (Table 2). The formula could be simplified to 17 (age ≥ 40) + 14 (WC) + 11 (HTN) + 7 (FDM). The probability values of having diabetes vary from 0 to 49, which are calculated as the sum of the scores of all individual risk factors.

Validating of risk scores

The performance of risk scores was verified by the area under the ROC curve (AUC), which showed the accuracy of the prediction of risk scores with AUC = 0.70 (95% confidence interval 0.58–0.81, p = 0.005), as shown in Fig. 1. The cut-off point of the risk scores was ≥ 29.5, with a sensitivity, specificity, and positive predictive value of 0.75, 0.55, and 17.8%, respectively. The increasing risk score was clearly correlated with an increasing prevalence of undiagnosed diabetes (chi-square for linear trend, p < 0.002). The exception was in the individuals with scores = 0–9 in the risk score validation subgroup, where the prevalence of undiagnosed diabetes was 6.7%.

Discussion

In Laos, it seems that this is the first study on the assessment of the prevalence of undiagnosed diabetes, as well as the first study developing and validating the risk score for predicting undiagnosed diabetes in the Lao population.

Prevalence of undiagnosed diabetes

This study found that the undiagnosed diabetes prevalence in Lao adults aged 30–70 years was 7%. It was high compared to the prevalence of only 4.4% estimated by the IDF for the Lao population aged 20–79 years [1] and to the prevalence found by previous studies of only 5 and 5.2% for rural ASEAN populations aged ≥ 25 and 15–85 years, respectively [6, 15]. This variation might be attributed to the different age ranges of the studied populations. However, this prevalence was smaller than the diabetes prevalence for urban ASEAN populations of

Table 1 Crude and adjusted odds ratios (ORs) of having undiagnosed diabetes

Characteristics	Undiagnosed diabetes				Undiagnosed diabetes			
	OR crude	95% CI		*p* value	$OR_{adjusted}$	95% CI		*p* value
		Lower	Upper			Lower	Upper	
Age ≥ 40								
No	1				1			
Yes	6.34	1.95	20.60	0.002	5.55	1.67	18.39	0.005
Family history of diabetes								
No	1				1			
Yes	2.10	1.17	3.76	0.013	2.08	1.12	3.86	0.02
Antihypertensive drug intake								
No	1				1			
Yes	1.98	1.07	3.68	0.031	0.90	0.44	1.83	0.762
History of dyslipidemia								
No	1				1			
Yes	1.37	0.66	2.88	0.4	1.20	0.55	2.64	0.645
Never test	2.77	1.33	5.78	0.007	0.83	0.12	5.98	0.855
Intake dyslipidemia lowering drug								
No	1				1			
Yes	2.88	1.36	6.08	0.006	1.31	0.58	2.95	0.511
BMI ≥ 25 kg/m^2								
No	1				1			
Yes	2.41	1.33	4.39	0.004	0.87	0.43	1.77	0.702
WC (cm); F ≥ 80, M ≥ 90								
No	1				1			
Yes	5.18	2.30	11.65	0.0001	4.13	1.81	9.41	0.001
WHR; F ≥ 0.85, M ≥ 0.9								
No	1				1			
Yes	3.44	1.45	8.19	0.005	1.36	0.52	3.61	0.532
Hypertension								
No	1				1			
Yes	4.15	2.29	7.49	0.0001	3.09	1.68	5.67	0.0003

Hypertension (SBP ≥ 140 or DBP ≥ 90 mmHg). Of all of the risk factors, four risk factors played a significant role in the increased prevalence of undiagnosed diabetes in the final model: hypertension (OR = 3.09, $p = 0.0003$), waist circumference ≥ 80 cm for females and ≥ 90 cm for males (OR = 4.13, $p = 0.001$), age ≥ 40 years (OR = 5.55, $p = 0.005$), and family history of diabetes (OR = 2.08, $p = 0.020$)

BMI body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

11 and 11.5%, respectively [15, 16]. However, it should be noted that our study was carried out in a rural area that has a lower risk of diabetes than the urban population [17].

The development of the risk score for predicting diabetes

Significant factors associated with undiagnosed diabetes were hypertension, waist circumference, age ≥ 40, and a family history of diabetes (parent, sibling). Therefore, these factors were selected to establish the equation for predicting the risk of undiagnosed diabetes.

Hypertension is a well-known diabetes risk factor, and its presence in the risk score models could improve the screening performance for diabetes prevalence. The findings from this study are consistent with the evidence from a prospective cohort study by Conen et al. [18]; it showed that hypertension was a strong and independent predictor of diabetes. Waist circumference also played a strong role in the diabetes risk score in this model at cut-off points recommended for Asian populations that were lower than those used for populations in Western countries [19]. Therefore, the risk functions could be applied for the studied population; those with dissimilar geographical and ethnic backgrounds could be invalid [20]. Age was widely put into the diabetes risk prediction model [6, 13].

Table 2 The undiagnosed diabetes risk score value

Characteristics	Undiagnosed diabetes		
	Beta coefficients	*p* value	Score (×10)
Family history of diabetes			
WC; F ≥ 80, M ≥ 90 cm	1.4	0.001	14
Hypertension (140/90 mmHg)	1.1	0.0001	11
Age ≥ 40	1.7	0.005	17
Total			49

The risk equation of the diabetes risk score value was derived from the β-coefficient as follows: 1.7 (age ≥ 40) + 1.4 (WC) + 1.1 (hypertension) + 0.7 (family history of diabetes)

WC waist circumference

This current study showed results similar to those of Chaturvedi et al. [14] and Pongchaiyakul et al. [6]; these studies found that an age > 49 was associated with type 2 diabetes. Several studies found that a family history of diabetes was an important risk factor of diabetes [21, 22]. It is a reflection of the genetic predisposition for the disease, and it is an essential marker for increasing the risk of type 2 diabetes [23]. Genetic predisposition may be necessary but insufficient for the occurrence of type 2 diabetes.

The risk score developed is quite suitable to the Lao context, as it is a simple, safe, inexpensive prediction tool without laboratory testing required at the screening stage. It might not be applicable for predicting the prevalence of undiagnosed diabetes, but it will be useful in categorizing the diabetes risk of individuals [24]. Our study developed a similar risk equation as Chaturvedi et al. [14] but used different cut-offs for the anthropometric measurements (WC in females was > 85 vs. ≥ 80 cm) and age scale (> 50 years vs. 40 years). The proportions of undiagnosed diabetes in the community are nearly 30–60% [25, 26]; thus, the diabetes risk score may benefit

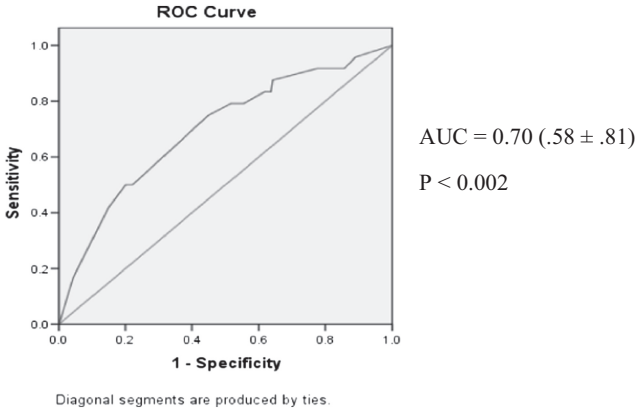

Fig 1 Risk score of undiagnosed diabetes analyzed by an ROC curve in the validation group. The performance of risk scores was verified by the area under the ROC curve (AUC), which showed the accuracy of the prediction of risk scores with AUC = 0.70 (95% confidence interval 0.58–0.81, $p = 0.005$)

the mitigation of the public health problem. The individuals identified with a high risk of diabetes would have an opportunity to increase their awareness of the modifiable risk factors and engage in a healthy lifestyle in order to delay the onset of diabetes; she/he may actually have unrecognized, asymptomatic diabetes that may involve further clinical assessment and therapy [27].

Validation of the risk score

The validation analysis of the risk score was done with 25% of all participants ($n = 1098$ participants). The performance of our risk score validation was AUC = 0.70 ($p = 0.002$). This performance was lower than one of developed risk scores for the studied population (AUC = 0.78 ($p = 0.0001$)). Our finding was similar to those of relatively recent systematic literature reviews of 23 different studies measuring the prevalence of diabetes risk scores without laboratory tests among different population groups (10 from Europe, 9 from Asia, 2 from the USA, and 2 from the Middle East). These reviews reported that the AUC in the development studies (range 0.65 to 0.88) was greater than in the validation studies (range 0.63 to 0.80) [28].

The findings of this study were specific and valid only for the studied population in Vientiane. For other Lao population, further research is needed, since previous studies have affirmed that the diabetes risk score developed among one population group might not be valid or generalizable when applied to another population group with distinct characteristics [29]. Limitation of this study included first the sample size used in the risk score validation that might be inappropriate, as the results of some important risk factors were not significantly associated with diabetes. Second, this study diagnosed type 2 diabetes through the FPG test instead of the OGTT. The FPG test is less precise than the OGTT, which could identify an overload of glucose, but it is not often done in routine clinical practice. Nevertheless, measuring FPG levels may be the best preliminary strategy for diabetes screening instead of a complex process to predict incidental diabetes.

Conclusion

A simple risk score for screening Lao people at a high risk of undiagnosed diabetes has been developed. The model equation is 17 (age ≥ 40) + 14 (WC) + 11 (HTN) + 7 (FDM). Its validity was a cut-off point of risk scores of 29.5 out of 49, which produced the optimal sum and a sensitivity of 0.75, a specificity of 0.55, a positive predictive value of 17.8%, and an AUC of 0.70 ($p = 0.002$) in the validation group. Primary prevention, including lifestyle modification and further blood testing, should be provided for populations with high-risk scores.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the National Institute of Public Health National Ethics Committee for Health Research (NECHR), the Lao People's Democratic Republic, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study. The clinical trial number is NCT03311802 (ClinicalTrials.gov).

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ORIGINAL ARTICLE

Biochemical and anthropometric changes during Ramadan among type 2 diabetes mellitus patients

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Abstract
In this study, our aim was to assess the impact of fasting during Ramadan on biochemical and anthropological factors in patients with type 2 diabetes and compare them with those who did not fast. This was a prospective study. It was carried out in the city of Yazd, Iran, during Ramadan of 2016. Body weight, height, waist circumference, blood pressure, body mass index (BMI), lipids, fasting plasma glucose (FPG), and fructosamine levels before and after Ramadan were assessed on 120 diabetic patients; 60 of whom had fasting, and 60 had no intention. Fifty-four patients from the fasting group (including 34 fasters with 10- to 25-day fasting (group 1) and 20 fasters with > 25-day fasting (group 2)) and 58 patients from the non-fasting group (group 3) completed the study. This study showed that the mean BMI in three groups significantly decreased, and the highest decrement was seen in group 1 at the end of Ramadan (p value < 0.001). Low-density lipoprotein (LDL) increased in fasting groups and decreased in the non-fasting group (p value > 0.01). Fructosamine was increased in all groups, and the highest increment was seen in group 2 after Ramadan (p value < 0.001). We observed that glycemic status and LDL-C levels worsened after Ramadan in T2DM patients, and beneficial effects of Ramadan on anthropometric factors are not limited to fasters, and other non-faster Muslims also showed weight reduction after Ramadan.

Keywords Fasting · Ramadan · Diabetic · Biochemical and anthropometric factors

Introduction

According to a recent study, approximately 1.6 billion (23%) of the worldwide population are followers of Islam [1]. Fasting during Ramadan, a holy month of Islam, is an obligatory duty for all of healthy adult Muslims, whereby they abstain from eating, drinking, using oral medications, and smoking [2]. It can be considered as a period of "intermittent fasting" or daily cycles of "alternating" fasting and feeding periods lasting between 11 and 19 h a day depending on geographical location during 28 to 30 days. Patients with type 2 diabetes mellitus (T2DM) usually should consume frequently low calorie meals (between 4 and 6 times a day), but in Ramadan, a faster should abstain drinking or eating from dawn to sunset. This condition leads to major changes in lifestyle, intakes, and activities that can affect general health. It can induce several complications in patients with T2DM. Islamic rules allow such patients not to fast, although they usually insist on doing so. A recent multi-centric large survey concerning patients with diabetes revealed that 78.7% with type 2 diabetes and 42.8% of patients with type 1 diabetes have fasted for at least 15 days [2]. In countries where Muslims are in preponderance, physicians are always challenged by inquiries of patients about their ability to fast and the effect of fasting on their plasma glucose control. Physicians should provide clear and evidence-based advice to their patients who intend to fast [3]. Yazd is a province in the center of Iran with high prevalence of T2DM [4] . Regarding the recently published and unpublished studies, T2DM prevalence in Yazd province among people of more than 30 years old is about 18% although prevalence of T2DM in Iran is 11% among 25–70 years [5]. Despite the high prevalence of T2DM and insist of patients for fasting during Ramadan, powerful studies that evaluate the effect of fasting on anthropometric indices, lipid profiles, and glycemic status

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of T2DM patients had not been performed in the past, and the results of studies in other parts of our country and other Islamic countries are controversial. Some studies showed that fasters lost significant weight during Ramadan, and some others reported no weight change [6–10]. It has been observed that the food containing pastas and calorie-filled fried foods during Ramadan are generally unhealthy. This is true even for people used to use a strict diet. Prolonged fasting, lifestyle changes, and medical challenge that the patients create for themselves may cause acute complications, such as hypoglycemia and hyperglycemia in subjects with diabetes during Ramadan. Most of the studies that evaluated biochemical changes in diabetic patients during Ramadan showed little changes in the glycemic control [11, 12]; however, the EPIDIAR study demonstrated a high rate of acute complications [2]. In the current study, our aim was to assess the impact of fasting and habitual habits during Ramadan on biochemical factors and anthropometric indices in patients with T2DM who fast and compare them with non-fasting patients.

Material and methods

Among them, 60 patients were eligible for fasting during Ramadan based on local guidelines [13], and others had no desire or were not in suitable condition for fasting. At the end of study, we observed a high diversity in number of fasting days among fasters, and for better evaluation of results, we divided fasting patients into two groups: those with 10–25-day fasting (group 1) and those with more than 25-day fasting (group 2). Details are given in Fig. 1. At the first visit, the following information was recorded: age, gender, duration of diabetes, education level, daily physical activity, smoking, complications and comorbidities, current treatment, anthropometric measurements, and laboratory marker. To perform second-order measurements, all the patients were invited to the center, during 3 days after the end of Ramadan.

While patients wore only light clothing, weight was measured without shoes using an electronic weighing scale (Glamor, BF-1041-A) and recorded to the nearest 100 g. Height was measured once at baseline without shoes with the subject stretching to the maximum height and the head positioned on the plane using a portable stadiometer and was recorded to the nearest 0.1 cm. Body mass index (BMI) was calculated (kg/m2) using weight and height values. Waist and hip circumferences were measured to the nearest 0.1 cm using a non-stretchable tape with an insertion buckle at one end. The waist was measured at the mid-point between the lower rib and the upper margin of the iliac crest in a horizontal plane, and the hip circumference was measured at the widest circumference of the buttock.

Number of fasting days, measurements of blood pressure (Citizen, AC-23OCZ), and any history of hospital admission during the Ramadan were recorded. Fasting plasma glucose (FPG), fructosamine, and lipid parameters were measured before and after the Ramadan.

Blood chemistry tests such as FPG, total cholesterol (Chol), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoproteins (HDL) were analyzed using an auto analyzer BA-400(Bio systems, European), and commercially available kits were used according to the manufacturer's instructions. The determination of fructosamine (Human fructosamine (FTA) ELIZA Kit, Cat Noc E3232Hu 96 Tests) levels was performed by enzyme-linked immunosorbent assay (ELIZA). The normal range of fructosamine with the above assessment method is 205–285 μmol/l. HgbA1C was measured by high-performance liquid chromatography on a Diamat Analyser (Bio-Rad, Munich, Germany).

Statistical analysis

SPSS version 20 software was used for statistical analysis. Results were expressed as mean ± standard deviation. The Mann-Whitney *U* test was used to compare the continuous variables and the chi-square test was used to compare categorical variables. The assumptions of ANCOVA for statistical analyses of this study were not realized. We compared the difference before and after the values between the three groups with the ANOVA. *p* value of less than 0.05 was considered to be statistically significant.

Result

We enrolled 120 patients with T2DM. For better evaluation and comparison of the results, we divided fasters into two groups: patients with 10–25-day fasting (group 1) and patients with more than 25-day fasting (group 2). Patients who had fasted for less than 10 days could not be assigned to any fasting or non-fasting groups. From the participants, 34 fasting patients with fasting days of 10–25 days (group 1), 20 patients with fasting for more than 25 days (group 2), and 58 non-fasting diabetic patients (group 3) completed the study. A total of eight patients could not be included in the analysis because of missing data or outliers. In group 1, 67% of the patients were female and the mean and SD of the age in this group was 49 ± 7 years. Group 2 was 51 ± 9 years and 6% were female, and group 3 was 55 ± 9 and 37% were female (*p* value: < 0.006). Mean duration of diabetes in the groups listed respectively was 4.08 ± 3.2 years, 3.65 ± 3.6 years, and 8.78 ± 6.6 years (*p* value > 0.001). The three groups at the beginning of the study had a significant difference in duration of diabetes (*p* value > 0.001), gender (*p* value > 0.001), FPG (*p* value > 0.017), history of cardiovascular complications (*p* value >0.01), and high blood pressure (*p* value > 0.003). HbA1C, BMI, WC and lipid profiles, and fructosamine did

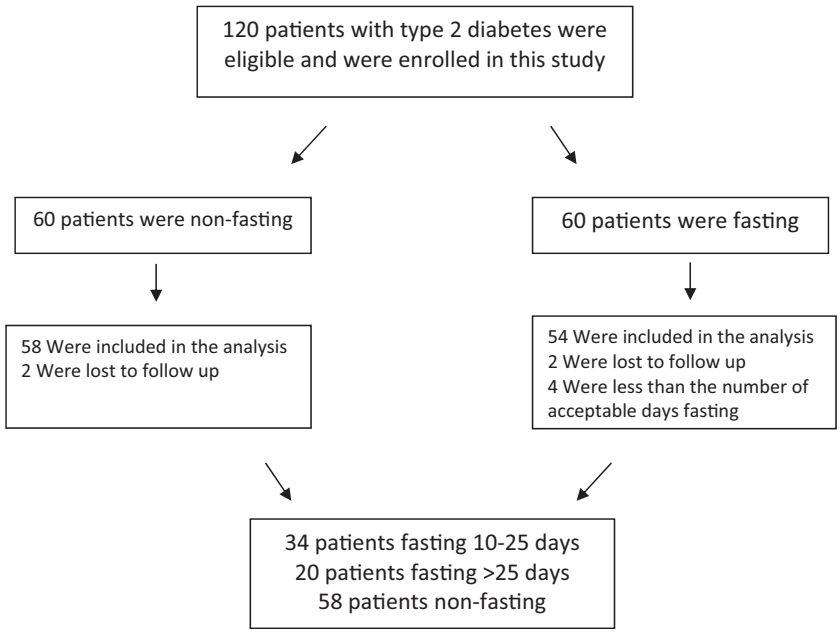

Fig. 1 Selection of the study population for analysis

not have significant difference at the baseline. The demographic and clinical features of the study population in baseline are shown in Table 1. The before and after values of the biochemical and anthropometric parameters were compared in each group, and the results are presented in Table 2.

The mean changes in fructosamine, LDL, and BMI were significantly different between groups (p value > 0.001). The description of the results of the changes is shown in Table 3. The mean of LDL and fructosamine among fasting participants after Ramadan was higher than before, (p value < 0.010), (p value > 0.001). BMI also decreased in all three groups, which was a significant decrease (p value > 0.001).

Discussion

In Iran, each year during holy Ramadan, some changes in habits of foods and activities occur that involve all people whether fasting or non-fasting. For example, some kinds of sweets, soups, and special desserts are commonly served in restaurants and homes. Because of these wide changes in Persian cuisine during Ramadan and for assessment of fasting effect on anthropometry and biochemical metabolic parameters, we designed to have two different Muslim groups: the first group included T2DM patients who had decided to fast during Ramadan, and the second group included the diabetic Muslims who were not going to fast during Ramadan, at all. We measured weight, height, waist circumference, fasting plasma glucose, fructosamine, and lipid profile a week before Ramadan of 2016 and during 3 days after the last day of Ramadan. The length of day during summer was about 16 h. We conducted this study in Yazd, a city in the center of Iran with high prevalence of T2DM and metabolic syndrome [1]. Both of the groups received dietitian education before Ramadan.

From the participants, we had 34 fasting patients with fasting days of 10–25 days (group 1), 20 patients with fasting for more than 25 days (group 2), and 58 non-fasting diabetic patients (group 3).

This study showed that the mean of BMI in three groups decreased significantly at the end of Ramadan, and between-group difference was significant. (p value < 0.001). The rate of BMI decrement was highest in group 1 and then in groups 2 and 3, respectively. However, it is obvious that favorable effects in decreasing BMI that were attributed to fasting during Ramadan in some previous studies is not limited to fasters and also is seen in non-fasters.

Group 3 at the end of Ramadan showed non-significant increase in waist circumference but groups 1 and 2 showed non-significant decrease in waist circumference (p value < 0.066).

Decrease in BMI without significant decrease in WC at the end of Ramadan in fasters (groups 1 and 2) may be attributed to dehydration and loss of total body fluid without any significant changes in central fat. Previous studies evaluating the effect of Ramadan fasting on anthropometry and metabolic factors have found no reduction in body fat [14] despite the decrease in body weight.

Inconsistent results have been reported about Ramadan effects on anthropometry of T2DM patients. Some of them showed weight reduction [15], and others showed increased BMI [6, 14, 16].

These mixed results about Ramadan effects on BMI and waist circumference may be secondary to different physical activities, meal habits, and fasting hours [6].

Table 1 Demographic and clinical features of the patients at baseline

	0 days	10–25 days	> 25 days	*p* value
Age (mean ± SD)	55.62 ± 9.6	49.01 ± 7.8	51.64 ± 9.4	0.006
Sex (female) *N*%	37 (63.8)	18 (90)	6 (17.6)	< 0.001
Family history of DM	37 (66.1)	18 (94.7)	24 (70.6)	0.052
Disease duration (mean ± SD)	8.78 ± 6.6	4.08 ± 3.2	3.65 ± 3.6	< 0.001
HbA1c %	7.05 ± 0.6	7.01 ± 0.6	6.96 ± 0.8	0.905
Waist circumference (cm)	102.24 ± 9.6	97.25 ± 10.4	101.11 ± 9.9	0.153
BMI	28.96 ± 4.0	28.62 ± 4.1	28.38 ± 3.5	0.789
LDL (mg/dl)	95.88 ± 32.7	88.92 ± 27.5	86.05 ± 29.4	0.335
Sys BP	132.34 ± 20.9	119.40 ± 14.0	128.17 ± 15.7	0.025
Dias BP	81.20 ± 10.4	79.35 ± 8.7	81.67 ± 10.0	0.534
FBS (mg/dl)	136.67 ± 38.6	149.61 ± 29.3	124.40 ± 23.5	0.017
Chol (mg/dl)	171.83 ± 39.3	168.44 ± 33.1	169.62 ± 44.7	0.939
HDL (mg/dl)	42.72 ± 8.6	45.33 ± 6.6	42.00 ± 6.1	0.192
TG (mg/dl)	175.18 ± 90.5	180.88 ± 86.5	191.78 ± 162.7	0.782
Fructosamine (mmol/L)	262.84 ± 119.2	238.73 ± 48.4	263.23 ± 131.0	0.902
HLP[a]	35 (60.3)	7 (35)	17 (50)	0.137
HTN[b]	36 (62.1)	6 (30)	10 (29.4)	0.003

[a] High lipid profile

[b] Hypertension

In the present study, we observed significant increase in FPG among T2DM patients who fasted during Ramadan (groups 1 and 2) (p value < 0.004) but without significant difference in group 3 (p value < 0.212). This may be the result of special food selection during Ramadan in Iran and consuming meals with higher amount of sugar in dawn and iftar meals. The increased FPG can have other causes in participating groups especially increased WC and elevated insulin resistance.

The other possible causes are meal pattern change (decrease in frequency and increase in amount of meals), unusual cortisol secretion during long-lasting fast [15], and decreased physical activity. These results are consistent with Sadiya et al. [15] and Momen et al. [17] observations and in contrast to Paul et al. [14] and Bener et al. [18] results.

Previous studies about lipid profiles and its changes during Ramadan fasting have shown inconsistent results. Our study did not show any significant change in total cholesterol, triglyceride, and HDL in groups. However, at the end of Ramadan, LDL was higher in fasting groups and decreased in the non-fasting group. These changes have been significant among participating groups (p value > 0.010).

Our results are in agreement with Barkia et al. observations [19]. Increasing LDL-C during Ramadan fasting is probably

Table 2 Comparison of changes in biochemical and anthropometric parameters before and after Ramadan

Groups →	0 days (group3)			10–25 days (group1)			> 25 days (group2)		
Variables ↓	Before	After	*p* value	Before	After	*p* value	Before	After	*p* value
FBS (mg/dl)	135.46 ± 35.9	136.69 ± 32.6	0.976	149.61 ± 29.3	153.88 ± 26.3	0.256	124.40 ± 23.5	136.06 ± 23.0	0.010
BMI	28.84 ± 3.8	28.77 ± 3.7	0.295	28.62 ± 4.1	28.13 ± 4.0	< 0.001	28.38 ± 3.5	28.15 ± 3.9	0.332
LDL (mg/dl)	94.59 ± 32.2	87.31 ± 29.0	0.140	88.92 ± 27.5	100.33 ± 30.4	0.008	86.05 ± 29.4	90.50 ± 29.4	0.305
Sys BP	130.08 ± 20.4	130.81 ± 20.5	0.732	119.40 ± 14.0	115.40 ± 15.5	0.135	128.17 ± 15.7	122.02 ± 14.7	0.011
Dias BP	80.83 ± 10.4	80.59 ± 8.8	0.862	79.35 ± 8.7	78.95 ± 9.5	0.717	81.67 ± 10.0	81.14 ± 10.2	0.684
Waist circumference (cm)	102.14 ± 9.2	101.55 ± 8.0	0.305	97.25 ± 10.4	98.8 ± 9.9	0.018	101.11 ± 9.9	102.92 ± 9.1	0.020
Chol (mg/dl)	172.00 ± 39.6	167.00 ± 40.7	0.502	168.44 ± 33.1	175.61 ± 36.0	0.083	169.62 ± 44.7	169.00 ± 39.0	0.939
HDL (mg/dl)	42.68 ± 8.4	39.85 ± 8.6	0.006	45.33 ± 6.6	46.77 ± 12.7	0.695	42.00 ± 7.1	38.37 ± 7.1	0.231
TG (mg/dl)	170.66 ± 81.2	195.43 ± 93.3	0.034	180.88 ± 86.5	152.88 ± 75.6	0.068	180.34 ± 121.0	187.12 ± 119.2	0.597
Fructosamine (mmol/L)	261.46 ± 127.4	290.19 ± 133.00	0.340	235.72 ± 48.0	313.16 ± 61.2	< 0.001	263.60 ± 133.0	344.75 ± 222.5	< 0.001

Table 3 Changes in anthropometric and clinical parameters in patients during Ramadan

Variables	0 day	10–25 days	> 25 days	*p* value
FBS diff*(mg/dl)	3.75 ± 34.0	4.27 ± 15.6	11.65 ± 24.0	0.212
BMI diff	− 0.07 ± 0.4	− 0.49 ± 0.4	− 0.22 ± 1.3	< 0.001
LDL diff (mg/dl)	− 6.97 ± 33.8	11.7 ± 15.8	4.77 ± 24.8	< 0.010
Sys BP diff	0.75 ± 15.0	− 4.00 ± 11.4	− 6.42 ± 13.4	0.070
Dias BP diff	− 0.77 ± 9.2	− 0.40 ± 9.2	− 0.60 ± 7.6	0.987
Waist diff (cm)	0.59 ± 3.9	1.55 ± 2.6	1.80 ± 4.3	0.066
Chol diff (mg/dl)	− 5.1 ± 35.8	13.72 ± 31.6	0.00 ± 47.3	0.129
HDL diff (mg/dl)	− 2.80 ± 7.0	− 5.44 ± 15.3	− 4.03 ± 6.0	0.673
TG diff (mg/dl)	21.27 ± 76.4	− 28.00 ± 60.8	17.10 ± 84.1	0.060
Fructosamine diff	1.48 ± 117.0	77.44 ± 66.4	81.15 ± 202.0	< 0.001

*Diff is "after value–before value"

the result of increased cholesterol consumption and decreased physical activity especially during warm months of the year.

It is important that 4 weeks after Ramadan, undesirable lipid changes have not restored to basal pre-Ramadan levels. There are some studies that have reported Ramadan fasting can decrease LDL-C between diabetic patients [15, 18, 20], and on the other hand, some studies have shown that Ramadan fasting cannot have any significant effect on blood lipids.

The main outcome at the end of Ramadan for diabetic people who fast is glycemic control. In this study, we evaluated fructosamine before and after Ramadan and observed that fructosamine at the end of Ramadan increased in all groups, but the increase was higher in people who fasted for the whole Ramadan. Fructosamine increased at the end of Ramadan in diabetic patients who fasted for 10–25 days and more than 25 days of a month as well as control group, and this showed that changes in habits in our region during Ramadan have negative effects on glycemic controls among patients with diabetes even in those who do not fast. Moreover, T2DM patients who desire to fast during Ramadan consume more simple sugars and fats to combat hunger and prevent hypoglycemia in long and warm days of summer that leads to uncontrolled glycemia. It is prudent to perform this study during years that daytime is shorter for example during winter or at locations where climate is more temperate to further evaluation of Ramadan effects on glycemic status. The results of this study can be used for guiding care givers of diabetic patients who consult about Ramadan effects on glycemic condition. According to our results, it is reasonable to exclude patients with poorly controlled T2DM from fasting during summer.

The strength of the present study is evaluating a group of patients with T2DM who did not fast and comparing them with Muslims with T2DM who fasted during Ramadan. Between Muslims during holy month of Ramadan, some changes in habits, physical activity, and diet happen and that some of these are not limited to fasters, as non-fasters are also exposed to those changes. Comparing a group of non-fasters with fasters, we tried to eliminate the effects of common changes and evaluate the fasting impact on lipids, anthropometric, and glycemic statuses of diabetic patients. One of our limitations is that we did not have sufficient data about patient diet and physical activity. T2DM patients who have plan for fasting had wide variety of conditions, and conducting them through definite investigational lines for better assessing, the impact of fasting was impossible.

Conclusion

With our best knowledge, this study is the first two-population cohort that evaluated the effect of Ramadan fasting on metabolic and anthropometric parameters among diabetic persons. We showed that glycemic control worsened at the end of Ramadan, and SBP decreased significantly, even in those who do not fast.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This was a prospective cohort study. Patients were included if they had a medical record at Yazd Medical Research Center. The study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences (17/1395/100). This study included 120 patients with type 2 diabetes who were evaluated before (1 week prior to the start of Ramadan) and after the Ramadan. We enrolled 120 patients with T2DM who were referred to Yazd Diabetes Research Center and signed written informed consent.

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ORIGINAL ARTICLE

The effect of consumption of low glycemic index, high fat content bread on anthropometric measurement and cardiometabolic risk factors in women with type 2 diabetes mellitus

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Abstract
The aim of the present study was to examine the effects of consumption of breads with different glycemic index as part of a balanced diabetic diet on anthropometric and biochemical parameters in patients with type 2 diabetes mellitus. The study was conducted on 24 diabetic women who consulted the Dilovası State Hospital Medicine Polyclinic; the patients had been diagnosed with type 2 diabetes with body mass index (BMI) ≥ 25 kg/m^2 and were aged 40.29 ± 6.81 years. A total of 24 subjects with type 2 diabetes were randomized to consume one of two breads as part of their diets, consuming whole grain bread or wheat bread for 4 weeks. Both groups' macronutrient distribution was planned so that about 45–65% of their calorie intake was from carbohydrates, 25–35% was from fat, and 15–20% was from protein. All food with glycemic response except bread was suggested similarly for both groups. Nutritional status, glycemic index values of foods, anthropometric values (weight, waist circumference, BMI, etc.), body composition, and biochemical analysis [fasting plasma glucose, fasting insulin, HbA1c, HOMA-IR, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and C-reactive protein (CRP)] were measured at the beginning and the end of the study. Decrease in the average daily glycemic index in the whole grain bread group was 33.45 ± 9.72%, while it was 21.79 ± 5.97% in the bread made from whole wheat flour. The decrease in glycemic index was statistically significant. There was a significant reduction in body weight, BMI, and waist circumference in both groups, but differences between groups were not significant ($p > 0.05$). A statistically significant, positive relationship was found between decrease in glycemic index and decrease in waist circumference ($r(22) = 0.46$, $p = 0.025$). While there was a significant decrease in HbA1c in patients who consume whole grain bread, there was a significant decrease in insulin and HOMA-IR in those who consume wheat bread. There were no statistically significant changes in plasma glucose, HDL cholesterol, total cholesterol, and triglyceride levels ($p > 0.05$). There is a need to further research to determine the effects of breads with different glycemic indices on glycemic control in patients with type 2 diabetes.

Keywords Bread · Cardiometabolic · Glycemic index · Type 2 diabetes mellitus

Introduction

Diabetes mellitus is a metabolic disease caused by lack of insulin which is necessary for regulation of blood glucose level. According to studies done in Turkey, 64% of patients do not achieve glycemic control [1]. In type 2 diabetes mellitus treatments, the main goal is to maintain glycemic control through nutritional therapy and to prevent complications resulting from type 2 diabetes.

Carbohydrate has a significant impact on postprandial glucose response. Studies defining the ideal intake of

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carbohydrates in patients with type 2 diabetes are limited. It is suggested that the daily carbohydrate intake should not be less than 130 g. Monitoring the amount of carbohydrate intake is a crucial strategy for postprandial glucose control. A meal plan is composed according to these measurements [2]. In medical nutrition therapy, the type of carbohydrates is as significant as the amount of the intake. The American Diabetes Association suggests that using glycemic index together with the carbohydrate intake provides better results than monitoring the carbohydrate intake by itself [3]. According to the 2009 Cochrane release, a diet with a low glycemic index is more effective for glycemic control [4]. A research that evaluated the results of 28 randomized controlled studies on the effect of low glycemic index on cardiovascular risk found that a low glycemic index diet causes a decline on total cholesterol and LDL cholesterol levels independently from weight loss [5].

The aim of this study was to examine the effects of consuming two types of breads with different glycemic index on anthropometric and biochemical parameters, compatible with a medical nutrition therapy protocol, for patients with type 2 diabetes mellitus.

Methods

Subjects

This research was conducted on female patients of Kocaeli Dilovası Public Hospital, Turkey İnternal Disease Department diagnosed with type 2 diabetes, who provided the full consent to be included in the study providing all the adequate criteria and all between the ages of 20–50.

Inclusion criteria of the participants were to be of 20–50 age, BMI index above 25 kg/m2, diagnosed and monitored with type 2 diabetes up to 10 years, using only oral diabetic agents, no complications of the type 2 diabetes, and no chronical diseases except dyslipidemia and hypertension. Patients with fluctuating weights within the last 6 months, pregnant, lactating, in menopause, who have endocrine, lipid disorders and liver disorders, and renal failure were excluded from the study.

Design

All the data from the patients such as their personal information, medical conditions, socio-demographic details, the duration of their type 2 diabetes diagnosis, oral anti-diabetic agents used, dietary habits, and daily psychical activities were collected by a questionnaire through face-to-face meetings. In the beginning of the study, the 3-day dietary intake of the participants was recorded. Each of the patient's dietary habits was closely monitored; their energy intake levels, macronutrient and micronutrient ingredients as well as the daily glycemic index were computed. Furthermore, blood samples were taken in the morning (following an overnight fasting) and fasting blood glucose, fasting insulin, HOMA-IR, HDL cholesterol, LDL cholesterol, total cholesterol, and triglyceride levels were analyzed at the Kocaeli Derince Public Hospital Biochemistry Lab.

Anthropometric measurements (height, body weight, and waist circumference) of all participants were taken and body mass index was calculated. Moreover, body fat percentages were measured through a Tanita TBF 418.

The patients daily energy requirement has been calculated in accordance to the 30–60 age-specific predictive equation of 8.126 × body weight + 658.5. For a patient who weighs 25% more than their ideal body weight, energy requirement has been calculated with adjusted body weight formula (adjusted weight = actual weight-ideal weight) × 0.25 + ideal weight) [6]. Patients were randomly divided into two groups. In the 4-week intervention period, diet planning for both groups was planned estimating an average of 4–5% weight loss. The macronutrient distribution range planned for both groups was for carbohydrate, 45–65% of energy; for protein, 15–20% of energy; and for fat, 22–35% of energy. Foods that have a glycemic response (except bread) were suggested similarly for both groups. Due to the differences in glycemic index of fruits, variety and amount of fruits given in the diet were the same for both groups. In the 4-week period, carbohydrates were taken only from bread; all bread substitutes were excluded from the diet.

The difference between the groups was only through consumption of different types of breads; while one group only consumed whole grain bread, the other group only consumed bread with whole wheat flour. The bread quality and ingredients were standardized through working with only one supplier. All patients' biochemical analysis, anthropometric measurements, and body compositions were re-assessed at the end of the intervention.

Results

The study was concluded with 24 females patients, with type 2 diabetes, and aged 40.29 ± 6.81 years old with no chronic diseases except dyslipidemia and hypertension. The average ages of diabetes for individuals were 4.09 ± 2.09 years and there were no significant differences between the groups (Table 1).

Table 1 Baseline characteristics of the study subjects

	All participants ($n = 24$)		Whole grain bread ($n = 12$)		Wheat bread ($n = 12$)		p value
	S	%	S	%	S	%	
Age (year)$\overline{X}$ ± SD (median)	40.29 ± 6.81 (40)		39.92 ± 6.87 (40.5)		40.67 ± 7.05 (39)		[c]0.977
Age of diabetes (year)	4.09 ± 2.09		4.25 ± 2.0		3.83 ± 2.24		[c]0.590
Diseases							
Hypertension	5	20.8	2	16.7	3	25.0	1.00
Dyslipidemia	19	79.2	10	83.3	9	75.0	
Oral anti-diabetic types							
Metformin	3	45.8	1	8.3	2	16.7	[f]0.403
Acarbose	8	12.5	8	66.7	–	–	
Pioglitazone	2	33.3	–	–	2	16.7	

[c] Mann-Whitney U test

[f] Chi-square

The mean body weight was 80.0 ± 12.6 kg for consuming whole grain bread and 80.34 ± 17.05 kg for the group consuming wheat bread. At the beginning of the study, BMI was 32.06 ± 3.93 kg/m^2 for the group consuming whole grain bread and 32.28 ± 6.01 kg/m^2 for the group consuming wheat bread. The changes critically observed, before and after the study, were the decline in BMI values in both groups ($p = 0.002$; $p < 0.01$; $p = 0.041$; $p < 0.05$). Furthermore, the waist circumference of the group consuming bread with whole grains was 104.92 ± 9.45 cm before and 100 ± 9.47 cm after the study ($p = 0.003$; $p < 0.01$), while the measurements were 101.83 ± 11.8 cm before and 98.5 ± 12.09 cm after the study in the group which consumed bread with whole wheat flour ($p = 0.011$; $p < 0.05$). The differences in weights and body fat percentages, before and after the study, of both groups that consumed different bread types were not statistically different (changes in weight $p = 0.671$; $p > 0.05$; changes in body fat $p = 0.298$; $p > 0.05$) (Table 2).

Table 2 Differences in body composition and anthropometric measurements of subjects

Anthropometric measurements		Whole grain bread ($n = 12$) $\overline{X}$ ± SS (median)	Wheat bread ($n = 12$) $\overline{X}$ ± SS (median)	[d]p value
Body weight (kg)	Baseline	80.0 ± 12.60 (79.5)	80.34 ± 17.05 (73.2)	*0.670*
	Week 6	77.71 ± 12.59 (77.2)	78.53 ± 16.79 (72.7)	*0.707*
[e]p value		0.002**	*0.045**	
Δ Body weight		2.33 ± 1.13	1.82 ± 2.46	*0.671*
BMI (kg/m^2)	Baseline	32.06 ± 3.93 (32.8)	32.28 ± 6.01 (31.3)	*0.799*
	Week 6	31.12 ± 3.9 (31.8)	31.52 ± 5.66 (29.9)	*0.902*
[e]p value		0.002**	0.041*	
Δ BMI		*0.94* ± 0.48	0.77 ± 0.98	*0.443*
Waist circumference (cm)	Baseline	104.92 ± 9.45 (105)	101.83 ± 11.8 (99)	*0.506*
	Week 6	100 ± 9.47 (98.5)	98.5 ± 12.09 (96.5)	*0.583*
[e]p value		*0.003***	*0.011**	
Δ Waist circumference		− 4.91 ± 3.33	− 3.33 ± 3.31	*0.294*
Body fat (%)	Baseline	38.91 ± 4.47 (39.2)	38.57 ± 6.62 (38.8)	*0.862*
	Week 6	37.78 ± 4.20 (37.9)	38.24 ± 6.47 (38.4)	*0.773*
[e]p value		*0.101*	*0.662*	
Δ Body fat		*− 1.12 ± 1.60*	*− 0.32 ± 0.83*	*0.298*

*$p < 0.05$; **$p < 0.01$

[d] Mann-Whitney U test

[e] Wilcoxon signed-rank test

There was no statistical difference between the groups in terms of biochemical parameters at the beginning of the study. A significant decrease in insulin was found in the group consuming wheat bread ($p = 0.0012$). When the HOMA-IR values were assessed for both groups, no considerable changes were observed in the group consuming whole grain bread ($p = 0.239$; $p > 0.05$); however, a significant statistical decline of 1.03 ± 1.37 was observed in the group consuming wheat bread 1.03 ± 1.37 ($p = 0.041$; $p < 0.05$). No significant changes were found on the patients' fasting blood glucose, LDL cholesterol, triglyceride, and total cholesterol values before and after the study ($p > 0.05$) (Table 3).

Table 4 summarizes the daily nutritional profile at baseline and end of study based on the bread they consumed. The energy intake was 1763.47 ± 251.8 kcal/day at the beginning of the study and 1317.63 ± 198.52 kcal/day at the end of the study for the group consuming whole bread; 1625.35 ± 229.1 kcal/day and 1163.87 ± 157.23 kcal/day at the end of the study for the group consuming wheat bread. There was a statistically important decline on average intake level of energy in both groups, but differences in this decline in both groups were not statistically important ($p = 0.862$). Similarly, at the beginning of the study, the amount of carbohydrate intake was 199.7 ± 35.37 g/day for the whole bread group, 200.15 ± 43.98 g/day for the whole bread group, and 200.15 ± 43.98 g/day for wheat bread group. Differences in decline in both groups were not statistically important ($p = 0.729$). There was an overall decline on the average intake levels of protein (g) and fiber (g) in both groups, with no significant differences in the decline amounts between two groups ($p > 0.05$).

Discussion

Bread and wheat products are an indispensable part of Turkish diet (Türkiye Beslenme ve Sağlık Araştırması—TBSA, 2010). The average daily energy intake comes 58% from bread and wheat products, while bread alone being 44% [7].

Table 3 Differences in biochemical parameters

Biochemical parameters		Whole grain bread ($n = 12$) $\overline{X} \pm$ SS (median)	Wheat bread ($n = 12$) $\overline{X} \pm$ SS (median)	[d]p value
Glucose (mg/dL)	Baseline	113.58 ± 33.96 (103.5)	128.75 ± 32.26 (117.5)	*0.166*
	Week 6	115.42 ± 20.75 (112)	120.17 ± 27.95 (118.5)	*0.506*
[e]p value		*0.964*	*0.154*	
Δ Glucose		-1.83 ± 20.26	-8.58 ± 25.96	*0.298*
Insulin (mIU/mL)	Baseline	11.49 ± 3.93 (10.5)	16.82 ± 12.45 (12.2)	*0.419*
	Week 6	9.83 ± 3.08 (9.5)	15.23 ± 12.22 (10.8)	*0.402*
[e]p value		*0.182*	*0.012**	
Δ Insulin		-1.66 ± 3.88	-1.58 ± 1.66	*0.931*
HOMA-IR	Baseline	3.43 ± 2.13 (2.80)	5.42 ± 4.89 (3.70)	*0.174*
	Week 6	2.85 ± 1.34 (2.46)	4.39 ± 3.52 (3.25)	*0.141*
[e]p value		*0.239*	*0.041**	
Δ HOMA-IR		-0.594 ± 1.47 (0.61)	-1.03 ± 2.01 (0.70)	*0.795*
LDL chol (mg/dL)	Baseline	116.25 ± 35.05 (106)	113.17 ± 49.43 (115)	*0.954*
	Week 6	109.33 ± 19.1 (108.5)	112.48 ± 33.5 (112)	*0.954*
[e]p value		0.695	0.424	
Δ *LDL*		-6.91 ± 32.82	-0.68 ± 60.74	*0.624*
Triglycerides (mg/dL)	Baseline	120.33 ± 49.86 (118)	198.75 ± 132.57 (180.5)	*0.088*
	Week 6	120.83 ± 68.06 (90.5)	148 ± 67.46 (128)	*0.112*
[e]p value		*0.906*	*0.142*	
Δ Triglycerides		0.50 ± 62.68	-50.75 ± 120.24	*0.470*
Cholesterol (mg/dL)	Baseline	183.92 ± 38.49 (171)	194.5 ± 45.29 (179.5)	*0.729*
	Week 6	161.58 ± 42.34 (172.5)	178 ± 36.2 (176.5)	*0.544*
[e]p value		*0.197*	*0.230*	
Δ Cholesterol		-22.33 ± 45.47	-16.50 ± 46.20	*0.977*

*$p < 0.05$; **$p < 0.01$

[d] Mann-Whitney U test

[e] Wilcoxon signed-rank test

Table 4 Daily nutritional profile at baseline and end of study

Daily dietary nutrients		Whole grain bread ($n = 12$) $\overline{X} \pm$ SS (median)	Wheat bread ($n = 12$) $\overline{X} \pm$ SS (median)	[d]p value
Energy (kcal/d)	Baseline	1763.47 ± 251.8 (1727.6)	1625.35 ± 229.14 (1678.1)	*0.248*
	Week 6	1317.63 ± 198.52 (1309.9)	1163.87 ± 157.23 (1161.6)	*0.065*
[e]p value		*0.006***	*0.004***	
Δ Energy		− 445.84 ± 349.81	− 461.48 ± 319.18	*0.862*
Protein (g)	Baseline	71.25 ± 14.95 (69.5)	64.29 ± 11.87 (67.7)	*0.419*
	Week 6	70.16 ± 15.17 (70.7)	63.96 ± 13.25 (60.5)	*0.106*
[e]p value		*0.583*	*0.638*	
Δ Protein		− 1.09 ± 23.31	− 0.33 ± 23.18	*0.773*
Carbohydrate (g)	Baseline	199.7 ± 35.37 (212.2)	200.15 ± 43.98 (215)	*0.773*
	Week 6	83.48 ± 12.41 (83.8)	89.45 ± 16.83 (89.8)	*0.356*
[e]p value		*0.002***	*0.002***	
Δ Carbohydrate		−116.21 ± 40.69	−110.69 ± 44.82	*0.729*
Fiber (g)	Baseline	21.56 ± 3.45 (21.5)	18.42 ± 3.7 (19.4)	*0.073*
	Week 6	12.48 ± 2.32 (12.5)	13.25 ± 4.73 (12.2)	*0.862*
[e]p value		*0.002***	*0.034***	
Δ Fiber		−9.07 ± 3.69	−5.17 ± 6.48	*0.050**
Fat (g)	Baseline	73.57 ± 17.66 (72)	60.93 ± 6.98 (59.9)	*0.065*
	Week 6	72.4 ± 15.93 (69)	63.38 ± 8.38 (63.9)	*0.166*
[e]p value		*0.937*	*0.433*	
Δ Fat		*− 1.11 ± 20.55*	*2.42 ± 10.13*	*0.686*

*$p < 0.05$

[d] Mann-Whitney U test

[e] Wilcoxon signed-rank test

The American Diabetes Association (ADA) 2016 position paper also recommends a low glycemic index food diet to all individuals with type 2 diabetes. The Turkish Endocrinology and Metabolism Foundation recommends staying away from foods that have high glycemic index and increase insulin secretion [8]. However, according to the Turkish Nutrition and Health Survey (TNHS), consumption of wheat bread is significantly lower than white bread. TNHS data further indicates that 71.4% of the individuals do not prefer wheat bread.

This is the first study comparing the effects of consuming two different glycemic index bread types with different fat content (whole grain bread with higher fat content vs. whole wheat flour bread with lower fat content) and measuring their impact on anthropometric measurements and biochemical blood parameters at the end of 4 weeks' term on patients with type 2 diabetes.

In literature, there is an impact of diets with low glycemic index on weight loss [9] and maintenance [10]. A research which was conducted on obese individuals assessed the effects of consuming hypocaloric diets with low or high glycemic index diet for 6 weeks. There were no differences between groups in terms of losing weight [11]. Similarly, some researchers observed a positive correlation between BMI and glycemic index [12, 13]. There are also studies showing that this relationship does not exist [14, 15]. In another study of 979 healthy and impaired glucose tolerance individuals obtained from the Insulin Resistance Atherosclerosis Study reports, no relationship was found between glycemic index and BMI or waist circumference [14]. Another study showed that a 1-unit decrease in daily glycemic index value resulted in a 0.12-cm decrease in waist circumference. This data is clinically important because of the fact that the waist circumference is a good indicator of abdominal fat and the risk factor of cardiometabolic disease [16]. In this study, body weight, BMI, and waist circumference decreased statistically significantly after 4 weeks in the group who consume whole grain bread. The weight loss that occurred in this study after 4 weeks in both groups was the result of the planned hypocaloric diet. In this study, a positive correlation was found between the percentage change in glycemic index and waist circumference ($p < 0.05$).

Low glycemic index diets are usually achieved by the administration of high-fiber foods [17]. In a meta-analysis conducted, the effect of using legumes as part of a low glycemic-indexed diet was examined for metabolic control. Consuming legumes as a component of a low glycemic index resulted in a

Table 5 Daily glycemic index values of individuals at baseline and end of study

Glycemic index	Whole grain bread ($n = 12$) $\overline{X} \pm$ SS (median)	Wheat bread ($n = 12$) $\overline{X} \pm$ SS (median)	[d]p
Baseline	58.55 ± 6.93 (60.3)	59.81 ± 3.03 (59.96)	*0.686*
Week 6	38.42 ± 3.03 (38.07)	46.69 ± 2.95 (47.18)	*0.001***
[e]p	*0.002***	*0.002***	
Δ GI %	*33.45 ± 9.72*	*21.79 ± 5.97*	*0.008***

*$p < 0.05$; **$p < 0.01$

[d] Mann-Whitney U test

[e] Wilcoxon signed-rank test

0.5% decrease in HbA1c [18]. In two cohort studies, intake of cereal fiber (dT2 29-31) also showed a decrease in incidence of type 2 diabetes and the cardiovascular events. In a study conducted in China, wild rice, known to have a low glycemic index, was given to eight individuals for 8 weeks. As a result, fasting blood glucose, fasting insulin, glucagon, and HOMA index decreased statistically in individuals consuming wild rice when compared to a group fed with rice flour [19] (Tables 5 and 6).

In this study, consumption of whole grains with low glycemic index for 4 weeks had no positive effect on fasting blood glucose, fasting insulin, and HOMA-IR index. However, a decrease in serum insulin and HOMA-IR levels of individuals consuming wheat bread was statistically significant ($p < 0.05$). We believe that the disparity between the two groups is due to the differences of fiber intake. Dietary fiber can affect carbohydrate metabolism. Insoluble fiber increases insulin sensitivity, but through which mechanisms are not yet clear. Soluble and insoluble fiber may have a role on regulation of hormones such as glucose-dependent insulin tropic peptide and glucagon-like peptide-1. This stimulates postprandial insulin secretion, increases glucose tolerance, and delays gastric emptying [20].

Increase in postprandial glucose and insulin levels creates a more atherogenic environment through a decrease in protein glycation and oxidative damage related to free radical generation resulting from the rise and fall in blood-borne fuels, glucose, and free fatty acids [21]. The effect of glycemic index on cholesterol is debatable. In randomized studies conducted on overweight and obese women and men, diets with low glycemic index have found to have no effect on LDL cholesterol, HDL cholesterol, and total cholesterol [22–24]. A study which evaluates the effect of low glycemic index on cardiovascular risk factors on 28 randomized controlled research shows that low glycemic index decreases total cholesterol and LDL cholesterol levels independent from weight loss [5]. In this study, the difference of LDL cholesterol and total cholesterol drop levels between wheat bread and whole grain bread is statistically not significant ($p = 0.624$; $p = 0.544$; $p > 0.05$ respectively).

This study contains various limitations. The breads were directly provided to all patients through the researcher and the amounts to be consumed were specified; however, the consumption was recorded by the patients and may not show exact accuracy. Many studies show that obese patients have the tendency to underreport their food consumption compared to normal-weight individuals. At the end of the 30-day intervention, patient's carbohydrate intake dropped significantly below the ADA carbohydrate intake benchmark. No detailed study was conducted on the factors causing the decline. The appetite trend of the patients was not taken into consideration. Furthermore, factors that may impact glycemic index such as chewing pace and water intake during meals were not taken into account.

Table 6 Composition of the breads

	Whole grain bread	Wheat bread
Energy (kcal)	270	227
Carbohydrate (g)	41.23	41.14
Protein (g)	8.09	7.92
Fat (g)	6.45	1.72
Fiber (g)	35.32	7.39
Glycemic index	39.6	58.6

Conclusions

Consumption of whole grain bread has more positive impact on anthropometric measurement and wheat bread has impact on insulin levels. Further studies are needed to confirm these effects of using these breads with different glycemic index on anthropometric and cardiometabolic risk factors in individuals with type 2 diabetes.

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Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study and the study received the approval of the Committee on Human Research of Acibadem University code of ethics 215-7/12 (Istanbul, Turkey).

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Effect of high-fat, low-carbohydrate enteral formula versus standard enteral formula in hyperglycemic critically ill patients: a randomized clinical trial

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Abstract
High-fat, low-carbohydrate diets may attenuate the hyperglycemia in critically ill patients. This study was performed to compare the effects of high-fat, low-carbohydrate enteral nutrition on glycemic control and clinical outcomes in new identified hyperglycemic patients. Eighty-eight new identified hyperglycemic patients with no history of diabetes or diagnosed hyperglycemia were randomly allocated to a standard (protein 20%, fat 30%, and carbohydrate 50%) or high-fat (protein 20%, fat as equal amount of olive and sunflower 45%, and carbohydrate 35%) kitchen formulas. Duration of intervention was 15 days. Baseline characteristics of patients were the same. Forty-one patients in the high fat and 39 patients in the standard group completed the study. Mean blood glucose, mean infused insulin, final blood glucose, and final infused insulin were not differed significantly between groups. Repeated measure showed that the average blood glucose declined in the high-fat formula group, as well as standard one, but with more decline at the end of the first week, and with a higher rate in the high-fat formula group. The mean blood triglyceride level on the final day was lower in the high-fat formula group ($p = 0.001$). There were no significant differences between groups in clinical outcomes. Although the high-fat formula declined blood glucose and triglyceride levels more than the standard group, yet the decrease in blood glucose was not significant. Also, it has no significant effect on clinical outcomes.

Keywords Olive · Lipid · ICU · Ventilator

Introduction

Hyperglycemia and glucose intolerance are common occurrences that occur in patients admitted to the intensive care unit via increased hepatic glucose production and reduced its consumption by peripheral tissues [1, 2]. It has been reported that hyperglycemia can be observed in 32–38% of critically ill patients with or without diabetes mellitus history [1, 3, 4]. Excessive fluctuation in blood glucose levels in ICU or inappropriate medical interventions can have detrimental effects on the patient's survival and outcomes, especially in non-diabetic cases [5, 6]. The prevalence of hyperglycemia in patients receiving nutritional support is much higher than other patients [1]. Its occurrence in patients with enteral nutrition (EN) in non-diabetic form has been reported about 12% [4, 7], although EN in comparison with parenteral nutrition (PN) can yield less hyperglycemia and infection episodes in consequence [8]. Studies about the EN formulas have demonstrated that liquid formulas can produce faster gastric emptying and absorption, higher serum glucose levels, and greater insulin needs than the solid-food diets. Enteral formulas can differ significantly with respect to their caloric content, osmolarity, and nutrient composition, so this can yield different hyperglycemic effect [9]. It has been proposed that an enteral nutritional formula, containing reduced carbohydrate, high concentration of fat, provides 35–45% of daily dietary energy from carbohydrate, 45–55% from fat, and 15–20% from protein,

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to attenuate postprandial blood glucose level in comparison with a standard formula containing about 55–65% of daily dietary energy from carbohydrate, 25–30% from fat, and 15–20% from protein [10]. Some studies have shown the preventive effect of the high-fat formulas, especially formulas containing olive oil without added fiber or antioxidants on glycemic control and lipid profile [11]. Because of the low number of studies [6, 12–15] with such formulas in the intensive care unit and their controversial results, there is not a consensus about their use. American Society of Parenteral and Enteral Nutrition has no recommendation at this time and practical application of using such formulas take grade: further research needed, in guidelines [16]. We therefore, conducted this study to compare the influence of fat-based enteral nutrition in comparison with glucose-based ones on the serum blood glucose levels and clinical outcomes in hyperglycemic critically ill patients.

Materials and methods

This prospective, randomized, controlled double-blind clinical trial was approved by Shahid Beheshti University of Medical Sciences ethical committee. Patients were recruited from six different ICU wards during October 2015 to September 2016. Eighty-eight new identified hyperglycemic patients who have inclusion criteria for the study were included. Inclusion criteria were the lack of past diagnosed diabetes history, age range between 18 and 65 years old, to be on enteral feeding (nasogastric, orogastric, and gastrostomy tube feeding), feeding initiation through of the first 48–72 h after hospitalization, receiving it at least for five consecutive days, and normal functioning of gastrointestinal tract. Excluded ones were liver and kidney failure, nephrotic syndrome, hyperlipidemia, obesity, participation in another interfering intervention study, receiving blood glucose elevating medications, and death or discharges earlier than 5 days of enteral feeding**.** Written, informed consent form was obtained from each patient or his/her legal guardian before the study enrollment. Patients received detailed information about the aim, risk, and the short summary of the study. Each patient had the right for withdrawing their consent, and to exit the study. Patients were divided into two groups. Randomization was performed according to a computer-generated random number table. The intervention group received a hospital handmade formula, 35% from carbohydrate (CHO), 45% from fat (equal amount of olive and sunflower oil), and 20% from protein, providing 1 kcal/cc, while non-intervention group received a hospital handmade isocaloric formula, 50% from CHO, 30% from fat, and 20% from protein for 15 days of intervention or up to the time of their discharge, death, or EN discontinuation. Formulas were prepared in the same bottles with the same size and appearance. Calorie needs were calculated as 25–30 kcal/kg for each patient. The amount of calorie provision was done according to patient status and attending physician order as a percent of calculated requirement. Administration of formula was done by the intermittent method. Feeding was done over 24 h with each two to three intervals and only interrupted for 6 h during the night. Capillary blood glucose measurements in all patients were done using the same glucometer (ACCU-CHECK; Roche diagnostics, Mannheim, Germany) for all patients. Blood glucose was measured every 6 h during the day. Patients received insulin according to protocol of insulin, if blood glucose level was higher than 200 mg/dL. Insulin was administered by continuous infusion in saline solution with a 1:1 ratio using a syringe pump. Each patient was evaluated daily for cardiovascular failure, central nervous system failure, coagulation failure, hepatic failure, and renal failure. The mean SOFA score was used to determine the extent of the patient organ function. To evaluate the number of days that patients were in ICU, and the number of ventilator-dependent days, we calculated the number of ICU-free days and ventilator-free days, as early mortality could result in skewed data. Gastrointestinal complications including high gastric residual volumes, diarrhea, vomiting, regurgitation, abdominal distension, constipation, and pulmonary aspiration were recorded every day. Lipid profiles (triglyceride, LDL, HDL, and total cholesterol) were measured by commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran) at first and 10th day of the intervention at 6 a.m. before feeding.

Statistical analysis Qualitative variables were presented by the frequencies and percentages, and continuous variables were presented by the mean ± standard deviation. The χ^2 test or alternatively Fisher's exact test was used to analyze the qualitative variables. Continuous data were assessed for normality by Kolmogorov-Smirnov test. The comparison was done with the independent sample's *t* test for normal data and comparing two independent variables and the Mann–Whitney test for non-parametric variables and comparing two independent variables. The paired *t* test was used to test the difference between the before and after means of blood glucose and infused insulin for each formula within the groups. Wilcoxon test was used to test the difference between the before and after medians of non-parametric variables. For detecting the exact effect of each formula on the level of hyperglycemia, another further analysis (ANCOVA) was recruited. In that analysis, adjustment based on the covariates such as age, Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II score, first blood glucose, and first infused insulin dose was done. For the main outcome measures, a repeated measure test was used for considering measurements taken at different time points within a group. Statistical significance was set at $p < 0.05$. The statistical power of 90% was used for this study. All analyses were performed using SPSS, Version 21.0.

Results

A total of 88 subjects were randomized into two groups: intervention (high-fat formula) and control (standard formula) group. Three patients in the high-fat formula group and five patients in the standard formula group did not achieve the minimum 5 days of receiving enteral nutrition. Finally, 41 patients in the high-fat group and 39 subjects in the standard group completed the study. Disposition of patients throughout the study is shown in Fig. 1.

The groups were homogenous and well matched. Baseline characteristics were similar with no statistically significant differences between them (Table 1).

The baseline blood glucose was not significantly different between the two groups ($p = 0.65$). Table 2 shows the data of outcomes during different phases of the study. The mean blood glucose level in both groups reduced during the study period. The average reduction was 66.75 mg/dL for the high-fat group and 51.74 mg/dL for the standard group. Paired *t* test showed significant difference within groups for comparing the blood glucose levels before and after the intervention in both groups (for both; $p < 0.001$). Although the reduction in the blood glucose level was higher in the high-fat formula group, but, statistical analysis showed no significant difference between two groups based on the final blood glucose ($p = 0.35$). Such similar result was observed for the infused insulin on final day ($p = 0.18$) (Table 2).

For detecting the exact effect of each formula on the level of hyperglycemia, another further analysis (ANCOVA) was performed. In that analysis, adjustment based on age, APACHE, first blood glucose, and first infused insulin dose was done. Again, results showed that the effect of different formulas on the glycemia status was not significant ($R^2 = 0.023$, $p = 0.33$).

Before and after the study, serum levels of blood lipids in both groups were measured. As it has been presented in the Table 3, there were no significant differences at the start and final day in the levels of lipids (cholesterol, triglycerides, HDL, and LDL) between the two groups except for final triglyceride (TG). The mean blood TG level on the final day was lower in the high-fat formula group in comparison with the standard one ($p = 0.001$).

Repeated measure test was used to compare the blood glucose measurements on different days (within 15 days). Data showed that the average blood glucose in the high-fat formula group, as well as standard formula group, consistently declined. This decline was more at the end of the first week, but with a higher reduction rate in high-fat formula than the standard group. In the high-fat group, mean blood glucose on the first day showed a significant difference in comparison with all other study days, except the second day. The average blood glucose level in the second day showed a significant difference with all other days except for the first and third day of the intervention. In the standard group, the average blood glucose on the first day just did not show significant difference with the mean blood glucose in days 5 and 7, while it had significant difference with all other days (Fig. 2).

In the same manner, repeated measures for comparing the mean infused insulin on different days (over 15 days) showed that the average infused insulin in the high-fat formula group, as well as the standard formula group, consistently declined. This decline was always more in the high-fat formula group than the standard group (Fig. 3).

Clinical outcomes are shown in Table 4. There were no statistically significant differences in the SOFA score between the two groups. The overall in ICU mortality was 12.5% ($n = 10$) with four deaths in the standard formula group and six deaths in the high-fat group.

At the end of study, in the high-fat formula group, 87.8% (36 patients) were ventilated, while in the standard group, 84.6% (33) were ventilator dependent. The results of analysis for comparing the patients based on the median days of ICU-free days and ventilator-free days showed no significant difference between the two groups. The proportion and an absolute number of subjects that were experienced, gastrointestinal complications were recorded which was not significant between two groups.

Discussion

The results of present study showed that the use of high MUFA low-carbohydrate enteral formula in new identified hyperglycemic critically ill patients was well tolerated and has insignificant improvement on carbohydrate metabolism and a significant decrease on serum TG levels compared with a control standard diet. Various studies have compared the benefits of different methods for controlling blood glucose, including dietary modifications. In comparative studies such as the de Azevedo et al. [12] and Mesejo et al. [17], like the present study, the effect of different formulas on the new identified hyperglycemic critically ill patients has been studied. In Nourmohammadi et al. study [18], the preventive effect of two types of high-fat formulas was evaluated in normoglycemic critically ill patients. In Wewalka et al. [19] and Tomoyoshi Mohri et al. [20] studies, a population of diabetic and newly diagnosed hyperglycemic critically ill patients have been studied. Other studies have been performed on type II diabetic patients [21–24].

Blood glucose and insulin use

In this study, the mean blood glucose level was higher in the standard formula group compared to the high-fat group during the all times except within the first days of the study, but with no significant difference. As shown in Fig. 2, after a 7-day

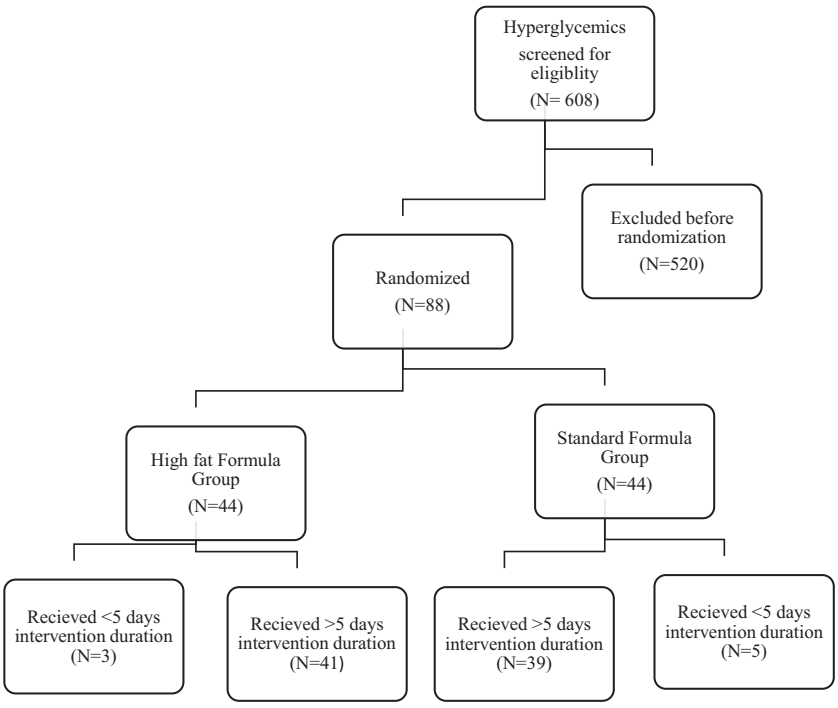

Fig. 1 Flow diagram of patients throughout the study

interval, the difference in blood glucose levels within the two groups has reached a significant level. In the final days of the intervention, the difference between the blood glucose level of the two groups has been reached to the near of each other. Partly, it may be related to the patient's passage from the catabolic phase and the collapse of inflammatory reactions

Table 1 Baseline subjects characteristics

Variables	Subgroups	Measure	High-fat formula group ($n = 41$)	Standard formula group ($n = 39$)	p value
Age (years)		M (Q_1–Q_3)	61 (53–64)	62 (57–64)	0.39[a]
Sex	Male	n (%)	24 (58.5)	16 (41)	0.90[b]
	Female	n (%)	17 (41.5)	23 (59)	
APACHI II Score		Mean ± SD	17.12 ± 4.32	15.66 ± 5.27	0.18[c]
First day SOFA Sore		Mean ± SD	7.95 ± 2.51	7.51 ± 1.87	0.37[c]
Ventilator dependency		n (%)	33 (84.6)	36 (87.8)	0.46[b]
Diseases	Medical	n (%)	21 (51.2)	26 (66.7)	0.18[b]
	Surgery	n (%)	8 (19.5)	7 (17)	
	Trauma	n (%)	4 (5.8)	0 (0)	
	Cancer	n (%)	8 (19.5)	6 (15.4)	
Albumin (mg/dL)		Mean ± SD	2.92 ± 0.69	2.97 ± 0.61	0.75[c]
Feeding route (%)	NGT	n (%)	36 (87.8)	34 (87.2)	0.16[b]
	OGT	n (%)	1 (2.4)	4 (10.3)	
	PEG	n (%)	4 (9.8)	1 (2.6)	
Energy requirement (kcal/day)		Mean ± SD	1807.95 ± 359.04	1664.6 ± 217.08	0.03[c]
Mean energy intake (kcal/day)		Mean ± SD	1726 ± 416	1593 ± 468.50	0.18[c]
Volume ratio (%)		Mean ± SD	99.21 ± 30.88	97.49 ± 31.50	0.8[c]

APACHE Acute Physiologic Assessment and Chronic Health Evaluation, *SOFA* Sequential Organ Failure Assessment, *NGT* nasogastric tube, *OGT* orogastric tube, *PEG* percutaneous endoscopic gastrostomy

[a] Mann-Whitney test

[b] Chi-square test

[c] Independent sample t test

Table 2 Results for comparing the blood glucose and infused insulin between the two groups before and after the intervention

Variable	Group	Before (mean ± SD)	After (mean ± SD)	*p* value
Blood glucose (mg/dL)	HFF group	226.73 ± 28.67	159.97 ± 49.99	< 0.001[b]
	SF group	222.92 ± 45.65	171.17 ± 56.87	< 0.001[b]
p value		0.65[a]	0.35[a]	
Infused Insulin (IU/day)	HFF group	2 (0–6)	0 (0–6)	0.007[c]
	SF group	6 (0–12)	0 (0–8)	0.04[c]
p value		0.35[d]	0.18[d]	

HFF high-fat formula, *SF* standard formula

[a] Indepedant sample *t* test

[b] Paired *t* test

[c] Mann-Whitney

[d] Wilcoxon

that has resulted in approaching to normalization of the blood glucose. Also, it can be possible that the effects of various formulations along with the blood glucose control protocol may be effective over a short period of time and have no longer ability to make more modifications.

The studies by Nourmohammadi et al. [18] and Mori et al. [6] have reported similar results regarding the effect of high-fat formulas on the level of blood glucose. In both studies, there have been observed no significant effects on the mean capillary and blood glucose levels. In the Nourmohammadi et al. study, the number of subjects and the type of patients have been differed from ours, while the duration of the intervention and the type of the used formula have been similar to the our applied formulas. They have compared the effects of three different types of formulas, including a high-fat olive oil based, a high-fat sunflower oil-based, and a standard formula one on the mean capillary and blood glucose levels. In Mesejo et al.'s study, patients who received high-fat formula had a significant reduction in plasma glucose levels, capillary glucose levels, and insulin requirements in comparison to patients on a conventional high-protein diet [17]. In their study, fiber intake and type of carbohydrate were different between two groups, and this difference might affect the results. de Azvedo et al. have studied a carbohydrate restrictive formula on 169 critically ill patients versus a standard formula for 168 patients for 2 weeks and found that at the end of the intervention, mean blood glucose level was significantly lower in the carbohydrate restrictive group compared to the control group [12]. In Tomoyoshi Mohri et al.'s study which was conducted on 147 traumatic mechanically ventilated

Table 3 Results for comparing the baseline and final values of blood lipids between two groups

Variable	Group	Before Mean ± SD	After Mean ± SD	*p* value[b]
Cholesterol (mg/dL)	HFF group	137.18 ± 52.42	144.71 ± 39.70	0.02
	SF group	125.23 ± 27.84	142.29 ± 23.23	0.05
p value[a]		0.34	0.84	
Triglyceride (mg/dL)	HFF group	144.59 ± 69.93	134.64 ± 37.02	0.46
	SF group	167.43 ± 69.63	196.00 ± 48.00	0.85
p value[a]		0.26	0.001	
LDL (mg/dL)	HFF group	109.90 ± 34.50	112.71 ± 43.00	0.98
	SF group	109.12 ± 34.78	115.06 ± 36.68	0.72
p value[a]		0.93	0.87	
HDL (mg/dL)	HFF group	39.8 ± 6.16	42.71 ± 2.51	0.61
	SF group	39.69 ± 5.76	40.09 ± 4.90	0.9
p value[a]		0.93	0.17	

HFF high-fat formula, *SF* standard formula

[a] Indepedant sample *t* test

[b] Paired *t* test

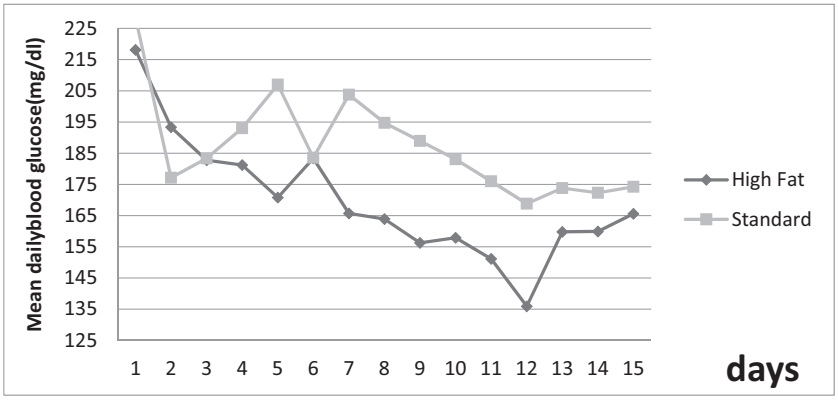

Fig. 2 Comparing the mean daily blood glucose between two groups during the different days of the study (repeated measures)

critically ill patients, low-carbohydrate, high-fat (monounsaturated fatty acid) enteral formula was more effective for glycemic control in critically ill patients compared with a standard enteral formula [20].

In our study, in parallel with the observed effects of formulas on the blood glucose level, similar effects on the insulin requirement were observed. The mean consumed insulin units at the start of the study was 5.34 ± 7.6 units/day in the high-fat formula group and 9.14 ± 6.82 units/day in the standard formula group. At the end of the intervention, for the high-fat group, it was 6.03 ± 3.21, and for the standard group was 6.21 ± 4.51 units/day, respectively. As we can see, following the use of high-fat formula in the corresponding group, an insulin reduction has been concurrent with a parallel decrease in blood glucose levels (66.75 ± 49.46). With the use of high-fat formula, insulin consumption was reduced as much as 1.73 ± 4.15 units/day, averagely. In the standard group, the use of the corresponding formula induced 51.74 ± 72. Eighty-seven milligrams/day reduction in blood glucose averagely yielded a reduction as much as 2.46 ± 7.46 units/day in insulin infusion need. The higher reduction in insulin consumption in the standard group rather than the high-fat group may be partly due to the higher levels of initial insulin infusion in the standard group (6.82 vs. 5.34). It means that in the standard group, the variation range has been wider.

The results from Nourmohammadi et al. [18] study coincided with the findings of the present study, which has showed that in the high-fat group, insulin need has been lower. In de Azvedo et al. [12] and Mesejo et al. [17] study, similar but significant changes in the need for insulin following the use of different formulas have been reported. In Wewalka et al. [19] study, irregular and non-significant changes in the mean blood glucose have been accompanied by similar and parallel changes in the amount of infused insulin.

Lipid profile

There are several arguments about the benefits of replacing some part of carbohydrate with fat and preparing high-fat formulations. Increasing the fat content of formula via reduction in the amount of carbohydrate intake in one hand, and partly because of slowing the gastrointestinal tract and slowly absorbing sugars in the diet in other hand, can help to improve the blood glucose control. In addition, several studies have discussed the effect of fatty acid types on the pattern of insulin secretion, insulin resistance, and cellular signaling [1, 11, 22, 25]. However, some studies have not recommended the use of high-fat formulas due to the possibility of lipid profile alterations and diarrhea occurrences [24, 25]. At the same time, some other studies have acknowledged that MUFA containing

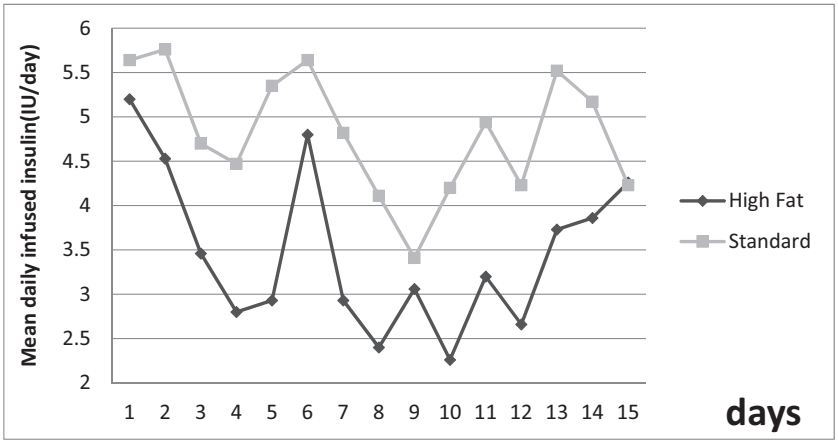

Fig. 3 Comparing the mean daily infused insulin between two groups during the different days of the study (repeated measures)

Table 4 Clinical outcomes in study groups

Variable	Measure	HFF group ($n = 41$)	SF group ($n = 39$)	p value
Mean SOFA	Mean ± SD	7.66 ± 2.39	6.91 ± 1.59	0.1[a]
ICU free days	M (Q_1–Q_2)	0 (0–0)	0 (0–1)	0.11[b]
Ventilator dependency	n (%)	35 (85.4)	34 (87.2)	0.81[c]
Sepsis	n (%)	0 (0)	1 (2.6)	0.48[d]
Bedsore	n (%)	5 (12.2)	6 (15.4)	0.46[c]
Death	n (%)	6 (14.6)	4 (10.2)	0. 51[c]
GI complications	n (%)	5 (14.6)	3 (7.7)	0.32[c]

HFF high-fat formula, *SF* standard formula, *BG* blood glucose, *ICU* intensive care unit, *LOS* length of stay

[a] Independent t test

[b] Wilcoxon

[c] Chi-square test

[d] Fisher exact test

formulas can be expected to increase HDL and reduce other lipid profile components [18, 26, 27]. Another proposed benefit for replacing some percentage of carbohydrate with fat is to help increase energy intake and improve nutritional status, especially in malnourished patients [24]. Recently, ideas have been put forward to replace some part of the fat with MUFA in such formulas. In the present study, the only significant difference that was seen based on the effects of two types of formulas on the lipid profile was the effect of high-fat MUFA containing formula on the reduction of TG. However, in some other studies, the different effects have been reported. For example, in Nourmohammadi et al. [18] study, it has been reported that high-fat MUFA-rich formulations (50% from MUFA) have been able to increase HDL levels in comparison with the standard formula. In the Mesejo et al. [17] study, it has been shown that triglyceride tends to decrease with the use of diabetes specific formulas. In Vaisman et al. [23] study, it has been pointed out that in high-fat group, HDL level has increased, while in standard group, it has decreased. There has been reported no other significant effect on the other components of the lipid profile among the groups.

Clinical outcomes

In this study, the duration of ICU stay was insignificantly longer in the fat-based formula group. The higher APACHE score in the fat-based formula group at admission may be the reason for this result. In the Nourmohammadi et al. study, the duration of ICU stay was significantly lower in the MUFA containing high-fat formula group compared to sunflower-based high-fat group, and carbohydrate-based formula group which may be contributed to antioxidant and anti-inflammatory components that can reduce inflammation in critically ill patient.

Mortality rate did not differ between two groups in our study. This result is consistent with the findings from the Mesejo et al. [17], Nourmohammadi et al. [18], Wewalka et al. [19] and Tomoyoshi Mohri et al. [20].

Furthermore, the comparison between the two groups based on the ventilator dependency did not show a significant difference at the end of the intervention. In Wewalka et al. [19], Mesejo et al. [17], and Tomoyoshi Mohri et al. [20] studies, all included ventilator-dependent patients at baseline. In Wewalka et al. study, no results about the mean duration of dependence on ventilator have been reported, but in two other studies, there was no difference in duration of mechanical ventilation between the two groups.

Gastrointestinal complications including dyspepsia, diarrhea, nausea, vomiting, high gastric residue volume, gastritis, bacterial colonization, regurgitation, and abdominal distension have been reported in several studies as most common occurrences during high-fat formula feedings. In our study, high-fat formula was well tolerated. Also, in the mentioned similar studies, no significant difference was seen with regard to gastrointestinal complications between the groups. Thus, it seems that intensive caregivers can take such formulas into consideration for such patients without any serious concerns about the occurrence of such complications.

Conclusion

Finally, it can be concluded from this study that using high-fat MUFA containing formula in comparison with standard CHO formula can produce some improvements in blood glucose level and lipid profile, but yet for verification of applying such formulas, designing studies with different fat and CHO composition in different fields and different forms (non-industrial) with a longer intervention duration will be needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of informed consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Determinants of self-monitoring of blood glucose with type 2 diabetes based on 496 questionnaire surveys in China

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Abstract
The purpose of the study was to investigate the current status quo of self-monitoring of blood glucose (SMBG) levels and to analyze the relationship between demographics and other characteristics of SMBG among people with type 2 diabetes (T2D) in China. In this multicenter, cross-sectional study, 496 individuals with T2D from five provinces across China voluntarily participated in the study and completed a standardized questionnaire that requested information on demographic data, clinic-related information, and glucose monitoring. Data was obtained via face-to-face interviews. Negative binomial regression analysis was used to identify factors associated with SMBG. Of the total sample, 99 (20.0%) participants had never performed SMBG and/or less than once. We found that 104 participants (21.0%) had tested just once. In addition 119 people (24.0%) had tested twice, 89 (18.0%) had tested between three and six times, and 85 participants (17.1%) had tested more than seven times in a week. Univariate analysis and multivariate analysis found that the patients that had high monitoring frequencies were those with URBMI, UEBMI, and GMI insurance; those who had previously experienced complications; inpatients; and those with a knowledge of HbA1c testing. The frequency of SMBG was suboptimal, compared with the once prior to each meal recommendation by the American Diabetes Association (ADA). Several factors influenced SMBG frequency: types of health insurance, complications, impatience, and knowledge of HbA1c.

Keywords Type 2 diabetes · Self-monitoring of blood glucose · Continuous glucose monitoring

Introduction

Diabetes is one of the major risk factors of high morbidity and mortality worldwide [1]. The development of this chronic condition severely reduces the quality of life for sufferers [2]. According to the latest statistics from the International Diabetes Federation (IDF), the prevalence of diabetes among adults aged 20–79 was 6.7% [3]. In 2015, the number of diabetes patients reached 418 million worldwide and is expected to rise to approximately 642 million by 2040. The new patients are mainly from China, Southeast Asia, Africa, and other developing countries. With a projected 109.6 million cases of adult diabetes, China is set to rank first in the world [4]. Today, type 2 diabetes (T2D) is a considerable public health and economic burden in China [5].

International diabetes organizations such as the IDF [6], the American Diabetes Association (ADA) [7], and the Chinese Diabetes Society (CDS) [8] each emphasizes self-management tools such as self-monitoring of blood glucose (SMBG) as a primary means of treating and controlling diabetes and its complications. SMBG provides the individual with immediate information on their current blood glucose status that can be used to adjust the self-care regimen and achieve the glycemic control necessary to prevent or delay diabetes-related complications [9]. SMBG is an important component of modern therapy for diabetes, thus ensuring the effectiveness of diet, exercise, and drug therapy that is tailored to meet the needs of the patient and varies depending on the treatment the patient is receiving [10, 11].

Numerous studies have demonstrated that effective SMBG can reduce the risk of medical complications and play an effective role in improving quality of care and clinical outcomes [12, 13].

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Despite the potential health benefits associated with SMBG in the management of diabetes, there is a high variability on the daily frequency of self-monitoring between patients but many diabetics do not perform it regularly and the adherence to blood glucose monitoring is suboptimal in general [14–17]. Studies have reported numerous factors to be associated with SMBG and frequency of testing: demographic data (for example, socioeconomic status, age, racial, and insurance coverage), clinic-related information (for example, diabetes type and duration, history of chronic respiratory disease, and HbA1c level), and medical service utilization (for example, medication regimen, frequency of hospitalization, healthcare provider recommendations, and environmental barriers) [18–22].

At present, the effect on glycemic control in patients with SMBG, SMBG status quo, and its factors have been relatively well studied in the countries previously mentioned above. In China, however, there is a paucity of research relating to the extent to which SMBG is used and factors influencing its use. The purpose of this cross-sectional study was to assess the frequency of SMBG and to identify patient characteristics that are associated with SMBG in patients with T2D in China and to provide theoretical basis and relevant support for the patients' management of diabetes.

Methods

Survey design

A multicenter, cross-sectional survey was conducted at general hospitals in the capital cities of five provinces (Beijing, Jiangsu, Heilongjiang, Sichuan, and Yunnan) across China. A stratified, convenient sampling of patients was taken consecutively from outpatients in all of the general hospitals. Face-to-face interviews by trained interviewers from the study team at the selected respondent hospitals were conducted and lasted approximately 30 min to an hour, in order to collect detailed information.

Study population

The inclusion criteria included (1) diagnosis with T2D based on World Health Organization (WHO) criteria [23], (2) at least 18 years old, and (3) were physically and mentally able to participate in the survey and agreed to participate in the study. We excluded patients who were pregnant, had a concomitant malignant disease, or were unable to complete the questionnaire.

The final sample consisted of 496 individuals, and the overall response rate for eligible participants was 87.3% (496/568). Of the 568 individuals, 72 individuals were abandoned due to missing data. A total of 496 participants were used for this analysis. The valid questionnaires showed no significant difference between different provinces ($\chi^2 = 3.982$, $p = 0.408$).

Questionnaire

The study questionnaire content was developed by the investigators based on a review of the literature, which included standardized items as well as items developed by consulting diabetes specialists and clinical pharmacists in endocrinology. A pilot was then tested by way of consecutively including outpatients from a general hospital in Beijing by the investigators. The final version was reviewed by diabetes specialist and clinical pharmacists. Elements of the survey included demographic background, treatment history, and the monitoring of blood glucose. Survey data were recorded on paper forms and translated using EpiData 3.1.

Measurement

Demographic data

The questionnaire included information on the individual's demographic characteristics, type of diabetes mellitus, age, gender, education level, employment, current residence, household income, and insurance coverage. Of all variables mentioned, education level was divided into three groups: (1) higher education, including postgraduate degrees; (2) mid-level education, including middle and high schools as well as vocational education; and (3) low education, including illiterate or semi-illiterate, under primary or home study. Employment status was divided into three categories: employed, retired, and unemployed. The questionnaire also included the current residence of participants, urban or rural. Health insurance was measured by having any form of health insurance: (1) urban employee basic medical insurance (UEBMI); (2) urban resident basic medical insurance (URBMI); (3) government medical insurance (GMI); (4) new cooperative medical scheme in rural areas (NCMS); (5) other health insurance, including private commercial medical insurance purchased by the respondents' employers, self-insured or uninsured.

Clinic-related information

The clinic-related information used in this study was a description of the patients' general health condition and treatment history. The duration of diabetes recorded was defined by the period from the time of diagnosis to present. The long-term complication field included hypertension, hyperlipidemia, retinopathy, peripheral neuropathy, autonomic neuropathy, diabetic feet, and hypoglycemia. In addition, the

questionnaire focused on whether the patient was an inpatient and whether the patient was aware of HbA1c levels.

Glucose monitoring

Self-reported glucose monitoring was based on the respondents' answer to the question "How many times did you measure your blood glucose per week?"

Data analysis

All demographic data and clinic-related information were summarized using counts or means and standard deviations as well as percentages.

Poisson regression model was usually adopted when the dependent variable was count variable, such as SMBG frequency. Nonetheless in this study, the distribution of the dependent variable was over dispersion (conditional variance of 34.8 was much greater than the conditional mean of 3.67). In this case, standard error estimates from the Poisson regression model may be biased downwards, but negative binomial regression which can be considered as a generalization of Poisson regression can be used to ease the bias [24]. As a result of this, a negative binomial regression was employed to model the association between SMBG frequency and independent variables, including univariate analysis and multiple analysis.

Data was processed by use of the STATA software package (version 12.0). The level of statistical significance was $p < 0.05$ (two sided).

Results

The study was based on a sample of 496 completed respondents aged 18 years and above with a broad range of characteristics (Table 1). Among the study population, the mean age was 63.21 and there were slightly fewer males than females. There was a majority proportion (90.9%) of people living in urban areas. In addition, the three major basic insurance coverage in China were also well represented: 46.0% by UEBMI, 22.6% by URBMI, and 8.1% by NCMS, another 16.7% by GMI and still 6.7% with other health insurance.

Of the diabetes-related health conditions reported by participants, the mean duration of diabetes was 7.54 years. In terms of long-term complications, most of the sample (90.0%) had various complications and 77.8% had more than two types of complications. Retinopathy, hypertension, and peripheral neuropathy accounted for first three. However, only around one quarter (26.0%) reported that they knew the value of their HbA1c when last measured. Additionally, when patients were asked whether they went to hospital, 36.1% reported they were inpatients.

Table 1 Characteristics of study participants with T2D in China ($N = 496$)

Characteristics	*n*/mean ± SD	Percentage (%)
Age	63.21 ± 11.81	
Gender		
Male	233	47.0
Female	263	53.0
Educational level		
High education	164	33.1
Medium education	129	26.0
Low education	203	40.9
Employment		
Employed	89	17.9
Retirement	357	72.0
Unemployed	50	10.1
Current residence		
Urban	451	90.9
Rural	45	9.1
Household income (ln*)	7.71 ± 1.39	
Insurance		
UEBMI	228	46.0
URBMI	112	22.6
GMI	83	16.7
NCMS	40	8.1
Other	33	6.7
Duration of diabetes (ln*)	2.02 ± 1.00	
Long-term complications		
Hypertension	263	53.0
Hyperlipidemia	233	47.0
Retinopathy	288	58.1
Peripheral neuropathy	238	48.0
Autonomic neuropathy	169	34.1
Diabetic foot	79	15.9
Hypoglycemia	179	36.1
Clinical characteristics		
Inpatient	179	36.1
HbA1c awareness	129	26.0

*ln means function $\log_e$

The patients included in this study performed various frequencies of weekly self-monitoring. As shown in Fig. 1, the adherence to SMBG variable had a skewed distribution. Of the total sample, 99 (20.0%) patients had never performed SMBG or less than once, 104 (21.0%) tested once, 119 (24.0%) twice, 89 (18.0%) three to six times, and 85 (17.1%) more than seven times a week. Of the total sample, the highest frequency of monitoring was up to 50.

The results of the univariate and multivariate analyses between SMBG frequency and the various variables are shown in Table 2. In the univariate regression, the SMBG frequencies

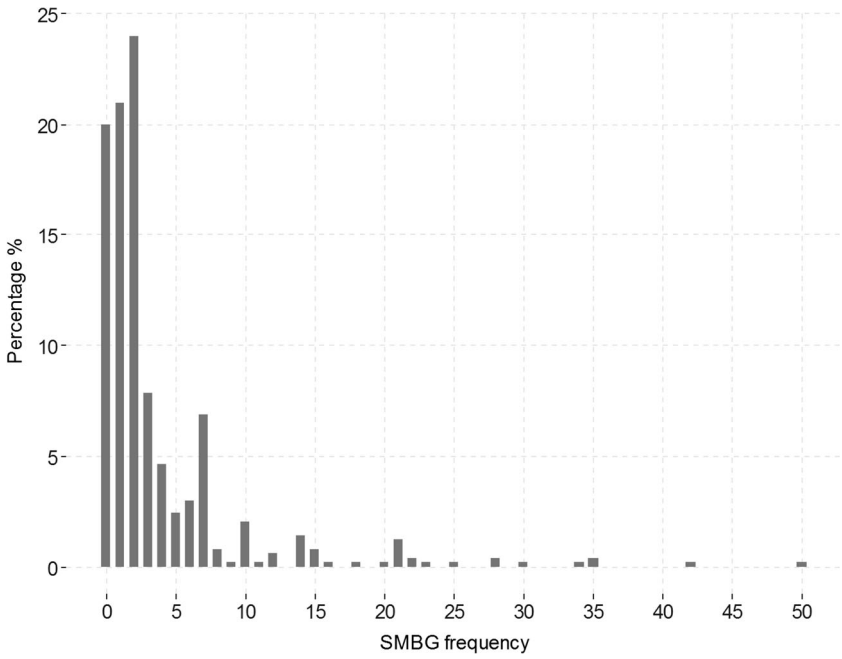

Fig. 1 Distribution of the SMBG frequency

of patients with URBMI, UEBMI, and GMI were 1.782, 2.150, and 2.341 times as much as the patients with other insurance, respectively. Patients with complications had more SMBG frequencies compared to those without complications.

Table 2 Negative binomial regression analysis of SMBG frequency ($N = 496$)

	Univariate			Multivariate		
	Odds ratio	95% CI	*p*	Odds ratio	95% CI	*p*
Age	1.002	0.99–1.01	0.690	0.991	0.98–1.00	0.180
Female	0.913	0.73–1.13	0.420	0.818	0.65–1.03	0.086
Medium education	1.280	0.98–1.69	0.077	1.141	0.87–1.50	0.338
High education	1.172	0.91–1.52	0.227	1.045	0.79–1.39	0.759
Employed	1.216	0.77–1.89	0.393	1.033	0.62–1.70	0.900
Retirement	1.456	0.99–2.12	0.056	1.408	0.89–2.20	0.159
Urban	1.185	0.80–1.72	0.386	0.735	0.40–1.31	0.247
Household income (ln*)	1.030	0.94–1.12	0.471	1.000	0.91–1.10	0.997
URBMI	1.782	1.06–2.95	*0.027*	2.084	1.24–3.46	*0.005*
UEBMI	2.150	1.31–3.45	*0.002*	2.402	1.49–3.82	*0.000*
GMI	2.341	1.37–3.94	*0.002*	2.491	1.47–4.18	*0.001*
NCMS	1.544	0.84–2.82	0.158	1.701	0.77–3.72	0.161
Duration of diabetes (ln*)	1.086	0.98–1.20	0.142	0.951	0.84–1.08	0.445
Hypertension	1.285	1.03–1.60	*0.026*	1.027	0.81–1.31	0.824
Hyperlipidemia	1.292	1.04–1.61	*0.022*	1.219	0.97–1.54	0.083
Retinopathy	1.439	1.15–1.80	*0.001*	1.363	1.07–1.74	*0.009*
Peripheral neuropathy	1.285	1.03–1.60	*0.025*	1.091	0.86–1.38	0.452
Autonomic neuropathy	0.976	0.78–1.24	0.839	0.665	0.52–0.86	*0.001*
Diabetic foot	1.399	1.045–1.90	*0.027*	1.293	0.97–1.75	0.080
Hypoglycemia	1.284	1.02–1.62	*0.031*	1.308	1.03–1.66	*0.021*
Inpatient	1.884	1.52–2.35	*0.000*	1.898	1.51–2.39	*0.000*
HbA1c awareness	1.314	1.03–1.69	*0.031*	1.366	1.07–1.76	*0.011*

The variable with an italic *p* value ($p<0.05$) is statistically significant

*ln means function $\log_e$

The SMBG frequency of inpatients was 1.884 times as much as outpatients, and the patients who were familiar with Hb1Ac were 1.314 times as much as those who knew nothing about Hb1Ac. Similar results could be seen in the multivariate analyses, but the results of patients with different complications varied. For example, patients with hypertension, hyperlipidemia, peripheral neuropathy, and diabetic foot had no significant difference.

Discussion

Our study showed that the frequency of SMBG was suboptimal, compared with one test before each meal as recommended by the ADA. The mean frequency of daily SMBG in our study was 3.67 per week, which was far lower than the data obtained in an observational study conducted in China, which calculated the mean self-monitoring at 2.08 times daily [17]. We also found that around one fifth of the patients did not perform any form of weekly self-monitoring. On average, only 6.9% monitored once a day and a few patients more than once daily. This helped to explain why our SMBG frequency, measured by the question "how many times did you perform SMBG a week," was only 3.67. While it should be noted that the proportion (80.0%) of participants in our study who performed SMBG was greater than two studies conducted in China, 65.0 [15] and 35.0% reported in another study published 10 years ago [25].

Among those demographic characteristics variables, health insurance was a significant independent predictor of SMBG frequency. Convincing evidence existed that persons with URBMI, UEBMI, and GMI were more adherent to SMBG than those with NCMS and no insurance coverage. It was similar to a survey conducted in China that the researcher found that SMBG adherence was significantly higher in the government subsidy subgroup (26.4%) than that of the national health insurance subgroup (18.9%) or in the self-payment subgroup (16.3%) [17]. This was also confirmed in Kenya that the payment for glucostrips was associated with poor adherence, though glucostrips were provided at subsidized cost, still for many patients this cost may be unaffordable due to other associated costs of DM management [23]. Therefore, it might be more critical to highlight that a population that does not have basic insurance is likely to have poor self-management for testing. The plausible explanation could be that diabetics with UEBMI, URBMI, and GMI were more likely to have a lower expenditure on SMBG than of those without medical insurance or with private commercial insurance.

There was no evidence suggesting that education status and income levels were significantly related to the frequency of SMBG in this study. Our results were inconsistent with many other studies, and several studies in the USA and the UK have already shown that low income and education can reduce SMBG frequency [26, 27]. This could be attributed to that the sample population was from general hospitals in the capital cities with relatively high education and income levels. Moreover, the frequency of SMBG in our study was generally low at present, and it may be hard to see the differences.

The present study showed that patients with various diabetes-related complications were more likely to perform SMBG. These findings are consistent with the qualitative data reported by other studies that showed that a higher level of comorbidity was associated with greater performance of SMBG [28]. This suggested that healthcare providers need to be more consistent and assertive when dealing with the issue of SMBG in newly diagnosed patients. In addition, educating inpatients about HbA1c and how to perform SMBG will result in positive self-care practices. However, the results of patients with different complications showed a variation in the univariate and multivariate analyses, the conflicting result could potentially be due to the number of complications, since nearly 80% percent of the patients suffered more than two complications, which caused a relatively huge impact on the study.

To further understand the determinants, the participants were asked additionally what most affected their SMBG frequency. We found that 61.8% of the population regarded the glycemic control as a primary factor and 20.2% were influenced by the price of glucose test strips. As mentioned previously, the frequency was generally low; therefore, the price and income did not contribute too much, but there was still a high expense to perform SMBG because it could not be reimbursed in most places. What is more, 71.2% of the respondents thought the current reimbursement policies were unreasonable and they considered 80% as a rational reimbursement rate. This was also verified by another study that many diabetics cannot afford a personal blood glucose testing meter as the cost is not covered by national health insurance, although many types of blood glucose self-monitoring equipment and supplies are readily available in China [29].

Limitations of the study

Some limitations of our study must be addressed. Firstly, the sample was extracted from five urban communities in China and the majority of the participants were from relatively high education and household income groups. This selection bias is a limitation of this study. Secondly, the data were derived from patient self-reports and not verified by a glucose meter download. We also were not aware of how accurately patients reported their own self-monitoring behavior or their healthcare professional recommendations for self-monitoring, thus raising the risk that our results were affected by recall bias. Lastly, we believe that the variables identified in this study were important, but they might not be a complete representation of the self-monitoring obstacles that patients face.

Conclusion

In conclusion, an important message for healthcare providers and diabetes educators is that more than one fifth of the study population did not conduct SMBG tests and that the mean frequency of SMBG was 3.67 times per week. Lower age, being male, living in a rural area, types of health insurance, health complications, and being an inpatient were factors positively associated with SMBG frequency. This has theoretical and practical implications that provide guidance for people with T2D and healthcare providers.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical standard Our study was a retrospective survey, using data from patients over the past period of time, so we simply obtained verbal consent from all patients during the interview.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Left ventricular mass in offspring of diabetic mothers: at 5–7 years old

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Abstract

Newborns of mothers with diabetes have increased risk for cardiac left ventricular (LV) hypertrophy. Diabetic pregnancy is also associated with in increased risk for obesity and hypertension, as well as for later cardiovascular morbidity and mortality. This study aimed to examine the connection between being the offspring from a diabetic pregnancy and having hypertension and obesity to the increased risk to have left ventricular mass (LVM) and altered LV geometry in childhood. We conducted a retrospective cohort study on 23 offspring of diabetic mothers and 23 sex- and age-matched control children at the age of 5–7 years. LVM and LV geometry were assessed using M-mode echocardiography and indexed for height$^{2.7}$. Data analyses were adjusted for birth weight, current overweight/obesity status and blood pressure. Prevalence of increased LVM/height$^{2.7}$ was higher in children of diabetic mothers, i.e. 43.5 vs. 8.7% in the control group (RR (95% CI) 5.0 (1.2–20.4), $p = 0.007$). The association between maternal diabetes and increased LVM persisted after adjustment for age, sex, birth weight, current overweight/obesity status and blood pressure, with regression coefficient of (95% CI) 5.7 (1.4–10.1), $p = 0.01$. Together, maternal diabetes, overweight/obesity status and blood pressure contributed 50% to the increase. Results showed that children of diabetic mothers were more likely to have altered LV geometry (RR (95% CI) 6.0 (1.5–23.9), $p < 0.001$). Maternal diabetes is a risk factor for increased LVM and altered LV geometry in childhood.

Keywords Diabetic pregnancy · Maternal diabetes · Childhood · Left ventricular mass · Left ventricular geometry · Left ventricular hypertrophy

Introduction

In the face of increasing global prevalence of obesity and diabetes mellitus (DM) type 2, the risk of a fetus to be exposed to hyperglycemia in pregnancy will increase. The International Diabetic Foundation (IDF) estimated that around 16.9% pregnancies were exposed to hyperglycemia, with more than 90% of the cases in the lower- to middle-income countries. The prevalence was worst in the Southeast Asia region, i.e. around 25% of the pregnancies [1].

Diabetic pregnancy was associated with several short- and long-term implications. Perinatal mortality was higher in diabetic pregnancy. The prevalence of stillbirth and neonatal mortality due to asphyxia, respiratory distress, hypoglycemia, congenital anomalies and other neonatal problems was higher in infants of diabetic mothers [2]. Echocardiography on fetuses and newborns of diabetic mothers had also observed an increase in left ventricular mass (LVM) and altered cardiac geometry [3–5].

Besides those adverse short-term effects, individuals born from a diabetic pregnancy had been observed to have increased risk for DM and cardiovascular disease (CVD) later in life [2, 6, 7]. An increase in LVM is a strong and independent predictor for later cardiovascular events [8]. However, one previous study on the offspring of mothers with type 1 diabetes observed no residual cardiac pathology in 7–8-year-old children who had hypertrophic cardiomyopathy in infancy [9].

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Several studies had revealed that children and adolescents born from mothers with diabetes were more likely to be obese and to suffer from insulin resistance and hypertension [10–12]. Since obesity and hypertension are also risk factors for increased LVM and alteration of cardiac left ventricular (LV) geometry patterns [13, 14], this study aimed to examine the connection between being the offspring from a diabetic pregnancy and having hypertension and obesity to the increased risk to have LVM and altered left ventricular (LV) geometry in childhood.

Methods

We performed a retrospective cohort study involving 5–7-year-old children born from a diabetic pregnancy in Dr. Sardjito Hospital Yogyakarta between January 2007 and December 2009. Maternal diabetes was defined as persistent hyperglycemia (random blood glucose > 200 mg/dL in more than one sample) detected during pregnancy [15]. We excluded children with congenital cardiac malformations or other severe congenital anomalies. Controls were children born with the same sex at the same day.

Data on birth weight were extracted from medical records. Birth weights were measured immediately after birth. Measurement of weight, height and blood pressure (BP) at the age of 5–7 years was performed by trained nurses. Overweight and obesity were defined as body mass index (BMI) for age above + 1 SDS (standard deviation scores) of the WHO child growth reference 2007 [16].

Blood pressure (BP) was measured using the standard techniques described by the fourth report of the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents. The percentiles of every subject's systolic and diastolic BPs were also computed using the formula given by the NHBPEP. Elevated BP was defined as systolic and diastolic BP at or above the 90th percentile for gender, age and height observed from three independent measurements [17].

A pediatric cardiologist measured LVM using M-mode echocardiography as recommended by the American Society of Echocardiography (ASE). LVM was calculated using the formula introduced by Devereux et al., [18] according to the ASE guidelines (Box 1). LVM were indexed for height (in $m^{2.7}$) into LVM/height$^{2.7}$. LVM/height$^{2.7}$ values were classified as high if they were above the 95th percentile of the reference introduced by Khoury et al. 2009 [19].

Subjects were classified into four groups of left ventricular (LV) geometry based upon LVM/height$^{2.7}$ and relative wall thickness (RWT). RWT was estimated and classified using methods by Hanevold et al. 2004 (Box 1) [13]. LV geometry was considered to be normal when LVM/height$^{2.7}$ was < 95th percentile and RWT < 0.41; concentric remodelling when LVM/height$^{2.7}$ was < 95th percentile and RWT ≥ 0.41; concentric hypertrophy when LVM/height$^{2.7}$ was ≥ 95th percentile and RWT ≥ 0.41; and eccentric hypertrophy when LVM/height$^{2.7}$ was ≥ 95th percentile and RWT < 0.41 [14].

This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Written informed consent was obtained from parents before data collection.

Mean difference of continuous data was analysed using independent sample *t* tests. Univariate regression analyses were used to assess the associations between LVM/height$^{2.7}$ as the dependent variable and maternal diabetes and other potential predictors for increased LVM/height$^{2.7}$, i.e. BMI for age SDS and blood pressures as the dependent variables. Multiple regression analyses were then performed to estimate the independent contribution of maternal diabetes to LVM/height$^{2.7}$ adjusted for sex, age, BMI for age SDS and blood pressures.

Results

Out of 27 diabetic pregnancies recorded in the hospital's medical records, we identified 24 live offspring (age range 5.2–7.8 years). One child was excluded because she had a congenital cardiac malformation. We included 23 children (48% males) born from mothers with diabetes, and 23 matched for sex and age controls. All of the diabetic mothers were first detected as having diabetes in the last semester of this pregnancy.

Offsprings of diabetic mothers were more likely to be born heavy (RR (95% CI) 8.0 (2.1–30.9), $p < 0.001$). At age 5–7 years, they were more likely to be overweight/obese (RR (95% CI) 2.0 (1.0–4.0), $p = 0.04$). Although not statistically significant, they were also more likely to have elevated blood pressure (BP), i.e. RR (95% CI) 3.5 (0.81–15.1), $p = 0.13$.

Children of diabetic mothers had larger measurements in all aspects of cardiac dimensions (Table 1). They were more likely to have high LVM/height$^{2.7}$, i.e. 43.5% in children of diabetic pregnancy vs. 8.7% in control children (RR (95% CI) 5.0 (1.2–20.4), $p < 0.007$).

Box 1 Formulas for calculating left ventricular mass (LVM) and relative wall thickness (RWT)

LVM (g)	$0.81 \times \{1.04 \times (\text{interventricular septal thickness at diastole} + \text{left ventricle posterior wall thickness at diastole} + \text{left ventricular internal dimension at diastole})^3 - (\text{left ventricular internal dimension at diastole})^3\} + 0.06$
RWT	2 × (posterior wall thickness left ventricle internal dimension at diastole)

Table 1 Results of M-mode echocardiography measurements

Parameter	Offspring of diabetic mothers ($n = 23$)	Control ($n = 23$)	Mean difference	95% CI	p
Mean (SD) LVIDd (cm)	3.65 (0.23)	3.39 (0.17)	0.25	0.13–0.37	< 0.001
Mean (SD) IVSd (cm)	0.65 (0.05)	0.58 (0.07)	0.07	0.03–0.10	0.001
Mean (SD) LVPWd (cm)	0.67 (0.07)	0.58 (0.06)	0.09	0.05–0.13	< 0.001
Mean (SD) LVM (g)	62.95 (12.76)	47.58 (8.56)	15.37	8.91–21.83	< 0.001
Mean (SD) LVM/height$^{2.7}$ (g/m$^{2.7}$)	40.96 (7.24)	31.79 (5.06)	9.17	5.45–12.88	< 0.001
Mean (SD) RWT (cm)	0.37 (0.04)	0.34 (0.04)	0.02	0.00–0.04	0.05

95% CI 95% confidence interval, *LVIDd* left ventricular internal dimension at diastole, *IVSd* interventricular septal thickness at diastole, *LVPWd* left ventricle posterior wall thickness at diastole, *LVM* left ventricular mass, *LVMi* left ventricular mass index, *RWT* relative wall thickness

Table 2 displays univariate and multivariate linear regression analyses of variables associated with LVM/height$^{2.7}$. The table shows that maternal diabetes, overweight/ obesity status and elevated BP were independent predictors for high LVM/ height$^{2.7}$.

Results showed that offspring of maternal diabetes were more likely to have altered left ventricular (LV) geometry, i.e. 12 (52.2%) out of 23 in the offspring of maternal diabetes compared to 2 out of 23 (0.1%) in the control group (RR (95% CI) 6.0 (1.5–23.9), $p < 0.001$). Distribution of LV geometry of the study subjects, stratified by maternal diabetes, overweight/ obesity and elevated BP status is displayed in Fig. 1. The figure also shows the association between the combinations of the presence and the absence of the three risk factors, i.e. maternal diabetes, obesity and elevated BP, with the types of the alteration of the LV geometry.

Discussion

Our study observed that maternal diabetes, along with overweight/obesity status and elevated blood pressure, were independent risk factors for high left ventricular mass (LVM) in childhood. Together, they contributed around 50% to the increase. To the best of our knowledge, this is the first research to report the persistence of increased LVM and altered LV geometry in offsprings of mothers with diabetes. Most previous studies did not observe any residual cardiac hypertrophy after the first months of life [9, 20].

In this study, we were not able to classify the types of maternal diabetes, whether they were gestational diabetes or pre-existing diabetes mellitus (DM), i.e. either type 1 or type 2, because diabetes was first diagnosed in the last part of the third semester of this pregnancy only by several random blood glucose measurements of above 200 mg/dL without any further workups [15]. Moreover, as measurement of glycosylated hemoglobin (HbA1c) was not performed, we were also unable to assess the glucose control of DM. A study in Egypt observed influence of diabetic control on fetal cardiac pathology. Thicker interventricular septum (IVS) and right and left myocardial wall were observed in newborns of uncontrolled diabetic pregnancy (HbA1c > 6.5) compared to newborns from well-controlled diabetic pregnancy (HbA1c < 6.5) [21]. However, a study in Spain observed a tendency towards mild IVS hypertrophy even in well-controlled diabetic pregnancies [4].

Table 2 Adjusted and unadjusted linear regression coefficient for LVM/height$^{2.7}$ as the dependent variable and maternal diabetes and BMI-for-age SDS and blood pressures as the independent variables

Variable	Unadjusted β			Adjusted β		
	β	95% CI	p	β	95% CI	p
Maternal diabetes (1 = yes, 0 = no)	9.16	5.43–12.88	< 0.001	5.77	1.42–10.11	0.01
Overweight/obesity (1 = yes, 0 = no)	7.86	3.85–11.88	< 0.001	4.37	0.58–8.16	0.03
Elevated blood pressure (1 = yes, 0 = no)	9.35	4.22–14.49	0.001	5.71	1.17–10.24	0.02
Birth weight (g)	0.01	0.00–0.01	0.002	0.001	− 0.002–0.004	0.56
Age (months)	0.04	− 0.22–0.29	0.77	− 0.04	− 0.23–0.14	0.64
Sex (1 = male, 0 = female)	− 1.29	− 5.93–3.35	0.58	− 2.21	− 5.62–1.21	0.20
Adjusted *R*-squared				0.50		

LVM left ventricular mass, *BMI-for-age SDS* body mass index-for-age standard deviation scores, *95% CI* 95% confidence interval

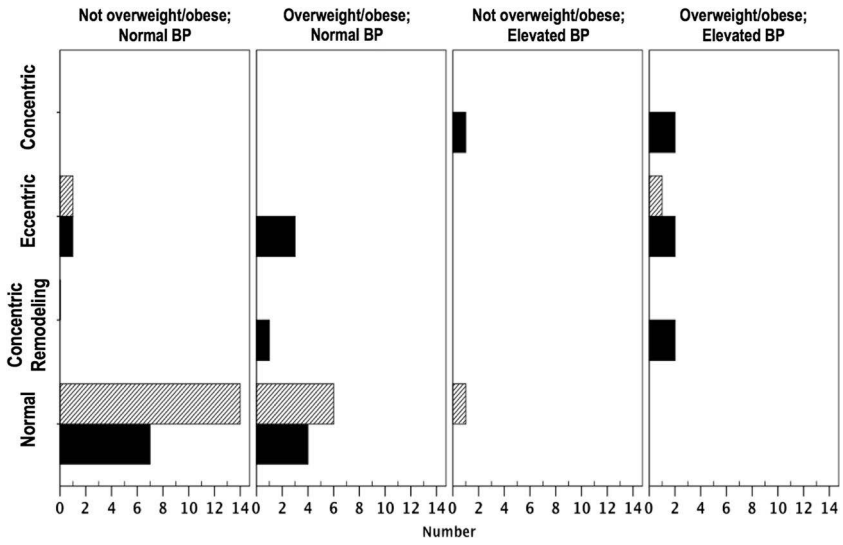

Fig. 1 Distribution of LV geometry of the study subjects stratified by maternal diabetes, overweight/obesity and elevated BP status. Solid black columns: offspring of diabetic mothers; striped columns: control group

The pathogenesis of increased LVM in infants of diabetic mothers is still unclear. Although fetal hyperinsulinism has long been suggested as the cause, the association between hypertrophic cardiomyopathy and high insulin levels in amniotic fluid has only recently been demonstrated [20]. The normalisation of post-natal insulin level might be the explanation of the post-natal regression of the cardiomyopathy observed in most studies [20]. As this study observed persistent cardiac hypertrophy at later age, it is interesting to know the insulin level of those children.

Despite the fact that offspring of mothers with diabetes were at increased risk for obesity and hypertension, the mechanism of how fetal exposure to diabetes increased the risk for later cardiovascular disease (CVD) remains unclear [7, 22]. Some argue that this was associated with higher risk for macrosomia, i.e. macrosomic infants were at higher risk for obesity. Others concluded that this was purely coincidence, i.e. diabetic women had genetic risks and life styles that were associated with higher risk for diabetes mellitus and cardiovascular risk. The risks, including the lifestyle, were inherited by her offspring [6]. This study, however, observed that the risk for increased LVM was independent from both birth weight and overweight/obesity status.

Most alterations in cardiac geometry reported prenatally or immediately after birth affected preferentially the interventricular septum (IVS), due to its abundance in insulin cardiac receptors [21]. However, at a lesser degree, left and right posterior free walls might also thicken [20, 21]. Our study observed increased dimension of both the IVS and the left ventricular posterior wall that lead to increased left ventricular mass (LVM) which was a strong and independent predictor for later cardiovascular events [8, 23]. It was not known, however, whether the thickening of the posterior wall was already present at birth or had developed thereafter because cardiac LVM and left ventricular (LV) geometry were not assessed in fetal life or at birth. This study could indicate, however, that the presence of post-natal factors, such as obesity and elevated BP, was associated with more severe alteration of LV geometry.

Conclusions

We concluded that diabetic pregnancy, along with overweight/obesity status and elevated BP, were independent risk factors for high left ventricular mass (LVM) in childhood. Together, they contributed 50% to the increase in LVM. As diabetic pregnancy was also associated with the offspring's increased risk for obesity and elevated BP, the presence of the three risk factors seemed to result in a more severe alteration of LV geometry.

Author contributions All the three authors' (RL, N, MJ) contributed to the conception and the design of the study. RL and N contributed to the acquisition of the data, and RL and MJ analysed the data. All authors (RL, N, MJ) contributed to the interpretation of the data. RL drafted the article. N and MJ critically revised the draft. All (RL, N, MJ) approved the version to be published and agreed to be accountable for all aspects of the work, including ensuring integrity and accuracy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and national regulations and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from parents before data collection.

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ORIGINAL ARTICLE

Levels of compliance of self-care practices of diabetes mellitus type 2 patients: a study from a tertiary care hospital of North India

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Abstract
Diabetes management strategies are interdependent and comprise of three basic key elements: self-care activities, effective drug treatment, and adequate follow-up for early detection of complications. There is a need to assess the levels of self-care practices among diabetic patients and factors influencing them for developing better educational strategies to address the gaps identified. A cross-sectional study was conducted to assess the compliance for self-care activities of type 2 diabetes mellitus patients, in a tertiary care hospital by using the Summary of Diabetes Self-Care Activities (SDSCA) scale among 60 patients aged > 18 years. More than 90% of respondents were compliant for not eating high-fat foods, were not smoking, and were monitoring glucose regularly. One third of respondents were not taking proper foot care. After adjusting for confounders, the proportion of individuals who eat five or more servings of fruits and vegetables were significantly lower in middle and low socioeconomic status (SES) individuals as compared to high SES (middle SES OR 0.06, 95% CI 0.01–0.39; lower SES OR 0.06, 95% CI 0.01–0.60). Females were less likely to be involved in physical activity-related self-care activities as compared to males (minimum 30 min of physical activity: OR 0.27, 95% CI 0.08–0.92; specific exercise sessions: OR 0.15, 95% CI 0.04–0.52). The proportion of diabetics who follow proper foot care instructions were significantly lower among middle SES as compared to upper SES (checking of feet: OR 0.08, 95% CI 0.01–0.47; inspection of footwear: OR 0.08, 95% CI 0.01–0.48). There is a need to emphasize the importance of diet, physical activity, and foot care during the counseling sessions of diabetes mellitus type 2 patients.

Keywords Type 2 diabetes · Self-care · Cross-sectional study · India

Introduction

India is progressively becoming the hub of diabetes mellitus in Southeast Asia and expected to have an increase in the number of diabetics from 49.8 million to 69.9 million by the year 2025 [1, 2]. Diabetes management strategies are highly interdependent and are mainly focused on three key elements: self-care education, effective drug treatment, and adequate follow-up for early detection of complications [3]. Primary management should be in patients' hands; they should be educated under the consistent guidance of doctors or health care team. This will help in having a better metabolic control over the disease and prevention of complications. There is substantial evidence to suggest that patient's self-care practices have a very strong association with their glycemic control [4–6]. Self-care is a dynamic and a continuous process which is influenced by the levels of knowledge regarding the diabetes mellitus and its complications and also by the levels of motivation towards self-care [7–11].

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The levels of self-care depend upon various factors like cultural, behavioral, social, geographical, and program effectiveness and quality of health care services in a region. Most of the research work has been done in the southern region so far. Thus, there is a need to assess the self-care practices among diabetic patients and factors influencing them so that better educational strategies can be developed to address the gaps identified. Hence, a cross-sectional study was planned to measure the compliance level of self-care practices among patients attending diabetic clinic in a tertiary care teaching hospital of North India with a validated questionnaire.

Methods

The study was conducted in a tertiary care institute from North India. A diabetic clinic is being conducted by the Department of Endocrinology on Wednesday and Thursday and about 500 patients visit the clinic on these days. The clinic caters to diabetic patients from the states of Jammu and Kashmir, Himachal Pradesh, Punjab, and Haryana and western parts of Uttarakhand and Chandigarh. A cross-sectional study was conducted among 60 patients aged > 18 years and diagnosed with type 2 diabetes and registered in the diabetic clinic of the Department of Endocrinology. Sample size of 60 was calculated assuming compliance of 60% for various self-care practices with an absolute error of 12.5%, at 95% confidence interval using Open Epi.

Data were collected from the eligible study subjects from 15th May 2011 to 30th July 2011. Participants were recruited from the waiting lounge area of the clinic after taking an informed written consent. Consecutive sampling was done during clinic days and about 15 patients were interviewed in a day. Patients with type 1 diabetes, patients who were not willing to enroll for the study, or critically ill patients were excluded.

Data were collected with the help of a pretested, structured interview schedule that was developed by adapting the Summary of Diabetes Self-Care Activities (SDSCA) questionnaire [12]. SDSCA is one of the most widely used psychometric tools for measuring the educational outcome of diabetic patients. It meets all the eight appraisal criteria (relevance, validity, reliability, responsiveness to change, burden, feasibility, and acceptability) identified by the Australian National Consensus on Outcomes and Indicators for Diabetes Patient Education [12–14]. The overall Cronbach's alpha for the questionnaire was 0.71 [15]. The frequency of self-care behavior over the past week was assessed by a revised version of SDSCA which measures a wide range of activities that includes healthy diet, physical activity, and blood glucose monitoring to foot care. Each self-care component measures were assessed separately, with higher scores indicating a greater level of self-care. Numerical scoring of items was based on the number of days in a week each of the self-care practices as observed by the patient (0–7) [12].

The questionnaire was translated to Hindi and back-translated into English to check for consistency and validity. The details of the factors that can affect the adherence to self-care practices such as demographic details, duration of illness, follow-up period, and socioeconomic status were also collected. A modified version of Kuppuswamy's socioeconomic status scale for 2010 was used to evaluate patient's socioeconomic profile [16]. The self-care activities were considered as compliant if planned calories were restricted in the meals as per the diet plan for more than 5 days in a week; ate five or more servings of fruits and vegetables on more than 3 days in a week; physical activity for at least 30 min per day and specific exercise sessions were followed for at least 4 days in a week; foot care practices like checking feet and inspecting shoes from inside were done for at least four times in a week and had blood glucose monitoring done at least once in 3 months [17]. The data was collected after obtaining written informed consent from the participants. The study has been duly approved by institute ethics committee.

Data entry was done in Microsoft Excel 2007 and data analysis was done using Social Package for Social Sciences (SPSS) version 16 for Windows. The compliance for the self-care activity was considered as an outcome variable, and age, sex, education, socioeconomic status, duration of illness, and the time since last follow-up visit were considered as independent variables. The adjusted odds ratios and 95% confidence intervals for the risk factors were calculated using logistic regression. p value of < 0.05 was considered statistically significant.

Results

About 50% of the respondents were males. The mean age of the respondents was 54.9 ± 9.2 years (range 33–76 years). Majority (81%) of the respondents were married. Though most of the respondents (82%) had completed at least primary education, there are about one fifth who did not have any formal education. More than half (56%) of the respondents were either unemployed or housewife. About 29% of people have income less than or equal to Rs 7000.

The average duration of diabetes was 8.3 ± 7.1 years (range 0.12–33 years). The average time since last follow-up was 2.5 months.

The compliance levels of self-care practices among diabetic patients were presented in Fig. 1. More than 90% of diabetic patients were compliant for not eating high-fat foods, glucose monitoring at least once in every 3 months, and quitting or not smoking as per the recommendations under self-care practices. About two thirds of respondents were checking their feet and shoes from inside at least four times a week. The

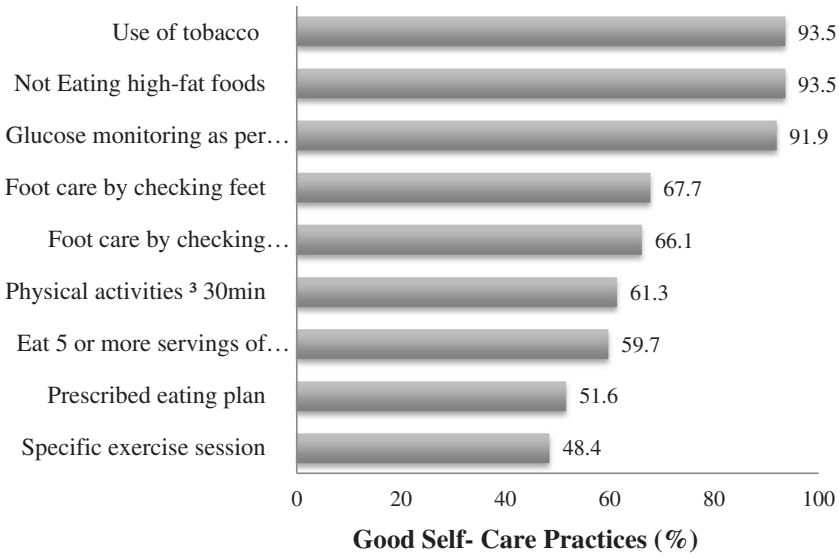

Fig. 1 Compliance levels of self-care practices by diabetic patients in the study

compliance was low (about 50–60%) mostly for self-care activities regarding physical activity and diet (except for eating high-fat foods).

The association of levels of compliance to various self-care activities and risk factors such as sex, socioeconomic status, literacy status, duration of illness, and time since last visit were presented in Table 1. Male diabetic patients have shown better compliance than females for the self-care practices like taking five or more servings of fruits and vegetables (OR 4.2; 95% CI 1.4–12.6), performing specific exercise sessions (OR 6.0; 95% CI 2.0–17.9), and taking good foot care by checking feet (OR 6.5; 95% CI 1.8–22.9) and inspecting shoes from inside (OR 5.0; 95% CI 1.5–16.3). The compliance was significantly higher among diabetic patients belonging to higher socioeconomic status as compared to lower socioeconomic status to self-care practices, namely taking five or more servings of fruits and vegetables (χ^2 9.3; $p < 0.001$), taking good foot care by checking feet (χ^2 15.9; $p < 0.001$), and inspecting shoes from inside (χ^2 15.8; $p < 0.001$).

Logistic regression analysis was done to adjust for the confounding factors. After adjusting for confounders, the compliance levels to eating five or more servings of fruits and vegetables for at least 5 days in a week were significantly lower in the middle (OR 0.06; 95% CI 0.01–0.39) and lower socioeconomic status (OR 0.06; 95% CI 0.01–0.60) individuals as compared to upper socioeconomic status individuals. Females were significantly less compliant to perform physical activity-related self-care activities, i.e., performing at least 30 min of physical activity (OR 0.27; 95% CI 0.08–0.92) and specific exercise sessions (OR 0.15; 95% CI 0.04–0.52) as compared to males. There was significant difference in the compliance levels regarding checking of feet (OR 0.08; 95% CI 0.01–0.47) and inspection of footwear (OR 0.08; 95% CI 0.01–0.48) between upper and middle socioeconomic status individuals (Table 2).

Discussion

After adjusting for all the risk factors, it was found that physical activity is significantly associated with sex. Females are less likely to involve than males in specific exercise sessions such as swimming, walking, and biking other than what anyone would do around the house or as a part of work.

Physical activity

It was observed that the awareness regarding the beneficial effect of physical activity is 70.9% in a study from a tertiary care hospital, Andhra Pradesh, but in reality, only 38.2% were actually performing the recommended physical exercise. Similar findings were obtained from Gujarat, Kolkata, and Karnataka [9, 18–20]. Major constraints to physical involvement are decreased awareness, decreased motivation, inaccessibility to the favorable environment, socio-cultural factors, and lack of political commitment.

Gender is a socio-cultural factor that has an impact over physical activity that is acknowledged in the present study. The potential barriers for women resulting in decreased participation in the physical activity depend upon the status of women in the society, secured and congenial environment, personal motivation, and time constraints. The perception of males and females regarding participation in physical activity is that females are more involved in the household tasks that engage them for all day long so there is lack of time for physical activity while males are more involved in rigorous physical activity even for a shorter span. Although the time of engagement in the physical activity is far more in case of females but when the metabolic equivalents (MET) are considered for the involved physical activity, females are found to be more inactive than males [21, 22]. Men were motivated more by the reports telling physical activity as a preventive

Table 1 Association of compliance of self-care practices with socio-demographic characteristics, duration of illness, and time since last visit

Risk factors		Prescribed eating plan, *N* (%)	Eat 5 or more servings of fruits and vegetables, *N* (%)	Not eat high-fat foods, *N* (%)	Physical activities ≥ 30 min, *N* (%)	Specific exercise session, *N* (%)	Glucose monitoring, *N* (%)	Foot care by checking feet, *N* (%)	Foot care by checking shoes from inside, *N* (%)	Tobacco consumption, *N* (%)
Sex	Male	17 (56.9)	23 (76.7)	28 (93.3)	22 (73.3)	21 (70.0)	29 (96.7)	26 (86.7)	25 (83.3)	26 (86.7)
	Female	15 (46.9)	14 (43.8)	30 (93.8)	16 (50.0)	9 (28.1)	28 (87.5)	16 (50.0)	16 (50.0)	32 (100.0)
	p value	*0.46*	*0.011*	*1.00*	*0.072*	*0.002*	*0.355*	*0.003*	*0.007*	*0.049*
Education	Illiterate	5 (45.5)	5 (45.5)	11 (100.0)	6 (54.5)	4 (36.4)	10 (90.9)	5 (45.5)	5 (45.5)	11 (100.0)
	Literate	27 (52.9)	32 (62.7)	47 (92.2)	32 (62.7)	26 (51.0)	47 (92.2)	37 (72.5)	36 (70.6)	47 (92.2)
	p value	*0.746*	*0.33*	*0.589*	*0.736*	*0.51*	*1.00*	*0.1513*	*0.16*	*0.589*
Socioeconomic status	Upper	17 (63.0)	21 (77.8)	25 (92.6)	16 (59.3)	14 (51.9)	24 (88.9)	24 (88.9)	24 (88.9)	25 (92.6)
	Middle	8 (61.5)	10 (76.9)	13 (100.0)	8 (61.5)	7 (53.8)	13 (100.0)	11 (84.6)	10 (76.9)	12 (92.3)
	Lower	6 (30.0)	6 (30.0)	18 (90.0)	12 (60.0)	8 (40.0)	18 (90.0)	6 (30.0)	6 (30.0)	19 (95.0)
	p value	*0.064*	*0.001*	*0.676*	*1.00*	*0.71*	*0.602*	*< 0.001*	*< 0.001*	*1.00*
Duration of illness (in years)	< 10	18 (43.9)	24 (58.5)	37 (90.2)	27 (65.9)	20 (48.8)	37 (90.2)	24 (58.5)	23 (56.1)	37 (90.2)
	≥ 10	14 (66.7)	13 (61.9)	21 (100.0)	11 (52.4)	10 (47.6)	20 (95.2)	18 (85.7)	18 (85.7)	21 (100.0)
	p value	*0.112*	*1.00*	*0.290*	*0.410*	*1.00*	*0.654*	*0.44*	*0.025*	*0.290*
Time since last follow-up (in months)	≤ 3	29 (56.9)	32 (62.7)	48 (94.1)	31 (60.8)	26 (51.0)	48 (94.1)	37 (72.5)	36 (70.6)	48 (94.1)
	> 3	3 (27.3)	5 (45.5)	10 (90.9)	7 (63.6)	4 (36.4)	9 (81.8)	5 (45.5)	5 (45.5)	10 (90.9)
	p value*	*0.101*	*0.33*	*1.00*	*1.00*	*0.51*	*0.212*	*0.15*	*0.16*	*1.00*

* *p* values have been italicized. *p* values < 0.05 are considered significant

Table 2 Adjusted odds ratios for association of compliance of self-care practices with socio-demographic characteristics, duration of illness, and time

Risk Factors		Eat 5 or more servings of fruits and vegetables		At least 30 min of physical activity		Specific exercise session		Checking feet		Inspecting shoes	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age		0.97 (0.90–1.05)	0.49	0.99 (0.93–1.06)	0.84	1.0 (0.94–1.07)	0.98	0.97 (0.89–1.05)	0.43	0.99 (0.91–1.07)	0.75
Sex	Male	Ref-		Ref-		Ref-		Ref-		Ref-	
	Female	0.39 (0.10–1.45)	0.16	0.27 (0.08–0.92)	0.04	0.15 (0.04–0.52)	0.003	0.23 (0.05–1.08)	0.06	0.29 (0.07–1.28)	0.10
Education	Illiterate	Ref-		Ref-		Ref-		Ref-		Ref-	
	Literate	0.41 (0.07–2.56)	0.34	1.60 (0.30–8.40)	0.58	1.74 (0.28–10.6)	0.55	0.81 (0.1–5.403)	0.83	0.67 (0.11–4.15)	0.67
Socioeconomic status	Upper	Ref-		Ref-		Ref-		Ref-		Ref-	
	Middle	0.06 (0.01–0.39)	0.003	1.38 (0.30–6.32)	0.68	1.36 (0.28–6.59)	0.70	0.08 (0.01–0.47)	0.006	0.08 (0.01–0.48)	0.006
	Lower	0.06 (0.01–0.60)	0.015	1.18 (0.19–7.18)	0.85	1.40 (0.22–8.93)	0.72	0.15 (0.02–1.34)	0.09	0.32 (0.05–2.22)	0.32
Duration of illness	< 10 years	Ref-		Ref-		Ref-		Ref-		Ref-	
	≥ 10	0.27 (0.11–3.11)	0.12	0.40 (0.10–1.61)	0.20	0.83 (0.20–3.38)	0.80	1.97 (0.27–14.57)	0.51	3.47 (0.50–24.29)	0.21
Time since last visit	≤ 3 month	Ref-		Ref-		Ref-		Ref-		Ref-	
	> 3 month	0.58 (0.11–3.11)	0.53	1.05 (0.23–4.77)	0.94	0.76 (0.16–3.70)	0.73	0.41 (0.07–2.52)	0.34	0.46 (0.08–2.62)	0.38

measure for reduction of diseases and also get motivated by watching others performing the exercise. There is also lack of motivation in females but they are motivated by monitoring their losing weight and comparing with other females [23].

Involvement of females in walking or organized specific exercise sessions in appropriately suited safe and secured environment and making small social clubs for females from the same community so as to get motivated by each other can contribute to an individual's fitness level. It also provides the opportunity for social interaction, reducing feelings of loneliness, and social exclusion that are seen as co-existing morbidities in this age group.

Dietary practices

The second factor contributing to NCDs is diet (unhealthy diet) and nutritional intervention is an integral part of self-care in type 2 diabetes. Although more than 90% of the participants were compliant with not eating high-fat food, only half of them were following the prescribed eating plan. Barriers to adherence in the nutritional management of type 2 diabetes patients were lack of individual level food planning, problems in identifying alternative food items, non-revision of the healthy eating plan from time to time leading to dissatisfaction and lack of knowledge regarding healthy choice of food for constant self-care [24]. Low compliance to prescribed eating plan could be due to difficulty in adoption of new food habits as these food items are usually considered additional to be the main family meal [25]. Some of the patients do not perceive the severity of the disease and had a poor understanding or misinformation regarding the diet-disease associations. A study conducted in the South India explored the barriers to the compliance for dietary modification and segregated them into two groups: patient related and health care system related. Age, health consciousness, and nuclear families are positive factors while duration of illness and complications play a negative role related to patient level. Facilitators identified at the health care facility level were the advices given by the dietician over health options, cooking methods, and practical approach to deal with day to day lifestyle issues and reinforcement [26]. Many studies focusing on the knowledge and practices opted by the diabetic patients found that patients have fairly good knowledge about the dietary modification but actual implementation was far less [27–29]. One of the studies from a developing country has shown that male patients were significantly more adherent to dietary advice as compared to females [30]. Socioeconomic status is also an important factor in determining the healthy choice for food items. People of low-income strata usually consume less protein, fresh fruits, and vegetables. This choice is made due to high cost of the food items and low taste. The cost of low-fat and sugar-free items was perceived as a major drawback to make health choices [31, 32].

The dietary educational sessions must be tailor-made as per the socio-demographic characteristics of the patients to make them more understandable and more culturally and socially acceptable. A standardized diabetic education program, training of health professionals, and the provision of unequivocal information to the mass media and the general public must be ensured to have uniformity in the information received by the patient [33].

About one third of the participants in the present study were not eating five or more servings of fruits and vegetables. Higher socioeconomic status had a positive impact on eating five or more servings of fruits and vegetables (after adjusting for various risk factors like age, sex, education, SES, duration of illness, and time since last visit). It is found that diabetic patients are more likely to report the food that is recommended rather than what they are eating [34]. So, it might be possible that even a lesser number of the patients were following the healthful eating plan or not eating high-fat food or not eating five or more servings of fruits and vegetables.

The dietary strategies are to be formulated in view to provide appropriate menus, identify low-cost foods, involve patients' families, and teach patients how to make healthy food choices. Promotion of kitchen garden and terrace gardens to grow fruits and vegetables will facilitate the compliance to healthful eating plan. Free distribution of fruit saplings to the families from the low socioeconomic group may be done through horticulture departments to promote kitchen gardens.

Foot care

Foot ulcer (diabetic foot) is most commonly associated with diabetic peripheral neuropathy (DPN). India has the maximum number of people living with diabetes so as the burden of foot problem and amputations. Reduction of morbidity and mortality from diabetic foot directly depends upon early identification of clinical manifestation for diabetic peripheral neuropathy. As recommended by the American Diabetic Association, all patients must be screened for DPN twice in a year with the start of diabetes type 2 and 5 years after the diagnosis of diabetes type I followed by yearly checkup via simple clinical test like 10-g monofilament. All patients with diabetes must undergo comprehensive foot examination annually to identify the risk factors for foot ulcers or amputation; this includes inspection and assessment of foot pulse [35].

Diabetic foot also has major economic consequences to society, diabetic patients, and their families. Foot infections are a major complication in patients with diabetes mellitus which are responsible for 24.4% of total expenditure incurred by diabetics [36]. A descriptive study conducted by Shahi SK et al. in the northern rural population had shown a prevalence of diabetic foot ulcer (DFU) to be 14.3% [37]. This huge burden of diabetic foot affecting both physical and economic well-being of a nation can be well controlled by self-care practices which are often neglected at both the ends, i.e., patient and health care provider team [38]. A study conducted in the Saurashtra region of Gujrat by Shah et al. to assess knowledge, attitude, and practice with type 2 diabetes stated that patients had very less knowledge regarding the disease and its complications. The reason behind this observation was found to be low literacy rate and very less number of trained endocrinologists in the region. Patients were very dissatisfied with the time given to them by the treating doctor. Only 56% of patients were checking their foot regularly [9]. In a cross-sectional study in Lahore in 2009, about one third of diabetic patients had poor knowledge and had poor foot care practices [39]. In Nigeria, 52 and 59% of diabetic patients were regularly inspecting feet and shoes, respectively [40]. In the present study, 68 and 66% of patients were compliant with checking their feet and inspecting their shoes, respectively.

In the present study after adjusting for the various risk factors, socioeconomic status was found to be directly related to good foot care by checking feet and inspecting shoes and that it was statistically significant. This finding was supported by a study conducted in a tertiary care center in Nigeria where poor educational and low socioeconomic status was found to be significantly associated with low knowledge of foot care [40].

Self-monitoring of blood glucose

As per ICMR guidelines, self-monitoring of blood glucose (SMBG) with glucose monitoring is indicated ideally for every diabetic but especially with patient having brittle diabetes mellitus and a complete clinical examination as well as biochemical examination minimum at the interval of 3 months [41]. The American Diabetic Association (ADA) recommends that patients with type 2 diabetes who are treated with insulin or oral hypoglycemic drugs should monitor blood glucose daily [35]. During the period in which the study was conducted, the availability of the glucometer to the people was minimal due to their cost and so was not considered while assessing self-care practices in the present study. As blood glucose monitoring is a salient marker in the self-care management of diabetes, the increasing use of glucometer presently gives a new scope to assess this self-care practice.

Tobacco consumption

As per National Family Health Survey 2015–2016 (NFHS-4), the tobacco consumption among men in Punjab was 19.2% as compared to National average of 44.5%; on the contrary, alcohol consumption was far higher (34.0%) than the national average (29.2%) [42]. The seemingly high compliance of 93.5% with regard to tobacco consumption in our study can be explained largely due to the lower tobacco consumption in general due to religious reasons.

The tool used in this study is one of the most widely used psychometric tools for measuring the educational outcome of diabetic patients, i.e., SDSCA (Summary of Diabetes Self-Care Activities). It meets the entire eight appraisal criteria (relevance, validity, reliability, responsiveness to change, burden, feasibility, and acceptability) identified in the (Australian) National Consensus on Outcomes and Indicators for Diabetes Patient Education and its overall Cronbach's alpha is 0.71 [12–14]. This tool allows better comparison of self-care activities across different domains like geographical regions and time. There is a need to emphasize the importance of diet, physical activity, and foot care during the counseling session of diabetes mellitus type 2 patients. Furthermore, interventional studies should be planned to address the gaps identified and evaluating the same can provide some concrete evidences on basis of which policy decisions can be made.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee. Informed consent was obtained from all the participants.

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Cost of illness (COI) of type-II diabetes mellitus in Shillong, Meghalaya

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Abstract
This study aimed at contributing to the evidence base of economic burden caused due to type-II DM. The data were obtained from the 158 diabetics from the three tertiary hospitals in Shillong, Meghalaya, during January–March 2017 with the use of a semi-structured questionnaire. The information received was cross-checked with the medical records from the finance department. Patients comprised of 86 (54.4%) females and 72 (45.6%) males. The individuals under the age group of 56–65 were 49 (31%). The total median cost of illness per month was Rs. 5375 (2524–18,968) which was made up of 70.92% direct cost and 29.08% indirect cost. 58.2% respondents used their savings for getting treatment. The cost of care is high and comparable to cost in other countries. An improved understanding of COI of type-II DM will help in informing and motivating the policy and health decision makers which will reduce the national burden of this disease.

Keywords Cost of illness · Type-II diabetes mellitus · Shillong · Meghalaya

Overview of non-insulin-dependent diabetes mellitus

Non-insulin-dependent diabetes mellitus (NIDDM) is a chronic non-communicable condition which disturbs the body from its proper physiological functioning due to impairment of the insulin hormone of the pancreas. The magnitude of the illness has been increasing steadfastly over the years. NID type-II DM is the most predominant form of diabetes mellitus which is primarily depicted by increase glucose in the blood, resistance in insulin production, and its deficiency. As a result of the change in trend from the previous decade, the burden has increased grossly with the number of vulnerables believed to be double in the years to come [1]. It is primarily contributed from the genetic makeup and the lifestyle factors and sedentary habits [2]. Although the illness usually affects older individuals, this has been diagnosed in younger age group too, those with family history of diabetes [3]. Type-II diabetics are more vulnerable to developing complications which can be of short term or long term and these could be life threatening if not controlled. These complications can affect any body organs, thereby causing disorder or failure of the target organ(s).

India stands world ranking with the biggest proportion of diabetics acquiring the questionable refinement of being named as "diabetes capital of the world." As indicated by the Universal Diabetes Alliance in its Chart book 2006, at present, the diabetics in India were 40.9 million approximately and by the year 2025, it is expected to ascend to 69.9 million unless there are pressing restoring and promoting strides being made available to the diseased ones [4]. A review in India [5] demonstrated that the annual normal expense per patient was at least Rs. 4500. The assessments proposed that the social insurance cost borne by people and their families from the aggregate family pay in India was 85–95% [6]. With the expanding predominance anticipated comprehensively, a local study in Meghalaya, North East India, was done by Syiem et al. evaluating the commonness of diabetes among the Urban Khasi and Jaintia populace which was observed to be 9.89 and 12.5% individually. Besides, there is a similarly huge pool of people with type-II and having impaired glucose tolerance (IGT), of which a number of them might developed the illness later on. Cost of lost productivity can far surpass diabetes-related medicinal cost among the working age populace [7].

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Rationale

In India, there are studies that estimate the cost burden of type-II DM at a macro level like a study conducted by Ramachandran et al. on increasing treatment cost incurred by the diabetics in India [5] and several other studies done on a national level, whereas very few are done at a micro level. The study aimed at contributing to the evidence base of economic burden caused due to type-II DM in India.

The aim of the study was to assess the cost of illness of type-II diabetes mellitus in Shillong, Meghalaya.

The objectives of the study were as follows:

- To estimate the direct and indirect cost of type-II diabetes mellitus
- To determine the incremental cost of those with comorbidities and complications developed from type-II diabetes mellitus
- To assess the coping strategies adopted by type-II diabetic patients to meet the economic burden due to it.

Materials and methods

The study design is a cross-sectional study.

The study sample includes subjects from the three different tertiary institutions in Shillong during the data collection period from January 2 to March 25, 2017.

The sampling technique used is a convenient sampling technique with time bound enumeration.

We conducted a cross-sectional study in three tertiary hospitals of Shillong. An approval from the three hospitals has been given prior to the start of the study (approval letter by the concerned hospitals attached along with). The study sites included *Dr. H. Gordon Roberts Hospital Shillong*, *Dr. Sethi's Hope Multispeciality Clinic Shillong*, and *SuperCare Hospital Shillong*. A consecutive sampling with time bound enumeration technique was used to select the study sites. The inclusion criteria were patients diagnosed with type-II DM on treatment for at least 1-year duration and the newly diagnosed type-II DM and seriously ill/bed-ridden patients were excluded. Ethical clearance for the study was procured from the Institutional Ethics Committee, Kasturba Medical College, tertiary care center in Manipal (IEC 789/2016). Consent from the study participants has been taken prior to the start of data collection. Only consented individuals have been included in the study. The tool that we used was a semi-structured questionnaire for collecting the data from the participants. The questionnaire has three sections for answering the three objectives of the study. Questions on the detail of the cost expenses have been used for assessing the cost component. The coping strategies were assessed with the help of questions from the questionnaire on the use of insurance coverage, selling of assets, loan, using of savings and the direct cost that were assessed through expense details spent on medicine, procedure, travel, food, consultation, and other drug charges in case of those with comorbidities/complications attributed to type-II DM**.** A standard WHO HPQ (Human and Work Performance Questionnaire) [8] has been used to calculate indirect cost in terms of productivity losses, e.g., absolute absenteeism using the 1-week estimates formula. Besides the information gathered through the use of a questionnaire from the three different sections including the socio-demography, the details of the cost component had been cross-checked with the details provided from the finance department of the different hospitals who had given the permission to access the MRD and finance of the hospital.

Statistical analysis

The Statistical Package for Social Sciences for Windows (IBM SPSS) was used to analyze the data. Through descriptive statistics, we described socio-demographic, cost of illness, and coping strategies and these were expressed in terms of frequencies and percentages (Table 1). Summary statistics of direct and indirect cost were reported using measures of central tendency. But since variables have skewed distribution, median and inter-quartile range has been reported. Descriptive statistics of indirect cost was carried out using the 1-week/7-day estimates formula of absolute absenteeism. The lost wages were calculated as the ratio of per capita monthly income/average number of lost working hours, and hence indirect cost was reported.

Results

The total of 158 patients comprised of 86 (54.4%) females and 72 (45.6%) males. The individuals under the age group of 36–65 were 114 (72.1%). On the basis of educational status, 37 (23.4%) have some secondary education but left school before the age of 16 years.

The total median cost of illness of type-II DM per month was Rs. 5375(2524–18,968). The median total cost of illness of type-II diabetes mellitus was made up of 70.92% of direct cost and 29.08% of indirect cost.

There were total of 133 patients who had hypertension arising from type-II DM. The median cost of hypertension was Rs. 428(249–872) (Table 2).

58.87 % of the respondents were having monthly income of Rs. 10,000–15,000. Most of the respondents were able to pay for their monthly expenses on their treatment and most of them preferred private institutions over government hospitals and 58.2 % of the respondents have used their savings/income.

Table 1 Distribution of the respondents according to the socio-demographic characteristics ($N = 158$)

Socio-demographic characteristics		Frequency	Percentage
Gender	Female	86	54.4
	Male	72	45.6
Age in years	25–35	4	2.5
	36–45	21	13.3
	46–55	44	27.8
	56–65	49	31
Educational status	No schooling	24	15.2
	Primary education only	30	19
	Some secondary education but left school before 16	37	23.4
	Secondary education	13	8.2
	At least 1 year of university but no degree	18	11.4
	University graduate	26	16.5
Monthly financial status	< Rs. 5000	7	4.43
	Rs. 5000–10,000	25	15.83
	Rs. 10,000–15,000	93	58.87
	> Rs. 15,000	33	20.87

Discussion

In the current study ($N = 158$), among the gender wise prevalence, there was a female predominance with 86 out of the total 158 patients and only 72 patients were male. These results resemble the findings of Riewpaiboon et al. [9] in his study which out of the total 475 study population, 354 were females and only 121 patients were males [9].

The median total cost of illness in this study was Rs. 5375/month while in a study conducted by Grover et al., [10] the total monthly cost was Rs. 1209 [11]; in a study conducted by Barcelo et al. [12, 13], the median total cost per month was 5434.67 million US$; and in a study conducted by Henriksson et al. [14, 15], the median total cost per month was 2083 SEK. These studies were found to have different total cost results.

Patients with no complications/comorbidities spent 4947 rupees as median total cost per month for their diabetes care; patients having complications/comorbidities spent an additional median cost of 428 rupees due to the cost arising from the current illness, i.e., type-II diabetes mellitus. These results were found to be different from the findings of the study led by Viswanathan et al. [16] in which the total mean direct cost was Rs. 2116 per month; patients having no complications spent only 544 rupees as average direct cost per month for their diabetes care and patients having complications/comorbidities spent an additional 2967 rupees as an average direct cost per month for their diabetes care [8]. This study shows that the illness affects not only the individual but the household and society as a whole because of its lost productivity and lost wages both in the developing [17] and developed countries [16].

In the study, majority of the respondents were having monthly income of Rs. 10,000–15,000 and were able to cope up with their financial strategies through the use of their monthly income (Table 3).

The cost of illness studies conducted in the USA [18] and Canada [19] showed similar findings that direct cost was comparatively more than indirect cost; the median total cost of illness of type-II diabetes mellitus was made up of 70.92%

Table 2 Tabulation of the total cost of illness of type-II DM and the incremental cost arising from presence of hypertension as a comorbidity ($N = 158$)

Cost component	Number	Median	IQR
Direct cost	158	3812	2077–6468
Indirect cost	158	1563	447–12,500
Total cost	158	5375	2524–18,968
Cost due to presence of hypertension	133	428	249–872

Table 3 Distribution of respondent's financial coping strategies in order to meet the economic burden ($N = 158$)

Financial coping strategies	Frequency	Percentage
Availability of health insurance	19	12.0
Loan from bank/relative or other sources	10	6.3
Used savings/income for treatment	92	58.2
Selling of assets	7	4.4
Children working	30	19.0
Total	158	100.0

of direct cost and 29.08% of indirect cost while in the study conducted by Barcelo et al., [12] it showed that indirect cost accounted for 82% of the total cost spent on treatment and management of type-II DM. A study led by Chatterjee et al. [20] resulted that 63% of the total cost was made up of 63% of the direct cost and 37% of the indirect.

To meet the cost of illness, poor households fall back on adapting methodologies for coping with the increasing burden of the illness that are potentially hazardous for their future welfare [21]. The utilization of savings was the most coping strategy used. These findings are similar to findings in previous studies where individuals fell back on savings earmarked for other needs to adapt to medicinal services payments. Incomes and savings have been reported as popular payment coping mechanisms in Zambia, Cote d'voire, Chad, and an average of 40% of West African countries cope with healthcare payment through them [22]. However, using money saved for other basic items like food as payment coping mechanisms could jeopardize the health of patients and further push them into poverty [23].

Conclusion

In this study, we have presented the cost of diabetes in Shillong. The findings are quite revealing. The cost of care is high as comparable to cost in other parts of India and in other countries and the impact of the burden of the illness on the individual and the household. This study shows that the considerable economic burden of diabetes in this setting has affected not just the diseased individuals and health providers but also employers, family/household, and society overall through the impact of lost productivity. Improved understanding of the economic cost of diabetes and the major determinants of costs help to inform and motivate decisions that can reduce the national burden of this disease. District program managers can utilize this information for developing cost-effective district management programs.

Recommendations

A non-parametric test can be done to compare the median cost between the groups like (1) who are on oral anti-diabetic drugs, (2) on insulin, (3) combination of oral drugs and insulin based on the prescription pattern.

Limitations

Besides the interview, hospital records were cross-checked for reliability of the data. The unavailability and inaccessibility of records from one of the hospital could lead to underestimation or over-estimation of the cost. Recall bias and social desirable bias are possible limitations, particularly in cases not cross-checked with medical records but only relying on verbal reporting during the interview.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the study involving human participants were in accordance with the Ethical standards of the Institutional Ethics Committee of Kasturba Medical College and Kasturba Hospital, Manipal bearing IEC project number: 789/2016.

Informed consent Prior information on the study objectives was given and informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

Diabetes Mellitus Knowledge Test: development, psychometric evaluation, and establishing norms for Indian population

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Abstract
The cornerstone of diabetes management is self-management—a set of skilled behaviors to manage one's own illness. Education for diabetes self-management is a vital component of overall management. Nevertheless, lack of knowledge concerning the various aspects of diabetes acts as one of the barriers to achieve optimal diabetes control. The objectives of the study were to develop a test to measure the knowledge of symptoms, causes and risk factors, complications, and management of type 2 diabetes and standardize the test initially by establishing the psychometric properties and norms on an Indian clinical sample. This new test named as Diabetes Mellitus Knowledge Test (DMKT) was developed through four phases—item writing, content validation, item analysis and reliability, and establishment of validity and development of norm—involving three clinical samples ($n_1 = 10$, $n_2 = 212$, $n_3 = 268$) basing on cross-sectional survey design. The DMKT consisted of 37 items having dichotomous response category that were distributed under four theoretical dimensions—symptoms (9 items), causes and risk factors (12 items), complications (11 items), and management (5 items). The reliability of the test was found to be .76. The convergent validity and norm were established. The implications and short-comings of the DMKT were discussed.

Keywords Knowledge of symptoms · Knowledge of causes and risk factors · Knowledge of complications · Knowledge of management · Convergent validity · Norm

Introduction

India is considered to be the diabetes capital of the world as 65.1 million Indians suffer from diabetes [1]. The chronic illness of diabetes mellitus, commonly called as diabetes, increases at an epidemic rate, with approximately 382 million people suffering from it across the world. Diabetes is not just an epidemic or public health burden, but on a personal level, it represents a daily challenge for those who are living with this condition. It is associated with psychological burden and can lead to diabetes-related distress and burnout. The treatment and daily management of the illness are dynamic and complex [2]. Apart from pharmacotherapy, the biopsychosocial treatment approach for diabetes requires a multifaceted treatment approach of lifestyle change, medication, psychosocial support, and self-management [3].

The cornerstone of diabetes management is self-management which is a set of skilled behaviors engaged to manage one's own illness [4]. Diabetes patients strive to maintain a high level of self-care in order to achieve the recommended and optimal diabetes control goals, which otherwise lead to multiple complications. Few of the common complications are neuropathy, retinopathy, coronary disease, and renal problems [5]. According to the Standards of Medical Care for Patients with Diabetes Mellitus [6], education for diabetes self-management is a vital component of overall management and emphasizes the need to address the individual's role in executing self-care at initial and ongoing visits. One of the main components of educating patients about diabetes self-management involves knowledge about the illness and its impact on various aspects of an individual.

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However, many diabetes patients lack the knowledge concerning the various aspects of diabetes which acts as one of the barriers to achieve optimal diabetes control. According to Merriam-Webster's Online Dictionary [7], the full definition of knowledge is "the fact or condition of knowing something with familiarity gained through experience or association". Knowledge of any illness—the range of one's information or understanding of the illness—essentially has four dimensions—symptoms, causes and risk factors, complications, and treatment or management strategies. All these dimensions are helpful in understanding and coping with the illness. Studies carried out in various communities with diverse socioeconomic and cultural backgrounds show that knowledge about self-care activities is a crucial element in diabetes treatment [8]. Studies conducted across the world [9] and especially in India [10] show that diabetes patients lack knowledge on various aspects of diabetes.

Considering the relationship between the knowledge of diabetes and psychological variables, such as self-efficacy, adherence, and self-management of the patients, the need to measure the knowledge of type 2 diabetes patients has been realized. After reviewing these instruments, it is observed that majority of the reviewed and used measures in diabetes research have been developed in advanced countries like USA, UK, and Australia where there are state-of-the-art health care facilities and information resources. It is observed that though the factual information about the disease remains constant across cultures, the individual differences are observed in understanding of the disease depending on the quality of health care system and the process of dissemination of information and socialization. This process of information dissemination is not as strong in India as it is in advanced countries. This brought a realization in authors to develop and standardize an instrument to measure knowledge of diabetes in the clinical population suitable of India.

Most of the existing instruments though widely used have certain limitations. For example, though the 23-item Michigan Diabetes Knowledge Test [11] though short contains 14 items of the test are relevant only to insulin-dependent patients. It contains items mentioning food items such as 'baked chicken', 'Swiss cheese', 'celery' that are less understood by people in India. Use of medical terms such as 'ketoacidosis' makes it difficult to comprehend by the lay persons. Similar limitations were found in the Diabetes Mellitus Knowledge ([DKN-A], [12]). This 40-item instrument, along with the 15-item parallel forms (DKNa, DKNb, and DKNc), contains long statements with multiple-choice response format. These instruments also contain terms such as 'protein', 'carbohydrates', items testing knowledge about 'one metric unit of energy', items testing if '5 oz milk is equal to 5 oz orange juice' are difficult for comprehension given the Indian scenario. Many such similar limitations have been observed in the existing measures, which led to the development of the present instrument which would be simple and relevant to Indian population.

Objectives

The main objectives of the study were to (i) develop a test to measure the knowledge of symptoms, causes and risk factors, complications, and management of type 2 diabetes and (ii) standardize the test initially by establishing the psychometric properties and norms on an Indian clinical sample.

For the development, psychometric evaluation, and establishing norm of a new test, the study was planned to be conducted through four phases—phase I: item writing, phase II: content validation, phase III: item analysis and establishing reliability, and phase IV: establishing validity and developing norms.

Phase I: item writing

Along with existing literature on different instruments measuring knowledge of diabetes like the Diabetes Mellitus Knowledge ([DKN-A] [12]); the Diabetes Knowledge Assessment (DKN) scale [13]; the Revised Diabetes Knowledge Scale [14]; the Diabetic Numeracy Test (DNT) [15]; the Diabetic Knowledge Questionnaire (DKQ) [16]; and Michigan Diabetes Knowledge Test [11] were referred. All these instruments were developed in the USA, UK, and Australia.

In addition, experts such as physicians, diabetologists, and health psychologists were consulted. The authors also interacted and interviewed with 15 patients from different hospitals under study. Basing upon these resources, initially 80 items pertaining to knowledge of diabetes were written in statement format, categorized into four dimensions such as symptoms, causes and risk factors, complications, and management. These items were to be responded by choosing the options of 'true' or 'false'. These items were given to three health psychologists and five diabetologists to assess the technicality of the items. It was suggested by the experts to use short phrases instead of statements. After converting the items to phrases, these were administered on a clinical sample of 10 persons with type 2 diabetes ($n_1 = 10$) for readability. Basing upon their comments, the use of jargons, complex words, and ambiguity in the items was eliminated. Any duplication or overlapping items across the dimensions of the test were removed. At the completion of the first phase of item writing and review process, the test consisted of 64 items, under four dimensions with 16 items in each dimension.

Phase II: content validation

To review whether the items of the test covered all the areas pertaining to knowledge of diabetes, it was given to a panel of eight doctors which included four diabetologists and four

physicians. The experts were asked to review each of the items of the test and decide whether the item was 'essential' or 'not essential' in order to measure knowledge about diabetes. Items which were considered as essential by all the eight experts were included. That is, those items with 100% agreement of the experts were taken as the criterion to include the items. According to the experts review, 58 items were retained—symptoms (13 items), causes and risk factors (14 items), complications (16 items), and management (15 items). Following this screening of the items, expert opinion was sought from two diabetologists, and 12 items were selected for each of the dimensions of symptoms, causes and risk factors, complications, and management and finalized for the testing of the developed measure.

It was planned to administer the newly developed test to persons suffering from type 2 diabetes, to conduct item analysis, measure its reliability, and validate it as a preliminary step in development of this instrument. As this scale was meant for the persons suffering from type 2 diabetes in India, the scale was named as Diabetes Mellitus Knowledge Test (DMKT) and the norm was established on Indian population. Two more phases were followed to standardize DMKT—phase III involved item analysis and establishing reliability whereas phase IV involved measuring the validity and development of the norms.

Phase III: item analysis and reliability

In phase III, the newly developed DMKT was administered on a clinical sample of type 2 diabetes. Item analysis was done basing on two criteria item—difficulty index and item-discrimination index. Since the response of each of the item of DMKT is dichotomous (true or false), Kuder-Richardson 20 (KR-20) formula was applied to find out the reliability coefficient of DMKT. The study was based on cross-sectional survey design.

Method

Participants

Purposive sampling method was used to select 250 patients for the administration of DMKT from out-patient units of two hospitals and four diabetic clinics in (in Hyderabad city of Telangana in India). Out of 250 participants, data of 212 participants ($n_2 = 212$) were retained because of withdrawal of participation by the patients and missing data. The sample comprised equal number of men and women, between the age range of 25–75 years ($M = 51.86$, $SD = 10.51$). The range of disease duration of the sample was from 1 month to 20 years ($M = 5.65$, $SD = 5.32$). The sample consisted of participants who were illiterate (10.8%), and with varied educational level—primary (4.7%), secondary (11.8%), high school (27.8%), intermediate (10.8%), graduation (20.3%), and post-graduation (13.7%). The sample was also found to consist of participants who were unemployed (0.5%), and those from various occupational backgrounds such as homemakers (40.1%), private or government employees (25.9%), entrepreneurs (15.6%), farmers (4.2%), retired persons (9.4%), and those working in corporate sector (4.2%). Persons suffering from type 2 diabetes for at least 1 month and between the age of 25–75 years were included in this phase of the study. Persons suffering from type 2 diabetes for more than 20 years and below the age of 25 years and above the age of 75 years, with co-morbid conditions like terminal illness, psychiatric illness, serious cardiac diseases, hepatic, and thyroid disorders, were excluded.

Description of the newly developed Diabetes Mellitus Knowledge Test

Diabetes Mellitus knowledge test The DMKT aims to measure knowledge specific to diabetes mellitus. The test consists of 48 items where each item has dichotomous response category—'true' or 'false' (Appendix A1). The DMKT comprises four theoretical dimensions—symptoms (e.g., frequent hunger), causes and risk factors (e.g., lack of physical activity), complications (e.g., kidney failure), and management (e.g., reduced consumption of rice). Each dimension consists of 12 items. To facilitate easy understanding of the items, simple and common language was used instead of medical jargons or complex terms pertaining to the four dimensions. The participants were to respond if each item was 'true' or 'false' with regard to diabetes.

Scoring of DMKT A score of 1 is assigned for each correct response (either true or false) and a score of 0 for any incorrect response. Out of 48 items, all the items except item numbers 1, 4, 8, 10, 16, 17, 18, 21, 22, 24, 25, 28, 31, 32, 36, 38, 42, and 46 are given a score of 1 when the answer is 'false' and for the rest of the items a score of 1 is given when the answer is 'true'. The total score of the test varies from 0 to 48, whereas the total score for each dimension varies between 0 and 12. The higher the score in a particular dimension, the higher the knowledge is. Similarly, the higher the score on the total test, the higher the person's knowledge of diabetes mellitus is.

Procedure

Before starting the study, approval was obtained from the institutional ethics committee. Initially, five hospitals and 10 diabetic clinics were selected from the city of (name of the location of the study has not been disclosed for blinded review). Permission was sought from these hospitals and clinics

out of which five clinics gave permission for data collection. The out-patient units of the clinics were visited every day for approximately 5 h, for the purpose of collecting data. Informed consents were taken from the patients and the test was then administered individually. Instructions were given in the regional language, Hindi or English as per the preference of the participants. Patients were encouraged to answer to each item as per their knowledge. It took approximately 10–15 min to complete the DMKT, after which the participants were debriefed.

Item analysis

The obtained quantitative data were analyzed by using IBM SPSS Statistics 20 for item analysis and reliability. According to Thompson and Levitov [17], item analysis "investigates the performance of items considered individually either in relation to some external criterion or in relation to the remaining items on the test". Item analysis aids in improving the test by revising and discarding the items that are deemed ineffective. Two methods were used for item analysis—(i) item difficulty value (p) and (ii) item discrimination value (d). These values were taken as criteria to identify relevant items for the test.

Item difficulty value The item difficulty value (p) is ratio between the number of respondents who correctly answer the item and the total number of respondents. The higher the difficulty value, the easier the item deemed to be [18]. In this study, item difficulty was calculated to identify and eliminate items that were either extremely difficult or extremely easy. This would help in determining individual differences and allow variability of test scores among different groups of participants. The formula adopted to calculate the item difficulty value [19] is as follows: $p = N_p/N$, where N_p indicated the number of test takers in the total group who answered the item correctly and N denoted the total number of test takers in the group. On the basis of the formula, the item difficulty value (p) was calculated for each item of the test and is presented in Table 1.

According to Lord [20], the ideal difficulty level for multiple-choice items involving dichotomous response category is .85, i.e., those items with difficulty value above .85 are considered 'easy' and those below .15 are considered very difficult for the respondents to answer correctly. Therefore, such items are to be deleted. The criterion of cut-off for the item difficulty level, however, is left to the discretion of the test developer based on the nature of the test. As the DMKT was not a classroom test or a test that was assessing knowledge acquired from prior instruction or training, the cut-off range for the item difficulty level was determined to be .10 to .90. This was considered to be so, as the knowledge of diabetes patients was being tested based on what they acquired from the doctors, family, friends, or from other patients and not from direct instructions or training. Based on the decided cut-off range of .10 to .90, item numbers 37, 40, 41, 42, 43, 47, and 48 were dropped, reducing the 48 original items to 41 items with 12 items each in the dimension of symptoms, causes and risk factors and complications, and 5 items in the dimension of management.

Item discrimination value Each item of the test needs to possess the power to discriminate between persons who performed well on the test and those who performed poorly. This discrimination value (d) was calculated for each item by identifying 27% top scorers and 27% bottom scorers [21]. The formula to calculate the item discrimination value was as follows: $d = U_p - L_p / U$, where U_p and L_p indicate the number of respondents in the upper and lower groups who pass the item and U is the total number of respondents in the upper group. Using the formula, the discrimination values were calculated for all the items of the test and are presented in Table 1. The higher the discrimination values of an item, the better the item's discriminating power. According to Ebel and Frisbie [22], items with discrimination value of .40 and above are considered to be 'good' items, those with .30 to .39 as 'reasonably good' items, those with .20 to .29 as 'marginal', and those below .19 as 'poor' items needing revision. As the test is not a classroom test, discriminating the merit of students but assessing the knowledge of the clinical population, the cut-off range for the discrimination value was taken as .10 to 1.0 considering the necessity of the items that measured the indicators of the disease. Based on this exclusive cut-off range of discrimination value, item numbers 1, 8, 10, and 28 were dropped. Item numbers 37, 41, 42, and 47 which were already dropped based on the cut-off range of item difficulty value were also found to have very low discrimination value. Thus, the process of item analysis gave rise to 37 items of DMKT showing variation of number of items in the dimensions—symptoms (9 items), causes and risk factors (12 items), complications (11 items), and management (5 items).

Reliability

It was intended to measure the reliability of the test to determine the internal consistency of the test to examine whether the items of the test measure the same construct. To meet this end, it was decided to calculate the reliability coefficient by using the Kuder-Richardson 20 ([KR-20] [23), as response category of each of the items was dichotomous and difficulty value varied between .20 and .90. The reliability coefficient was found to be .76. Hence, this 37-item DMKT was accepted for establishing validity and developing preliminary norm which was done with a new clinical sample under phase IV of the study.

Table 1 Item difficulty index (p) and discrimination index (d) of the Diabetes Mellitus Knowledge Test

Test dimension	Score	Diff (*p*)	27% Top (58)	27% Bottom (58)	Dis (*d*)
Symptoms					
Item 1[b]	179	0.85	51	46	0.09
Item 2	177	0.84	55	38	0.3
Item 3	180	0.85	55	38	0.3
Item 4	157	0.75	50	38	0.21
Item 5	160	0.76	53	35	0.32
Item 6	143	0.68	53	22	0.54
Item 7	161	0.76	55	32	0.4
Item 8[b]	187	0.89	52	52	0
Item 9	177	0.84	56	36	0.35
Item 10[b]	74	0.35	21	24	− 0.06
Item 11	186	0.88	58	37	0.37
Item 12	171	0.81	55	36	0.33
Causes and risk factors					
Item 13	172	0.82	55	35	0.35
Item 14	143	0.68	56	16	0.69
Item 15	138	0.66	51	22	0.5
Item 16	186	0.88	57	42	0.26
Item 17	91	0.43	38	13	0.44
Item 18	121	0.58	39	26	0.23
Item 19	138	0.66	50	18	0.56
Item 20	109	0.52	35	18	0.3
Item 21	74	0.35	31	13	0.32
Item 22	41	0.2	18	7	0.19
Item 23	161	0.76	49	31	0.32
Item 24	127	0.6	49	23	0.45
Complications					
Item 25	149	0.71	48	39	0.16
Item 26	126	0.6	45	22	0.4
Item 27	173	0.82	51	41	0.18
Item 28[b]	69	0.33	20	16	0.07
Item 29	169	0.8	57	32	0.44
Item 30	169	0.8	54	35	0.33
Item 31	104	0.5	43	14	0.5
Item 32	109	0.52	45	20	0.44
Item 33	149	0.71	49	32	0.3
Item 34	149	0.71	49	26	0.4
Item 35	151	0.72	47	30	0.3
Item 36	78	0.37	30	19	0.19
Management					
Item 37[a, b]	208	0.99	58	57	0.02
Item 38	132	0.63	40	30	0.18
Item 39	190	0.90	57	45	0.21
Item 40[a]	204	0.97	58	52	0.11
Item 41[a, b]	206	0.98	58	54	0.07
Item 42[a, b]	208	0.99	57	53	0.07
Item 43[a]	194	0.92	57	47	0.18
Item 44	161	0.76	48	40	0.14
Item 45	163	0.77	52	37	0.26
Item 46	54	0.26	23	10	0.23
Item 47[a, b]	202	0.96	56	55	0.02
Item 48[a]	197	0.93	58	48	0.18

[a] Items deleted from the test basing on the cut-off determined for the item difficulty index

[b] Items deleted from test basing on the cut-off determined for the item discrimination index

Phase IV: establishing validity and development of norms

In phase IV, convergent validity of the DMKT was established on a new group of clinical sample by identifying relevant constructs—adherence, self-efficacy, and social support. Research has shown that acquiring knowledge about diabetes influences the persons to make better health choices and improves their adherence to medical recommendations [24]. Possessing better knowledge about diabetes has been associated with increased inclination to perform self-care activities, such as regular exercise, and sticking to diabetes relevant diet, perception of fewer

barriers to blood glucose monitoring, better adherence to medication [25], and better glycemic control [26].

To evaluate the effect of patient education on their knowledge level, self-management, and self-efficacy, involving 80 type 2 diabetes patients, researchers found a marked improvement in outcome measures after the intervention involving the education program [27]. They also found a limited effect on patient's knowledge and their self-management behaviors; however, there was a significant effect in their self-efficacy. Research has also found a positive relationship between social support, patient education, and self-management of type 2 diabetes patients [28, 29].

Like phase III, this phase also involved cross-sectional study design to establish validity and develop norm for the Indian clinical population.

Method

Participants

For establishing validity and norm of DMKT, data were gathered from 300 patients suffering with type 2 diabetes using purposive sampling method from five diabetic clinics in (name of the location of the study has not been disclosed for blinded review). Due to reasons such as missing data and withdrawal by the participants, data gathered from 168 patients ($n_3 = 268$) were taken into consideration. The final sample consisted of 136 male and 132 female patients, between the age of 25–75 years ($M = 51.84$, $SD = 10.97$). The range of duration of disease of the sample was found to be from 1 month to 35 years ($M = 6.48$, $SD = 6.42$). The sample consisted of participants who were illiterate (9.3%) and those with different educational level—primary (6%), secondary (12.7%), high school (26.1%), intermediate (12.7%), graduation (19%), and post-graduation (14.2%). The sample was also found to consist of participants who were unemployed (0.4%) and those from various occupational backgrounds such as homemakers (40.3%), private or government employees (25.7%), entrepreneurs (15.7%), farmers (4.1%), retired persons (9.7%), and those working in corporate sector (4.1%). A similar inclusion and exclusion criterion that was followed in phase III of the study was applied in this phase.

Instruments

The following instruments were used to measure the criterion constructs—Interpersonal Support Evaluation List (ISEL), Self-efficacy for Diabetes, and Diabetes Adherence Scale, in addition to the newly developed DMKT.

Interpersonal support evaluation list To measure the level of social support of the patients, the ISEL [30] was administered. It has 40 statements concerning the perceived availability of potential social resources, which fall into four 10-item subscales. It measures four dimensions of social support—tangible support, appraisal support, self-esteem support, and belongingness support. Half of the items are positive statements about social relationships while half are negative statements. Respondents were asked to indicate whether each statement is 'definitely true' or 'probably true' or 'probably false' or 'definitely false', a score of 3, 2, 1, and 0, were given, respectively, to these options. Out of 40 items, 20 items are reverse scored. The higher the score, the higher the perceived support by the individual on that particular subscale is. The total ISEL score is obtained by adding the scores of all the subscales. The range of score for each subscale would be 0–30; similarly, the range for total ISEL score would be 0–120. Internal reliability coefficient of the total general population ISEL ranges from .88 to .90, and ranges for subscales are .70 to .82 for appraisal, .62–.73 for self-esteem, .73–.78 for belonging, and .73–.81 for tangible support. The scales have been validated with other measures; the desire for verbal intimacy subscale of the Colwill and Spinner Privacy Measure correlated .40 ($p < .001$) with the appraisal scale and .80 and .24 ($p < .01$) with the tangible and belonging scales, respectively. The self-esteem support subscale from the ISEL was correlated .74 ($p < .001$) with the Rosenberg Self-esteem Scale (1965).

Self-efficacy for diabetes This scale was developed by funded by the National Institute of Nursing Research (NINR) in United States of America. The scale contained eight items, which measured the confidence of the patients in doing certain diabetes-related activities like managing their diet, exercising, monitoring their blood glucose levels, visiting the doctor, and daily functioning. The respondent rated his/her response for each item by circling a number on a 10-point scale where a score of 1 indicates 'not at all confident' and a score of 10 indicates 'totally confident'. The score for each item is the number circled. If two consecutive numbers are circled, the lower number is coded. If the numbers which are circled are not consecutive, the item is not scored. If more than two items are missing, the scale is not scored. The observed range of the score is 1–10. The higher score indicates higher self-efficacy. The internal consistency reliability was found to be .82, as given by the developers.

Diabetes adherence scale This scale is the adapted version [31] of the original 14-item Hill Bone High Blood Pressure Compliance Scale [32]. The scale consists of five domains of adherence namely—reduced sucrose intake (diet), keeping doctor's appointment, taking medication, monitoring blood glucose level, and following exercise regime. The scale contains 15 items and has a five-point rating scale. The patient rates his/her response for each item by ticking on one of the

five options—'None of the time', 'Some of the time', 'Most of the time', 'All the time', and 'Not applicable/Do not know' which are given a score of 4, 3, 2, 1, and 0, respectively. Items numbered 6, 12, and 13 are reverse scored. The total score is calculated by adding the score of each item based on the response given by the subject and score allotted to the option. The higher the score, the better the adherence, and the score can range from 0 to 60. Item numbers 7, 8, and 9 from the original scale have been omitted, and items 13, 14, and 15 have been added to the Diabetes Adherence Scale in order to include items on exercise. The standardized alpha of the original scale for the total scale ranged between 0.74 and 0.84 and the average inter-item correlations were 0.18 and 0.28, respectively.

Diabetes Mellitus Knowledge Test The 37-item final version of DMKT developed in phase III was used to test knowledge of diabetes. The originally developed measure had 48 items, and after item analysis, the items were reduced to 37 items, with the different number of items in each dimension—symptoms (9 items), causes and risk factors (12 items), complications (11 items), and management (5 items).

Scoring of DMKT A score of 1 is assigned for each correct response (either true or false) and a score of 0 for any incorrect response. Out of 37 items, all the items except item numbers 4, 16, 17, 18, 21, 22, 24, 25, 31, 32, 36, 38, and 46 are given a score of 1 when the answer is 'false', and for the rest of the items, a score of 1 is given when the answer is 'true'. The total score of the test varies from 0 to 37, the total score on each dimension ranges between 0 and 9 for symptoms, 0 and 12 for causes and risk factors, 0 and 11 for complications, and 0 and 5 for management. The higher the score in a particular dimension, the higher the knowledge is. Similarly, the higher the score on the total test, the higher the person's knowledge of diabetes mellitus is.

Procedure

In this phase, a new clinical sample of 268 participants was selected from basing upon purposive sampling method. Data were collected from the same six clinics as before. To collect data, the out-patient units of the clinics were visited every day for approximately 5 h every day. Informed consents were taken from the patients, and the test was then administered individually. Instructions were given in the regional language, Hindi or English as per the preference of the participants. Patients were encouraged to answer to each item as per their knowledge. It took approximately 10–15 min to complete the DMKT, after which the participants were debriefed.

Establishing validity

Product-moment correlation coefficients were conducted in order to analyze and compare the relationship between the constructs and thereby establish the validity. The correlation coefficients are mentioned in Table 2.

Relationship between diabetes knowledge and adherence Table 2 reveals that there was a significant positive correlation between different dimensions of knowledge of diabetes and adherence. The dimension of adherence to medicine was found to have a significant positive correlation with knowledge of management, $r(266) = .13$, $p < .05$. Adherence to diet was found to have a significant positive correlation with knowledge of symptoms, $r(266) = .15$, $p < .05$; and with knowledge of management, $r(266) = .12$, $p < .05$.

The dimension of adherence to exercise was found to have a significant positive relationship with all dimension of knowledge of diabetes: knowledge of symptoms, $r(266) = .17$, $p < .01$; knowledge of causes and risk factors, $r(266) = .21$, $p < .01$; knowledge of complications, $r(266) = .22$, $p < .01$; knowledge of management, $r(266) = .12$, $p < .01$; and the total knowledge related to diabetes, $r(266) = .26$, $p < .01$. The dimension of adherence to blood glucose monitoring was found to have significant positive correlation with the dimension of knowledge of causes and risk factors, $r(266) = .16$, $p < .05$; and with total knowledge of diabetes, $r(266) = .14$, $p < .05$.

A significant positive correlation was found between total adherence and all dimensions of knowledge related to diabetes except for the dimension of knowledge of causes and risk factors. Adherence total was found to be correlated with the dimension of knowledge of symptoms, $r(266) = .22$, $p < .0$; with dimension of knowledge of complications, $r(266) = .17$, $p < .01$; with dimension of knowledge of management, $r(266) = .19$, $p < .01$; and with total knowledge of diabetes, $r(266) = .22$, $p < .01$. The only significant negative correlation was found to be between the dimension of adherence to doctor's consultation and knowledge of causes and risk factors, $r(266) = -.14$, $p < .05$.

Relationship between diabetes knowledge and self-efficacy It can be observed from Table 2 that self-efficacy was found to have significant positive correlation with all dimensions of knowledge of diabetes namely knowledge of symptoms, $r(266) = .30$, $p < .01$; knowledge of causes and risk factors, $r(266) = .36$, $p < .01$; knowledge of complications, $r(266) = .30$, $p < .01$; knowledge of management, $r(266) = .28$, $p < .01$; and with the total knowledge of diabetes at $r(266) = .43$, $p < .01$.

Relationship between diabetes knowledge and social support Table 2 reveals that there was a significant positive correlation

Table 2 Relationship between diabetes knowledge, social support, self-efficacy, and adherence

Dimensions	AM	AD	AE	ABGM	ADC	AT	SE	SA	ST	SB	SSE	STO	*M*	SD
DMKT-T	.02	.03	.26**	.14*	− .05	.22**	.43**	.16*	.06	.07	.01	.14*	24.65	5.24
DMKT-S	.11	.15*	.17**	.11	− .01	.22**	.30**	.09	− .03	.01	.02	.05	7.11	1.77
DMKT-CR	− .07	− .09	.21**	.16*	− .14*	.11	.36**	.15*	.08	.11	.04	.15*	7.09	2.37
DMKT-C	− .02	.01	.22**	.06	.01	.17**	.30**	.14*	.06	.05	− .04	.12*	7.18	1.91
DMKT-M	.13*	.12*	.12*	.08	.06	.19**	.28**	.01	.08	.01	− .06	.02	3.27	0.96
M	27.06	11.09	8.13	2.16	3.75	52.19	57.27	21.54	21.88	22.93	25.92	92.27		
SD	1.63	1.07	3.53	0.54	0.64	4.57	10.12	8.36	4.91	2.55	2.17	12.78		

Note. All analyses are two tailed. *$p < .05$, **$p < .01$

Row: *DMKT-T* DMKT total score, *DMKT-S* DMKT symptoms, *DMKT-CR* DMKT causes and risk factors, *DMKT-C* DMKT complications, *DMKT-M* DMKT management. Column: *AM* adherence to medication, *AD* adherence to diet, *AE* adherence to exercise, *ABGM* adherence to blood glucose monitoring, *ADC* adherence to doctor's consultation, *AT* total adherence, *SE* self-efficacy, *SA* social support-appraisal support, *ST* social support-tangible support, *SB* social support-belongingness support, *SSE* social support-self-esteem support, *STO* total social support, *M* mean, *SD* standard deviation

between the two constructs on few dimensions. The dimension of appraisal support was found to have significant positive correlation with knowledge of causes and risk factors, $r(266) = .15$, $p < .05$; also with the dimension of knowledge of complications, $r(266) = .14$, $p < .05$, and with the total knowledge of diabetes at $r(266) = .16$, $p < .05$. Significant positive correlation was also found between the total social support and the dimension of knowledge of causes and risk factors, $r(266) = .15$, $p < .05$; with the dimension of knowledge of complication, $r(266) = .12$, $p < .05$; and with the total knowledge of diabetes at, $r(266) = .14$, $p < .05$. Nevertheless, there was no significant correlation found between knowledge of diabetes and other dimension of social support, which were tangible support, belongingness support, and self-esteem support.

Therefore, it can be observed from the results that knowledge of diabetes has a strong and significant correlation with the constructs on self-efficacy and adherence. Persons having knowledge about diabetes symptoms, causes and risk factors, complications, and management tend to have better/greater self-efficacy and show better adherence on the whole and specifically to exercise. Persons with better knowledge of symptoms have better adherence to diet, those with better knowledge of diabetes causes and risk factors have better adherence to blood glucose monitoring and lower adherence to keeping appointment with their doctor, those with better knowledge of diabetes management are shown to have better adherence to medication and diet, and those with overall knowledge of diabetes has shown to have better adherence to blood glucose monitoring.

Likewise, knowledge about causes and risk factors and complications of diabetes was found to have significant positive correlation with total social support and specifically with the dimension of appraisal support. In other words, those having better social support and appraisal support in specific have better knowledge about diabetes causes, risk factors, and complications. No correlation was found between other dimensions of knowledge of diabetes and social support. These results help in validating the newly developed measure DMKT with the significant relationship that it was found have with self-efficacy, adherence, appraisal support, and overall social support. These findings are validated with the help of previous literature which found similar results and relationship among the constructs.

Table 3 Mean, standard deviation, and confidence intervals for the total sample, men and women of both the total test and dimensions scores

Dimensions	Total sample	95% CI		Men	95% CI		Women	95% CI	
	M (SD)	L	U	*M (SD)*	L	U	*M (SD)*	L	U
DMKT-T	24.65 (5.24)	24.02	25.28	25.76 (4.61)	24.98	26.54	23.52 (5.61)	22.55	24.48
DMKT-S	7.11(1.77)	6.90	7.32	7.40 (1.60)	7.13	7.68	6.81(1.88)	6.49	7.13
DMKT-CR	7.09 (2.37)	6.81	7.38	7.79 (1.99)	7.46	8.13	6.37 (2.51)	5.94	6.80
DMKT-C	7.18 (1.92)	6.95	7.41	7.37 (1.84)	7.06	7.68	6.98 (1.98)	6.64	7.33
DMKT-M	3.27 (0.96)	3.15	3.38	3.19 (0.95)	3.03	3.35	3.35 (0.98)	3.18	3.52

DMKT-T DMKT total score, *DMKT-S* DMKT symptoms, *DMKT-CR* DMKT causes and risk factors, *DMKT-C* DMKT complications, *DMKT-M* DMKT management, *CI* class interval, *L* lower limit, *U* upper limit

Table 4 Norm for the total sample, men and women on the total test and dimension scores

Dimensions	Total sample			Men			Women		
	Low	Average	High	Low	Average	High	Low	Average	High
DMKT-T	0–19.40	19.41–29.88	29.89–37.00	0–21.14	21.15–30.36	30.37–37.00	0–17.90	17.91–29.12	29.13–37.00
DMKT-S	0–5.33	5.34–8.87	8.88–9.00	0–5.79	5.80–8.90	8.91–9.00	0–4.92	4.93–8.68	8.69–9.00
DMKT-CR	0–4.71	4.72–9.45	9.46–12.00	0–5.79	5.80–9.77	9.78–12.00	0–3.85	3.86–8.87	8.88–12.00
DMKT-C	0–5.25	5.26–9.00	9.01–11.00	0–5.52	5.53–9.20	9.21–11.00	0–4.90	4.91–8.95	8.96–11.00
DMKT-M	0–2.30	2.31–4.22	4.23–5.00	0–2.23	2.24–4.13	4.14–5.00	0–2.36	2.37–4.32	4.33–5.00

DMKT-T DMKT total score, *DMKT-S* DMKT symptoms, *DMKT-CR* DMKT causes and risk factors, *DMKT-C* DMKT complications, *DMKT-M* DMKT management

Development of norms

Another important task of this phase of developing the DMKT was to develop the preliminary norms for the clinical population in India. Statistical analysis such as *M*, *SD*, and percentile scores was done to develop norm for the total sample. The independent *t* test results revealed that there was a significant difference between male and female patients in respect of knowledge of diabetes, $t(266) = 3.58$, $p < .001$, indicating that male patients have higher knowledge ($M = 25.76$, $SD = 4.61$) than female patients ($M = 23.52$, $SD = 5.61$). For this, gender-based norm was developed not only for the overall knowledge, but also for each of the four dimensions and presented in Tables 3, 4, and 5.

From Table 3, it is revealed that the mean score of the total sample on DMKT total was found to be $M = 24.65$, $SD = 5.24$, on the dimensions of symptoms $M = 7.11$, $SD = 1.77$, causes and risk factors $M = 7.09$, $SD = 2.37$, complications $M = 7.18$, $SD = 1.92$, and management $M = 3.27$, $SD = 0.96$. The 95% confidence interval was also calculated and presented in the table.

Table 4 presents the norm for the total sample, men and women on the total test, and the dimension scores. The range of the low score was determined by subtracting 1 *SD* from the *M* ($M - 1$ *SD*), whereas the high score was calculated by adding 1 *SD* to the *M* ($M + 1$ *SD*). In this manner, the norm was calculated for overall knowledge and dimension specific knowledge in respect of the total sample and each gender. The norm has been developed by classifying the range of scores under three categories—high, average, and low. When the patients score between 29.89 and 37.00 in overall knowledge, they are expected to have high level of knowledge. Likewise, if the patients score between 0 and 19.40, they are expected to have low knowledge, whereas those who score between 19.41 and 29.88 are expected to have average knowledge in diabetes mellitus. Likewise, the interpretation of the norm is to be done for each dimension and gender.

Figure 1 represents the profile of male and female patients as well as the total sample so far as their overall knowledge and dimension-wise knowledge of diabetes are concerned.

Table 5 Percentile scores for the total sample, men and women for both the total test and dimension scores

Dimensions	Total sample			Men			Women		
	25th	50th	75th	25th	50th	75th	25th	50th	75th
DMKT-T	22	25	28	23	26	29	20	24	28
DMKT-S	6	8	8	7	8	9	6	7	8
DMKT-CR	6	7	9	6	8	9	5	7	8
DMKT-C	6	7	9	6	7	9	6	7	8
DMKT-M	3	3	4	3	3	4	3	4	4

DMKT-T DMKT total score, *DMKT-S* DMKT symptoms, *DMKT-CR* DMKT causes and risk factors, *DMKT-C* DMKT complications, *DMKT-M* DMKT management

Discussion

The main objectives of the study were to develop a test for Indian population to measure knowledge of symptoms, causes and risk factors, complications, and management of diabetes mellitus, and also to standardize the test by establishing the psychometric properties and norm through initial assessment on clinical population of India. By following the rigorous scientific process of test construction—item writing, content validation, item analysis and reliability, and establishing validity and norm—a new test named the DMKT has been developed.

The newly developed instrument is short with easily understandable phrases which do not involve the usage of jargons. The usage of simple terms can be understood by both literate and non-literate test-takers in rural and urban India, and other developing and under-developed countries. The nature of items and the dichotomous response pattern of the test are simple, making the test easy to administer, score, and interpret. The test DMKT may either be administered as an individual test or a group test in both clinical and non-clinical settings.

The test proves to be an efficient instrument to measure knowledge of diabetes of newly diagnosed patients and also

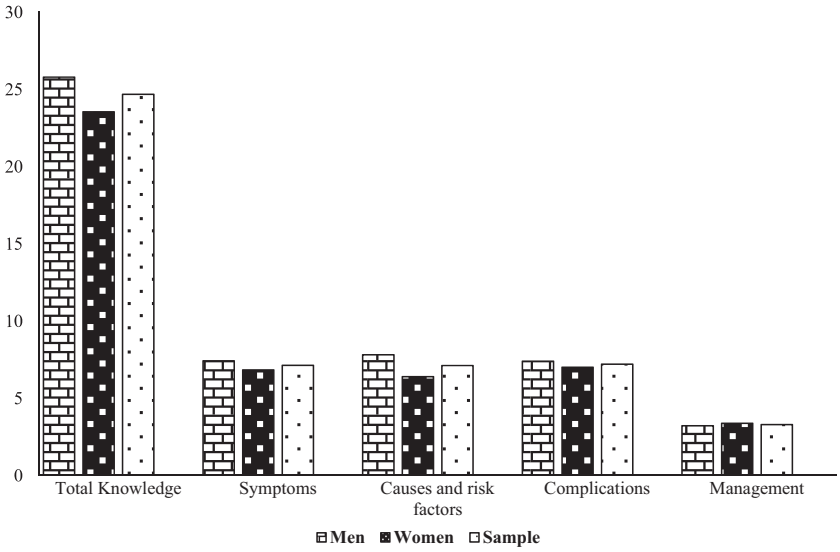

Fig. 1 The profile of male and female patients as well as the total sample so far as their overall knowledge and dimension-wise knowledge of diabetes are concerned

for those with longer duration of disease. The test can be administered by psychologist and paramedical support professionals (nurses, physiotherapists, and dietician) having minimum knowledge in assessment. The test can be administered to both insulin-dependent and non-insulin-dependent patients. The DMKT may also be used as a self-report measure by the diabetes patients.

Each dimension of the test gives insight about the person's knowledge on that specific dimension of diabetes. This is beneficial for the patients, their caregivers, and health professionals as they may look into specific areas in which they lack knowledge based on their duration of disease. This would help in developing individualized intervention program. The assessment of DMKT is also expected to provide information about people at-risk, newly diagnosed patients, and those patients with longer duration of illness. For those who are at risk for diabetes, the dimensions of causes and risk factors, and complications of the condition may both inform and forewarn them of the harm. For those who are recently diagnosed with diabetes, all the dimensions give the required information in terms of, whether they know the kind of symptoms they are likely to experience, if they know what causes the condition so that they may reduce the harmful health behaviors, are they aware of the complications that are likely to occur, and do they know how to manage the condition. For those with longer duration of disease could be benefitted from the dimension of management.

Considering the rapid increase in the number of diabetes patients across the country, DMKT may be used for screening patients with low knowledge level and for diagnostic purpose to find out on which dimension a patient lacks knowledge in and they can be provided with information about the illness. The DMKT may be used during health check-up camps or diabetic camps as it is handy for quick measurement and eventually an awareness program could be planned as an intervention to educate the patients with the knowledge of the disease.

The DMKT is a contribution to the body of research and test construction, as it is unique when compared to already existing measures. As mentioned before, the instrument has practical implications of applicability and usability given the rural and urban settings in India even if there is a lack of accessibility to health information and therefore meets the existing need.

Nevertheless, there are a few shortcomings concerning the test. The norm was calculated based on a limited clinical sample. The present norm may be used as preliminary information and follow-up study may be conducted to standardize DMKT on a larger sample considering the higher incidence and prevalence of diabetes mellitus. The convergent validity which was established by finding out the correlation between theoretically related constructs of adherence, self-efficacy, and appraisal support may be replaced with construct validity using the design of multitrait-multimethod matrix in the follow-up studies.

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Compliance with ethical standards

Before starting the study, approval was obtained from the institutional ethics committee.

Conflict of interest The authors declare that they have no conflict of interest.

APPENDIX 1

Diabetes Mellitus Knowledge Test (DMKT)

Instructions: "This test measures a person's knowledge of diabetes mellitus and consists of right and wrong answers. Below are certain terms/phrases written under four sections—Section A (Symptoms), Section B (Causes and risk factors), Section C (Complications), and Section D (Management). Please read each of the following terms/phrases carefully. If the term/phrase is true for diabetes mellitus please encircle Ⓣ and if you know that it is not true for diabetes mellitus, please encircle Ⓕ. Please respond to all the terms/phrases one by one and be genuine in your answer."

Section A: Symptoms		Response	Section C: Complications		Response
#1.	Frequent cold	T/ F	**25.	Loss of appetite	T / F
*2.	Frequent hunger	T/ F	*26.	Coma	T / F
*3.	Blurry vision	T/ F	*27.	Foot ulcer	T / F
**4.	Difficulty in breathing	T / F	#28.	Chest pain	T / F
*5.	Dizziness	T / F	*29.	Kidney failure	T / F
*6.	Excessive thirst	T / F	*30.	Gangrene	T / F
*7.	Weight loss	T / F	**31.	Tonsillitis	T / F
#8.	Bleeding from the nose	T / F	**32.	Jaundice	T / F
*9.	Frequent urination	T / F	*33.	Urinary infection	T / F
#10	Joint pains	T / F	*34.	Heart disease	T / F
*11.	Slow healing of wounds	T / F	*35.	Cataract	T / F
*12.	Physical weakness	T / F	**36.	Headache	T / F
Section B: Causes and Risk Factors		Response	Section D: Management		Response
*13.	Heredity	T / F	#37.	Regular medication	T / F
*14.	Inadequate secretion of insulin	T / F	**38.	Excessive consumption of white bread	T / F
*15.	Overweight	T / F	*39.	Low fat diet	T / F
**16.	Contact with diabetic people	T / F	#40.	Regular physical exercise	T / F
**17.	Blood transfusion	T / F	#41.	Maintenance of body weight	T / F
**18.	Old age	T / F	#42.	Consumption of sweets	T / F
*19.	Lack of physical activity	T / F	#43.	Regular check up of blood sugar level	T / F
*20.	Poor diet	T / F	*44.	Low salt diet	T / F
**21.	Consumption of red meat	T / F	*45.	Usage of footwear inside the house	T / F
**22.	High cholesterol	T / F	**46.	Avoidance of cold weather	T / F
*23.	Stress	T / F	#47.	Reduced consumption of rice	T / F
**24.	Head injury	T / F	#48.	High fiber diet	T / F

Details about the DMKT

- The final version of DMKT consists of 37 items after deleting items marked with # basing on item analysis.
- The items marked with * are to be positively scored (True = 1, False = 0).
- The items marked with ** are to be negatively scored (True = 0, False = 1).
- Items in each dimension: Symptoms (Items No: 2, 3, 4, 5, 6, 7, 9, 11, and 12), Causes and risk factors (Items No: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24), Complication (Items No: 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, and 36), and Management (Items No: 38, 39, 44, 45, and 46).

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ORIGINAL ARTICLE

Psychometric properties of Persian Diabetes-Mellitus Specific Quality of Life (DMQoL) questionnaire in a population-based sample of Iranians

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Abstract

Health-related quality of life (HRQoL) among patients with diabetes mellitus is often poorer than in those with other chronic medical conditions. Appropriate disease specific measures are needed to measure HRQoL in these patients. This study sought to validate a culturally adapted version of the Diabetes-Mellitus Specific Quality of Life (DMQoL) questionnaire module in Persian. Concurrent validity of the scale was assessed by the Diabetes Quality of Life (DQOL) questionnaire. Convergent and discriminative validity of the DMQoL was determined using a brief version of World Health Organization's Quality of Life Scale Brief version (WHOQOL-BREF), Hospital Anxiety and Depression Scales (HADS), and Medication Adherence Report Scale (MARS). Construct validity was examined using confirmatory factor analysis. Rasch analysis was also performed to examine the unidimensionality of the DMQoL. Known-group method was used to examine the ability of the scale to differentiate between different categories of patients. A sample of 824 patients (512 females) with diabetes mellitus was recruited from diabetic care centers located in Qazvin, Iran. The mean age of participants was 54.1 (SD 6.3) and 27% were smokers. All items loaded on a single factor (factor loadings ≥ 0.6) and internal consistency of the scale was acceptable ($\alpha = 0.89$). Significant associations were found between the scale and DQOL, indicating concurrent validity ($p < 0.001$). The DMQoL was able to differentiate subgroups of patients with hypertension, HbA1c, cholesterol, and diabetic diet. All items were appropriate with regard to difficulty level and confirmatory factor analysis verified the scale's single dimension (CFI = 0.927; RMSEA = 0.067). Persian DMQoL is a reliable and valid measure of HRQoL in a Persian-speaking population with type II diabetes. Further assessment is needed to confirm the psychometric properties of the scale in other cultures and languages. Future studies are needed to determine the sensitivity of the scale to change over time in response to treatment.

Keywords Diabetes mellitus · Health-related quality of life · Psychometrics · Validity · Reliability

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Introduction

Diabetes mellitus (DM) is a global health problem that threatens the lives of many people, particularly those who pursue a sedentary lifestyle, are overweight or obese, and those with a familial history of diabetes [1]. The prevalence of DM is increasing worldwide and is estimated by 2035 to affect 592 million people (i.e., 7% of world population) [2]. The mortality rate attributed to DM is also considerable and those with this disease experience an age-adjusted death rate that is nearly twice that of healthy people [3]. DM is not limited to a particular region, ethnicity, culture, or country, and those in both developed and developing countries are at risk. In the UK, 6–9% of people over age 65 years suffer from DM [4] and the prevalence is even higher in the US population with more than one fourth of older adults affected [5]. Among Asian countries, the prevalence is also high, particularly in countries such as China, Malaysia, Taiwan, and Turkey that reported a DM prevalence of 8–15% [6–8]. The prevalence of the disease in Iran has increased substantially over the past two decades and is now estimated to be about 5% in general population and nearly 14% in older adults [9, 10].

Chronic diseases such as diabetes include various physical, psychological, social, and cultural dimensions that may affect the development and progression of the disease. Consequently, monitoring diabetics only by laboratory assessments such as fasting blood sugar, hemoglobin A1C, lipid profile, and blood pressure does not provide a holistic approach to this disease [1]. Health-related quality of life (HRQOL) is a patient-centered measure of health that informs health care providers about patients' perceptions of how the disease is affecting them. This construct describes the individual's perception of their health in the context of their social, cultural, and value systems and in light of their goals, standards, and life concerns [11]. Utilizing HRQOL as a measure of subjective health is a common practice for chronic diseases such as diabetes particularly since many studies have shown that people with DM have poorer HRQOL than those with other chronic diseases or those who are healthy [12–14]. This construct is also useful for describing the overall effectiveness of all interventions implemented to improve health [11].

There are two primary categories of HRQOL instruments, those that assess this construct more generally and disease-specific measures. Although both types of measure may provide valuable information regarding patients perceptions of their health, disease-specific measures have been recognized as more efficient in assessing different aspects of a disease that may not be captured by more general measures [15]. Measures assessing disease-specific HRQOL also have higher sensitivity to change in response to therapeutic interventions because the items are adapted to the particular disease. Thus, disease-specific measures are more likely to capture the effects that a specific disease has on an individual's performance and functioning [16].

To date, many disease-specific measures have been developed for people living with DM. Examples of these are the Diabetes Quality of Life (DQOL) Scale [17], health-related quality of life measure in older African American women with type II diabetes [18], Audit of Diabetes-Dependent Quality of Life (ADDQoL) Scale [19], and Diabetes Quality of Life Clinical Trial Questionnaire (DQLCTQ) [20]. However, these measures are usually available only in English and are specifically developed for those who live in Western countries. The cultural and contextual factors that may influence the use of these measures in different regions or countries have not yet been determined. Recently, a new disease-specific measure of the HRQOL in diabetics has been translated and culturally adapted for use among Persian speaking people (i.e., DQOL) [21]. Adapting an instrument such as this one (originally developed in one particular region) for those in other regions of the world will enrich the measure's use more globally.

The Diabetes-Mellitus Specific Quality of Life (DMQoL) scale was initially developed to assess HRQOL among Taiwanese patients with DM. This is a short disease-specific measure with only 10 items derived from recommended guidelines for the disease. The authors used objective measures such as HbA1c, lipid profile, and glomerular filtration rate, along with the World Health Organization Quality of Life Scale Brief version (WHOQOL-BREF) to develop the scale. The DMQoL has been used for two purposes, both individually as a stand-alone disease-specific measure and as a module of the WHOQOL-BREF [22]. Thus, this measure allows for the determination of both general and specific profiles of HRQol for a target population. Assessment of the DMQoL's psychometric properties has shown it to be a reliable valid measure for use in other languages and populations. The limited number of items allows researchers to determine the quality of life of participants in a brief manner.

Given the relatively few scales available to measure HRQOL in Iranian patients, we decided to conduct a linguistic and cultural validation of the DMQoL in Persian-speaking diabetics in Iran.

Methods

Design

The study was conducted in two phases. In the first phase, the DMQoL was translated into Persian and culturally adapted. The second phase involved evaluating the psychometric properties of the DMQoL in Iranian patients with diabetes mellitus. The study was approved by the Ethic Committee at Qazvin University of Medical Sciences, and all patients gave written informed consent prior to participation.

Participants

Participants were selected based on consecutive sampling technique. The patients with type II diabetes being seen at specialty outpatient clinics of the Qazvin University of Medical Sciences in Qazvin (a city near to Tehran, Iran) were selected from August until September 2017. Patients were eligible if they were 18 years of age or older with a diagnosis of diabetes mellitus type II determined by a physician, spoke Persian, and were willing to participate in the study. Patients were excluded if they had significant cognitive impairment (Mini-Mental State Examination [MMSE] less than 26), major psychiatric disorders (psychotic and bipolar disorders), or were pregnant.

Translation procedure

The translation procedure was performed based on international guidelines for cross-cultural adaptation of measures [23]. The following steps were taken to adapt the English version of the DMQoL into Persian/Farsi. First, two bilingual translators translated the DMQoL into Persian independently (forward translation). The translators had different academic backgrounds and training (in medicine and in history) as recommended. The two translated versions were then compared and synthesized into one Persian version by consensus of the translators. The resulting Persian version was then translated back into English by two different translators whose native language was English (backward translation). The translators worked independently and were blinded to the original English version of the DMQoL. An expert committee (endocrinologist, nurse, psychologist, epidemiologist, and the translators) was then convened to construct the semi-final version of the Persian DMQoL. This version was then piloted in 37 diabetic patients (21 women and 16 men, mean age 51.2 years). Patients were interviewed to determine their views regarding how easy the items were to understand in terms of phrasing, response options, and initial instructions. Additional revisions were performed on the Persian DMQoL based on the pilot testing, and the final version was then administered to 824 patients with DM to assess its psychometric properties.

Measures

Socio-demographic factors

Data on age, gender, education, living situation, duration of diabetes, type of treatment, and smoking status were collected.

Anthropometric measures

Participants' height (cm) and weight (kg) were measured in the standard fashion to calculate body mass index (BMI).

Biochemical measures

Overnight fasting (12 h) blood samples were taken from patients to determine blood sugar (FBS) and were assessed by a HbA1cbya glucometer (YSI 2700 Select, YSI, Inc., Yellow Springs, OH) and by ion exchange chromatography (DS5 Analyzer, Drew Scientific Limited, Cumbria, UK). In addition, triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine (Cr), and blood urea nitrogen (BUN) were assessed using an auto analyzer (Liasys, AMS, Italy). Glomerular filtration rate (eGFR) was then calculated based on age, gender, blood creatinine, and body size using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. An eGFR < 60 ml/min is defined as chronic kidney disease.

Blood pressure and related complications

Systolic and diastolic blood pressures were measured using the left arm (mmHg) by a mercury sphygmomanometer after 15 min of rest in the seated position.

The presence of diabetes-related complications (e.g., retinopathy, coronary heart disease (CHD), and neuropathy) was determined based on the International Classification of Diseases, ninth version (ICD-9), using patients' medical records.

WHOQOL-BREF

The WHOQOL-BREF is a brief version of WHOQOL-100 that is used to assess quality of life. The WHOQOL-BREF consists of 26 items that cover four domains: physical health, psychological health, social relationships, and environment. Higher scores on each domain and on the overall measure indicate better quality of life. The WHOQOL-BREF has been translated into several languages including Persian [24].

DQOL

The DQOL is a diabetes-specific measure of quality of life used in diabetic patients. It contains 46 items with four subscales that assess satisfaction with treatment, impact of treatment, worry about the future effects of diabetes, and worry about social/vocational issues. All items are rated on a five-point Likert scale, with higher scores indicating more complications or greater dissatisfaction. In addition, a total score is computed. The Persian version of the DQOL has been shown

to have acceptable psychometric properties for use in adult Iranian diabetics [21].

Hospital Anxiety and Depression Scale

Psychological distress was assessed using the Hospital Anxiety and Depression Scale (HADS), a 14-item self-report measure that assesses symptoms of depression and anxiety. All items are scored on a four-point Likert scale (range 0–3) with higher scores indicating higher distress. The psychometric properties of the Persian version of the HADS have been examined and found to be acceptable in Iranian patients [25].

Medication Adherence Report Scale

The Medication Adherence Report Scale (MARS) is a self-report measure of medication adherence. It contains five items, each rated on a five-point Likert scale with an overall score ranging from 5 to 25, where higher scores indicate better adherence. The psychometric properties of the Persian version of the MARS have been examined among Iranian patients and found to be acceptable [26].

DMQoL

The DMQoL was recently developed to assess quality of life among patients with DM. The DMQoL consists of 10 items scored on a five-point Likert scale with higher scores indicating better quality of life [22].

Statistical analysis

Two categories of measurement properties were examined: classical test theory (CTT) and the Rasch model. The CTT is a traditional quantitative approach for assessing the reliability and validity of a measure (e.g., corrected item-total correlation, factor analysis). However, the CTT uses an inappropriate scoring system (e.g., means and standard deviations) and does not assess item difficulty and person ability (influence of respondent's abilities, attitudes, or personality traits). The Rasch model is a modern psychometric model that can estimate item difficulty and person ability. Therefore, both CTT and Rasch analyses were performed. CTT analyses were conducted using MPLUS version 7 (Muthén and Muthén 2012), and Rasch analysis was performed using WINSTEPS version 4.0.1. The Persian version of the DMQoL was examined for response rate, floor and ceiling effects, construct validity, internal consistency, concurrent validity, test-retest reliability, known-group validity, item difficulty, and item and person separation reliability.

Floor effects (percentage of the sample achieving the lowest possible scores) and ceiling effects (percentage of the sample achieving the highest possible scores) were computed. Floor and ceiling effects are present if 20% of the participants provide minimum or maximum possible scores.

Internal consistency was assessed by the Cronbach's alpha coefficient and corrected item-total correlation. A Cronbach's alpha > 0.70 is considered satisfactory, as are corrected item-total correlations > 0.40. Test-retest reliability was evaluated in 783 patients who completed the Persian DMQoL on two occasions (baseline and 3 weeks later). The intra-class correlation coefficient (ICC) between the scores at baseline and 3 weeks later was used to assess reliability across time.

In order to confirm the factor structure of the Persian DMQoL, a confirmatory factor analysis (CFA) was performed to assess construct validity and confirm the factor structure reported in the original study. Due to the ordinal nature of the data, weighted least squares estimation (WLS) with poly choric correlations and an asymptotic covariance matrix were computed. Goodness of fit was assessed using the following fit indices: chi-squared test (χ^2), comparative fit index (CFI), Tucker-Lewis index (TLI), root-mean-square error of approximation (RMSEA), and standardized root-mean-square residual (SRMR). A nonsignificant χ^2, CFI, TLI > 0.90, RMSEA, and SRMR < 0.80 support the construct validity of a measure. Additionally, a series of multigroup CFA's were conducted to examine structural invariance across subgroups of the patients based on gender and living situation. These models were examined for factorial invariance, configural invariance (pattern of factor loadings), metric invariance (the magnitude of factor loadings), and scalar invariance (the magnitude of item intercepts). According to Chen, factorial invariance is confirmed if ΔCFI > − 0.01, ΔSRMR < 0.01, and ΔRMSEA < 0.015 [27].

Pearson correlation coefficients were computed between the DMQoL and other instruments (WHOQOL-BREF, HADS, DQOL, and MARS) to measure concurrent validity. These correlations were controlled for age and gender.

To examine known-group validity, an independent *t* test was performed to determine whether the DMQoL total score differed among subgroup of patients based on specific characteristics of diabetic patients. It was hypothesized that patients with higher HbA1c, higher cholesterol, and the presence of diabetic complications (e.g., hypertension, diabetic foot, neuropathy, and retinopathy) would show differences in quality of life.

Rasch rating scale models were used to examine item difficulty and person separation reliability. Information-weighted fit statistic (in fit) mean square (MnSq) and outlier-sensitive fit statistic (outfit) MnSq were used to determine item fit. Item fit is supported if in fit or outfit MnSq are between 0.5 and 1.5. Differential item functioning (DIF) was also examined for DMQoL in terms of gender and situation to further investigate the measurement variance of the DMQoL at the item level. A DIF contrast (the difficulty for group 1 minus the difficulty for group 2) < 0.5 logits is considered small or absent.

Results

The mean (SD) age of participants was 54.1 (6.3) years, and the majority were female. More than half of participants were illiterate or had only an elementary school education. Nearly 70% of subjects were from rural areas. Hypertension (37%), ischemic heart disease (35%), and nephropathy (31%) were the most prevalent health problems. The mean (SD) for laboratory parameters were HbA1c = 7.9 (2.1%), triglyceride = 161.6 (11.0 mg/dl), and cholesterol = 183.4 (30.2), all of which exceeded international normal ranges. Details of other clinical and demographic characteristics are presented in Table 1.

There was no floor (ranged from 0.7 to 3.1%) or ceiling effects (ranged from 0.4 to 10.4%) for any of the items or for the total score.

As shown in Table 2, all items of the DMQoL loaded on a single factor with high factor loadings (≥ 0.6) and corrected item-total correlations (≥ 0.64). Test-retest reliability was excellent for all items (ICC ≥ 0.78). Rasch analysis revealed that participants in different genders or in different living situations (rural vs. urban) interpreted the ten-item descriptions in a similar fashion (DIF < 0.5). Multigroup CFA further confirmed that participants in different gender (ΔCFI = − 0.005 and − 0.001; ΔRMSEA = 0.002 and 0.005) or in different living situations (ΔCFI = − 0.001 and − 0.004; ΔRMSEA = − 0.004 and 0.005) interpreted the DMQoL similarly (Table 3).

Internal consistency was high (α = 0.89), as was separation reliability based on the Rasch analysis (person separation = 0.88; item separation = 0.95). Measures for the CFA were in all in acceptable ranges (χ^2 = 159.2; CFI = 0.927; TLI = 0.916; RMSEA = 0.067; SRMR = 0.049) (Table 4). In addition, the DMQoL total score demonstrated acceptable average variance extracted (0.51), satisfactory composite reliability (0.91), and low standard error of measurement (0.202).

Associations between the DMQoL and the DQOL (total score and subscale scores) were robust (*r* = − 0.398 to − 0.512), indicating concurrent validity for the DMQoL. Associations between the DMQoL and other instruments were likewise significant (*r* = 0.241 to 0.467 for WHOQOL-BREF; − 0.301 and − 0.382 for HADS; 0.341 for MARS), again supporting the concurrent validity of the DMQoL (Tables 5 and 6).

Similar to the DQOL, the DMQoL differentiated patients with and without hypertension, those having high HbA1c and low HbA1c, those having high and low cholesterol, and those consuming diabetic foods and those not ($p < 0.05$). However, the DMQoL could not differentiate those with and without nephropathy (Table 7).

Table 1 Participant characteristics (*n* = 824)

	Mean (SD)	*n* (%)
Age (year)	54.1 (6.3)	
Gender (female)		512 (62.1)
Educational year	6.9 (4.1)	
Body mass index (kg/m ^{2})	28.2 (4.5)	
Currently smoker (yes)		223 (27.1)
Living situation		
Rural		585 (71.0)
Urban		239 (29.0)
Diabetes-related complications		
Hypertension		304 (36.9)
Neuropathy		255 (30.9)
Nephropathy		171 (20.8)
Retinopathy		102 (12.4)
Diabetic foot		181 (22.0)
Ischemic heart disease		288 (35.0)
Systolic blood pressure (mmHg)	135.4 (19.1)	
Diastolic blood pressure (mmHg)	87.8 (15.2)	
Fasting blood sugar (mg/dl)	162.8 (85.2)	
HbA1c, percentage,	7.9 (2.1)	
Creatinine (mg/dl)	0.9 (0.3)	
Blood urea nitrogen (mg/dl)	18.1 (7.6)	
Triglyceride (mg/dl)	161.60 (11.0)	
Total cholesterol (mg/dl)	183.4 (30.2)	
LDL- cholesterol (mg/dl)	118.1 (61.6)	
HDL- cholesterol (mg/dl)	56.4 (41.2)	
Duration of diabetes (years)	13.1 (5.7)	
eGFR	89.2 (3.2)	
Oral agent, n (%)		596 (72.3)
Insulin		98 (11.9)

Discussion

The present study examined the psychometric properties of the Persian version of the DMQoL among a sample of Iranian patients with DM. We found that this measure had acceptable concurrent and construct validity as well as high internal consistency and test-retest reliability. In addition, known-group validity revealed its value in differentiating between patients with different diabetic characteristics indicating high sensitivity of the DMQoL across various conditions. Measurement invariance analyses confirmed the scale's ability to assess HRQOL among different groups of patients. Finally, the unidimensional nature for the DMQoL was confirmed using CFA and Rasch analyses.

Although not an epidemiological study designed to determine prevalence rates, the sex distribution of the sample was consistent with current rates of type II diabetes in women more generally [28, 29]. Furthermore, females usually access health services at a higher rate than males, which may explain the higher number of women in this sample (62%). CFA in a sample of 200 subjects (5–10 persons per item) would have been sufficient for the

Table 2 Psychometric properties of DMQoL at the item level

Item no.	Analyses from classical test theory			Analyses from Rasch				
	Factor loading[a]	Item-total correlation	Test-retest reliability[b]	InfitMnSq	Outfit MnSq	Difficulty	DIF contrast across gender[c, d]	DIF contrast across living situation[c, e]
DMQoL-1	0.70	0.73	0.92	0.96	0.94	− 0.51	0.02	− 0.08
DMQoL-2	0.60	0.64	0.81	1.30	1.35	0.38	− 0.43	− 0.03
DMQoL-3	0.78	0.79	0.84	0.81	0.74	0.03	− 0.03	− 0.05
DMQoL-4	0.77	0.77	0.86	0.90	0.88	− 0.06	0.02	− 0.36
DMQoL-5	0.73	0.74	0.85	0.87	0.93	0.34	− 0.38	− 0.15
DMQoL-6	0.69	0.73	0.82	1.16	1.06	− 0.28	0.28	− 0.04
DMQoL-7	0.63	0.66	0.81	1.06	1.06	0.05	0.07	0.20
DMQoL-8	0.80	0.77	0.78	0.68	0.70	− 0.37	− 0.02	0.01
DMQoL-9	0.74	0.80	0.87	1.11	1.09	0.05	0.37	0.07
DMQoL-10	0.67	0.69	0.79	1.12	1.13	0.38	0.15	0.08

DMQoL diabetes-specific quality of life questionnaire, *MnSq* mean square error, *DIF* differential item functioning

[a] Based on the first-order confirmatory factor analysis

[b] Using intraclass correlation coefficient (ICC)

[c] DIF contrast > 0.5 indicates substantial DIF

[d] DIF contrast across gender = difficulty for females − difficulty for males

[e] DIF contrast across accommodation = difficulty for participants living in rural − difficulty for participants living in urban

present study [11]. Therefore, including a large number of participants here may be considered as strength because it increases the study's power. In the initial study that developed the DMQoL, only 117 participants were included due to limitations involving the costs of laboratory tests [22]. In other studies which have done psychometric assessment of new instruments for disease-specific measures of HRQOL among patients with DM, the sample size has ranged between 100 and 500 subjects [18–20, 30].

Obesity, hyperlipidemia, hypertension, and poor health behavior such as smoking are modifiable risk factors in DM [1]. These conditions were also prevalent in our

Table 3 Measurement invariance across gender and across living situation (rural vs. urban) by confirmatory factor analysis

Model and comparisons	Fit statistics							
	χ^2 (*df*)	$\Delta\chi^2$ (Δdf)	CFI	ΔCFI	SRMR	ΔSRMR	RMSEA	ΔRMSEA
Gender (male vs. female)								
M1: configural	280.03 (70)*		0.925		0.051		0.063	
M2: plus all loadings constrained	295.35 (80)*		0.920		0.060		0.065	
M3: plus all intercepts constrained	310.58 (90)*		0.919		0.064		0.070	
M1 vs. M2		15.35 (10)		− 0.005		0.009		0.002
M1 vs. M3		15.23 (10)		− 0.001		0.004		0.005
Accommodation (rural vs. urban)								
M1: configural	341.99 (70)*		0.915		0.039		0.075	
M2: plus all loadings constrained	352.72 (80)*		0.914		0.048		0.071	
M3: plus all intercepts constrained	366.62 (90)*		0.910		0.057		0.076	
M1 vs. M2		10.81 (10)		− 0.001		0.009		− 0.004
M1 vs. M3		13.9 (10)		− 0.004		0.009		0.005

M1 model 1, a configural model; *M2* model 2, a model based on M1 with all factor loadings constrained being equal across groups; *M3* model 3, a model based on M2 or M2P with all item intercepts constrained being equal across groups; *CFI* comparative fit index; *SRMR* standardized root-mean-square residual; *RMSEA* root-mean-square error of approximation

*$p < 0.05$

Table 4 Psychometric properties of the DMQoLat the scale level

Psychometric testing	Value	Suggested cutoff
Ceiling effects (%)	5.8	< 20
Floor effects (%)	0.3	< 20
Internal consistency (Cronbach's α)	0.89	> 0.7
Person separation reliability from Rasch	0.88	> 0.7
Item separation reliability from Rasch	0.95	0.7
Confirmatory factor analysis		
χ^2 (*df*)	159.19 (35)*	Nonsignificant
Comparative fit index	0.927	> 0.9
Tucker-Lewis index	0.916	> 0.9
Root-mean-square error of approximation	0.067	< 0.08
Standardized root-mean-square residual	0.049	< 0.08
Average variance extracted	0.51	> 0.5
Composite reliability	0.91	> 0.6
Standard error of measurement	0.202	The smaller the better

*$p < 0.001$

sample and indicate poor disease control. We also found in our test of known-group validity that those with hypertension or hyperlipidemia experienced poorer HRQoL than did other participants. This finding suggests that the scale is sensitive enough to differentiate between patients with various health conditions and DM-related risk factors.

We assessed the psychometric properties of the DMQoL at both the item and the scale level. At the item level, all 10 items loaded on a single factor, confirming that the measure is assessing a single dimension. According to Stevens, when the number of items is 10 or lower, finding more than one dimension is difficult because related items tend to concentrate around a single concept [31]. Finding a single dimension in the DMQoL also suggests that disease-specific quality of life in diabetic patients may be a relatively simple and does not require developing long and complex instruments. However, longer scales with more items may identify different dimensions of HRQoL providing further insight with regard to this construct in diabetic patients.

The strong associations between the DMQoL and DQOL, along with relatively weak correlations with other measures (HADS and MARS), supported the convergent and divergent validity of the scale. Rasch model analyses also supported the construct validity of the scale. When comparing our assessment of the DMQoL with studies of other diabetes-specific measures of HRQoL, many of these other scales were validated using a less comprehensive package of validation tools than were employed in

Table 5 Concurrent validity of the DMQoL using Pearson correlation and partial correlation adjusted for age and gender

Criterion	Pearson correlation *r* (*p* value)	Partial correlation *r* (*p* value)
WHOQOL-BREF overall QoL	0.375 (< 0.001)	0.360 (< 0.001)
WHOQOL-BREF general health	0.241 (< 0.001)	0.234 (< 0.001)
WHOQOL-BREF physical domain	0.398 (< 0.001)	0.382 (< 0.001)
WHOQOL-BREF psychological domain	0.467 (< 0.001)	0.460 (< 0.001)
WHOQOL-BREF social domain	0.410 (< 0.001)	0.392 (< 0.001)
WHOQOL-BREF environment domain	0.333 (< 0.001)	0.281 (< 0.001)
WHOQOL-BREF total score	0.290 (< 0.001)	0.269 (< 0.001)
HADS anxiety	− 0.301 (< 0.001)	− 0.274 (< 0.001)
HADS depression	− 0.382 (< 0.001)	− 0.368 (< 0.001)
MARS	0.341 (< 0.001)	0.327 (< 0.001)

HADS Hospital Anxiety and Depression Scale, *MARS* Medication Adherence Report Scale, *WHOQOL-BREF* World Health Organization Quality of Life scale brief version

Table 6 Construct validity of the DMQoL using Pearson correlation and partial correlation adjusted for age and gender

Diabetes Quality of Life (DQOL)	Pearson correlation *r* (*p* value)	Partial correlation *r* (*p* value)
Satisfaction	− 0.483 (< 0.001)	− 0.451 (< 0.001)
Impact	− 0.512 (< 0.001)	− 0.493 (< 0.001)
Diabetes-related worry	− 0.398 (< 0.001)	− 0.337 (< 0.001)
Social/vocational worry	− 0.402 (< 0.001)	− 0.374 (< 0.001)
Total DQOL	− 0.436 (< 0.001)	− 0.401 (< 0.001)

the present study. For example, in a recent assessment of the Appraisal of Diabetes Scale, Hara and colleagues examined only concurrent validity and internal consistency when validating the Japanese version [32]. Similarly, Chin et al. in their evaluation of the English version of the Diabetes Distress Scale examined only convergent and discriminant validity along with internal consistency [33].

Also measured here were a variety of objective measures of health status such as HbA1c, eGFR, BUN, creatinine, and lipid profile. Because of the poor HRQoL reported by participants, it was expected that these parameters might also indicate poor health status and diabetic control (as an indicator of criterion validity of the DMQoL). HbA1c and lipid profile values were indeed out of range among those with low HRQoL (normal range for HbA1c is defined as less than 5.6% and less than 6.5% indicates good diabetic control; likewise, the normal range for total cholesterol is less than 100 mg/dl). However, the other laboratory values were surprisingly within the normal range. The relationship between poor HRQoL and increased HbA1c has been demonstrated in prior studies [34, 35].

The findings from this study also underscore the need for education to improve self-care behaviors among those with low education. The majority of our sample was illiterate or had only an elementary school education. Lack of education can negatively impact lifestyle choices, resulting in poorer disease prognosis and increased diabetic complications [36].

The present study had a number of limitations that should be considered when interpreting the results reported here. First, we recruited only clinic patients that may influence the generalizability of the findings. However, use of a large sample and comprehensive assessment of psychometric properties may help to diminish this concern. Second, we only included patients with type II diabetes because the scale was initially developed for such patients. However, the prevalence of individuals with type I diabetes is growing and developing similar scales appropriate for this population is also necessary. Future studies should focus on developing measures of HRQoL that may be relevant for those with both types of disorders. Finally, the DMQoL's sensitivity to change over time was not examined in the present study. Therefore, future studies will need to address this issue in order to demonstrate the usefulness of this scale for both clinical and research purposes.

Table 7 Known-group validity for DMQoL compared with that of DQOL

		Satisfaction, *M* (SD)	Impact, *M* (SD)	Diabetes-related worry, *M* (SD)	Social/ vocational worry, *M* (SD)	Total DQOL, *M* (SD)	DMQoL, *M* (SD)
Hypertension	Yes	2.37 (0.61)	2.46 (0.71)*	2.79 (0.65)*	2.47 (0.58)*	2.42 (0.77)*	3.51 (0.70)*
	No	2.21 (0.55)	2.10 (0.45)*	2.31 (0.62)*	2.13 (0.76)*	2.11 (0.59)*	3.81 (0.87)*
Retinopathy	Yes	2.54 (0.51)	2.35 (0.42)	2.63 (0.38)	2.58 (0.56)*	2.48 (0.60)	3.27 (0.41)
	No	2.22 (0.57)	2.21 (0.52)	2.40 (0.44)	2.39 (0.62)*	2.22 (0.47)	3.44 (0.44)
Nephropathy	Yes	2.42 (0.75)*	2.50 (0.78)	2.31 (0.34)	2.39 (0.58)	2.36 (0.62	3.19 (0.51)
	No	2.04 (0.50)*	2.32 (0.37)	2.23 (0.42)	2.21 (0.42)	2.25 (0.44	3.30 (0.53)
Neuropathy	Yes	2.67 (0.59)*	2.59 (0.47)*	2.62 (0.60)	2.71 (0.61)*	2.54 (0.51*	3.42 (0.52)
	No	2.29 (0.50)*	2.23 (0.45)*	2.22 (0.49)	2.43 (0.55)*	2.27 (0.48*	3.57 (0.49)
HbA1c	> 8%	2.63 (0.37)*	2.61 (0.51)*	2.57 (0.64)*	2.78 (0.51)*	2.61 (0.49*	3.40 (0.49)*
	≤ 7%	2.11 (0.59)*	2.03 (0.42)*	2.09 (0.55)*	2.15 (0.44)*	2.01 (0.52*	3.71 (0.55)*
Cholesterol	> 200 mg/dl	2.74 (0.43)*	2.49 (0.39)*	2.72 (0.59)*	2.79 (0.67)*	2.61 (0.47*	3.37 (0.46)*
	≤ 200 mg/dl	2.19 (0.68)*	2.01 (0.29)*	2.19 (0.58)*	2.12 (0.25)*	2.07 (0.33*	3.78 (0.53)*
Diabetic food	Yes	2.69 (0.42)*	2.63 (0.34)*	2.59 (0.55)*	2.48 (0.38)*	2.55 (0.41*	3.19 (0.41)*
	No	2.28 (0.36)*	2.12 (0.31)*	2.19 (0.47)*	2.18 (0.33)*	2.19 (0.30*	3.64 (0.48)*

*$p < 0.05$

Conclusion

The DMQoL is a brief, reliable, and valid measure of disease-specific HRQoL among Persian-speaking patients with type II diabetes. Use of this measure along with other more general measures of HRQoL may deepen our understanding of the perceptions of diabetic patients in Iran regarding their health state. Further evaluation of this scale in diabetic patients from different cultures, regions of the world, and those speaking other languages will help to determine whether the DMQoL may be useful more generally. Finally, we recommend that the DMQoL be examined over time and in response to medical and psychological treatments to determine whether it is sensitive in detecting clinically relevant changes in HRQoL among diabetic patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethic Committee at Qazvin University of Medical Sciences, and all patients gave written informed consent prior to participation.

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CASE REPORT

Dorsal pancreatic agenesis in newly diagnosed type one diabetes mellitus: case report and review of literature

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Abstract
Dorsal pancreatic agenesis is a rare congenital anomaly that may be associated with recurrent bouts of pancreatitis and diabetes mellitus. We report the case of a 21-year-old man newly diagnosed with diabetes mellitus and agenesis of dorsal pancreas. A brief review of literature and association of both entities is presented.

Keywords Pancreas · Dorsal agenesis · Diabetes mellitus

Introduction

Pancreatic developmental anomalies are rare. The most common anomaly seen is pancreas divisum among others like annular pancreas, heterotopic pancreas, ansa pancreatica, anomalous pancreaticobiliary union, and partial pancreatic agenesis [1].

Dorsal pancreatic agenesis, defined as the absence of the neck, body, and tail of the pancreas, is rarely seen; it is usually asymptomatic and incidentally detected on imaging; however, an association with pancreatitis or diabetes mellitus has been described.

Case report

A 21-year-old man, previously healthy, presented on May 2015 to the emergency department with a three-week history of polyuria, polydipsia, and nocturia, associated with weight loss of 10 kg during that period. Additional symptoms included feet tingling, mainly at night with headache and dizziness. History was negative for abdominal pain, nausea, and vomiting. Past medical history and past surgical history were unremarkable, and he denied smoking and drug or alcohol intake. Family history was positive for diabetes mellitus type 2 in maternal grandfather diagnosed at an old age. On physical exam, his body mass index was 23.7 kg/m^2, he had stable vital signs without tachycardia or hypotension, and an unremarkable examination with moist mucus membranes, and no abdominal tenderness.

Laboratory studies were: arterial blood gases = 7.39/31.2/91.9/18.6/97.62; lipase = 19 U/L; glucose 501 mg/dL; Na = 137 mmol/L, K = 4 mmol/L, Cl = 92 mmol/L, CO^2 = 19 mmol/L, BUN = 14 mg/dL, creatinine = 0.7 mg/dL, and ketones = 65.8 mg/dL; and urinalysis: 4+ glucose and 3+ ketones.

He was diagnosed with mild diabetic ketosis which was managed during his admission. Further lab tests showed negative anti-glutamic acid decarboxylase antibodies (anti-GAD), HbA1c of 12.4%, normal liver enzymes, normal TSH and anti-thyroglobulin, and elevated anti-peroxidase. The patient was diagnosed as diabetes type 1b [2] and discharged on insulin therapy.

On June 2015, he presented again to the emergency department with right lower quadrant pain not associated with any other complaints. Physical exam revealed a positive McBurney's sign. Work-up diagnosis was done to rule out appendicitis. Multi-slice CT of the abdomen-pelvis with oral and intravenous contrast was performed. It showed absence of the pancreatic body and tail with preserved pancreatic head and remnant of Santorini duct, consistent with partial dorsal pancreatic agenesis (Figs. 1 and 2).

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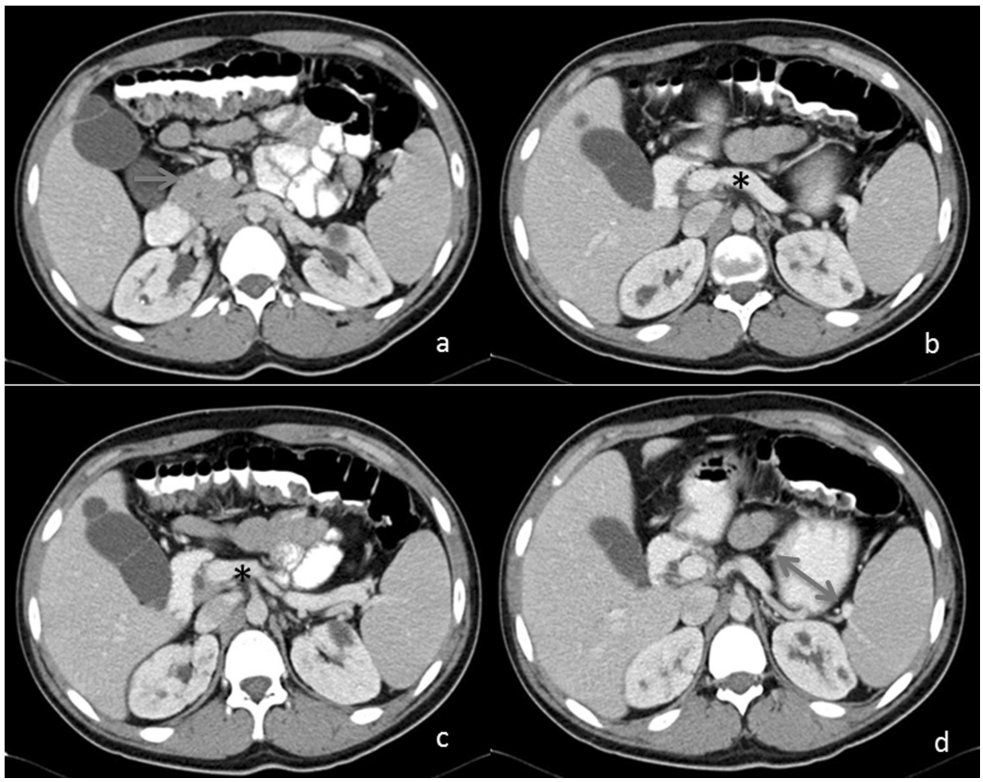

Fig. 1 **a**–**d** Axial CT views showing preserved pancreatic head (blue arrow) and remnant of Santorini duct, with absence of the pancreatic body and tail in the pancreatic bed that was occupied by the stomach (red arrow) anterior to the splenic vein (asterisk). Findings are compatible with partial dorsal pancreatic agenesis

Discussion

The present case is an association of diabetes mellitus with agenesis of the dorsal pancreas in a young man.

The pancreas is formed embryologically from ventral and dorsal buds that normally fuse at eighth week of gestation; the ventral bud is the origin of inferior pancreatic head and uncinate process and drains through the duct of Wirsung. The dorsal bud is the origin of pancreatic body, tail, superior head, and minor papilla and drains through the Santorini duct [3].

Dorsal pancreatic agenesis can be complete or partial. It is complete when there is complete absence of dorsal bud elements and it is partial when there is remnant pancreatic body, Santorini duct, and minor papilla. In our patient, the agenesis was partial.

It is associated with polysplenia and bowel malrotation [4].

Dorsal pancreatic agenesis is a rare entity with 64 cases reported from 1913 till 2018. It has a higher predominance in males and has been reported in both children and adults. While this abnormality has been reported in many areas to date, there have been no studies about ethnic or regional distributions. The vast majority of cases we came across in the literature had been reported in Turkey, India, and Japan showing a clear predominance in Asians and Middle Easterns. It is most often asymptomatic. When symptomatic, it most commonly presents as nonspecific abdominal pain, followed by diabetes mellitus and pancreatitis [5]. In our case, the patient had been diagnosed with diabetes mellitus during a previous admission, and was later found to have dorsal pancreatic agenesis when presenting with vague abdominal pain. Our patient had no evidence of pancreatitis based on normal lipase levels and CT findings. The etiology of abdominal pain has been suggested to be papillary dysfunction and may be related to underdevelopment of the papillary muscle.

The diagnosis is usually made in adults during investigation of acute abdominal pain by means of different imaging modalities such as CT, ultrasound, and MR that raise the possibility of dorsal pancreatic agenesis. The latter imaging modalities help in delineating the detailed anatomy of the pancreatic ducts [6]. There are two helpful CT signs, the dependent stomach sign and dependent intestine sign, whereby the distal pancreatic bed is occupied by stomach or loops of small bowel, directly anterior to the splenic vein (Figs. 2 and 3). A similar presenting pathology is pseudoagenesis due to chronic pancreatitis, obstructing pancreatic mass, and trauma. The main difference is the absence of Santorini duct in dorsal pancreatic agenesis and its presence in pseudoagenesis [7].

There is a high prevalence of diabetes (50%), often insulin-dependent, in those with pancreatic agenesis [4]. The association with diabetes mellitus has long been

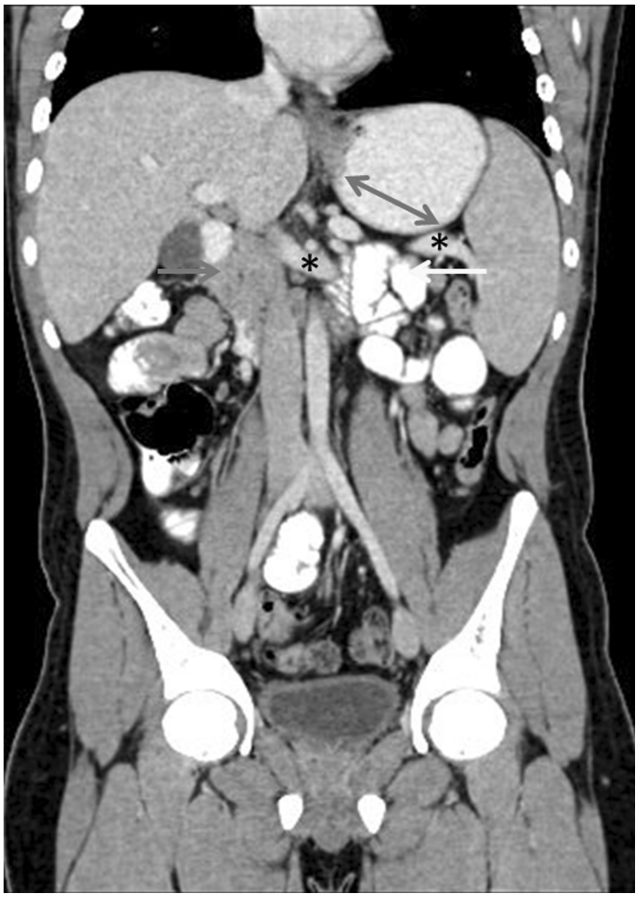

Fig. 2 Coronal CT view showing pancreatic head (blue arrow), with the stomach (red arrow) and jejunum (yellow arrow) occupying the pancreatic bed replacing the body and tail, anterior to splenic vein (asterisk), demonstrating the dependent stomach and dependent intestine signs

thought to be related to the absence of Langerhans islets that are usually found in the pancreatic body which is replaced by fatty tissue. This microscopic finding was described in the case report presented by Wang et al. [1] in which the patient underwent a laparotomy with a pancreatic biopsy that showed normal pancreatic head tissue and fatty replacement of pancreatic body and tail. The presence of diabetes mellitus may therefore reflect the asymmetric distribution of insulin-producing cells. On the other hand, the significantly elevated anti-peroxidase level in our patient may also suggest the involvement of an autoimmune mechanism in the development of his diabetes mellitus. In fact, the most common autoimmune disorder related with type 1 diabetes mellitus is autoimmune thyroid disease [8], while the frequency of thyroid autoantibody positivity in children with type 1 diabetes mellitus has been reported to be up to 50% [9]. To our knowledge, this is the first case of dorsal pancreatic agenesis associated with elevated thyroid autoantibodies, although the patient reported by Gilinsky et al. did have hypothyroidism [10]. While the absence of anti-GAD antibodies in our patient favors the first mechanism as the major culprit, an autoimmune etiology can still possibly be simultaneously involved.

While most cases are sporadic, some reports of familial inheritance suggest a genetic etiology, with an X-linked or autosomal dominant mode of transmission [5]. Several genetic studies linked a gene mutation to the dorsal pancreatic agenesis and its association with diabetes mellitus. One of the mice experiments, done by Harrison et al. [11], showed that the dorsal pancreas failed to form with a mutation of the homeodomain protein HB9 (Hlxb9) and also showed significant reduction in the size of the Langerhans islets and number of insulin-producing beta-cells. Other studies have suggested that the etiology is an autosomal dominant mutation of the hepatocyte nuclear factor 1B (HNF1B) gene [12]. In our patient, there was no known family history of dorsal pancreatic agenesis, nor even a family history of pancreatitis. The association with pancreatitis has also been studied, possibly due to sphincter of Oddi dysfunction; however, other theories have been proposed, such as hypertrophy of the remnant ventral gland with higher intrapancreatic duct pressures [13]. Moreover, ten cases in the literature have been reported with development of pancreatic tumors, which is an important association to recognize in the follow-up of patients diagnosed with this pathology [14, 15].

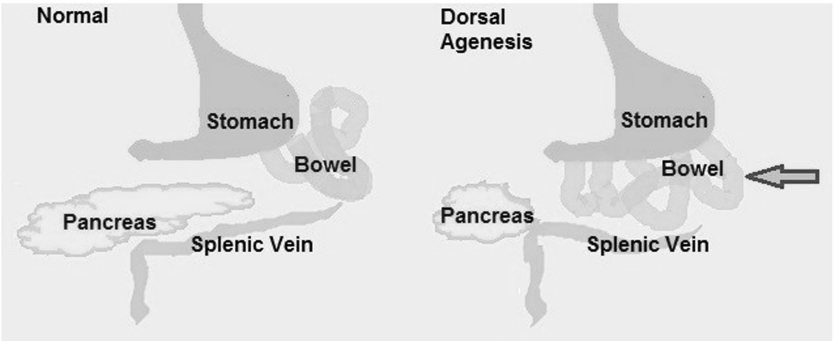

Fig. 3 Depiction of the dependent stomach and dependent intestine signs (arrow): distal pancreatic bed occupied by the stomach or intestine (which abuts the splenic vein) in patients with dorsal pancreatic agenesis

Conclusion

We report a case of a newly diagnosed young man with diabetes mellitus type 1 in association with dorsal pancreatic agenesis, which is a very rare developmental pancreatic anomaly.

We have reviewed the literature on agenesis of the dorsal pancreas and its genetic association with diabetes mellitus.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the participant included in this study.

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CASE REPORT

c.2425G>A mutation in the *WFS1* gene associated with Wolfram syndrome: a case report

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Abstract
Wolfram syndrome is a rare hereditary disease characterized by clinical congenital progressive hearing impairment, diabetes mellitus, optic atrophy, and diabetes insipidus. A girl at the age of 35 months with diabetes mellitus was diagnosed as incomplete Wolfram syndrome; the patient also had optic atrophy, deafness, and diabetes mellitus. Molecular genetic examinations revealed a de novo mutation (c.2425G>A) in the *WFS1* gene. No mutations were detected in the biological parents. The mutation c.2425G>A in the *WFS1* gene is associated with the occurrence of Wolfram syndrome. This newly discovered mutation in the *WFS1* gene may contribute to the diagnosis of Wolfram syndrome.

Keywords Wolfram syndrome · Mutation · Genotype

Introduction

Wolfram syndrome was first described in 1938 by Wolfram [1] and it is known as diabetes insipidus, diabetes mellitus, optic atrophy, deafness (DIDMOAD) syndrome. It is a rare autosomal genetic neurodegenerative disease. The prevalence of Wolfram syndrome is about 1 in 770,000 live births, with a 1 in 354 carrier frequency. According to a global report, the number of patients in 2010 was 219 [2]. The prognosis of this syndrome is currently poor. *WFS1* gene mutation is the main cause of this disease. Determining the phenotype-genotype correlation is difficult, as the same mutation exhibits very different phenotypes. Currently, numerous gene mutations have been identified. In this article, we described the clinical features of a 35-month incomplete Wolfram syndrome patient and the concomitantly discovered new gene mutations and reported the genetic results of her parents.

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Case report

As results of "polydipsia, polyuria, polyphagia, and weight loss for 20 days," a 35-month-old girl of Han nationality was admitted to the Second Hospital West China Medical College on November 27, 2015. Twenty days before admission, the child began to experience symptoms of increased water consumption, food intake, and urine void. The water consumption was approximately 1500 mL per day and the body weight loss was approximately 1 kg. Laboratory tests at a local hospital found a positive urine glucose (3+) and blood glucose level as high as 26.11 mM. She was then referred to this hospital for further diagnosis and treatment.

Illness history At the age of 12 months, the patient received a cochlear implant because of neurogenic deafness. At the age of 16 months, she had undergone congenital cataract surgery and intraocular lens implantation for visual impairment.

Birth history The patient's birth history was G1P1, 39 weeks of gestation, cesarean section, and birth weight 3000 g. Her parents denied birth history of asphyxia rescue and denied consanguineous marriage.

Physical examination Her consciousness was clear and moderately nourished with normal skin in color and no

subcutaneous bleeding. Superficial lymph nodes were not found enlarged. Her heart, lung, and abdomen examinations were normal.

Laboratory investigation Urine specific gravity was 1.022, urine glucose was positive (3+), and urine ketone was positive. Fasting blood glucose was 31.1 mM (normal range 2.97–6.80 mM), fasting insulin was 0.9 uIU/mL (3.0–25.0 uIU/mL), fasting C-peptide was < 0.05 nM (0.27–1.28 nM), HbA1c was 13.5% (4.27–6.07%), thyroid stimulating hormone was 3.963 mIU/L (0.55–4.78 mIU/L), free triiodothyronine was 3.67 pM (3.5–6.5 pM), free thyroxin was 12.52 pM (11.5–22.7 pM), thyroglobulin antibody and thyroid peroxidase antibody tests were negative, and immunologic tests for antinuclear antibodies and anti-double-stranded DNA antibodies were negative. Chest X-ray, electrocardiogram, and abdominal color ultrasound were normal.

Genetic tests finding Exome sequencing revealed a de novo mutation in exon 8 of the *WFS1* gene c.2425G>A, p.(Glu809Lys). The mutation was not detected in her parents (Fig. 1).

Based on our results, the patient was diagnosed with Wolfram syndrome. The treatment regimen included subcutaneous injection of 3 U of short-acting insulin 30 min before each meal and 4 U of isophane insulin before bed time. The child has been followed up for 6 months. During the monthly visit in the first half of this year, she showed good glycemic control and HbA1C recently reached 7.8%. She currently does not show symptoms of diabetes insipidus and there is no abnormality in mental development.

Discussion

Wolfram syndrome is a rare autosomal genetic disease with four main symptoms, namely diabetes mellitus, ocular symptoms, deafness, and central diabetes insipidus. Insulin-dependent diabetes mellitus is the first symptom at onset, typically occurring at the age of 6 years [3]. In China, the average age of onset is 5 years, while the youngest patient reported was 3 years old. Ocular symptoms typically begin at 6–7 years of age; visual acuity is decreased and some patients show visual field defects. Optic atrophy is observed in 98% of cases, while other patients develop cataract. Ocular symptoms appear 2–3 years after the diagnosis of diabetes. Deafness is frequently observed, suggesting neurogenic deafness with an incidence of approximately 70%. The incidence of central diabetes insipidus is low, about 32%. In addition to the above symptoms, DIDMOAD patients may also suffer from ataxia, neurogenic bladder, mania, depression, organic brain syndrome, hypothyroidism, and sexual developmental delay, among others [4]. The diagnosis of DIDMOAD is based primarily on clinical manifestations; patients with all four major symptoms are diagnosed as the complete type, whereas those without all four symptoms are diagnosed as the incomplete type. In this case, the patient presented with diabetes mellitus, impaired vision, and neurogenic deafness, but no diabetes insipidus, and therefore she was considered to have an incomplete type of Wolfram syndrome. The patient's uniqueness lies in her congenital cataract. Unlike other reported cases, this occurred before the onset of diabetes mellitus. In addition, the patient is the youngest case of DIDMOAD reported in China.

In 1998, *WFS1* gene mutations were identified to contribute to the molecular mechanism of Wolfram syndrome. This

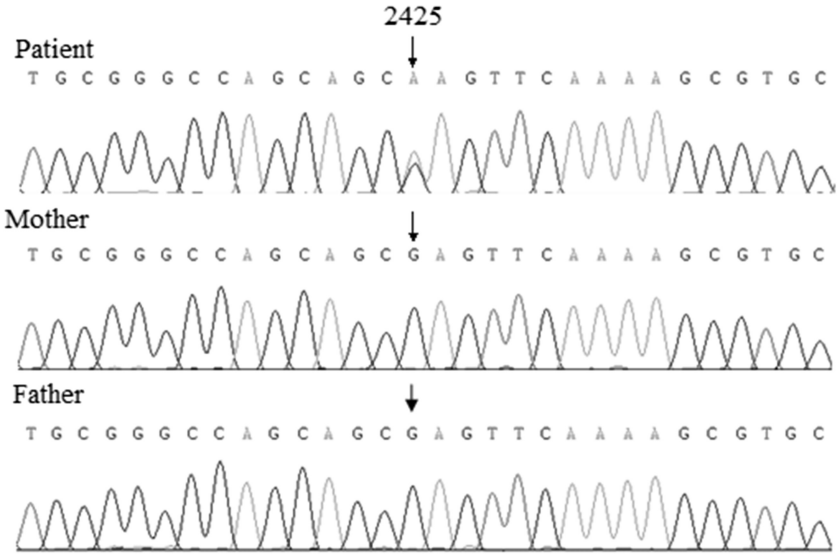

Fig. 1 c.2425G>A mutation sequence in the *WFS1* gene

gene is located in the short arm of the fourth chromosome (4p16.1), encoding the 890-amino acid transporter membrane glycoprotein wolframin [1, 5]. The function of this protein is currently unclear. It is generally thought to be a transmembrane protein and may be related to membrane transport, protein processing, and apoptosis inhibition of pancreatic islet cells [6]. Numerous gene mutations in the *WFS1* gene have been reported. Rigoli et al. described a novel missense mutation (G107R) in the *WFS1* gene in two siblings from Southern Italy [7]. Sobhani et al. found a frameshift alteration c.2177_2178insTCTTC (or c.2173_2177dupTCTTC) in exon 8 in a study conducted in Iran [8]. In addition, various gene mutation loci have been reported in many countries. It is caused by mutations in the *WFS1* gene that are inherited in an autosomal recessive way in most affected individuals, although dominant forms exist [9]. In this study, gene scanning of the parents revealed no abnormalities, but the patient was found to have a novel mutation in the *WFS1* gene. The de novo mutation occurred in the coding region of 2425 base G to A, leading to a missense mutation from glutamic acid to lysine. This gene mutation has not been reported previously in China. In May of 2016, Prochazkova et al. reported a case of a 3.25-year-old boy diagnosed as Wolfram-like syndrome [9]. Molecular genetic examinations revealed a de novo mutation p. (Glu809Lys) in the *WFS1* gene. Although the abovementioned case and the case reported in this paper showed the same level of amino acid mutations, their clinical manifestations were very different and may be related to race. The boy was diagnosed with WFSL, an autosomal dominant disease, and the girl was diagnosed with incomplete Wolfram syndrome. Thus, the same genetic mutation may have different effects. This is due to the fact that the amino acid sequence in this region is highly conserved across species and therefore may affect the normal function of the protein, leading to disease [10]. The effect of this mutation on the function of wolframin remains unclear, but may explain the difference between this patient's clinical presentation and other reported cases. Çelmeli et al. present the clinical and genetic characteristics of three WS patients from three unrelated Turkish families, of which one had a novel homozygous missense mutation (c.2534T>A) [11]. In a recent research in Japan, massively parallel DNA sequencing (MPS) was used for the mutational analysis of the WFS1 gene among a larger series of 2549 unrelated Japanese hereditary HL patients. Thirteen WFS1 variants were identified in 19 probands: eight of the 13 variants were previously reported mutations, including three mutations (p.A684V, p.K836N, and p.E864K) known to cause Wolfram-like syndrome, and five were novel mutations [12]. A mutation (c.376G>A, p.A126T) has been found in the patients diagnosed with Wolfram syndrome; however, this mutation probably does not cause deafness in affected individuals [13].

Currently, there is no special treatment for Wolfram syndrome. Clinical managements include the use of insulin for glycemia control cochlear implant for hearing improvement, artificial lens for cataract, and regular monitoring. Patients have poor prognosis. They usually die around the age of 35 because of severe neurological complications [14].

Conclusion

Detection of gene mutations can help diagnose Wolfram syndrome. The correlation between phenotype and genotype is difficult to determine, and the same mutation can cause very different phenotypes. The de novo mutation c.2425G>A in the *WFS1* gene may be associated with Wolfram syndrome. Further protein function tests are needed to determine the importance of this mutation.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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CASE REPORT

Fibrocalculous pancreatic diabetes with hemorrhagic pericardial effusion in a female—a rare case report

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Abstract
Fibrocalculous pancreatic diabetes (FCPD) is a unique form of diabetes due to non-alcoholic chronic pancreatitis peculiar to tropical countries. The hallmark features are younger age of onset pain abdomen and presence of pancreatic calculi. Though etiology remains unclear but on long-term follow-up, patient may develop microvascular and macrovascular complication. Chances of progression to malignancy are also high which carries poor prognosis. We present a case of a 36-year-old female presenting with recurrent abdominal pain, diabetes and chronic calcific pancreatitis. She was diagnosed to have FCPD based on her findings. She was managed with insulin, pancreatic enzyme supplementation and analgesics for pain relief. Due to her intractable pain poorly responding to medical management, she underwent Roux-en-Y pancreaticojejunostomy but later developed massive pericardial effusion hemorrhagic in nature with pleural effusion and ascitis. Analysis showed high possibility of adenocarcinoma though the primary focus could not be confirmed as the patient developed sepsis and unfortunately succumbed to her illness. Though FCPD is a rare entity, it is important to rule out chronic calcific pancreatitis as underlying etiology in all patients of diabetes presenting with recurrent pain abdomen and also to periodically screen FCPD patients for pancreatic malignancy. This helps to prevent progression to complications by an early medical intervention. Surgical intervention may be considered an option in case of failure of medical management, though data of its usefulness is very rare.

Keywords Fibrocalculous pancreatic diabetes · Hemorrhagic pericardial effusion · Roux-en-Y pancreatojejunostomy · Adenocarcinoma

Introduction

Fibrocalculous pancreatic diabetes (FCPD) is a rare form of secondary diabetes reported mainly from the tropical countries [1]. In absence of alcohol intake, there occurs exocrine and endocrine pancreatic failure due to the chronic pancreatic inflammation and calcification associated with dilatation of the pancreatic duct with intraductal calculi. Kerala, Orissa and Chennai report highest prevalence. Prevalence of tropical chronic pancreatitis was reported to be 0.09% by Balaji in a population of 28,507 in Kerala [2]. Kini reported the first case in 1937 from India [3]. As per our knowledge, one case of FCPD has been reported from Gujarat until now [4], and this will be the first unusual case of FCPD from South Gujarat where surgical intervention was done to allay the pain and improve quality of life but later patient presented with massive pericardial effusion, ascitis and pleural effusion suspected to be malignancy.

Case history

A 36-year-old female was admitted to the hospital with recurrent pain abdomen for 2 years and is aggravated for 1 day. The pain was of moderate to severe intensity, located in epigastric region and radiating to the back. The pain used to get aggravated by meals and relieved by stooping forward. There is also history of polyuria, polydipsia and polyphagia for last 6 months. There was no history of diabetes in the parents or any other family member. There was no history of consumption of alcohol and tobacco. She consumed a vegetarian diet and belongs to low socioeconomic status.

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On examination, she was afebrile; conscious alert; responsive to commands; and pale but no icterus, cyanosis, no clubbing, lymphadenopathy or parotid gland enlargement. Her BP was 110/70 mmHg, pulse 78/min, RR 18/min and Spo2 100%. Her BMI was 17.8 kg/m2. On systemic examination, her neurological, cardiorespiratory and abdomen examinations were normal.

Her random blood sugar was 364 mg/dl. Fasting sugar-220 post prandial sugar was 300 mg/dl. Her urine analysis showed glycosuria with negative urine ketones. Her CBC revealed hemoglobin 9.3 g/dl, TLC 9800/cumm, platelet count 276,000/cumm, HbA1c 14.3%, serum protein and lipid profile were normal. Serum calcium was 8.4 mg/dl. PTH level was 43.18 pg/ml. Her kidney functions and liver functions were normal. Her C-peptide level was 0.41 ng/ml (0.9–7.1, normal range). Serum amylase was 42 IU/l and serum lipase was 23 IU/l.

Stools examination revealed no fat globules. ECG and fundus examination were normal. Her chest X-ray PA view revealed obliteration of right costophrenic angle suggestive of mild pleural effusion which was not tappable (Fig. 1). X-ray of the abdomen was suggestive of pancreatic calcification at the level of L1-L2 vertebrae (Fig. 2). USG of the abdomen showed acute on chronic pancreatitis with dilated MPD and calcification. CT of the abdomen revealed generalised reduced volume of the body, tail of pancreas with multiple calcifications throughout pancreatic head, body with dilated tortuous main pancreatic duct (8–10 mm) with calculus in pancreatic duct and subcentimeter peripancreatic lymph nodes with mild right-sided pleural effusion (Fig. 3). So on the basis of clinical features and investigations, a diagnosis of FCPD was made. She was discharged on human mixtard insulin (30/70) 12 units in the morning before breakfast and 6 units before dinner along with pancreatic enzymes and calcium supplementation. Over the next 6 months, her sugar remained fairly controlled,

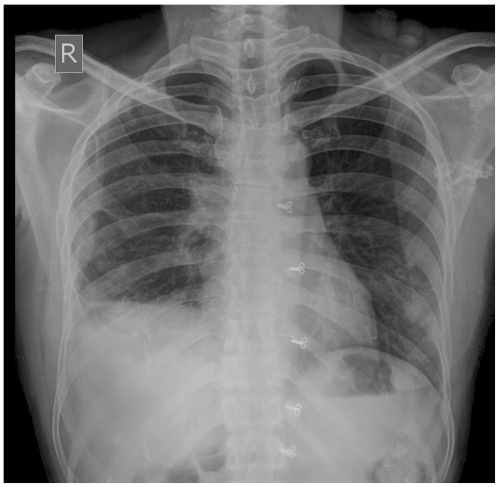

Fig. 1 Chest X-ray PA view revealing obliteration of right costophrenic angle suggestive of mild pleural effusion

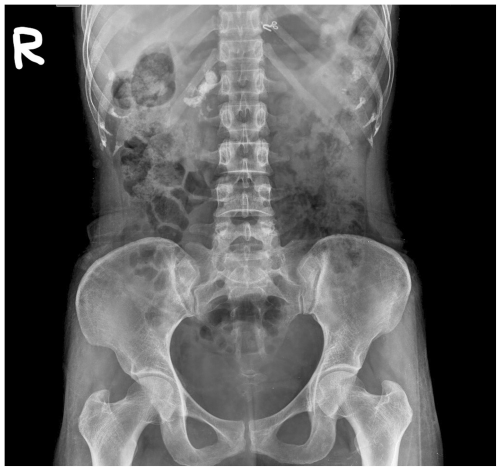

Fig. 2 X-ray of the abdomen suggestive of pancreatic calcification at the level of L1-L2 vertebrae

but she had 2–3 episodes of pain abdomen requiring hospitalisation, and hence, a surgical intervention was planned by the surgical department to allay her symptoms and improve quality of life. Roux-en-Y pancreaticojejunostomy was performed and after 2 weeks of postoperative care and diabetes management, patient was discharged in stable condition on human mixtard insulin (30/70) 12 units in the morning before breakfast and 6 units before dinner. She was counselled about symptoms of both hypo- and hyperglycaemia and also was given dietary advice on discharge. However, during her recent follow-up, she presented for her complaints of breathlessness. Her chest X-ray was repeated to see for any progression of effusion. X-ray was suggestive of massive effusion on the

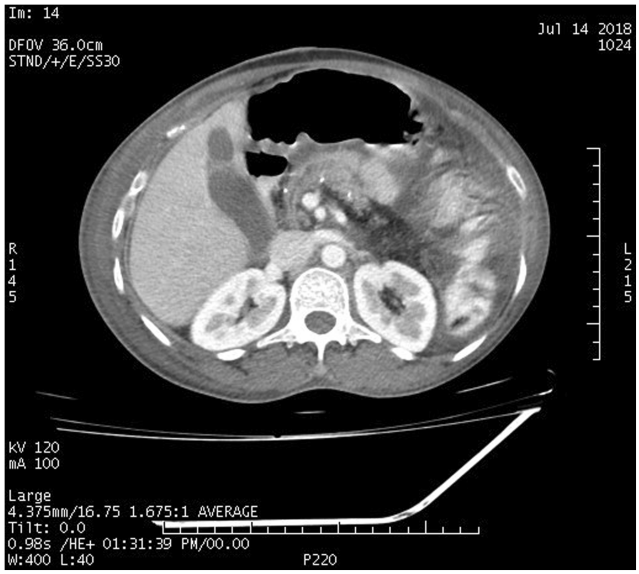

Fig. 3 CT of the abdomen revealed generalised reduced volume of the body, tail of pancreas with multiple calcifications throughout pancreatic head, body with dilated tortuous main pancreatic duct (8–10 mm) with calculus in pancreatic duct and subcentimeter peripancreatic lymph nodes with mild right-sided pleural effusion

right side for which a therapeutic tapping was done and almost 500 ml of fluid was aspirated which was hemorrhagic and transudative in nature. Post this visit, her breathlessness was reduced, and repeat X-ray on follow-up visits showed minimal non-progressive effusion on the right side. During her recent visit, her blood sugars were controlled with fasting blood sugar 120 mg/dl, PPBS 180 mg/dl and HbA1C 7.6%. But this time, she got admitted for abdominal distension. USG showed gross ascitis with chronic pancreatitis and few intraluminal calculi of size 4–6 mm at the body and tail, with moderate pericardial effusion. 2D echo showed moderate pericardial effusion of strip 3.19 cm (Fig. 4). Her ascitic fluid tap was done 600 ml in amount, hemorrhagic in nature. Fluid was transudative in nature with SAAG of 1.3; serum albumin, 3.7; fluid albumin, 2.4; fluid amylase, 21.3 and fluid lipase was 4.3. Her pericardial fluid was also aspirated 500 ml in quantity, hemorrhagic in nature. Pericardial fluid amylase was 33 U/l and lipase was 22 U/l (normal range, 0–60 U/l. Pericardial fluid ADA was 13.9 U/l which was normal. Pericardial fluid cytology showed high suspicion of adenocarcinoma. So her repeat CT for abdomen and chest was planned to see for any signs of malignancy. CT of the abdomen showed changes of chronic pancreatitis and spotty calcifications in pancreatic head and uncinate process with dilated MPD (5 mm) with gross ascitis and few lymph nodes in the mesentery and periportal region. CT of the chest showed partial volume loss of right lung with multiple scattered patchy soft tissue opacities and scattered nodular opacities with peribronchovascular interstitial thickening. Based on the cytology report, tumor markers were done. Her CA 19.9 was 6.81 μ/ml (normal range, 0–37 μ/ml), while carcinoembryonic antigen was raised to 91.15 ng/ml (normal, < 2.5 ng/ml). In view of all reports, we planned to send patient for PET CT scan to look for the primary cause and biopsy if needed but before that patient developed signs of sepsis. Her condition deteriorated and she succumbed to her illness.

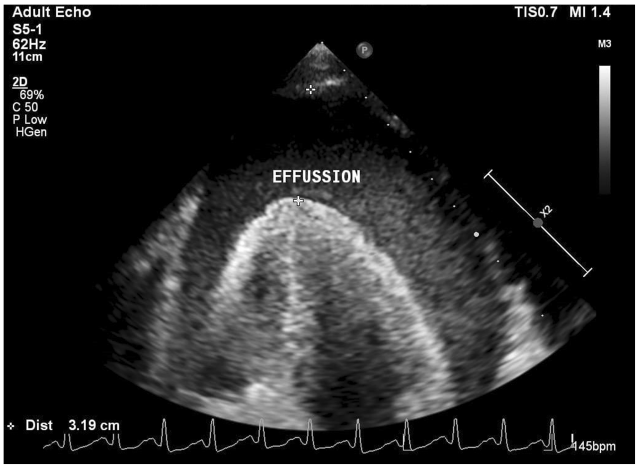

Fig. 4 2D echo showing moderate pericardial effusion of strip 3.19 cm

Discussion

FCPD is a rare form of diabetes secondary to pancreatic calcification in non-alcoholics, mostly seen in young population between the age of 10 and 40 with male preponderance [5], though here, the patient is 36 years old female. The several factors which play a role in the pathogenesis of FCPD are malnutrition, increased oxidant stress from vitamin C and A deficiencies [5] and genetic association like serine protease inhibitor, Kazal type 1 (SPINK 1) gene that prevents unregulated activation of the pancreatic enzyme cascade by inhibiting trypsin activity [6].

FCPD patients have history of pancreatitis, severe epigastric pain abdomen radiating to back with intermittent episodes of remissions and exacerbations [7]. Initially, overt diabetes may not be present but as beta cell deficiency progresses, diabetes sets in. Abdominal X-ray is diagnostic of pancreatic calculi. Ultrasonography and computed tomography scans are helpful to look for calculi, atrophy, fibrosis, ductal dilatation and "Bag of stones" appearance [8]. The peculiar finding is absence of ketosis despite high sugars which can be attributed to the residual beta cell function, reflected by the intermediate levels of C-peptide [9].

Our patient had recurrent episodes of abdominal pain, diabetes with absent ketosis and evidence of pancreatic calculi on X-ray abdomen, ultrasonography and abdominal computed tomography which confirmed the diagnosis of FCPD.

The complications follow the same pattern as type 2 DM with microvascular complications like retinopathy [10], neuropathy [11], and nephropathy [12]. Macrovascular complications are less common possibly due to young age, their lean body mass and the low cholesterol levels [13].

The mainstay of management is insulin therapy to achieve an adequate glycemic control. Pancreatic enzyme for malabsorption and fat-soluble vitamins supplementation are also helpful. In cases of intractable abdominal pain where medical management fails, surgical intervention Peustow's procedure may be considered an option. In this procedure, the pancreatic duct is opened up longitudinally, then stones are removed and pancreaticojejunostomy is performed. The residual beta cell and exocrine pancreatic mass are salvaged. In a prospective study conducted by Sidhu et al., it was observed that Peustow's procedure not only provides pain relief but also improvement in diabetes [14].

There is no data to suggest the incidence of pancreatic ascitis or pleural effusion in patients with tropical pancreatitis. It has been reported that the ruptured pseudocyst occurs in 80% of patients, disruption of pancreatic duct exists in 10% of cases and an obscure leakage site exists in the remaining 10% [15]. There have been case reports of recurrent pericardial effusion with tamponade due to pancreatic-pericardial fistula which is an extremely rare complication of chronic pancreatitis [16–18]. Pericardial

effusion has also been observed in acute pancreatitis due to several mechanisms like chemical pericarditis due to pancreatic enzymes carried by lymphatic vessels, necrosis of vascular walls in fatty areas, necrosis of subepicardial fat and fistulous connection between abdominal and pericardial cavities [19]. Internal pancreatic fistulas result from erosion of pancreatic fluid into adjacent or distant organs like thoracopancreatic communication leading to pleural effusion while external fistulas drain into defects in skin. If the communication occurs anteriorly, pancreatic ascitis may occur [18]. The presenting symptoms may vary depending upon the location and size of communication ranging from dyspnea, cough, hemoptysis, chest pain, palpitations, acute respiratory compromise and cardiogenic shock. In our case, ascitic fluid and pericardial fluid amylase were not raised, and cytology also suggested high suspicion of adenocarcinoma though we could not get any confirmatory findings on CT abdomen and chest. Other finding supportive of malignancy was tumor markers. The sensitivity and specificity of CA19-9 is higher than CEA in diagnosis of early stage of pancreatic cancer [20], but in this case, CA19-9 was normal and only CEA was raised, keeping possibilities of other malignancies also high like colorectal, pancreatic, lung, breast, liver, stomach and ovary. Pericardial effusion can be a marker of occult malignancies of lung, lymphoma, pancreas and other unspecified metastatic cancer. The risk of pancreatic carcinoma is significantly high in tropical pancreatitis as observed in an epidemiological study in Madras [21], and hence, it is considered as a premalignant condition [22]. On the background of hemorrhagic pericardial effusion, ascitis and pleural effusion with cytology suggesting adenocarcinoma, raised CEA in a patient of fibrocalculous pancreatic diabetes, likelihood of pancreatic carcinoma was high though other malignancies could not be ruled out. This case highlights the fact that though FCPD is a rare entity, it should be suspected in all diabetic patients presenting with pain abdomen and also all FCPD patients should be periodically screened for pancreatic malignancy as there are high chances of malignant transformation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the participant involved.

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VISION STATEMENT

To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

MISSION STATEMENT

1. Promotion of excellence in diabetes care to make India the Diabetes Care Capital
2. Empowerment of persons living with diabetes
3. Support for diabetes research
4. Dissemination of information and knowledge in diabetes care
5. Advocacy for the cause of diabetology

RSSDI Research Grants

- For providing research grants, RSSDI invites proposals from Indian scientists, interested in conducting original research in the field of diabetes mellitus. Furthermore, limited grants are also available for the students of medical colleges for smaller projects.
- There is no deadline for submission of the proposals, which can be sent throughout the year. These proposals may fall into one of the following three categories:

1. Projects involving funding up to Rs 40,000 per project (preference will be given to young scientists <40 years).
2. Projects involving funding up to 10 lakhs.
3. We also invite proposals for more than 10 Lakhs as major projects but these have to be preferably multicentric.

- The detailed proposals should include the following:
  - ◊ Title, names of principal and co-investigators, summary, introduction/background, review of literature, aims, methodology, study design, and detailed plan of work and bibliography. Brief biodata of principal investigator and other co-investigators
  - ◊ Importance of work in the context of national priorities. Detailed budget sought along with full justification/ proposed utilization, of funding sought from RSSDI
  - ◊ Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.
  - ◊ Ethical committee clearance of the institution or other bonafide body.

Travel grants for young diabetes researchers to attend International Conferences

Criteria's for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

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(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 18 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

List of RSSDI Accredited Centres

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2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Totall Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	G D Hospitals and Diabetes Institute	Kolkata, West Bengal
10.	Aditya Diagnostics & Hospital	Dibrugarh, Assam
11.	Sunil's Diabetes Care N' Research Centre Pvt Ltd.	Nagpur, Maharashtra
12.	Marwari Hospital and Research Centre	Guwahati, Assam
13.	Down Town Hospital	Guwahati, Assam
14.	St.Theresa's Hospital	Hyderabad, Telangana
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Name of the Course: Advanced Certificate Course in Diabetology

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Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given preference.

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- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

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