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EDITORIAL

Glycaemic index: challenges in translating concept to practice

SV Madhu[1]

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Dietary prescribing in diabetes mellitus based on glycaemic index (GI) of foods has always been an attractive concept. Food choices with lower GI are associated with lower glucose responses after consumption and hence would be the preferred choices in patients with diabetes. While this idea has been very promising, the real challenge over the years has been its implementation in day to day practice. Several issues like validity and reproducibility of GI measurements, factors affecting glycaemic responses, differences when consumed as single foods or in mixed meals and amount of absorbable/digestable carbohydrate need consideration besides other methodological concerns.

The concept of GI has evolved over time. When it was first proposed [1], it measured the glycaemic response of a test food as a percentage of the glycaemic response of a reference food containing a similar amount of carbohydrate. The reference food has traditionally been glucose or white bread. Even this lead to variability of GI values depending on whether it was glucose or white bread that was the reference. Individual foods have been tested and categorized as low, medium or high GI foods based on their glycaemic responses [2] when consumed alone. However, these values lose significance when the foods are consumed as part of a mixed meal [3].

The concept of GI does not take into account the amount of carbohydrate consumed even though this is a major determinant of the glycaemic response. This lead to the extension of the concept of GI to glycaemic load (GL) which is the product of GI and the total amount of carbohydrate consumed [4]. GL gives a fair idea of the glucose load or burden that results from the ingestion of a carbohydrate containing meal. A high GI food can have a low GL if the portion size consumed is small and a low GI food can have a high GL if the serving size is big.

It has also been realized that all carbohydrate in food is not available for conversion to glucose and hence only the amount of "available" carbohydrate also called glycaemic carbohydrate and not total carbohydrate should be used for calculation of GI. What constitutes available carbohydrate has also been a subject of debate and the consensus view is that the undigestable carbohydrate which includes the fibre and all resistant starch is taken as unavailable [5].

There are several limitations to the usefulness of GI and GL. Studies suggest that GI could be affected by many factors including the amounts of other nutrients such as fat, protein and fibre, structure of the carbohydrate, particle size, food form, food processing and cooking method [5]. These factors lead to a lot of intra and inter individual variability of GI values and GL making these estimates less reliable and raise questions about their validity [6]. The rates of digestion of carbohydrates also vary with health status, race, gender and underlying insulin resistance all of which can affect GI [5]. Similarly, methodological differences such as the amount of tested food which contains 50 g of carbohydrate (available, absorbable, digestable), method of glucose measurement, time of day when the test was performed and the method of calculation of glucose response also affect GI values further complicating interpretation and limiting their value for day to day use [7].

In vitro models for estimation of GI of different foods have been proposed to overcome the problem of variability. While these models have been accurate and reproducible, they do not take into account biological factors like differences of digestion, absorption and insulin resistance that can significantly impact GI [5].

✉ SV Madhu
drsvmadhu@gmail.com

[1] Department of Endocrinology, Centre for Diabetes, Endocrinology and Metabolism, University College of Medical Sciences and GTB Hospital, Delhi, India

In the current issue, Dharmendra et al [8] from NIN, Hyderabad, have for the first time accurately estimated the glycaemic or available carbohydrate content of different food items in vitro by carefully mimicking their digestion in the human body in controlled laboratory conditions. These values were then used to calculate the portion sizes needed for in vivo GI testing against a glucose reference as per recent guidelines [9]. Using this method, they estimated GI for a variety of foods including rice, wheat and legumes. The methods followed reliably account for the resistant starch contents in the food items also and hence are more accurate than calculations of available carbohydrate content from existing charts of total carbohydrate and fibre contents of foods. It is important to create our own database of GI of different Indian foods using acceptable and accurate methods. This study by Dharmendra et al. is an important addition in this direction. Use of standard methods will ensure that a major cause of variability of GI measurements is eliminated and we can use these values with more confidence in clinical practice.

Studies on high GI diets and risk of insulin resistance and diabetes have given inconsistent results with a few of them pointing to a higher risk [10, 11]while others have found no additional risk [12, 13]. Similarly, low Gi diets have been shown to be associated with a greater reduction in HbA1C than with high GI diets [14, 15]. However, the difference in HbA1C between the two groups is small and may not affect outcomes in the long term. Questions have also been raised on the instruments of dietary assessment that may not have been designed to capture reliably the information on GI [16]. Overall, it would appear that low GI foods can favourably affect glycaemic control in diabetic patients.

Despite all the limitations, GI continues to capture the attention of physicians and nutritionists alike as it does offer a rational way of ranking carbohydrate containing foods that has the potential to favourably affect the prevention and management of diabetes. However, one has to exercise caution and refrain from using GI as the sole basis for diabetic diet prescribing. Low GI foods like ice cream may have a high content of undesirable fat which delays carbohydrate absorption and results in lower GI but adversely affects health outcomes. GI can be used judiciously in addition to the other nutritional health needs of the patient to further enhance the quality of the food choices and improve diabetes management. RSSDI recommends that in addition to other measures, low GI foods may be incorporated [17] as healthier carbohydrate choices in diabetic diets to achieve better glycaemic control.

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GUIDELINES

RSSDI clinical practice recommendations for diagnosis, prevention, and control of the diabetes mellitus-tuberculosis double burden

Vijay Viswanathan[1] · Sarita Bajaj[2] · Sanjay Kalra[3] · Sameer Aggarwal[4] · Atulya Atreja[5] · Dhruva Chaudhry[4] · D. J. Christopher[6] · A. K. Das[7] · Sujoy Ghosh[8] · Jubbin Jacob[9] · Anil Kapur[10] · M. V. Ajay Kumar[11] · Satyavani Kumpatla[1] · S. V. Madhu[12] · B. M. Makkar[13] · Salam Ranabir[14] · Rakesh Sahay[15] · P. K. Thomas[16] · Mangesh Tiwaskar[17] · Srikanth Tripathy[18] · Zarir Udwadia[19] · Sunny Virdi[20] · Nevin Wilson[11]

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Background

The International Diabetes Federation (IDF) estimates for 2015 revealed that 8.8% of the world's population had diabetes mellitus (DM) and with 69.2 million people with DM, India ranked second in the world [1]. Globally, 10.4 million new cases of tuberculosis (TB) were estimated to have occurred in 2015 with India,

Extended working group Sanjay Agarwal, Sunil Agarwal, S.R. Aravind, Rajeev Chawla, Jayaprakashsai Jana, Vasanth Kumar, Anuj Maheshwari, Anand Moses, Jayant Panda, Vijay Panikar, P.V. Rao, Banshi Saboo

✉ Vijay Viswanathan
drvijay@mvdiabetes.com

[1] M.V. Hospital for Diabetes and Prof. M. Viswanathan Diabetes Research Centre (WHO Collaborating Centre for Research, Education and Training in Diabetes), No. 4, West Mada Chruch Road, Royapuram, Chennai, Tamil Nadu 600013, India

[2] MLN Medical College, Allahabad, Uttar Pradesh, India

[3] Bharti Hospital, Karnal, Haryana, India

[4] Pt Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India

[5] Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Haryana, India

[6] Christian Medical College, Vellore, Tamil Nadu, India

[7] Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

[8] The Institute of Post-Graduate Medical Education and Research, Kolkata, West Bengal, India

[9] Christian Medical College and Hospital, Ludhiana, Punjab, India

[10] World Diabetes Foundation, 2820 Gentofte, Denmark

[11] International Union Against Tuberculosis and Lung Disease, South-East Asia Regional Office, New Delhi, India

[12] University College of Medical Sciences and GTB Hospital, New Delhi, India

[13] Diabetes & Obesity Center, New Delhi, India

[14] Regional Institute of Medical Sciences, Imphal, Manipur, India

[15] Osmania Medical College, Osmania General Hospital, Hyderabad, Telangana, India

[16] Apollo Hospital, Chennai, Tamil Nadu, India

[17] Shilpa Medical Research Centre, Mumbai, Maharashtra, India

[18] National Institute for Research in Tuberculosis (ICMR), Chennai, India

[19] P.D. Hinduja National Hospital and Medical Research Center, Mumbai, Maharashtra, India

[20] Ivy Hospital, Mohali, Punjab, India

Indonesia, China, Nigeria, Pakistan, and South Africa accounting for 60% of them [2]. There exists a strong epidemiological evidence base demonstrating the coexistence of DM and TB [3–8]. This association between a globally prevalent non-communicable disease such as DM and a serious infectious disease (TB), endemic in developing nations is known to adversely impact the course of progression and treatment outcome for both diseases and is transforming into a "syndemic" necessitating synergistic management [4, 5, 9, 10]. A recently published systematic review identified several risk factors associated with the DM-TB comorbidity that included older age, sedentary occupation, cigarette smoking, alcohol consumption, both lower and higher body mass index (BMI), human immunodeficiency virus (HIV) co-infection, weight loss, hypertension, and familial history of DM or TB [11].

Epidemiology

As per the WHO estimates for TB and DM double burden, 15% TB cases worldwide are associated with DM, and India and China account for more than 40% of these cases. India alone accounts for the highest number of adult TB cases associated with DM followed by China [12, 13]. Although the prevalence of DM and TB coexistence in India is not yet determined, prevalence studies have been conducted and reported from different regions. In a survey from five randomly selected TB units in Tamil Nadu, around 25% of TB patients had DM of which 9.3% were newly detected cases of DM. A similar percentage of TB patients had prediabetes. Common risk factors including age, BMI, family history of DM, and sedentary lifestyle were identified as possible risk factors for DM in TB patients [14]. Further, the prevalence of DM was significantly higher in men compared to women which was attributed to smoking, tobacco consumption, and alcohol use that are known risk factors for both TB and DM [14]. In the state of Kerala, the prevalence of DM in patients with TB was nearly double than that observed in Tamil Nadu. Among the total patients with TB in this study, 44% had DM, of which, 21% patients had newly diagnosed DM. The demographic characteristics of the investigated population was in line with those observed in Tamil Nadu and had a preponderance of men and patient aged above 50 years [15]. The risk of DM in TB patients was relatively higher in sputum positive TB patients in both Kerala and Tamil Nadu [14, 15]. In a recent cross-sectional study from Odisha, 13.9% tribal patients with TB had DM and 8.9% had impaired fasting glucose (IFG) [16]. In another retrospective study, among 1000 patients with respiratory diseases from Punjab, 11.6% had DM and TB coexistence, majority of which were men (56.5%), in the age group of 51 to 60 years and belonging to rural areas (68.4%) [17]. From studies conducted in patients with TB from urban centers, 29.0% (8.3%, newly diagnosed DM) from Puducherry and 15.3% (8.23%, newly diagnosed DM) from Ahmedabad had diabetes [18, 19]. A recent study conducted among patients with established TB and registered under the national program in Gwalior district of Madhya Pradesh reported the prevalence of DM as 15.5%, comparable to most reports from India [20]. Overall, the prevalence of DM among Indian patients with active TB appears to be at least two to three times higher than the national average or comparative regional data in the general population [21].

Biological links

Numerous plausible biological links between TB and DM have been elucidated. Chronic hyperglycemia in DM is known to increase the susceptibility of patients to infections by direct suppression of the innate and adaptive immunity [4, 7]. Compromised cell-mediated immunity has been particularly shown to facilitate *Mycobacterium tuberculosis* infection via disruption of key defense mechanisms such as monocyte chemotaxis, neutrophil recruitment, phagocytosis by alveolar macrophages, and antigen-specific cytokine responses (interferon-gamma release) due to depressed T helper cell activation [22, 23]. This immunosuppression in poorly controlled DM increases susceptibility to infections such as TB along with an increased baseline load of the mycobacilli and protracted time for sputum culture conversion in response to antibiotics, thus adversely impacting treatment outcomes [24]. The presence of DM also threatens TB control by worsening the outcomes of TB treatment and increasing the risk of treatment failure, deaths during treatment, recurrences, and development of resistance, highlighting the severity of this association [10, 25].

Further, studies reporting the development of glucose intolerance in patients with active TB implicate the two-way association between TB and DM [26, 27]. An endocrine-linked metabolic response to stress such as an acute infection has been postulated to severely hamper glycemic control; however, the underlying mechanism specific to TB infection is uncertain [7, 27, 28].

Need for clinical practice recommendations

In the light of this surging danger, the World Health Organization (WHO) along with the International Union against

Tuberculosis and Lung Disease (the Union) proposed the *Collaborative Framework for Care and Control of Tuberculosis and Diabetes* that emphasizes the need for collaborative control efforts through bi-directional screening and efficient co-management of both diseases [29, 30]. The "National Framework for Joint TB-Diabetes Collaborative Activities," released in March 2017 highlights the need and measures for strengthening operational consensus and integration of services between the two national programs for DM (National Program For Prevention and Control of Cancer, Diabetes, Cardiovascular Disease and Stroke [NPCDCS]) and TB (Revised National Tuberculosis Control Program [RNTCP]) [31]. Although these documents outline a systematic approach for bi-directional screening within routine health care settings, the guidance for clinical management of TB and DM at the individual patient level is missing.

The current article aims to fill this gap and provide evidence-based recommendations to clinicians, researchers, policy makers, patients, and all other stakeholders' for the screening, diagnosis, and management of the double burden. These recommendations are convened to facilitate clinical decision making and should be appropriately adjusted when required for individual patient needs, comorbidities, and other factors based on good clinical judgment and practice.

Diagnosis and management of tuberculosis in patients with diabetes mellitus

Diagnosing tuberculosis in patients with diabetes mellitus

Diabetes mellitus increases the risk of developing TB by 2.5- to 3-fold underscoring the need for effective screening and diagnosis of TB in patients with DM [3, 4, 7, 32]. In general, the approach of screening activities should focus on the early detection of TB, prevention, and control of the transmission with an ultimate goal of improving overall treatment outcome and lowering associated morbidity and mortality [5]. Results from a systematic review that evaluated the studies implementing a bi-directional screening of DM-TB conducted before the release of the WHO framework assert the significance of such a screening approach [29, 33]. The high risk of TB affliction in patients with DM was observed in all of the 12 studies that screened patients with DM for TB despite heterogeneity in geographic area, baseline disease burden, and techniques of screening and diagnosis. Most often, the diagnosis was a combination of screening of clinical signs and symptoms, radiological changes in chest, and positive smear and/or culture of *M. tuberculosis* complex [33].

A pilot project describing the implementation of TB screening in patients with DM within tertiary healthcare setting in India outlines the systematic screening work-up for TB [34]. The diagnosis of active TB followed the national guidelines (RNTCP) and was carried out at every patient visit [35]. Patients with a history of TB and/or having common symptoms of TB were referred for sputum smear microscopy (Ziehl-Neelsen staining followed by examination for acid fast bacilli) and chest radiography. Diagnostically sensitive techniques such as mycobacterial culture tests or nucleic acid amplification were not used. Despite several operational limitations of this study the findings demonstrated the higher incidence of TB in DM patients and more importantly the feasibility of TB screening in routine DM clinics [34, 35]. Subsequently, several studies reporting the double burden conducted across India screened patients based on the presence of clinical symptoms of TB and diagnostic investigations (chest X-ray, sputum smear microscopy) in different patient populations and healthcare settings [16, 17].

More recently, under the expanding laboratory services strategy, the RNTCP has widely implemented state-of-the-art diagnostic methods such as the cartridge based nucleic acid amplification test (CB-NAAT) and line probe assay (LPA) at TB units nationally [36]. The RNTCP technical and operational guidelines for TB control advocate the use of CB-NAAT in high-risk groups that include patients with DM [37]. The Gene Xpert MTB/RIF assay or CB-NAAT is a WHO-endorsed assay to detect TB infection along with resistance to rifampicin [38, 39]. The assay has a high degree of accuracy and sensitivity with a single direct sputum sample and offers rapid diagnosis with a turnaround time of less than 2 h. In contrast to conventional solid culture techniques that need a minimum of three sputum samples and a turnaround time of several weeks, the CB-NAAT offers significant operational benefits for employment at a program level [40, 41].

Although the national TB program mandates screening of TB in patients with DM at every visit, this approach may not be possible and pragmatic in a program setting. It is recommended that patients should be screened for TB as soon as the diagnosis of DM is made. Thereafter, a symptom screen should be undertaken annually. In addition, patients with DM should be screened for TB when metabolic control worsens and cannot to be explained by other known causes.

At the outset, screening of active TB in all patients with DM should be based on the identification of any one of the recognized symptoms (cough of any duration, fever, unexplained weight loss, night sweats, hemoptysis) or suggestive chest X-ray in patients not currently receiving anti-TB medications (Fig. 1) [37]. It must be noted that patients with DM and having autonomic neuropathy may not manifest cough; on the other hand, DM patients receiving angiotensin-converting-enzyme (ACE) inhibitors may manifest cough as side effect of the medication. The initial screening of TB

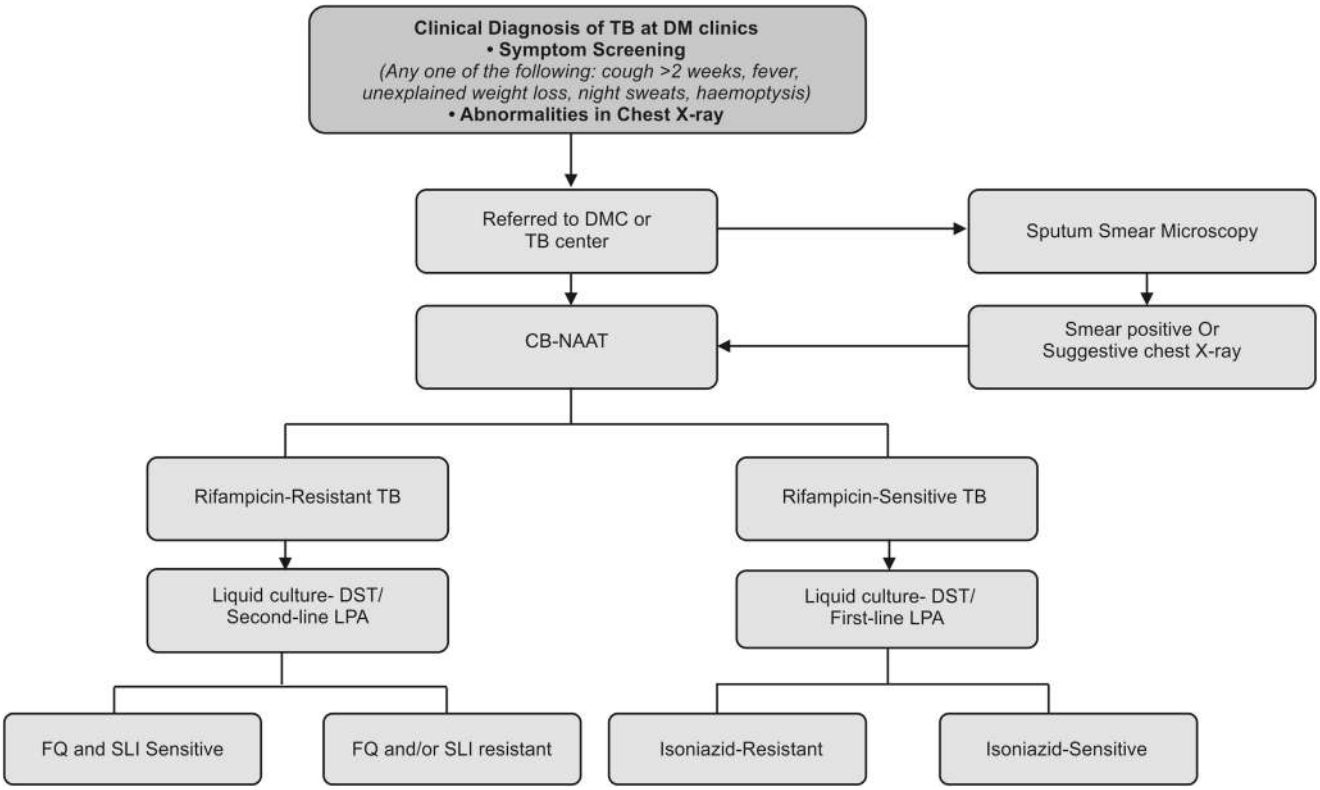

Fig. 1 Diagnostic algorithm for tuberculosis in patients with diabetes mellitus. CB-NAAT, cartridge-based nucleic acid amplification test; DM, diabetes mellitus; DMC, designated microscopy center; FQ, fluoroquinolone; LPA line probe assay; SLI, second-line injectables; TB, tuberculosis

should also involve identification of presumptive cases to facilitate diagnostic strategy and better clinical management (Box 1) [37].

Box.1: Presumptive Tuberculosis

1. Presumptive Pulmonary TB
   Patients with any of the following symptoms
   - Cough >2 weeks
   - Fever >2 weeks
   - Significant weight loss
   - Haemoptysis
   - Abnormal chest radiography
2. Presumptive extra pulmonary TB
   Organ-specific symptoms in addition to constitutional symptoms of pulmonary TB
   - Swelling of lymph nodes
   - Pain and swelling in joints
   - Neck stiffness
   - Disorientation etc.
3. Presumptive paediatric TB
   - Persistent cough for >2 weeks
   - Weight loss or no weight gain
   - Contact with TB-infected individuals
4. Presumptive drug resistant TB
   - First-line treatment failure previously
   - Pediatric non-responders
   - Contact with drug resistant TB patients
   - Culture positive during first-line treatment
   - TB-HIV co-infected

Patients with clinically diagnosed TB should be referred to a designated microscopy center (DMC) or testing laboratory for microbiological confirmation of TB, if such a facility is not available within the clinic/hospital. Microbiological confirmation of TB infection is essential in all suspected cases. In patients tested as screening positive, the RNTCP endorses the use of sputum smear microscopy, culture-based drug susceptibility testing or rapid molecular assays that involve different levels of infrastructural sophistication and technical complexities (Box 2) [37].

Box.2: RNTCP endorsed TB diagnostic tools

1. **Sputum Smear microscopy (AFB)**
   - Zeihl-Neelson staining
   - Fluorescence staining

   Most commonly used
   Limited sensitivity in children and HIV-co-infected patients
2. **Culture**
   - Solid culture (Lowenstein Jensen [LJ])
   - Automated liquid culture system (e.g. BACTEC MGIT-960, BacT/ALERT, VersaTREK etc.)
   - Modified LJ and MGIT-960 systems

   Highly specific and sensitive
   Turnaround time of 2 to 8 weeks
   Useful for assessing response to treatment during follow-up of MDR-TB
3. **Rapid Molecular Diagnostic Testing**
   - Line probe assay for MTB complex and detection of RIF and INH resistance
   - Gene Xpert MTB/RIF assay or CB-NAAT

   High specificity and sensitivity
   Rapid diagnosis
   Recommended for diagnosis of TB in key population such as children, HIV co-infected patients and for extra-pulmonary TB and DR-TB

Positive conventional sputum smear microscopy (for AFB) or CB-NAAT or growth of MTB on culture may be sufficient for the diagnosis of TB. Patients unable to produce a good sputum sample for testing may need sputum induction using nebulized hypertonic saline. In patients with RIF-resistant strains detected by CB-NAAT (Fig. 1), and in those suspected to have drug resistance, liquid culture (BACTEC MGIT 960)-

based drug susceptibility testing (DST) should be employed for rapid detection of MDR-TB in resource-limited settings where LPA may not be feasible [42–44]. A solid culture-based DST may be used if liquid culture is not available, although the turnaround time to yield results will be increased by several additional weeks [45].

Plain chest radiograph is recommended in all patients with presumptive pulmonary TB [46]. Although there is a lack of consensus among TB experts over the differences in the radiological manifestations of TB in patients with DM as compared to the general population, conflicting manifestations specific to this population have been observed [47–51]. The differences reported include the presence of greater frequency of pulmonary lesions in the lower lung field in contrast to the typical upper lobe lesions observed in normoglycemic patients with pulmonary TB. A significantly higher occurrence of cavitary lesions have also been noted in patients with TB having uncontrolled DM [50, 52, 53]. These atypical radiographic presentations of TB in patients with DM pose a problem of misdiagnosis of the mycobacterial infection for community-acquired pneumonia or lung cancer [54]. Similar uncommon presentation of pulmonary TB has also been observed in chest computer tomography (CT) assessments in patients with DM [55]. A bilateral and multilobar pulmonary involvement was noted along with substantially greater lymphadenopathy in patients with TB having underlying DM [52, 55, 56]. Observations of aggressive pulmonary manifestations, greater parenchymal lesions, and multiple cavities have been potentially linked to higher bacillary load in sputa, leading to higher positive smear rates at diagnosis in patients with poor glycemic control [14, 48, 51, 57, 58].

For patients who have pulmonary lesions and fail to produce sputum even with induction or in cases where the suspicion of pulmonary TB persists despite a negative sputum test, more complex diagnostic methods using the flexible bronchoscope such as bronchoalveolar lavage and transbroncheal lung biopsy should be considered [54].

Management of tuberculosis in patients with diabetes mellitus

Tuberculosis, an immunopathological complication of DM should not be underestimated and managed with equal rigor as the management of other complications of DM (neuropathy, nephropathy, and retinopathy). Patients with DM and TB (RIF-sensitive as confirmed by Gene Xpert MTB/RIF assay) must be initiated on the standard directly observed treatment (DOT) with the four-drug regimen similar to the treatment presented to the general TB population [29, 37]. The standard therapy for tuberculosis includes the daily dosing of anti-TB drugs that include isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (Box 3 outlines the regimen) [37]. It should be noted that patients who have previously failed or defaulted the first-line TB regimen or relapsed after treatment are at a greater risk of lodging drug-resistant or MDR strains and have reported lower success rates with the retreatment TB regimen (Box 3) [59–62]. Therefore, in accordance with the recent WHO update on the guidelines for retreatment of TB in drug-susceptible cases, it is highly recommended that patients with TB being retreated after a previously failed anti-TB regimen should first undergo DST to determine the appropriate choice of drug regimen [63].

Owing to the established vulnerability and treatment challenges in patients with DM, WHO recommends optimization of the standard algorithm depending on the pharmacokinetic alterations and metabolic differences in patients with the twin burden [29].

Box.3: Treatment of tuberculosis in patients with diabetes mellitus

1. **Newly diagnosed tuberculosis**
   - **Intensive phase (8 weeks)**
     Isoniazid, Rifampicin, Pyrazinamide, Ethambutol
   - **Continuation phase (16 weeks)**
     Isoniazid, Rifampicin, Ethambutol
2. **Previously treated tuberculosis**
   - **Intensive phase (12 weeks)**
     Isoniazid, Rifampicin, Pyrazinamide, Ethambutol + Streptomycin (8 weeks)
   - **Continuation phase (20 weeks)**
     Isoniazid, Rifampicin, Ethambutol

Diabetes is often associated with numerous complications such as peripheral neuropathy, retinopathy, and compromised kidney function [64]. Therefore, management of concurring toxicities such as INH-induced peripheral neuropathy, EMB-induced optic neuritis, and EMB-induced impaired kidney function must be kept in mind during treatment of TB in patients with DM [65]. The use of pyridoxine (vitamin B6) along with INH is therefore mandatory in patients with DM [7, 66].

Changes in drug absorption due to DM can lead to inadequate blood level of first-line anti-TB drugs in these patients that can eventually culminate into drug resistance. It has been observed that patients with DM exhibit suboptimal response to anti-TB agents particularly RIF due to lower serum concentrations [67]. In a cross-sectional study conducted in South India, a significantly higher proportion of patients with DM demonstrated resistance to RIF (27%) as compared to patients without diabetes (8.8%, $p < 0.01$) [68]. A Turkish study reported that plasma INH and RIF concentrations in TB patients with diabetes were 50% lower than those observed in TB patients without DM [69]. Rifampicin serum concentrations were 53% lower in patients with DM as compared to

normoglycemic TB patients in a study from Indonesia [67]. In a recent study from India, TB patients with DM had lower serum concentration of INH and PZA as compared to patients without DM and the effect was linked to hyperglycemia [70].

Reduced exposure to anti-TB drugs is also attributed to the higher body weight observed in patients with concomitant TB and DM and is regarded as a serious impediment to clinical recovery and contributing to therapeutic failure and acquired drug resistance [67, 69]. Therefore, appropriate dose adjustments based on body weight is recommended while prescribing anti-TB drugs and should be in accordance with good clinical sense [71]. Routine monitoring of therapeutic drug responses and fortification of TB regimen such as use of higher doses or extended treatment duration is advocated especially in patients at risk of inferior treatment outcome [10, 72].

Drug interactions with anti-TB drugs

Caution should be exercised when prescribing anti-TB drugs in patients receiving oral anti-diabetic drugs (OADs). Most commonly prescribed OADs (sulfonylureas and thiazolidinedione) are mainly metabolized by enzymes of the cytochrome P450 system. Rifampicin is a potent inducer of the several isoenzymes of the cytochrome P450 system and by activating these enzymes, RIF accelerates the systemic elimination of concomitantly administered drugs significantly reducing their therapeutic efficacy [73]. Initiation of RIF in patients receiving sulfonylureas—glipizide and glyburide (CYP2C-mediated metabolism)—has been associated with 39 and 22% lower serum concentration, respectively, hampering their hypoglycemic effects [74]. Among thiazolidinediones, RIF decreased plasma concentrations of rosiglitazone by 54 to 65% and induced a 54% decrease in levels of pioglitazone via increased CYP2C8-catalyzed metabolism [75–77]. Modest reductions in plasma levels of nateglinide and substantial decrease in repaglinide concentrations have been observed following co-administration with RIF with potential effects on hypoglycemic effects of these drugs [78, 79]. Isoniazid on the other hand is an inhibitor of the cytochrome system and delays the biotransformation of glimepiride, a CYP2C9 substrate, and increases the risk of hypoglycemia due to accumulation of the sulfonylurea [80].

Diagnosis and management of MDR-TB in patients with DM

Delays in mycobacterium TB clearance during treatment and treatment failures predispose patients with DM to an increased risk of primary multidrug-resistant TB (MDR-TB) [81]. Numerous studies have identified a 2- to 9-fold increase in the risk of MDR-TB among patients with DM as compared to normoglycemic individuals [82–85]. A recent meta-analysis addressing the comorbid relationship between DM and MDR-TB reported significantly higher odds of MDR-TB in patients with DM (OR = 1.71, 95% CI = 1.32; 2.22) [86]. Concurrent DM was significantly associated with MDR-TB in both Caucasians and Asian subgroups and was identified as an independent risk factor especially for primary MDR-TB [86].

As recommended for the general population with TB, patients with DM and receiving treatment for TB should also be monitored through the time course of treatment for the development of drug resistance [82, 87, 88]. Chest CT with pulmonary lobe consolidation and multiple mouth-eaten cavities and bronchial damage are characteristic of MDR in patients with DM and TB [89]. Rapid molecular techniques (CB-NAAT and LPA) or liquid and culture DST should be employed for prompt diagnosis of resistance (Fig. 1) [35, 90]. Patients already receiving first-line anti-TB therapy and who continue to be smear positive at the end of the intensive treatment phase (first 2 months) should be assessed for drug resistance. It is imperative that confirmed cases of MDR-TB be referred to specialized centers (under respiratory physicians, infectious disease specialists or TB specialists with experience in the management of MDR-TB) for treatment [35].

The treatment of MDR-TB comprises an extensive 24- to 27-month chemotherapy [35, 91]. The WHO and RNTCP prescribed treatment for MDR-TB or RIF-resistant TB includes the use of at least five effective anti-TB drugs. The RNTCP-recommended regimen for MDR-TB is as shown in Box 4 [37].

Box.4: Treatment of multi-drug resistant tuberculosis in patients with diabetes mellitus

Intensive phase (6 to 9 months)	Continuation phase (18 months)
Fluoroquinolone	• Levofloxacin
• Levofloxacin	• Ethionamide
Second-line injectable	• Ethambutol
• Kanamycin	• Cycloserine
Core second-line agents	
• Ethionamide	
• Cycloserine	
Add-on agents	
• Pyrazinamide	
• Ethambutol	

In RIF-resistant and isoniazid-sensitive cases, isoniazid should be added to the regimen (Box 4). Screening using liquid culture DST or second-line LPA (Fig. 1) should be carried out for all cases at baseline to detect sensitivity to kanamycin and levofloxacin. Appropriate alterations in second-line drug regimen may be considered if resistance is detected. In routine clinical practice, standardization of second-line regimen based on individual patient's drug resistance (using DST) and tolerance profile has been adopted to improve treatment success rates [92, 93]. In cases of additional drug resistance, the inclusion of clofazimine, linezolid, and paraaminosalicylic acid may be considered [37]. Although potential drug interactions with most prescribed second-line

drugs are minimal, consideration of DM-related changes in pharmacokinetics that can influence therapeutic outcome should be considered in patients with DM.

Bedaquiline and delamanid are recent additions to the MDR-TB therapy. The use of bedaquiline has been indicated as a part of MDR-TB regimen by the RNTCP [37]. Therapeutic drug monitoring is advised in patients on anti-diabetic therapy and receiving bedaquiline owing to CYP450-mediated pharmacokinetic alterations, drug-drug interactions, and adverse events. Concerns of electrolyte imbalance, QTc prolongation, and gastrointestinal toxicity following concurrent use of bedaquiline with sulfonylureas, insulin analogs, and meglitinides have been reported [94]. Overall, metformin has a favorable pharmacokinetic and safety profile for use with bedaquiline. Metformin is also known to restrict intracellular replication of MDR strains and can therefore be a potentially viable option for the co-management of DM and MDR-TB [95].

Diagnosis and management of diabetes mellitus in patients with tuberculosis

Diagnosis of diabetes mellitus in patients with tuberculosis

Diabetes mellitus is associated with greater risk of treatment failure, morbidity, death, and relapse in TB [6, 7, 10]. The higher mycobacterial load at diagnosis and delayed sputum culture conversion during the initial intensive treatment phase (first 2 months) are regarded as strong determinants of this increased risk [24]. A study from India that assessed sputum conversion rate reported positive sputum smear in about 14.7% of patients with TB and DM at the end of the intensive phase of DOTS therapy. The relative risk to remain sputum smear positive at the end of intensive phase was estimated to be 3.9 (95% CI = 1.5; 10.6) in patients with DM [96].

Other confounding factors associated with coexisting DM include the development of adverse events that negatively influence treatment adherence hampering treatment success [25, 97]. Detection of overt DM, previously undiagnosed DM or inadequate glycemic control in patients with TB is crucial for achieving optimal treatment outcomes. Convergence of active screening and monitoring of DM with routine TB screening at national-level programs can greatly aid current TB control efforts [29]. This approach of routine DM screening at the time of TB registration has been implemented at national-level TB programs in China and India [98, 99].

The systematic screening and diagnosis approach suggested here is based on the experiences from the pilot project that implemented a standardized procedure for DM screening at TB units in India and is in accordance with the RSSDI recommendations (Fig. 2) [99–101]. Patients with TB are first interviewed and those with known DM should be referred to diabetes care centers for the management of glucose control. For patients without a history of DM, RSSDI-endorsed screening methods such as random blood glucose (RBG), oral glucose tolerance test (OGTT), or estimation of glycated hemoglobin (HbA1c) can be employed. RBG and fasting blood glucose (FBG) using capillary (using glucometer) or venous sampling are the common, widely accepted and convenient diagnostic methods that are adopted for the mass screening of DM among patients with TB [29]. A FBG $\geq$ 126 mg/dL is diagnosed as DM; FBG 110 to 125 mg/dL is considered as impaired fasting glucose. Patients with RBG $\geq$ 200 mg/dL should be diagnosed as diabetes and referred for management of DM [101]. RBG between $\geq$ 100 and < 200 mg/dL is suggestive of retesting and FBG should be utilized for screening in the next patient visit and a FBG $\geq$ 126 mg/dL should be regarded as diabetes [101, 102]. Confirmatory assays such as OGTT and estimation of HbA1c can be used for cases primarily diagnosed using glucometers [29]. A recent interim report from South India studying the effect of DM on TB severity (EDOTS) reported high prevalence of DM and prediabetes in adults with pulmonary TB and also noted considerable heterogeneity in the severity of DM among patients with TB. This heterogeneity has implications for clinically relevant TB-DM interaction and the interpretation of DM diagnosis in TB studies [103].

Although both OGTT and HbA1c are expensive diagnostic methods that involve tedious procedures limiting their feasibility for community-based screening activities, they offer a higher degree of sensitivity compared to FBG and RBG [29, 104]. The OGTT, a WHO recommended measure for DM is particularly of value for the detection of previously undiagnosed cases and patients with impaired glucose tolerance [100]. OGTT was used for patients without a history of DM in a study conducted across TB units of the RNTCP in Tamil Nadu that reported a prevalence of 24.7% prediabetes among TB patients in South India [14]. A study from South India that compared the performance of HbA1c and FPG evaluated HbA1c as a better diagnostic tool for the identification of newly diagnosed DM among patients with TB [104].

HbA1c has been used for screening newly diagnosed cases of DM among patients with TB in India [15, 104] and few other resource-limited countries [105, 106]. In more recent studies, the severity of hyperglycemia measured using HbA1c has been associated with risk of TB occurrence. HbA1c > 7.0 has been associated with a three times increased risk of active TB (Hazard ratio: 3.11) [51] whereas HbA1c < 7.0 is associated with protective effects

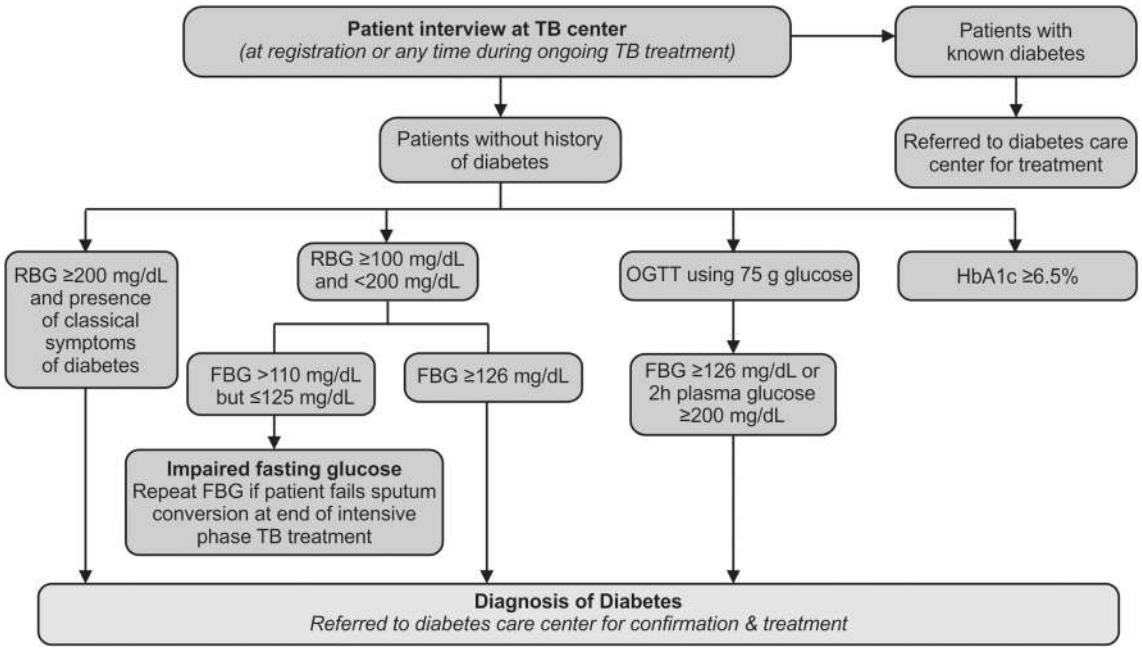

Fig. 2 Diagnostic algorithm for diabetes mellitus in patients with tuberculosis. CB-NAAT, cartridge-based nucleic acid amplification test; DM, diabetes mellitus; DMC, designated microscopy center; FQ, fluoroquinolone; LPA line probe assay; SLI, second-line injectables; TB, tuberculosis

against TB (odds ratio 0.52) [107]. HbA1c value of ≥ 6.5% is recognized as the clinical cut-off for the diagnosis of DM by the RSSDI [101]. The HbA1c gives a cumulative index of the blood glucose profile over a period of 2 to 3 months in contrast to time point FBG and RBG measures and therefore eliminates risk of possible false diagnosis of stress-induced hyperglycemia [15, 104]. In addition, it can be measured irrespective of the patient's fasting state and is devoid of rapid fluctuations that are common with RBG and FBG estimations. The absence of a prerequisite fasting state is major advantage of HbA1c measure especially in a country like India where most patients with TB visiting community centers are unlikely to visit the centers in a fasting state and may not come back when asked to return for the test. On the other hand, there are issues interpreting the results in presence of concomitant anemia, likely in patients with TB. Both methods, OGTT and HbA1c, are recognized as standard screening techniques for DM by the RSSDI and have clinical cut-off for diagnosis (Figs. 2 and 3) [101].

The time of screening of DM in patients with TB may impact the result of diagnosis, however, clinical management of DM is imperative and should be regardless of the severity or stage (diabetes, prediabetes) of glucose abnormality [15, 29]. Transient hyperglycemia possibly induced by infection-related inflammation is commonly detected at the time of TB diagnosis and is linked with adverse outcomes of TB treatment [102]. Although normalization of this temporary glucose elevation is largely achieved during TB treatment, it may be suggestive of a reverse causality and may precede clinical onset of DM [102, 108]. Therefore, multiple glucose measurements during the course of TB treatment and a repeat investigation towards completion of treatment is recommended [15]. Frequent follow-ups after resolution of TB are advised especially in patients on single-drug anti-diabetic therapy [102].

Management of diabetes mellitus in patients with tuberculosis

Infections tend to deteriorate glucose regulation and poor glucose regulation worsens infection in patients with DM and TB is no exception; hence, intensive management of DM in patients concomitantly suffering from TB is critical [66].

Insulin

Insulin is the most preferred treatment in patients with severe TB because of its anabolic actions, regulation of appetite, promotion of weight gain, low potential for drug interactions with the anti-TB agents, and effect on producing a general sense of well-being [47, 66, 109]. In addition, it provides the practical advantage of reducing the pill burden thereby improving chances of treatment adherence to the TB regimen. Insulin is recommended for the management of DM in all TB patients and should be used wherever feasible. As recommended by the RSSDI, insulin must be used in newly diagnosed DM patients with

HbA1c > 9.0%, FBG > 250 mg/dL, ketone bodies, symptoms of hyperosmolar state, and for those in a catabolic state. In patients already on OADs, insulin may be added to ongoing therapy if the patient is poorly controlled and has a low BMI. Regular monitoring of glycemia and adoption of lifestyle interventions are advised to complement ongoing insulin treatment for better management of DM. Appropriate dose adjustments for insulin are critical as requirements vary during the course of treatment: after attainment of glucose control, reduction in infection load, and improvements in appetite [66, 110]. As in the general cases of DM (without TB), postponement of insulin therapy should be avoided in patients with concomitant DM-TB.

Metformin

The use of OADs is permissible in less severe cases of TB especially when insulin treatment is not available or acceptable to the patient [66]. Among the OADs, the current first-line of treatment for patients with DM is the use of biguanide agent metformin [111, 112]. Metformin is not metabolized by the CYP450 enzymes and thus its exposure is not influenced by RIF or any anti-TB agents making it a viable treatment option for patients with concurrent DM-TB [109]. Owing to its additional immunomodulatory properties described below, metformin is the preferred therapy for DM in patients with TB either alone (as a first-line OAD) or in combination with insulin or other OADs and in the absence of specific contraindications. Dose adjustments to minimize gastrointestinal intolerance and regular monitoring of renal and hepatic functions are recommended during metformin treatment [101]. Metformin should be discontinued in patients with abnormal liver function tests (> 3 times upper limit of normal [ULN]) and patients with an estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2. Signs of metformin-associated lactic acidosis (MALA) should be observed especially in hypoxic conditions [109, 113].

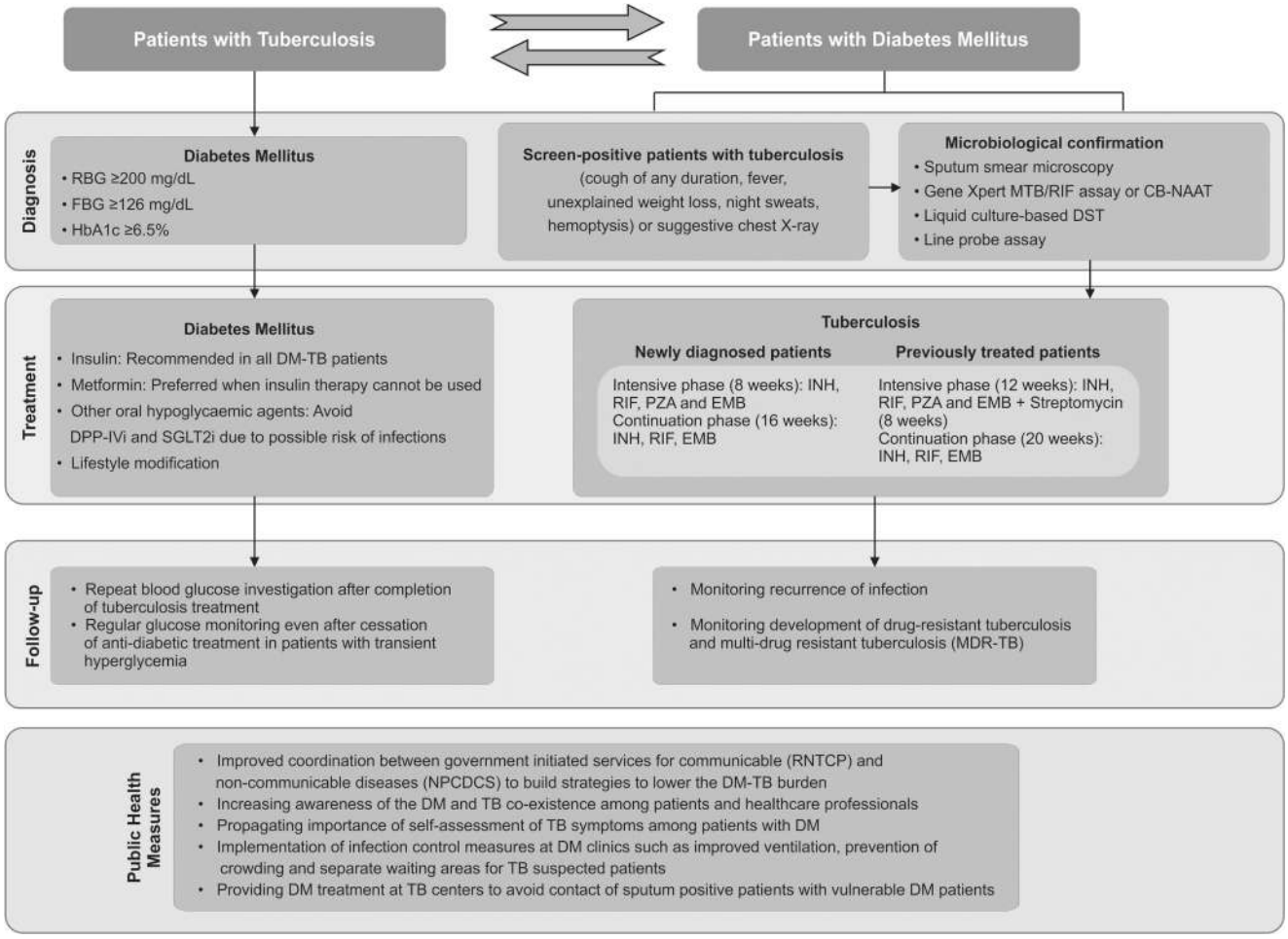

Fig. 3 Algorithm for bi-directional management of DM-TB. CB-NAAT, cartridge based nucleic acid amplification test; DM, diabetes mellitus; DPP-IVi, dipeptidyl peptidase-IV inhibitor; DST, drug susceptibility testing; EMB, ethambutol; FBG, fasting blood glucose; INH, isoniazid; PZA, pyrazinamide; RBG, random blood glucose; RIF, rifampicin; SGLT2i, sodium-glucose cotransporter-2 inhibitor; TB, tuberculosis

In addition to its antihyperglycemic effects, metformin is also a mitochondrial toxin and this effect has been recently researched as a clinical benefit in the treatment of TB. By blocking the NDH-1 complex, an intermediate in the respiratory chain, metformin inhibits important metabolic mechanisms that have shown to induce antibiotic tolerance in *M. tuberculosis* [114]. Metformin also reduces the activation of inflammatory genes and enhances host immunity against *M. tuberculosis* and has been proposed as an adjunct to improve anti-TB treatment outcomes [95]. Activation of adenosine monophosphate-activated protein kinase (AMPK)-mediated phagolyses of *M. tuberculosis* by the host cell is regarded as one of the underlying mechanisms for metformin-induced modulation of host immunity [95, 107]. In a retrospective case-control study, the protective effect of metformin at doses 500 and 1000 mg was 3.9-fold as compared to OADs [107]. Thus, metformin has a favorable pharmacokinetic, has safety and efficacy profile, and should be included in the co-management of DM-TB if not contraindicated and tolerated by patients.

Other oral anti-diabetic drugs

As for the choice of OADs, it is imperative to consider the possible drug-drug interactions with anti-TB drugs to avoid toxicity and potential clinical failure of either therapy [30, 66, 74, 77, 79, 80]. Sulfonylureas may be used as the second-line treatment for DM in patients with TB when metformin is contraindicated. These agents may be used to rapidly achieve glucose targets in patients with high blood glucose and in patients who cannot tolerate metformin [101]. Shorter acting sulfonylureas such as gliclazide and glipizide may be used owing to the lower magnitude of adverse events as compared to longer acting molecules of this class [115]. As with the other OADs, there exists inadequate data for use of dipeptidyl peptidase-4 (DPP-4) inhibitors, alpha glucosidase inhibitors (acarbose), thiazolidinediones, and sodium-glucose co-transporter 2 inhibitors (SGLT2i) in patients with concurrent TB. The immunomodulating effect of DPP-4 inhibitors (e.g., sitagliptin, vildagliptin, saxagliptin) and associated risk of infections, mainly respiratory, raises concerns over their use in patients with DM-TB; although recent reports do not indicate significant risk [116, 117]. Appearance of clinical features of drug-induced hepatotoxicity should be monitored during concurrent use of thiazolidinediones or acarbose with anti-TB drugs [118–120]. The higher risk of infections and diabetic ketoacidosis associated with SGLT2i therapy poses a major challenge in the use of these newer glucose-lowering agents in patients with DM-TB (Box 5) [121, 122].

Box.5: Pharmacotherapy of Diabetes in Patients with Tuberculosis

Insulin

- Recommended in all patients with TB
- Potential benefits in patients with DM-TB
  - Anabolic actions
  - Low potential for drug interactions with the anti-TB agents
- Use of insulin is advised in newly diagnosed DM patients with
  - HbA1c >9.0%
  - FBG > 250 mg/dL
  - Presence of ketone bodies, symptoms of hyperosmolar state and for those in a catabolic state
- Dose adjustments may be needed during the course of treatment

Metformin

- Recommended in less severe cases of TB especially when insulin treatment is not available or acceptable to the patient
- Potential benefits in patients with DM-TB
  - Low potential for drug interactions with the anti-TB agents
  - Immunomodulatory effects: Enhances host immunity against *M. tuberculosis*
- Discontinue metformin in patients with
  - Abnormal liver function tests (>3 times upper limit of normal)
  - Estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2

Other Oral Antidiabetic Agents

- Avoid possible drug–drug interactions
- Shorter acting sulfonylureas such as gliclazide and glipizide may be used
- Possible risks of infections with DPP-4 inhibitors and SGLT2 inhibitors should be considered

Adjunctive lifestyle therapy

Lifestyle modifications (LSM) along with ongoing DM treatment can greatly aid treatment outcomes in patients with the DM-TB double burden. Assessment of quality and quantity of nutrition is important. Evidence for correlation of nutritional deficiency with lower immunity and increasing susceptibility to TB is of metabolic significance in patients with DM [123, 124]. Protein malnutrition and depletion of protein reserves in DM is known to increase risk of recurrent TB infections [125]. Further, by triggering malabsorption of TB drugs, protein insufficiency negatively affects treatment outcomes in TB [126]. Therefore, emphasis on nutrition management is highly recommended in patients with concomitant DM and TB. The RSSDI recommends a protein intake accounting for a minimum of 15% of total daily calorie consumption in patients with DM [101]. Although a calorie intake threshold is not established for patients with DM and concomitant TB, it is recommended that a major portion of calorie intake should come from proteins in patients without any renal or hepatic comorbidity. In hyper catabolic patients with DM-TB and experiencing weight loss of > 10% within 3 to 6 months, addition of 500 calories essentially from protein sources is recommended. Inclusion of complex carbohydrates and moderate fats in diet is also advised in patients with the double burden. Among micronutrients, appropriate intake of minerals and vitamins should be maintained especially throughout the course of TB treatment in patients with DM [127]. Vitamin D supplementation may be initiated in patients with TB and underlying DM to resolve immunopathological inflammatory responses and improve outcome of ongoing TB treatment [128–130]. In addition, supplementation with vitamin B6 and B12 for management of neuropathy is strongly recommended in patients with DM and receiving TB treatment. Use of vitamin A for improving immune cell responses to mycobacterial infection may also be considered [131, 132].

In obese people with DM and concomitant TB, weight reduction may be considered as an adjunctive measure; however, this should be secondary to achieving optimal glycemic control, preventing infections and ensuring macro- and micronutrient adequacy.

Mild to moderate physical activity as per the patient's ability and tolerance is also advised in patients with DM-TB. Outdoor activity is preferred to increase skin exposure to sunlight and increase vitamin D synthesis. The intensity of physical activity should be increased gradually as the patient's condition improves.

Attenuation of immune responses due to direct or indirect effects of alcohol is known to substantially increase the risk of acquiring TB infections, reactivation of latent TB infections, and a higher risk of TB recurrence [133, 134]. Cessation of alcohol intake is therefore recommended during treatment of TB as well as post treatment [135].

Tobacco use in patients with DM has been associated with an increased risk of TB occurrence and transmission; hence, permanent discontinuation of tobacco use in all forms (smoking, chewing, etc.) is considered critical (Box 6) [136].

The impact of LSM on treatment outcome should be monitored and tailored as per individual patient needs. It is important that patients are educated about the benefits of continued LSM and timely and regular blood glucose measurements. Regular blood glucose monitoring should be emphasized even after TB is cured and upon completion of TB treatment and reversal to normoglycemic state along with cessation of antidiabetic treatment. Even if hyperglycemia was transitory due to stress of infection, patients manifesting hyperglycemia under stress are extremely vulnerable for future diabetes and integrated lifestyle measures may delay the onset of diabetes in these cases.

Box 6. Recommended Lifestyle Modifications

- Lifestyle measures should be tailored to individual patient needs
- Improvement of nutrition
  - Increase protein intake in patients with significant weight loss
  - Weight reduction may be considered in obese patients
  - Ensure appropriate intake of minerals and vitamins
  - Vitamin D supplementation: for improved TB outcomes
  - Vitamin B6 and B12 supplementation: for management of neuropathy
  - Vitamin A supplementation: for improved immune cell responses
- Mild to moderate physical activity
- Cessation of alcohol intake
- Permanent discontinuation of tobacco use in all forms
- Continue lifestyle modifications and regular blood glucose monitoring even after completion of TB treatment and attainment of normoglycemic status.

Management of TB in patients with diabetes complications

Chronic kidney disease

Chronic kidney disease (CKD) is a common comorbidity and complication of DM with high morbidity and mortality [137]. CKD is known to adversely impact multiple immune functions (phagocytosis, B cell and T cell response) and substantially increase the risk of acute and chronic infections such as TB, HIV, and hepatitis B virus (HBV) [138, 139]. Several studies report the high incidences of TB in high-risk renal patients and patients with end-stage renal disease (ESRD) and advocate periodic screening of TB in these patients [139–144]. The compounded effect of CKD and DM not only increases the risk and severity of TB but also complicates its treatment.

Although there is dearth of studies reporting the prevalence of TB-DM and comorbid CKD, the occurrence of TB has been found to be higher in patients with DM undergoing dialysis [145, 146]. In a nationwide, 10-year study from Taiwan, patients with TB having chronic kidney or liver diseases were observed to have a higher chance of developing DM underlining the two-way association between the comorbidities [147]. The same study reported a high prevalence of DM among patients with TB and the prevalence of CKD among patients with TB and DM was 11.7% [147]. A study conducted in a tertiary care hospital from North India showed that patients with type 1 DM had high rates of positive *M. tuberculosis* culture and a significant number of patients among these had CKD (31.2%; $p < 0.001$) [148].

The standard quadruple regimen for active TB (RIF, INH, PZA, and EMB) is recommended in patients with CKD [149]. Dose reduction of up to 50% for drugs EMB and PZA that are majorly metabolized and eliminated by the kidneys is recommended in patients with compromised renal function and advanced renal impairment having creatinine clearance < 10 mL/min [150, 151]. The risk of under-dosing can jeopardize TB outcomes and regular monitoring of treatment outcomes and adverse events is therefore essential to achieve optimal therapy [144, 149]. To reduce cross-interactions of RIF with common concomitant drugs such as OADs, immunosuppressants, and antihypertensives, substitution of RIF with rifabutin may be considered.

Among interventions for DM, insulin is the preferred therapy and the use of metformin should be avoided in patients with eGFR < 30 mL/min/1.73 m^2 [152]. Insulin dose must be carefully titrated as the risk of hypoglycemia is increased due to prolonged insulin half-life in patients with DM and concurrent CKD. Diagnostic difficulties may be encountered in patients with CKD and chronic liver disease (CLD) that include misdiagnosis due to exaggerated symptoms of the comorbid conditions. False-positive elevation in HbA1c as a result of analytical interference due to high concentration of urea in patients with ESRD attenuates the reliability of HbA1c for glucose monitoring in these patients (Box 7) [153]. Also, a false-positive diagnosis of ketoacidosis due to INH or PZA treatment may also interfere with the management. Monitoring of renal adverse events including hyperuricemia

is recommended in patients with compromised renal function and receiving treatment for concurrent DM-TB. Assessment of calorie intake and restrictions in protein intake (instead of the usually recommended high protein intake) is advised in patients with DM-TB and comorbid CKD. Vitamin B6 supplementation is recommended in these patients.

Box 7. Recommendations for DM-TB in Patients with Comorbid Chronic Kidney Disease

- Management of TB
  - Standard quadruple regimen (RIF, INH, PZA and EMB) is recommended in patients with CKD
  - Up to 50% dose reduction for EMB and PZA may be needed in patients with creatinine clearance <10 ml/min
  - Regular monitoring of treatment outcomes and adverse events is advised to ensure optimal therapy
- Management of DM
  - Insulin is the most preferred therapy
    - Careful dose titration is advised to avoid risk of hypoglycaemia
  - Use of metformin should be avoided in patients with eGFR <30 mL/min/1.73 m^2
- Regular monitoring of renal function is advised during ongoing DM-TB therapy
- Restricted protein intake is recommended

Chronic liver disease

As with CKD, there are limited studies reporting the coexistence of DM-TB in patients with CLD. The treatment of TB in patients with CLD is challenging due to the lower threshold of drug-induced hepatitis and hepatotoxic events in these patients with preexisting liver impairment [154, 155]. The three core first-line anti-TB drugs, INH, RIF, and PZA, are associated with hepatotoxicity with RIF being least and PZA having the highest potential. Most second-line anti-TB drugs are safe and associated with mild and reversible hepatic adverse events [156–158]. The time of initiation and cut-off for cessation of anti-TB treatment are pivotal factors in the management of DM-TB in patients with CLD. It is recommended that the treatment for TB be delayed and deferred until acute hepatitis is resolved. If acute hepatitis develops during TB treatment, all potentially hepatotoxic drugs (INH, RIF, and PZA) should be discontinued till complete clinical and biochemical resolution of hepatotoxicity. Non-hepatotoxic anti-TB drugs such as EMB, streptomycin, ofloxacin, and levofloxacin may be used in the interim period along with periodic monitoring of renal and hepatic function. Both HBV and hepatitis C virus (HCV) infections are significant risk factors for TB treatment-induced hepatotoxicity. Monotherapy with INH is advised and multidrug TB therapy should be avoided in patients with HBV or HCV infection [159–162], Antiviral therapy for HBV (entecavir and tenofovir) and HCV (pegylated interferon and ribavirin) may be initiated to lower viral load in patients who need anti-TB treatment [156]. Monthly monitoring of liver function is recommended in these patients upon reintroduction of anti-TB treatment. Anti-TB regimen in patients with cirrhosis should be introduced with caution keeping in mind the elevated risk of liver failure in these patients [156, 163]. Treatment with most anti-TB dugs may be restarted upon normalization of elevated transaminases (AST concentration < 2 times ULN) [156]. It is recommended that the therapy should be restarted with RIF due to its lower potential for hepatotoxicity. Reintroduction of anti-TB therapy should be implemented in a phased manner starting with lower doses of INH (50 mg) and RIF (150 mg) and up-titrated every 3 to 4 days to the recommended therapeutic doses. Pyrazinamide should be permanently discontinued in patients with liver dysfunction. The treatment should be reassessed upon recurrence of symptoms [156].

Insulin is the drug of choice for treatment of DM in patients with TB and comorbid CLD, more so in patients with cirrhosis [164]. Insulin requirements are higher in patients with impaired hepatic function due to insulin resistance and may be lower in patients with decompensated liver disease due to reduced breakdown of insulin [165]. Close monitoring of blood glucose and frequent dose adjustments are recommended during insulin therapy [166]. Metformin can be used in patients with stable liver disease (Child-Pugh score < 7) and should be avoided in patients with cirrhosis due to higher risk of MALA [167]. Use of metformin may be beneficial in treatment of simple hepatic steatosis and non-alcoholic steatohepatitis (NASH) and has been associated with normalization of transaminases [168, 169]. Reductions in hepatocellular carcinoma and hepatic complications in patients with DM and cirrhosis have been observed. Sulfonylureas have limited benefits in patients with CLD due to their pharmacodynamic and pharmacokinetic profile and should be avoided in patients with CLD [164]. Alpha-glucosidase inhibitors effectively lower both fasting and postprandial hyperglycemia and have an acceptable safety profile in patients with CLD [170]. Thiazolidinediones should be administered with caution in patients with elevated transaminases (> 2.5 times ULN) and periodic monitoring is advised [164, 171]. Treatment with pioglitazone and rosiglitazone has been associated with lowering of serum transaminases and insulin resistance and may also be useful in non-alcoholic fatty liver disease (NAFLD) [164, 172–174]. Regardless of the therapy, drug-drug interactions should be monitored and dose titrations should be reassessed in patients receiving concomitant treatment for DM-TB. Assessment of calorie intake is important in patients with DM-TB and coexisting CLD. Higher protein intake is recommended in cases of protein malnutrition that is common in patients with liver disease. In cases of NAFLD, calorie restriction and weight loss is advised although weight loss due to TB should also be taken into account. Cessation of alcohol consumption is strongly recommended due to toxic liver effects. Although beneficial,

physical exercise may not be advisable in patients with active liver disease [164, 175].

As with CKD, inconsistencies in HbA1c measure pose diagnostic challenges that further confound management of DM-TB in patients with CLD. Falsely elevated HbA1c levels due to nutritional anemia and increased RBC survival and misleading reductions due to bleeding and hemolysis hamper accuracy of results (Box 8) [176].

Box 8. Recommendations for DM-TB in Patients with Comorbid Chronic Liver Disease

- Management of TB
  - Delay and defer TB treatment until acute hepatitis is resolved
  - Discontinue hepatotoxic drugs (INH, RIF, and PZA) if acute hepatitis develops during TB treatment. Non-hepatotoxic anti-TB drugs such as EMB, streptomycin, ofloxacin, levofloxacin may be used in the interim period
  - Use INH monotherapy and avoid multidrug TB therapy in patients co-affected with hepatitis B and C virus infections
  - Restart TB treatment upon normalization of elevated transaminases (AST concentration <2 times upper limit of normal)
    - Start TB treatment with lower doses of INH (50 mg) and RIF (150 mg) and up-titrate every three to four days to the recommended therapeutic doses
  - Discontinue pyrazinamide in patients with liver dysfunction
- Management of DM
  - Use of insulin is recommended for treatment of DM
  - Insulin requirements may be higher in patients with impaired hepatic function and lower in patients with decompensated liver disease. Monitoring of blood glucose and frequent dose adjustments are therefore recommended during insulin therapy.
  - Metformin can be used in patients with stable liver disease (Child-Pugh score <7) and should be avoided in patients with cirrhosis
- Cessation of alcohol consumption is strongly recommended

Human immunodeficiency virus

The confluence of HIV and TB is a recognized global syndemic and TB is regarded as the second leading cause of death due to infectious diseases among patients with HIV [177, 178]. Several studies from India have also acknowledged the high prevalence of TB-HIV co-infection [179]. Estimates for DM-TB and HIV coexistence is currently not reported. A study examining comorbidities in TB patients from South India identified the preponderance of DM coexistence over HIV [179]. The WHO recommends symptom screening (persistent cough or fever, weight loss, night sweats, and lymphadenopathy) for TB in all patients with HIV followed by a confirmatory assessment using Gene Xpert MTB/RIF assay in suspected cases. It has been observed that TB-HIV co-infection confounds the clinical diagnosis of TB and typical chest radiographic presentations may be absent in sputum culture-positive patients with HIV and low $CD4^+$ count [180]. Screening of latent TB using methods such as interferon-gamma release assays (IGRA) and skin tuberculin are also advised to facilitate preventive care in vulnerable cases. On the other hand, HIV testing is advocated in all patients with presumptive TB. The feasibility of HIV testing was demonstrated in India through a joint effort by RNTCP and the National AIDS Control Organization (NACO) in two districts from India where the HIV status was ascertained in 70% patients with TB [181].

Anti-retroviral therapy (ART) is strongly recommended in all patients with TB and HIV irrespective of the $CD4^+$ count. It is recommended that TB treatment should be started first followed by the initiation of ART as soon as possible and preferably within the first 8 weeks of TB treatment [63]. In co-affected patients with severe immunosuppression ($CD4^+$ count < 50 cells/mm^3) ART should be started within the first 2 weeks of TB treatment [63]. A paradoxical worsening of TB symptom shortly after initiation of ART may be suggestive of the development of TB-immune reconstitution inflammatory syndrome (TB-IRIS) and should be monitored closely [182, 183]. The symptoms of TB-IRIS commonly include lymphadenopathy, recurrent fever, worsening respiratory symptoms, and radiological manifestations of TB. The possible misclassification of TB-IRIS as a superinfection, TB treatment failure, and development of drug resistance or TB relapse should be eliminated before confirming diagnosis. The management of TB-IRIS usually involves systemic administration of corticosteroids and anti-inflammatory drugs [182, 183].

Anti-retroviral therapy, the mainstay in the treatment of HIV, has been associated with increased risk of metabolic derailments that include hyperglycemia. The presence of coexisting TB and poor nutrition may further stress the compensatory mechanisms and overwhelm the ability to increase insulin production in the face of rising insulin resistance resulting in a fully decompensated diabetic state. Alternatively, DM induced by ARTs can further enhance the risk of TB in these patients and complicate management of comorbidities [184, 185]. It is recommended that patients with HIV should be screened for DM at the onset of ART and at an interval of 3 to 6 months. The use of venous sampling is advised as opposed to finger prick for blood glucose estimations and HbA1c is not recommended in patients with HIV (Box 9) [186]. By virtue of its anabolic effects, favorable effects on inflammatory markers and limited effects on renal and hepatic functions, insulin is recommended for treatment of DM in patients with HIV and TB [186]. The use of metformin should be avoided in cachexic patients with TB and concurrent DM. Metformin-related incidences of diarrhea, reductions in subcutaneous fat, and subsequent worsening of lipodystrophy can complicate management of comorbidities [187]. Among other OADs, meglitinides and sulfonylureas may be used for rapid improvements in cases of severe insulin resistance. Close supervision of adverse events is essential during treatment with thiazolidinediones; however, poor responses to these agents have been reported in patients with HIV limiting their use. LSM including adoption of healthy balanced diet, regular physical activity, and restricted tobacco use is recommended for improved management of comorbidities [186].

Box 9. Recommendations for DM-TB in patients with comorbid HIV

- Use Gene Xpert MTB/RIF assay for diagnosis of TB in all patients with HIV
- Use of Interferon-Gamma Release Assays and skin tuberculin is advised for screening latent TB in vulnerable patients
- HIV testing is recommended in all patients with presumptive TB
- Initiate antiretroviral therapy in all patients with TB and HIV irrespective of the CD4+ count
- Start TB treatment first followed by antiretroviral therapy preferably within the first 8 weeks of TB treatment
- In patients with CD4+ count <50 cells/mm3, antiretroviral therapy should be started within 2 weeks of TB treatment
- Patients with HIV should be screened for DM at the onset of antiretroviral therapy and every 3 to 6 months subsequently
- Treatment with insulin is recommended for DM
- Avoid use of metformin in cachexic patients

Public health implications of the co-epidemic

The escalating prevalence of both DM and TB and the syndemic association of their coexistence is a growing concern in India. Coordination between government initiated services for communicable (RNTCP) and non-communicable diseases (NPCDCS) to build a synergistic strategy to reduce the burden of TB and DM is essential. Although these independent bodies have efficiently raised awareness of TB and DM as individual issues, there is limited understanding about the DM-TB comorbidity among patients and healthcare professionals. This lack of awareness can seriously jeopardize treatment outcomes of both diseases, increasing related morbidity and mortality. It is therefore imperative to defy barriers that reduce availability, affordability, dissemination, and efficacy of optimal diagnosis and treatment of both diseases in public settings.

The efforts towards lowering the DM-TB burden start with increasing awareness of the DM and TB coexistence. It is essential to educate patients with DM about the common symptoms of TB and the importance of self-identifying the symptoms of TB. Patients should be made aware of practical indicators of weight loss such as change in clothing size and belt size, and this should be promptly communicated to health care professionals for further investigations or dose adjustments during ongoing treatment. Displaying posters of TB awareness, symptoms screening, and treatment at DM clinics and vice versa would help educate patients. Involvement of media should be promoted to disseminate health information to a wider audience and propagate the intensity of the problem as well as publicize ongoing efforts for control and management.

It is also essential to assess the awareness and level of clinical knowledge of DM among TB health workers. A study evaluating the impact of a training program on screening, diagnosis, and management of DM, designed for TB health care providers across 22 tuberculosis units in Tamil Nadu reported enhancement of knowledge and improvement in attitude and practice sense for screening and primary care for DM [188]. Similarly, health care professionals at DM clinics should be made aware of the increasing incidences of TB among patients with DM and measures to prevent transmission of infection. Implementation of key infection control strategies should be mandated at all public health facilities attended by vulnerable patients with DM. At DM clinics, measures such as installation of proper ventilation systems, improving natural ventilation and air circulation, minimizing patient waiting time, and providing separate waiting rooms for suspected patients with TB (sputum-positive patients or patients with chronic cough) should be encouraged [31, 189]. Considering the high propensity of TB infections among patients with DM and to minimize the risk of transmission, it is recommended that consultation, follow-up, and treatment for DM in sputum-positive patients be conducted at TB facilities until the patients become sputum negative.

The National Framework for Joint TB-Diabetes Collaborative Activities summarizes collaborative efforts between the two national bodies NPCDCS and RNTCP [31]. Recommendations include implementation of TB symptom screening at DM clinics and DM screening (RBG/FBG) at peripheral health institutes. Strengthening of referral systems between the two bodies and generating effective referral and feedback mechanism are important to ensure every referred patient reaches the DMC or diabetes clinic. Suggested measures include provisions in the NIKSHAY database to capture diabetes-related information for each reported and referred patient. Training and familiarization of staff members including medical officers, counselors, nurses, and data entry operators at both centers is recommended for smooth coordination and execution of screening, referrals, and treatment. Organization of state-level, district-level, and subdistrict-level workshops and trainings for collaborative activities has been proposed [31].

Promoting operational research in the prevention, diagnosis, and management of TB and diabetes co-epidemic: establishing a multicenter study on the bi-directional screening and management of DM-TB

Operational research is needed to determine the feasibility, challenges, and opportunity of bi-directional screening and management of DM-TB. Such approaches will greatly aid collaborative mechanism between the RNTCP and NPCDCS. Establishing multicenter study involving key stakeholders from both national programs (RNTCP and NPCDCS) will help to achieve consensus and wide ownership of the results, thus creating several advocates for policy change. The overall objective of the prospective research should be to identify gaps in operational activities (pertaining to screening and management) and design appropriate studies to address shortcomings and enhance understanding of the DM-TB syndemic (Table 1).

Table 1 Proposed studies to support bi-directional screening and management of diabetes mellitus-tuberculosis

Key research question	Study design and methodology	Need
• Screening patients with DM for active TB • Screening patients with active TB for DM	Prospective observational cohort studies of patients with DM routinely attending DM clinics and screened for TB, and patients with TB starting anti-TB treatment and screened for DM both in public and private setting • Testing protocols for feasibility and carrying out needs assessment and resources required for large scale program implementation • Research to determine the most effective type of screening algorithm for TB among DM patients and vice versa • Research to determine the appropriate time to perform screening/testing for diabetes: at registration, or during the course of treatment • Research to determine the most appropriate screening tool for TB among diabetes patients and for diabetes among TB patients	High
Use of the community to improve diagnosis, management, and care of patients with DM and TB	Prospective observational studies to determine feasibility and yield of screening household contacts and "at risk" individuals. These include family members of index patients with pulmonary TB and DM for TB infection, active TB, and DM	Medium
Evaluating TB treatment outcomes in patients with DM and strategies to improve outcome	Prospective observational cohort studies using standardized TB regimens and standardized treatment outcomes based on • Death in relation to start of TB treatment and cause • Duration of sputum conversion • Cure rate • Relapse and reinfection All outcomes to be analyzed in relation to DM control If improved DM control is not associated with better outcomes, studies to determine if modification to TB drug regimens, duration of therapy, and TB drug doses are required	High
Protocols for treating hyperglycemia in people with TB	Designing and testing protocols for treating hyperglycemia in people with TB through observational case cohorts and case control studies • Newly diagnosed diabetes: Initiating diabetes treatment, referrals, and follow-up. To understand if TB outcomes are better with insulin therapy or OADs. To analyze which cases need insulin • Previously known diabetes: to analyze if patients not previously on insulin be shifted to insulin • Prediabetes: need for treatment initiation RCTs to determine whether all DM patients with TB be initiated on metformin given its potential impact on improving immune functions RCT to determine the role of metformin for prevention of TB treatment failure and reinfection/relapse in DM patients with TB and prevention of DM and active TB disease in patients with prediabetes and latent TB	High
Evaluation of point-of-care diagnostic and monitoring tests for DM in primary care settings where TB treatment is delivered	Designing multicenter study to assessing the feasibility, acceptance, sensitivity, specificity and cost effectiveness of FBG, OGTT, and HbA1c to guide large scale adaptation Developmental work to produce a reliable low cost finger stick test for measuring blood glucose and HbA1c in rural areas, which then needs to be tested for efficacy and feasibility in the field	High
• Evaluating risk of MDR-TB in patients with DM	Prospective MDR-TB case cohort studies where screening for DM is done	High
• Evaluating MDR-TB in patients with poorly controlled DM	Prospective DM-TB cohort studies	
Establishing Care Delivery Model	Developing and testing approaches to care delivery—observational cohort studies • Initial treatment—in TB center • Long-term follow-up	High
Rates of hospitalization and additional medical costs associated with diagnosis and management of dual disease	Cross-sectional and case-control studies and activity based cost analysis (ABC)	Medium
• Population attributable risk of DM on TB rates in India • Effect of the DM epidemic on the TB epidemic	Epidemiological and mathematical modeling to understand spatial spread of the epidemic	High
Evaluating strategies to improve diabetes care delivery at primary care setting Implementing and evaluating the "DOTS" model for standardized care management of DM	Operational research that includes quarterly cohort reporting of new cases and treatment outcomes of cumulative cases including frequency of comorbidities and survival analysis	High
Treatment protocols in special situations	Literature review and small observational studies • Pregnancy, hyperglycemia, and TB • Diabetic renal disease and TB • HIV on ART, TB, and hyperglycemia • Diabetes, CLD/CKD, and TB	Medium

ART anti-retroviral therapy; *CKD* chronic kidney disease; *CLD* chronic liver disease; *DM* diabetes mellitus; *DOTS* directly observed treatment, short course; *FBG* fasting blood glucose; *HbA1c* glycated hemoglobin; *HIV* human immunodeficiency virus; *MDR-TB* multidrug-resistant tuberculosis; *OAD* oral anti-diabetic drugs; *OGTT* oral glucose tolerance test; *RCT* randomized controlled trial; *TB* tuberculosis

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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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POSITION STATEMENT

Diabetes and driving

Sujoy Ghosh[1] · Sarita Bajaj[2] · Kaushik Pandit[3] · Sanjay Agarwal[4] · SR Aravind[5] · Rajeev Chawla[6,7] · Sunil Gupta[8] · J Jayaprakashsai[9] · Sanjay Kalra[10] · Ch Vasanth Kumar[11] · Anuj Maheshwari[12] · BM Makkar[13] · CR Anand Moses[14] · Jayanta Panda[15] · Vijay Panikar[16] · PV Rao[17] · Banshi Saboo[18] · Rakesh Sahay[19] · KR Narasimha Setty[20] · Vijay Viswanathan[21]

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Abstract More than 69 million Indians are suffering from diabetes, of which a substantial proportion of the population are currently holding or will seek in the future the license to drive. Driving essentially requires multitasking with visuospatial skills at the same time and thus the management of diabetes in individuals which should demonstrate a proper detection and treatment of diabetes-related hypoglycemia will predict the capacity of driving any motor vehicle. Repeated hypoglycemia-related neuroglycopenia causes increased risk of neurocognitive dysfunction leading to visuospatial skills deficiency. Eight percent of dementia may be attributed to diabetes. Potential causes of driving impairment associated with diabetes are acute hypoglycemia, and its unawareness, retinopathy, neuropathy related to foot that affects ability to use pedals, IHD, cerebrovascular disease, somnolence and sleep disorder associated with obesity, use of pain relieving medications and antidepressant, and cognitive dysfunction and thus should be reviewed properly before issuing a driving license. Medical evaluation and documentation of acute and chronic complications of drivers by a registered medical practitioner at pre-determined intervals may be considered as a legal necessity to identify at-risk drivers. Secretagogues have a higher incidence of hypoglycemia compared to someone who is on metformin alone. On the other hand, hypoglycemia is more frequent in an insulin-treated patient of both type 1 and type 2 diabetes. In many countries as well as in European Union (EU), it is necessary to review medical fitness in every 3 years by the authority; a person should not have any severe hypoglycemic event in preceding 12 months and a driver must have awareness

✉ Sujoy Ghosh
drsujoyghosh2000@gmail.com

[1] Department of Endocrinology, Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India

[2] Department of Medicine, Moti Lal Nehru Medical College, Allahabad, Uttar Pradesh, India

[3] Fortis Medical Centre and Belle Vue Clinic, Kolkata, India

[4] Department of Diabetes, Aegle Clinic, Pune, Maharashtra, India

[5] Sunil's Diabetes Care n' Research Centre, Nagpur, Maharashtra, India

[6] Diacon Hospital, Bengaluru, India

[7] Columbia Asia Hospital, Karnataka, Bengaluru, India

[8] North Delhi Diabetes Centre, New Delhi, India

[9] Apollo Sugar Clinic, Banjara Hills, Hyderabad, India

[10] Department of Endocrinology, Bharti Hospital, Karnal, Haryana, India

[11] Apollo Hospitals, Hyderabad, India

[12] Department of General Medicine, Babu Banarasi Das University, Lucknow, Uttar Pradesh, India

[13] Department of Diabetes and Obesity, Diabetes and Obesity Centre, New Delhi, India

[14] Institute of Diabetology, Madras Medical College, Chennai, India

[15] Department of Medicine, Shri Ramachandra Bhanj Medical College, Cuttack, Odisha, India

[16] Dr. Panikar's Specialty Care Centre, KJ Somaiya Medical College, Sion, Mumbai, India

[17] Nizam's Institute of Medical Sciences, Hyderabad, India

[18] Department of Endocrinology, DIACARE, Ahmedabad, Gujarat, India

[19] Department of Endocrinology, Osmania Medical College, Hyderabad, Telangana, India

[20] Founder Director Karnataka Institute of Diabetology, Bengaluru, Karnataka, India

[21] Head and Chief Diabetologist, MV Hospital for Diabetes, Chennai, Tamil Nadu, India

of hypoglycemia and its management. According to Canadian diabetes association consensus statement, review should be done every 2 years; a person should not have any severe hypoglycemic event in preceding 6 months, and according to ADA position statement evaluation should be done every 2–5 years. Medical fitness certificate should be reviewed at frequent intervals; the authorities should enforce strict regulation on suspension and revocation of driving license. Information to the authorities should be promptly done by doctors or patients. Decision should be based on medical evaluation, but hypoglycemia that occurs due to medication change and during sleep does not warrant for disqualification as it may be corrected with proper dietary changes and dose adjustments. Any driver with suspended license should be re-assessed in the next 6 months for their medical fitness and hypoglycemic profile and if found suitable, the license can be revoked. Physicians should participate and should assess patient's physical and mental status, medical condition and treatment, list of medications which may impair driving performance, and any disease-related complication that lead to impaired driving or dangerous driving. Patient education is the most important factor to prevent any motor accident related to their medical condition and should be trained to prevent acute and chronic complications of diabetes.

Keywords Patient education · Hypoglycemic profile · Fitness certificate

Recommendations

1. Driving essentially requires proper visuospatial skills, thus repeated hypoglycemia-related neuroglycopenia leading to neurocognitive dysfunction.
2. Diabetes-related complications—hypoglycemia and its unawareness, retinopathy, neuropathy, foot-related problems, CVD, IHD, sleep disorders, and side effects of medicines interferes with driving and needs to be evaluated before issuing a license.
3. Evaluation and documentation should be done at predetermined frequency by registered medical practitioner.
4. Drugs like secretagogue and insulin have an increased risk for hypoglycemia.
5. There are no Indian Guidelines, but EU guidelines suggests that every 3 years a checkup is required, person should not have any severe hypoglycemic event in preceding 12 months, and a driver must have awareness of hypoglycemia and its management.
6. According to the Canadian diabetes association consensus statement, review should be done every 2 years, a person should not have any severe hypoglycemic event in preceding 6 months, and according to ADA position statement evaluation should be done every 2–5 years.
7. Authorities should review medical fitness and can revoke or suspend license.
8. Any driver with suspended license should be re-assessed in the next 6 months for their medical fitness.
9. Patients' education is of utmost importance, and should be trained to prevent acute and chronic complications of diabetes.

Diabetes mellitus is a chronic metabolic condition that is characterized by persistently elevated glucose in the blood due to insulin secretory defect or defect in insulin action or both. More than 69 million Indians are suffering from diabetes and it is estimated to increase to more than 123 million by 2040 [1]. In a country like India witnessing rapid economic transition, a substantial chunk of India's diabetes population are currently holding or will seek in the future the license to drive.

- More than 69 million Indians are suffering from diabetes, of which a substantial proportion of the population are currently holding or will seek in the future the license to drive.

Driving

For many, it is essential for their daily work, taking care of their family, accessing to public and private facilities, and performing important functions of their daily routine [2].

In most of the instances, people who are highly concerned about the road safety like motor vehicle owners, employers, and other road users link diabetes with unsafe driving but in reality, most of the people with diabetes can operate a motor vehicle without any risk to themselves and others. In this case, the main concern is not being a diabetic but assessing the management of diabetes in individuals which should demonstrate a proper detection and treatment of diabetes-related hypoglycemia which may predict the capacity of driving any motor vehicle [2].

Driving is common in the adult community but it is a highly complex task that involves maneuvering a complex projectile with perfect timing and space at high speed, judging road traffic signals in different weather conditions. It essentially requires multitasking with visuospatial skills at the same time. If a person fails to maintain coordination, it may impair his ability to drive consciously [3].

- Driving essentially requires multitasking with visuospatial skills at the same time and thus the management of diabetes in individuals which should demonstrate a proper detection and treatment of diabetes-related hypoglycemia will predict the capacity of driving any motor vehicle [2].

Diabetes-related neurocognitive changes

Diabetes-related hypoglycemia is defined as any blood glucose level ≤ 70 mg/dL. Clinically significant hypoglycemia is characterized by < 54 mg/dL in which patient requires assistance for correction of symptoms [4, 5].

Acute hypoglycemic episodes are a rate-limiting side effect in the treatment of diabetes. When severe, hypoglycemia can lead to coma and seizure while mild to moderate is responsible for impairing cognitive function like working memory [6].

There is a longstanding debate on the clinical benefit and associated risk of tight glycemic control in diabetes patients. Nonetheless, it is a matter of fact that hypoglycemia is a major side effect of strict blood glucose control. However, many guidelines are available to minimize hypoglycemia and related mortality and permanent neurocognitive damages. Hypoglycemia-related neuroglycopenia causes increased risk of neurocognitive dysfunction when it occurs repetitively [7]. These patients also have a deficiency in visuospatial skills which are the ability to identify a visual and spatial relationship in objects and measured by three ways namely, imagination ability of objects, making a global appearance by identifying small components, or by understanding the difference and similarity between objects [8].

According to Duarte JMN, neurocognitive dysfunction or dementia is raising in the world. While Alzheimer's disease is most common, it accounts for 60–80% of total cases and among them, about 8% is attributed to diabetes mellitus and is supposed to increase further with the growing diabetes prevalence [9]. Furthermore, cognitive dysfunction impairs the ability to control glucose level in patients with diabetes and is associated with increased risk of severe hypoglycemic episodes and cardiovascular disease when compared with patients without cognitive dysfunction [10].

As previously stated, hypoglycemia is one of the key factors in this situation that leads to decreased blood glucose level in brain which is responsible for neuroglycopenia, that results in decreased supply of energy and this leads to metabolism of another substrate for ATP generation to cover energy demand [9]. Kerr D and Olateju T have reported potential causes of driving impairment associated with diabetes—they are as follows: acute hypoglycemia, hypoglycemia unawareness, retinopathy, neuropathy related to foot that affects ability to use pedals, ischemic heart disease, cerebrovascular disease, somnolence and sleep disorder associated with obesity, use of pain relieving medications and antidepressant, and cognitive dysfunction. In order to maintain seamless driving, it is necessary to correct all the above medical conditions before driving and thereby agencies responsible for giving license to such people should warrant the same before awarding license [11].

- Repeated hypoglycemia-related neuroglycopenia causes increased risk of neurocognitive dysfunction leading to visuospatial skills deficiency. Eight percent of dementia may be attributed to diabetes. Potential causes of driving impairment associated with diabetes are acute hypoglycemia, and its unawareness, retinopathy, neuropathy related to foot that affects ability to use pedals, IHD, cerebrovascular disease, somnolence and sleep disorder associated with obesity, use of pain relieving medications and antidepressant, and cognitive dysfunction and thus should be reviewed properly before issuing a driving license.

Medical evaluation

The evaluation should include an assessment by the treating physician or any physician knowledgeable in the field of diabetes having access to the recent diabetes history of the driver. The input of the concerned physician is essential to decide whether the driving would be safe or practicable. If any concern arises with regard to evaluation of chronic complications of diabetes, it is sensible to refer the individual to a specialist with expertise in assessing the diabetes-related problem for specific recommendations [12].

There are different scenarios which may demand the medical evaluation of the person to demonstrate their driving ability. These include hypoglycemic episodes while driving (even resulting in no motor vehicle accident), severe hypoglycemia, and alteration in vision. The medical evaluation process must include assessment of hypoglycemia risk, evaluation of cataract formation or retinopathy, and neuropathy in feet which may affect the ability to feel and maneuver the brake and clutch pedals. Certain medical conditions which have the propensity of driving mishaps, namely unstable coronary heart disease, obstructive sleep apnea, epilepsy, Parkinson's disease, or alcohol and substance abuse should also be thoroughly checked [12].

It is prudent to frame a questionnaire for identifying at risk potential drivers who may require further detailed evaluation. The questions should inquire for episodes of hypoglycemia leading to loss of consciousness within the past 12 months, required hypoglycemia correction assistance from other person, episodes of hypoglycemia interfering driving, hypoglycemia without warning, loss of visual acuity or peripheral vision, or loss of sensation in the right foot. Any response in affirmative should trigger an evaluation to ascertain whether a restriction on the license or mechanical modifications to the vehicle is required or not [12]. The vital information that has to be gathered during a medical evaluation is enlisted in Table 1.

Medical evaluation of drivers by a registered medical practitioner at pre-determined intervals may be considered as a legal necessity to identify at-risk drivers. These intervals may differ between commercial and non-commercial drivers.

Table 1 Information to be gathered during medical evaluation from a person seeking driving license

Q1	Episodes of severe hypoglycemia necessitating assistance from another person within previous two years
Q2	Explanation for the hypoglycemic episode (if available)
Q3	Whether there is an increased risk of hypoglycemia
Q4	Ability of the driver to recognize nascent hypoglycemia and ability to correct the same
Q5	Ability to perform self-monitoring of blood glucose (SMBG)
Q6	Presence of any diabetes related complication (requiring further assessment) that may affect driving
Q7	Understanding of diabetes, its management, measures to avoid hypoglycemia while driving, compliance with a suggested treatment plan

Adapted from the American Diabetes Association. Diabetes and Driving. *Diabetes Care*. 2014; 37 (Suppl 1): S97–S103

Licensing authorities may debar the driving permission if medical conditions suggestive of substantially affecting the driving ability of the person. The restricted period may range from 3 to 6 months or even longer. Nevertheless, special considerations must be allowed if the hypoglycemia is attributed to alteration in medications or severe hypoglycemia occurs in sleep [12].

- Medical evaluation and documentation of acute and chronic complications of drivers by a registered medical practitioner at pre-determined intervals may be considered as a legal necessity to identify at-risk drivers.

Driving and anti-hyperglycemic agents

Treatment of any type of diabetes depends upon how the active drug is lowering the blood glucose level, which may act by different mechanisms.

Metformin is used as first line therapy due to fewer side effects and less hypoglycemia risk; subsequently, sulfonylurea is one of the second choice drugs to add to metformin when it fails to control blood glucose alone in patients with type 2 diabetes. Sulfonylureas, the classic secretagogues, increase the risk of hypoglycemia. Another approach is adding insulin to metformin, where the concern of hypoglycemia is further elevated. In type 1 diabetes patients, insulin is the only agent which is used to control blood glucose and any mismatch between carbohydrate intake and insulin may cause a serious hypoglycemia.

Relation between AHAs and driving

Oral antihyperglycemic drugs (OADs) are known to either increase insulin secretion from beta cell or insulin action. Insulin and incretin mimetics are injectable AHAs which are known to fulfill insulin demand in the body. AGIs and DPP4 inhibitors act by a mechanism that regulates glucose absorption in intestinal lumen or nephrons to regulate overall glucose circulation in the blood. Among these, secretagogues are responsible for having a larger impact on the possibility of hypoglycemia compared to someone who is on metformin alone. On the other hand, hypoglycemia is more frequent in an insulin-treated patient of both type 1 and type 2 diabetes. In a study done by LeRoy and Morse, hypoglycemic medications are stratified by their physiologic action, insulin (OR—1.80), sulfonylureas (OR—1.50), and biguanides (OR—1.49) [13]. In another study done by Heller et al., it was reported that patients with type 2 diabetes initiated on insulin therapy earlier in life, having a similar frequency of hypoglycemic episodes when compared with patients treated with sulfonylureas [10]. Majority of patients with hypoglycemia are on sulfonylurea and biguanide, 38.2 and 56.3% respectively; α-glucosidase inhibitors, sitagliptin, incretin mimetics, and thiazolidinediones were less related to hypoglycemic events. The author concluded that hypoglycemia was related to higher risk of accidents in patients treated with non-insulin agents and require hospital visits related to driving and falls [14].

It is generally stated that the frequency of hypoglycemia in a patient taking insulin less than 2 years is equivalent to non-insulin-treated patient of type 2 diabetes. Around 7% in both groups reported severe hypoglycemia and 39 and 51% respectively, reported mild hypoglycemia [10]. According to Bodmer and colleagues, the ratio of hypoglycemia among the people taking sulfonylurea is more than twice that among those taking metformin [15].

As mentioned above, several studies have revealed that diabetes when treated with insulin and oral drugs has the serious side effect of hypoglycemia which impairs the neurocognitive ability and visuospatial skill to concentrate while driving. As diabetes, if not treated properly, itself cause serious chronic complications like neuropathy and retinopathy and when it come up with the hypoglycemic event, the risk of a road accident can increase several times if not treated within time. It will continue to happen if proper awareness is not created in the community by treating physicians and governing body.

- Secretagogues have a higher incidence of hypoglycemia compared to someone who is on metformin alone. On the

other hand, hypoglycemia is more frequent in an insulin-treated patient of both type 1 and type 2 diabetes.

Law stating driving with diabetes

Driving is a very essential and necessary activity in this era to complete time-specific needs in daily life of everyone but when it is compromised with some ailment, the task may become dangerous for driver and others. Hence there should be strict rule by the driving authority to control these mishaps. Unfortunately, there is no such law in India by driving authority or the concerned ministry which can evaluate the medical condition of an applicant or existing drivers. Thus it is very difficult to ensure proper control on road accident. A substantial number of road mishaps can be minimized by those drivers who are taking AHAs.

Countries like Canada, America, Australia, New Zealand, Ireland, United Kingdom, and many more have regulations in their driving department to evaluate diabetes and related complications of hypoglycemia for safe driving. In the European Union (EU), it is necessary to review medical fitness in every 3 years by the authority; a person should not have any severe hypoglycemic event in preceding 12 months and a driver must have awareness of hypoglycemia and its management. Drivers on insulin are advised to carry glucometers and blood glucose strips with them and is required to check blood glucose every time before driving and every 2 h while driving; if hypoglycemia persist, driving must be stopped and restart after 45 min when blood glucose returns to normal; it is mandatory for drivers to carry fast-acting carbohydrate and a personal identification indicating their medical condition [16].

The Canadian diabetes association has released a consensus statement as Diabetes and Driving for their private and commercial drivers in 2015. It has a similar kind of statement as of UK but there are some other points to consider while driving like drivers have to be reviewed every 2 years for medical evaluation of hypoglycemia frequency and other diabetes-related complications; assessment of hypoglycemic frequency in preceding 6 months; while driving SMBG should be performed every 4 h; if a driver experienced moderate to severe hypoglycemia, a driver must refrain from driving at the time and inform their doctor and driving license body immediately within 72 h. For commercial drivers, a complete eye examination should be done by an ophthalmologist or optometrist, assessment of hypoglycemic frequency in preceding 12 months, and a detailed questioner for assessment of diabetes and hypoglycemia awareness [17].

The American diabetes association has also released a position statement in 2012 and in 2014 on driving which is nearly the same as previously stated [18]. Other countries like Australia, New Zealand, and Ireland have a similar kind of law enforced by driving authority stating that driver should inform about any medical condition, including diabetes, which can impair driving performance; they should be medically evaluated in every 2 to 5 years by authority depending upon medical illness and treatment and if they are found to be hypoglycemic, should refrain from driving until their treating physician gives clarity of their fitness.

- In many countries as well as in EU, it is necessary to review medical fitness in every 3 years by the authority; a person should not have any severe hypoglycemic event in preceding 12 months and a driver must have awareness of hypoglycemia and its management. According to the Canadian diabetes association consensus statement, review should be done every 2 years; a person should not have any severe hypoglycemic event in preceding 6 months and according to ADA position statement evaluation should be done every 2–5 years.

Necessity of law related to diabetes and driving in India

The diabetic population of India is crossing over 69 million and increasing very rapidly over time because of change in culture and routine in daily life of people. Healthcare sector is trying to spread awareness but it is not sufficient to impart all the proper knowledge to every patient. It is a prime responsibility of the healthcare practitioners to inform the patient on their medical condition and also to state driving authority so that mishaps can be minimized. In addition, driving authority should come up with such kind of regulations while giving driving license to people with diabetes with following points.

Screening medical condition in applicants and existing drivers

One should present a proper medical fitness certificate by Diabetologist or Endocrinologist while applying for license and routine submission of medical fitness certificate a regular interval of 2 to 5 years; if diabetes is insulin-treated, the follow-up should be earlier compared to OADs-treated patients. The parameters should include medical history, diabetes and hypoglycemia awareness, duration of existing diabetes, number of hypoglycemic events in preceding 6 to 12 months and management, and diabetic complications like retinopathy and neuropathy. The authority should enforce strict regulation on suspension and revocation of driving license that should depend upon hypoglycemic episodes during driving due to antihyperglycemic medications. Such events should be voluntarily informed to driving authority by

concerned doctor or patient continuously in a smooth and convenient manner.

- Medical fitness certificate should be reviewed at frequent intervals; the authorities should enforce strict regulation on suspension and revocation of driving license. Information to the authorities should be promptly done by doctors or patients.

Decision based upon medical evaluation should be strictly implemented, whether it requires suspension or continuation. It should be based upon driver's hypoglycemic profile and medical condition in case of new application. Since any history of complicated and uncomplicated hypoglycemia does not warrant for unsafe driving unless it is not managed properly to prevent reoccurrence and if reoccur, proper evaluation should be done based upon underlying cause and necessary action should be taken to renew the license. Hypoglycemia that occur due to medication change and during sleep does not warrant for disqualification as it may be corrected with proper dietary changes and dose adjustments. Any driver with suspended license should be re-assessed in next 6 months for their medical fitness and hypoglycemic profile and if found suitable the license can be revoked.

- Decision should be based on medical evaluation, but hypoglycemia that occur due to medication change and during sleep does not warrant for disqualification as it may be corrected with proper dietary changes and dose adjustments. Any driver with suspended license should be re-assessed in next 6 months for their medical fitness and hypoglycemic profile and if found suitable the license can be revoked.

Role of physicians who are treating diabetes is important for minimizing road accidents

A new regulation can be enforced in which physician participation should be important by sharing patient detail indicating medical condition to driving authority. This should include patient's physical and mental status, medical condition and treatment, list of medications which may impair driving performance, and any disease-related complication that lead to impaired driving or dangerous driving.

Patient education is the most important factor to prevent any motor accident related to their medical condition. One should be properly educated by the treating doctor about patient's medical condition and his driving performance if his treatment includes drugs like insulin and insulin secretagogue that may cause serious hypoglycemia. Patient should be trained to manage hypoglycemia when it is mild or moderate and if severe, a detailed instruction should be given. It may include checking of blood glucose before starting to drive first time in a day and if continuing, should check on regular intervals to prevent hypoglycemia while driving. He should be advised to carry blood glucose meter and immediate source of carbohydrate while driving and if hypoglycemia persist, should inform the doctor immediately.

- Physicians should participate and should assess patient's physical and mental status, medical condition and treatment, list of medications which may impair driving performance, and any disease-related complication that lead to impaired driving or dangerous driving. Patient education is the most important factor to prevent any motor accident related to their medical condition and should be trained to prevent acute and chronic complications of diabetes.

Conclusion

To summarize, diabetic patients seeking driving license should undergo a through medical evaluation by the treating physician to detect any potential risk associated with driving. If found, corrective actions should be taken not only the healthcare professional but also by the licensing authority to ensure minimization of hazards which may lead to road mishaps.

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REVIEW ARTICLE

From obesity through immunity to type 2 diabetes mellitus

Leszek Szablewski[1]

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Abstract Diabetes mellitus is a metabolic disorder that leads to the development of a number of complications. The etiology of each metabolic complication is undoubtedly multifactorial. Patients with diabetes have increased susceptibility to and severity of infections. The course of infections is also more complicated in the patient group. One of the possible causes of this increased prevalence of infections is defect in immunity. Different disturbances in humoral innate immunity have been described in patients with diabetes. Concerning cellular innate immunity, most studies show decreased functions of polymorphonuclear cells and monocyte/macrophages of these patients compared to cells of healthy subjects. Several studies have shown alterations in peripheral blood mononuclear cells from patients with type 2 diabetes, an effect that contributes to the high incidence of infections in these patients. The gut microbiota plays different roles such as the following: protects against pathogens, helps in the maturation of the immune system, regulates the intestinal hormone secretion, synthesizes vitamin K and several vitamins B, and produces short-chain fatty acids (SCFAs). It also plays a role in immunomodulation and might contribute to the alterations in glucose metabolism. In the present review, I focused on the role of obesity, the immune system, and the gut microbiota in the pathogenesis of type 2 diabetes mellitus, and as a second point, how type 2 diabetes impairs the immune system.

Keywords Adipose tissue · Innate immunity · Adaptive immunity · Gut microbiota · Diabetes mellitus

✉ Leszek Szablewski
leszek.szablewski@wum.edu.pl

[1] Chair & Department of General Biology and Parasitology, Centre for Biostructure Research, Medical University of Warsaw, 5 Chalubinskiego Str, 02-004 Warsaw, Poland

Introduction

Type 1 diabetes mellitus, in most cases, is due to autoimmune-induced inflammation with destruction and apoptosis of pancreatic islets β-cells. This process is usually associated with infiltration of the innate immune cells [1, 2]. These cells produce cytokines which promote β-cell apoptosis and increase infiltration of islet-specific T cells which attack and destroy β-cells [3]. Type 2 diabetes is not caused by autoimmune destruction of the islet cells and is not therefore associated with circulating immune markers. On the other hand, some researchers have hypothesized that type 2 diabetes evolves from an innate immune response to an autoimmune condition [3, 4]. Furthermore, susceptibility to type 2 diabetes is not associated with the human leukocyte antigen genes within the major histocompatibility complex.

Patients with diabetes mellitus have infections and sepsis more often than those without diabetes. The overall mortality due to infections is nearly twice as higher when compared to patients without diabetes [5]. One of the possible causes of this increased prevalence of infections is defect in immunity [6]. This risk may be revealed to dysregulated humoral and cellular responses to infection agents.

Components of the immune system are altered in type 2 diabetes and insulin resistance [7, 8]. These immunological changes include altered levels of specific cytokines and chemokines, as well as changes in the number and activation state of the various leukocyte populations. Type 2 diabetes is associated with perturbed innate immunity. Macrophages bridging innate immunity and metabolic disturbances play important roles in controlling immune homeostasis. The macrophage system dysfunction leads to the major alterations among these innate abnormalities [9]. Peripheral blood mononuclear cells from patients with Type 2 diabetes show a reduced proliferative response to mitogens such as phytohemagglutinin and

lipopolysaccharides (LPS). In addition, the different adaptive immune system cells also play an important role in this process.

The gut microbiota plays different roles: protects against pathogens, helps in the maturation of the immune system, regulates the intestinal hormone secretion, synthesizes vitamin K and several vitamins B, and produces short-chain fatty acids (SCFAs). It also plays a role in immunomodulation and the gut microbiota might contribute to alterations in glucose metabolism. Numerous studies showed the link between the gut microbiota composition and obesity; however, the contribution of gut microbiota to obesity in humans remains unclear. The obesity is correlated to changes in proportions of *Firmicutes* (gram-positive bacteria) and *Bacteroidetes* (gram-negative bacteria). The effects of the changes in diet on the composition of the intestinal microbiota were obtained from human studies and confirmed in animal studies. Fat-rich/low-fiber diet, so called "western diet", produces an increase in the gram-negative gut microbiota that plays a role in the development of the insulin resistance and diabetes and decrease in the gram-positive microbiota that plays the protective role against the development of metabolic diseases.

An important role in pathophysiology of type 2 diabetes plays obesity and changes in composition of gut microbiota that destroys the immune system [10–12].

Damages of immunity due to obesity as a cause of type 2 diabetes mellitus

Obesity is considered as the pandemia of the twenty-first century. In 2011, ~500 million people worldwide suffered from obesity, and these numbers are estimated to at least double until 2030 [13]. Obesity-associated morbidity and mortality affect > 1 billion individuals worldwide [14]. The pathogenesis of obesity and its complications remain incompletely understood.

Obesity is a major predisposing factor for the development of type 2 diabetes [15, 16]. Adipose tissue consists of adipocytes that secrete adipokines such as adiponectin and leptin [17]. These hormones influence insulin sensitivity and therefore play a role in maintaining normal glucose levels. On the other hand, leptin activates monocyte function or regulates cytokine balance [18]. Leptin regulates the production of inflammatory cytokine by monocytes, such as tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12) [19]. Adipose tissue also contains macrophages. When macrophages move into the blood stream, they take on different characteristics and are called monocytes.

The nomenclature of different defined macrophage populations is a variable [20]. According to Gordon's classification [21], macrophages have been segregated into two broad groups: M1s (or classically activated), these macrophages secrete proinflammatory cytokines and M2s (or alternatively activated) that secrete anti-inflammatory cytokines. Therefore, classically activated macrophages (M1) are referred to as proinflammatory macrophages, and alternatively activated macrophages (M2) are referred to as anti-inflammatory macrophages [21]. Classically activated macrophages secrete a large amount of cytokines such as interleukins (IL-1β, IL-6, IL-8, IL-12) TNF-α and express high levels of costimulatory molecules important in the T cell activation (e.g., MHC, CD40, CD86) [22]. The results obtained by Nieto-Vazquez et al. [23] and Vanderford [24] showed that these cytokines can impair insulin signaling or induce β-cell apoptosis. On the other hand, according to the results obtained by Odegaard and Chawla [25] and Espinoza-Jeménez et al. [26], alternatively activated macrophages secrete, for example, IL-10 and transforming growth factor-β (TGF-β). Proinflammatory cytokines are derived primarily from activated macrophages and can directly enhance insulin resistance in adipocytes, muscle, and liver cells [27, 28]. Cytokines also trigger the synthesis of acute-phase inflammatory proteins such as C-reactive protein (CRP) and serum amyloid A. As was shown by Arora et al. [29], proinflammatory cytokines and acute-phase reactants play a role in initiating the early stages of type 2 diabetes and are known to increase with disease progression. In adult obesity, the proinflammatory cytokines are overexpressed and are linked to the metabolic syndrome [28].

Macrophage/monocyte interacts through receptors on their surface including toll-like receptors (TLRs) that are cell-surface receptors. These cells protect mammals from pathogenic organisms by generating an innate immune response to the products of the pathogenic organisms. The innate immune response increases genes of several inflammatory cytokines and therefore is critical for the development of antigen-specific adaptive immunity, both humoral and cell mediated. TLRs are present in monocytes, macrophages, and immune cells. TLR4 protects the body from bacterial infections. It binds lipopolysaccharides on the surface gram-negative bacteria. The binding TLR4 to LPS in bacterial infections results in the macrophage/monocytes becoming activated allowing them to produce proinflammatory cytokines. It is to note that upon consumption of diet, high in lipids can occur a chronic cytokine exposure.

Adipose tissue acts as an inflammatory immune tissue [10, 13, 30–34, 46, 47]. As an individual becomes obese, several changes occur within the adipose tissue, leading to a shift from an anti-inflammatory to an inflammatory milieu. It was observed [35] that in lean individuals, M2 macrophages are the predominant resident adipose tissue macrophages (ATMs). In obese individuals, the local population shifts from M2 macrophages to M1 ATMs [35]. This occurs via two main mechanisms: phenotypic conversion of M2 to M1 and recruitment of M1 macrophages into adipose tissue [9]. Obesity results in increased levels of circulating saturated fatty acids that activate TLR2 and TLR4 to promote classical activation of adipose tissue macrophages. In obese individuals is observed infiltration of proinflammatory macrophages (M1) and infiltration proinflammatory $CD8^+$ and

$CD4^+$ T cells into the adipose tissue [36]. Adipose tissue inflammation is now recognized as a crucial process leading to diabetes, and macrophage infiltration of adipose tissue has been described in both animal models and human diseases by Hotamisligil [28]. Macrophages comprise about 10–15% of all cells within a lean visceral adipose tissue and expand tremendously with obesity, where they account for a staggering 45–60% of all cells [37]. Accumulation of T cells has been observed in an obese adipose tissue [38]. Monney et al. [39] suggest that T lymphocytes are known to interact with macrophages and regulate the inflammatory cascade. Thus, adipose tissue is an important inflammatory source in obesity and type 2 diabetes, because adipocytes produce cytokines and infiltration by proinflammatory macrophages [50]. The results obtained by Rempel et al. [40] suggest that certain immunological parameters are common in obese youth independent of type 2 diabetes. The immune cell count is altered in obese individuals in relation to metabolic parameters [51, 52, 62]. It was found [52] that obese individuals have significantly higher CD4% and CD4 count, white blood cells, and absolute lymphocyte count, as compared to lean subjects. On the other hand, obese subjects have significantly lower expression of CD8 and natural killer cells (NK cells). The decreased CD8+ T cells and increased or decreased CD4+ T cells in obese subjects were also observed by other authors [127]. Of note, obese subjects showed reduced lymphocyte proliferation, and the number of NK cells was diminished [62]. Carolan et al. [128] investigated the effect of childhood obesity on immune cell frequency, macrophage activation, and cytokine production. Authors have found that in obese subjects, compared to nonobese children, there was a higher degree of insulin resistance; soluble CD163, that is a marker of macrophage polarization to a proinflammatory profile, was elevated, and invariant NK T cells were reduced. The levels of TNF-α and leptin were elevated and adiponectin was reduced. On the basis of obtained results, authors conclude that childhood obesity is associated with changes in immune cell frequency and inflammatory environment. These changes may be a cause of metabolic disease, as for example type 2 diabetes, and premature cardiovascular disease. Schipper et al. [126] investigated the inflammatory mediators in children aged 6 to 16 years. In obese group, authors reported increased levels of chemerin, IL-18, EGF, and TNF-R2 as compared to the lean objects.

It is suggested that human adipose tissue represents an important site of innate immune activation, because human adipocytes can significantly increase the secretion of the potentially diabetogenic proinflammatory cytokines [41–45, 64]. The potential pathways for proinflammatory cytokine release in obesity and type 2 diabetes may occur through activation of the innate immune pathway [48]. Innate immune system acts via TLRs. Muzzio et al. [49] observed that activation of TLRs leads to the translocation of nuclear factor κB (NF-κB) into the nucleus to initiate the transcription of IL-1, IL-8, and TNF-α. According to Dinarello et al. [53], one of the components leading to the development of type 2 diabetes is the activation of the innate immune system in obesity and insulin resistance by IL-1 signaling. IL-1β is believed to initiate the arrival of macrophages to "inflamed" adipose tissue and islets, further amplifying islet inflammation [36]. It was found that IL-1β is cytotoxic to insulin-producing β-cells [54]. Therefore, it is suggested that type 2 diabetes mellitus is a disease of the innate immune system [55, 56]. The acute-phase response is part of this system. In response to a variety of stress are pronounced changes in the concentration of plasma proteins, such as C-reactive protein, fibrinogen, serum amyloid A, albumin, and transferrin. Pickup and Crook suggested [55] that a long-term activation of the innate immune system, resulting in chronic inflammation, elicits disease instead of repair in individuals who develop type 2 diabetes. The obtained results showed that increased inflammatory markers were associated with the risk of developing type 2 diabetes [57]. It was observed that serum concentrations of CRP were associated with the development of diabetes in elderly [58], and increased serum γ-globulin concentrations, another expression of the acute-phase response, predict risk of developing type 2 diabetes in the Pima Indian population [59]. The association between altered levels of acute-phase reactants and the development of diabetes seems to be weaker if adjusted for obesity and is independent of age, sex, and blood glucose concentrations. [60].

Supporting the roles of metabolic inflammation has been also ascribed to the adaptive immune system [61, 63]. Cells of the adaptive immune system are present in adipose tissue. These cells may contribute to the metabolic disruption. It was shown that T cells in adipose tissues play a role in the regulation of adipose tissue macrophage numbers and activation state. It appears that $CD8^+$ T cells and $CD4^+$ T helper (Th 1) cells promote insulin resistance [65, 66], whereas $CD4^+$ regulatory T (Treg) and Th 2 cells tend to counter it [67]. $CD8^+$ T cells that accumulate in inflamed adipose tissue contribute to local inflammation and are capable of exacerbating existing disease [66].

As mentioned above, adipose tissue can activate $CD8^+$ T cells, which then promote the recruitment and activation of macrophages resulting in the metabolic syndrome [66]. Treg cells prevent the development of autoimmunity. As was observed in the animal models by Donath and Shoelson [67], in adipose tissue of lean mice, the number of Treg cells is higher than that of obese mice. On the other hand, the number of macrophages in adipose tissue of obese mice was higher than in lean mice [67]. Treg cells in adipose tissue secrete an unusually high amount of the anti-inflammatory cytokine IL-10. In patients with metabolic syndrome and type 2 diabetes was observed low production capacity of IL-10 [68]. Targeted induction of Treg cells improves circulating glucose levels and insulin sensitivity in obese mice and reduces macrophage numbers and TNF levels in adipose tissue [69]. On the other hand, according to the results obtained by Xu et al. [70], the expression rate of $CD4^+CD25^+Foxp3^+$ Treg cells between the

control group and the patients with type 2 diabetes yielded no significant difference. However, in these patients with microalbuminuria and macroalbuminuria, the expression of the abovementioned cells was significantly lowered, as compared to the control group. The above cited authors observed that patients with macroalbuminuria showed a lower expression of these cells than the patients with microalbuminuria [70]. In type 2 diabetes, Treg cells seem to play a fundamental part in the regulation of body weight, adipocyte hypertrophy, glucose tolerance, insulin resistance, and thus in the disease progression [65, 71]. Ohmura et al. [72] observed that during the development of insulin resistance and glucose intolerance in obese mice, natural killer T cells were reported to infiltrate into the visceral adipose tissue in associations with adipose tissue macrophages.

The role of B cells in immunity is very interesting. Shu et al. [35] observed that B cells accumulated in the visceral fat of obese mice produce and secrete of IgG antibodies that activate proinflammatory macrophages and T cells. The transfer of B cells or serum IgG from high-feed diet fed mice into B-cell-deficient recipients resulted in the transfer of insulin resistance [35].

The maternal obesity is also a problem. In this case, obesity is an obstetric problem that increases mortality and morbidity in both mother and offspring. In these women is a higher risk for the development of the gestational diabetes mellitus, and children are more likely to develop cardiovascular and metabolic disease in later life [73].

Damages of immunity due to type 2 diabetes mellitus

Hyperglycemia, a state observed in patients with diabetes, may induce the production of IL-12 by macrophage, which can stimulate the production of interferon-γ (IFN-γ) by CD4 cells [74] and may activate NF-κB through protein kinase C (PKC) and reactive oxygen species to rapidly stimulate the expression of cytokines [75, 76]. Long-term of disease results in increased advanced glycosylation end (AGE) products and AGE-modified proteins. These products could bind to the receptor for AGE on macrophages and T cells, stimulating synthesis and release of proinflammatory cytokines [77]. Hyperglycemia stimulates synthesis and secretion of Il-6 and TNF-α by human monocytes in vitro [78]. It was found increased production of TNF-α by adipocytes, and therefore, significantly higher serum TNF-α concentrations in both obese individuals and in patients with type 2 diabetes in comparison to lean healthy subjects was observed [79]. Secretion of IFN-γ by T cells can initiate and induce further inflammation and oxidative stress [80]. It is to note that TNF-α acts in different ways: impairs tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1), activates the phosphoinositide-3-kinase (PI-3 K) in muscle cells [81], as well as reduces synthesis of glucose transporter 4 (GLUT 4) in adipocytes [82].

Results obtained by Luo et al. [83] showed that diabetes induces rapid suppression of adaptive immunity. Authors observed that untreated acute diabetes causes rapid lymphopenia followed by homeostatic T cell proliferation. Lymphopenia, on the other hand, was associated with an immunosuppressed state. There are also many other damages of immunity due to diabetes mellitus. High glucose levels impair natural killer cell functions, as an effect of oxidative stress and endoplasmic reticulum stress [84, 85]. In patients with type 2 diabetes, the results of immunoglobulin profiles IgG, IgM, and IgA were found to be significantly lowered, whereas the levels of serum complement components C3 and C4 were higher significantly decreased as compared to the healthy control group [86]. Ex vivo studies on immune cells from patients with type 2 diabetes have shown pronounced, as well as attenuated cytokine responses to LPS stimulation in comparison with cells from healthy subjects [87, 88], and these patients exhibit an attenuated increase in plasma levels of interleukin-1 receptor antagonist (IL-1RA), as well as attenuated up-regulation of vascular adhesion molecule (VCAM-1) and intracellular adhesion molecule (ICAM-1) to LPS in vivo.

Gut microbiota as a risk factor for obesity and type 2 diabetes mellitus

The gut microbiota inhabit the distal gut, which contains an estimated 10^{11}–10^{12} bacterial concentrations per gram of content [89], and they together weight about 1.5 kg [90]. These organisms are dominated by anaerobic bacteria. More of these bacterial phyla are *Firmicutes* and *Actinobacteria* (gram-positive) and *Bacteroidetes* (gram-negative) [91, 94]. There are more than 1000 different species in the gut. The composition of our microbiota changes within our life [92]. The newborn tract, that is sterile until birth, begins to be colonized. The composition of microbiota depends on the following: type of delivery (vaginally or Caesarian section) and the method of feeding (breast-fed or formula-fed). The introduction of solid foods changes the composition of the gut microbiota. The stable community similar to the adult microbiota becomes established at 2–3 years of age [95]. In healthy adults, the microbiota composition is relatively stable. It is altered, however, transiently, by external disturbances such as diet, disease, and environment. The important alterations in gut microbiota are observed after antibiotic treatment.

The gut microbiota plays different roles: protects against pathogens, helps in the maturation of the immune system, regulates the intestinal hormone secretion, synthesizes vitamin K and several vitamins B, and produces short-chain fatty acids (SCFAs). It also plays a role in immunomodulation [93].

The first evidence that the gut microbiota might contribute to alterations in glucose metabolism was published by Backhed et al. in 2004 [96]. Colonization of germ-free mice with the gut microbiota from conventionally raised mice resulted in an increase in body fat mass and insulin resistance [96, 97]. Murine studies have shown that in vegetable type diet, low in fat and rich in vegetable polysaccharides or rich in fat and low in plant polysaccharides, so called "western diet", influences gut microbiota [98]. Animal studies showed that western diet increases in the abundance of bacteria of the phylum *Firmicutes* and decreases in the abundance of the bacteria of the phylum *Bacteroidetes* [98]. The composition of gut microbiota also depends on carbohydrate-reduced diet and calorie-restricted diets [99, 100]. It was found that an increase in fat intake produces an increase in the gram-negative bacteria and decrease gram-positive bacteria [92]. Therefore, several studies have investigated the role of the human gut microbiota in type 2 diabetes mellitus and the link between the gut microbiota composition and obesity and metabolic disorders [101]. On the other hand, a limited number of human clinical trials have assessed the effects of diet on the composition of the gut microbiota. The result obtained by De Filippo et al. [102] is an example of this investigation. Authors showed that European children, in comparison to rural African children, have a microbiota depleted of *Bacteroidetes* and enriched in *Enterobacteriaceae*. According to the authors' suggestion, these differences are due to a diet: the European children intake a low dietary fiber, and diet of the African children is plant-rich.

Numerous studies showed the link between the gut microbiota composition and obesity; however, the contribution of gut microbiota to obesity in humans is unclear [92]. As mentioned above, obesity is correlated to changes in proportions of *Firmicutes* and *Bacteroidetes*. The effects of changes in diet on the composition of the intestinal microbiota were confirmed in animal studies [98, 103]. Fat-rich diet produces an increase in the gram-negative gut microbiota and decrease in the gram-positive microbiota [92]. Food ingredients consumed by the host can be absorbed and utilized by gut microbiota. This microbiota degrades polysaccharides that cannot be digested by the host, resulting in a higher production of short-chain fatty acids such as acetate, propionate, and butyrate. These SCFAs are converted to triglycerides in the liver [92]. Short-chain fatty acids activate G-protein-coupled receptors (GPCRs): GPR41 (expressed in the gut epithelium) and GPR43 (expressed in the intestine, adipocytes, and immune cells). Activated receptors induce secretion of peptide YY (PYY, peptide tyrosine-tyrosine) that belongs to the gut hormones. PYY suppresses gut motility and retards intestinal transit. Expression of GPR43 in the mentioned cells suggests an involvement in lipid and immune regulation. On the other hand, activation of GPCRs may promote energy absorption and synthesis of triglycerides and may affect immune and inflammatory responses [104, 105]. As was shown by Backhed et al. [106], gut microbiota decreases the production of the fasting-induced adipose factor (FIAF, also called angiopoietin-like protein 4 (ANGPTL4) that is a circulating lipoprotein lipase inhibitor) by the intestinal cells. FIAF inhibits activity of lipoprotein lipase (LPL) resulting in increasing the storage of liver-derived triglycerides. Gut microbiota downregulates the expression of AMP-activated protein kinase (AMPK). Downregulation of AMPK induces the inhibition of fatty-acid oxidation resulting in obesity [106].

Western diet, that is high-fat/low fiber, promotes the overgrowth of gram-negative bacteria. Lipopolysaccharide (LPS) is a compound from gram-negative bacterial cell walls. It was defined as a factor involved in the early development of inflammation and metabolic diseases [107]. Concentration of LPS is up along with an increase in the proportion of gram-negative bacteria. An increase in plasma LPS and excessive high level of lipopolysaccharide is defined as metabolic endotoxemia [107]. In animal studies, it was shown that LPS interacts with TLR-4/CD-14, specific receptor of the host's immune system. This interaction triggers an inflammatory cascade [107–109]. Therefore, metabolic endotoxemia induced by LPS is the first step for the development of insulin resistance and diabetes [107]. In humans, increased levels of circulating LPS stimulates the TLR-2 mediated inflammatory response and increases the secretion of proinflammatory cytokines by the adipose tissue [110]. Another mechanism was proposed by de La Serre et al. [111]. According to this suggestion, high-fat diet changes in gut microbiota composition resulting in an increase in luminal LPS and gut permeability. Enhanced gut permeability increases plasma levels, resulting in an appearance of hyperphagia and obesity.

Guerts et al. [112] suggest two physiological systems linked with the gut microbiota: the endocannabinoid system (eCB) and apelinergic system. According to the authors' suggestion, the endocannabinoid system is one of the mediators of communication between the gut and adipose tissue. This system consists of bioactive lipids, such as N-arachidonoylglycerol (AEA) and 2-arachidonylglycerol (2-AG) that bind to cannabinoid receptors (G-coupled-protein receptors, namely CB1 and CB2) and elicit cell signaling. The tight control of eCB levels depends on the balance between synthesis and degradation. Endocannabinoid system is widely expressed in tissues and organs such as pancreas, muscle, gut, and adipose tissue that controls the energy balance. This system regulates feeding behaviors and metabolism [113] and facilitates energy intake and storage. Dysregulation of this control may result in obesity and/or type 2 diabetes mellitus. The stimulation of the eCB system increases food intake and treatment with CB1 (an eCB receptor) antagonist in rodents and humans, reduces food intake, and decreases body weight [112, 114]. The eCB system is overactivated during obesity in rodents and humans

and was demonstrated that this system increases gut permeability observed in obesity [115]. For details on influence of particular bacterial phylum on eCB system, see [112].

The apelinergic system comprises apelin, adipokine produced and secreted by adipocytes, and the apelin receptor (APJ). This system is widely expressed in mammals. The results obtained by Dray et al. [116] showed that intravenous injection of apelin in mice lowered glucose levels by stimulating glucose utilization in skeletal muscle via eNOS, AMPK, and Akt-dependent pathways. This adipokine restored glucose tolerance in obese and insulin-resistant mice fed a high-fat diet. Duparc et al. [117] demonstrated in animal studies that acute intracerebroventricular injection of apelin improves glucose homeostasis via nitric oxide-dependent pathway. On the other hand, the administration of the high doses of apelin either acute or chronic that is observed in obese/diabetic mice provokes hyperinsulinemia, hyperglycemia, glucose intolerance, and insulin resistance in fasted normal mice [117]. Similar effects were observed by the authors in high-fat fed mice. Guerts et al. [118] demonstrated that expression of apelin and apelin receptors are downregulated by eCB in physiological conditions, and acute stimulation of eCB system decreases apelin and apelin receptor in adipose tissue. On the other hand, LPS increases apelin and APJ mRNA expression in adipose tissue [118]. Authors discovered more than 20 positive and negative correlations between gut microbes and apelinergic system. Therefore, authors postulate that gut microbiota composition has direct impacts on the adipose tissue and the apelinergic system [118].

LPS also plays a role in the development of insulin resistance and diabetes. As described above, the first step for the development of these pathologies is an LPS-induced metabolic endotoxemia promoted by gut microbiota. Animal studies showed that infusion of LPS leads to fasting hyperglycemia and hyperinsulinemia [109]. In patients with diabetes were observed specific changes in the composition of gut microbiota. It was observed an increase of *Bacteroidetes* and *Prevotella* and proportional decrease of *Firmicutes* and *Clostridia* [119]. It is to note that *Akkermansia muciniphila* and *Bifidobacteria* play the protective role against the development of metabolic diseases. *Bifidobacteria* and *Lactobacillus* produce butyrate and conjugated linolenic acid. In the presence of these bacteria, were observed: decreased endotoxemia, circulating proinflammatory cytokines, and intestinal permeability [120]. It was found a decrease of bacteria that play a protective role against insulin resistance and diabetes.

Several studies have investigated the role of the human gut microbiota in type 2 diabetes mellitus. Two studies with Chinese [121] and Swedish [122] individuals have shown that the gut microbiota composition might be an important contributor to the development of type 2 diabetes. The obtained results showed that patients with type 2 diabetes had less butyrate producing bacteria (*Clostridales sp.*, *Roseburia intestinalis*, *R. inulinivorans*, *Eubacterium rectale*, *Faecalibacterium prausnitzii*), and some opportunistic pathogens had an increased number (*Bacteroides caccae*, *Clostridium hathewayi*, *C. ramosum*, *C. symbiosum*, *Eggerthella lenta*, *Escherichia coli*) [101]. Several studies confirmed the changes in the composition of the gut microbiota in patients with type 2 diabetes [92]. Several studies also link the intestinal microbiota composition to metabolic disorders such as insulin resistance and diabetes mellitus [123, 124].

It was observed that children with type 1 diabetes mellitus showed changes in the amount of *Bifidobacterium*, *Lactobacillus*, and *Clostridium* and a reduction in *Firmicutes* to *Bacteroidetes* ratio [125].

How to explain the influence of the gut microbiota on the development of type 2 diabetes mellitus? Various mechanisms have been proposed. The obtained results suggest that an important role in this dependence plays metabolic endotoxemia, modifications in the secretion of incretins, and butyrate production [92].

The role of insulin receptors in adipose tissue and its mechanics in obesity

In addition to muscle and the liver, adipose tissues are relevant sites of insulin action. Understanding the signal pathways involved in insulin action could lead to a better understanding of the role in obesity.

Two types of adipose tissues have been described. White adipose tissue stores triglycerides and releases free fatty acids in response to changing energy requirements. White adipocytes constitute the classical fat cell and represent the majority of cells in visceral and subcutaneous adipose depots. Brown adipose tissue plays a role in thermogenesis in most mammalian species. The presence of brown adipose tissue has recently been described in adult humans [129]. The thermogenic activity is mainly controlled by the sympathetic nervous system.

White adipose tissue plays a critical role in the maintenance of insulin sensitivity and in the development of insulin resistance. Adipose tissue, that is an endocrine organ, can secrete the modulators of insulin action. Adipocytokines positively or negatively regulate insulin action, whereas free fatty acids can accumulate the insulin-sensitive tissues, such as muscle and adipose tissue, and impair insulin sensitivity.

Intracellular insulin signaling plays an important role in adipocyte metabolism. It stimulates, for example, synthesis and storage of glucagon and inhibits its catabolism [130]. Insulin activates different signaling pathways. Two of these signal transduction cascades are implicated in obesity and/or in type 2 diabetes: the phosphoinositide-3-kinase and the mitogen-activated protein kinase (MAPK). Insulin binds to

the insulin receptor (IR) on adipocytes, triggering its autophosphorylation. The binding of insulin to IR results in the tyrosine phosphorylation of insulin receptor substrates by the insulin receptor tyrosine kinase. The next step is the activation of PI-3 K by phosphorylated IRS. Active PI-3 K catalyzes the formation of lipid second messenger, phosphatidylinositol-3,4,5-triphosphate (PIP_3). PI-3 K activates protein kinase B (PKB), known as AKT that targets several downstream proteins. AKT regulates the activity of the glycogen synthase kinase-3 α/β (GSK-3α/β), which leads to the activation of glycogen synthase and subsequent glycogen synthesis. Akt also regulates transcription of genes involved in gluconeogenesis, glycolysis, protein synthesis, and lipogenesis, as well as stimulates translocation of GLUT4 from intracellular compartments into the cell membrane thereby enhanced flux of glucose into the cell [131, 132, 145].

Mitogen-activated protein kinase has multiple cellular substrates. Activation of MAPK pathway by insulin signaling is responsible for gene expression and mitogenesis, however, this pathway is generally considered to be important in the insulin regulation of growth [133]. The most data do not implicate the MAPK pathway in the well-studied metabolic actions of insulin [134].

Defects in PI3-K pathway have been demonstrated in type 2 diabetes [135]. Mice lacking Akt2 demonstrate diabetes mellitus-like syndrome: insulin resistance, hyperlipidemia, and hyperglycemia [136]. Mice with adipocyte-specific deletion of insulin receptor are resistant to diet-induced and age-related obesity [137]. Cariou et al. [138] have studied insulin action in white adipose tissue during hyperinsulinemic euglycemic clamp. The experiments were performed on muscle-specific insulin receptor knockout (MIRKO) mice. These mice do not develop insulin resistance or diabetes under physiological conditions despite a marked increase in adiposity. Authors have found that in experimental conditions, there is no alteration in the expression or activation of molecules in the PI3-K pathway; however, these mice display muscle insulin resistance, visceral obesity and dyslipidemia, but does not develop hyperinsulinemia or diabetes. Blüher et al. [139] observed that fat-specific insulin receptor knockout (FIRKO) mice were found to have an increase in a mean life-span of ~134 days. These animals have reduced their fat mass, although their food intake is normal. Authors suggest that increased longevity in these animals depends on insulin signaling. The role of insulin receptor and insulin signaling in adipose tissue has been studied by many authors, as for example [140–143]. In adipose tissue of obese women with gestational diabetes and type 2 diabetes, were observed reduced IRS-1 protein levels and reduced GLUT4 translocation as compared to healthy controls [144].

Downregulation of insulin signaling protein levels, as observed in obesity, can result in insulin resistance [131]. In obesity, plasma insulin levels are increased [146]. Hyperinsulinemia itself can reduce IRS protein levels via transcriptional regulation [147]. There were also other described defects in insulin signaling [148–150]. Several models have been put forward to explain the mechanism of obesity-induced insulin resistance [151].

In obesity, adipose function is impaired. The obesity-related insulin resistance is due to the invasion of white adipose tissue by mononuclear cells that release proinflammatory cytokines. These cytokines promote a whole-body insulin resistance. Insulin plays an important role in the regulation of macrophage of adipose tissue and development of obesity-associated insulin resistance [152]. Mauer et al. [153] have been observed that deletion of insulin receptor in myeloid cells reduces accumulation of macrophages in adipose tissue during high-fat diet. As mentioned above, mice with adipocyte-specific knockout of the insulin receptor have a decreased ability of insulin to suppress lipolysis, insulin-stimulated glucose uptake, and synthesis of triglyceride in adipocytes [137].

Besides macrophages-derived proinflammatory cytokines, adipocytes also may contribute to the development of obesity-related insulin resistance. Adipose tissue expansion is caused by adipocyte hypertrophy. These hypertrophic adipocytes display elevated expression and secretion of proinflammatory adipokines [154]. It was observed [155, 156] that in obese individuals, the plasma levels of adiponectin, that are insulin sensitizing, are reduced. The reduction in adiponectin levels were found inversely correlated with the degree of insulin resistance and hyperinsulinemia.

Adipose tissue secrete numerous peptides, such as hormones (leptin, adiponectin, resistin, apelin, visfatin), chemokines (monocyte chemotactic protein (MCP)-1, IL-8), proinflammatory cytokines (IL-1, IL-6, angiotensin-II, TNF-α), and anti-inflammatory cytokines (IL-10); for details, see [151]. Increased proinflammatory cytokines induce the expression of suppressor of cytokine signaling (SOCS)-1 that blocks the interaction between IR and IRS and contributes to insulin resistance [148]. Of note, SOCS-1 and SOCS-3 can induce degradation of IRS [157]. TNF-α impairs insulin signaling through serine phosphorylation of IR and IRS (inactivate) both of which result in diminishes activation of PI3-K and reduction of GLUT4 gene expression [158–160]. TNF-α increases circulating free fatty acid levels that inhibit insulin signaling through serine phosphorylation in IRS [149]. Interleukin-6, like as TNF-α, increases free fatty acid concentrations that can affect insulin signaling [161]. In adipose tissue and in the liver, IL-6 upregulates SOCS-3 [162]. The higher expression of the p85 regulatory subunit of PI3-K is also associated with insulin resistance [163]. Amyloid-β (Aβ) precursor protein is expressed in adipose tissue. It is upregulated with obesity and correlated to insulin resistance. In adipose tissue, Aβ may affect insulin signaling pathway. It was observed that Aβ decreases the expression of IRS-2 and reduces the Akt-1 phosphorylation [164].

The above-presented examples of defective insulin signaling at various levels of this cascade, due to obesity, can cause insulin resistance. Of note, studies have demonstrated reduced IRS-1 protein levels and reduced GLUT4 translocation in adipose tissue of obese women with gestational diabetes and type 2 diabetes [165]. Mahesan et al. [166] have observed that in pregnancy, the insulin resistance correlates with the decrease of the expression of insulin receptor gene in omental adipose.

Conclusion

Obesity and gut microbiota play an important role in pathogenesis of type 2 diabetes mellitus. As an effect of obesity and western diet is an impairment of immunity and the development of insulin resistance and type 2 diabetes. On the other hand, type 2 diabetes impairs the immune system. This is a cause why patients with diabetes have increased susceptibility to and severity of infections. This is also an answer why the course of infections is also more complicated in the patient group.

Compliance with ethical standards

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ORIGINAL ARTICLE

Molecular analysis of the gut microbiome of diabetic rats supplemented with prebiotic, probiotic, and synbiotic foods

Muhammad U. Sohail[1] · Muhammad Z. Shabbir[2] · Jörg M. Steiner[3] · Shakeel Ahmad[4] · Zahid Kamran[4] · Haseeb Anwar[1] · Ghulam Hussain[1] · Arsalan Shaukat[1] · Muhammad Irfan ullah[1] · Jan S. Suchodolski[3]

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Abstract Beneficial symbionts residing in our gut have positive therapeutic effects on several metabolic disorders including diabetes. Oral administration of probiotic and prebiotic foods strengthens the beneficial symbiont populations in the gut and may prevent immune-mediated destruction of pancreatic β-cells. The present study was designed to elucidate the gut microbiome of diabetic rats supplemented with a *Lactobacillus* probiotic and a *Saccharomyces cerevisiae* (SC) cell wall prebiotic. Diabetes mellitus was induced in male Wistar rats with allaxon monohydrate (150 mg/kg). The rats were fed chow maintenance diet (control and diabetic control groups) or the same diet supplemented with a SC prebiotic (1 %), probiotic (multispecies *Lactobacillus* @10^8 CFU), or synbiotic. On d30, DNA was extracted from colon digesta for 16S ribosomal RNA (rRNA) gene sequencing. Serum was obtained to estimate total oxidant and anti-oxidant concentrations. A distinct clustering pattern (Unifrac distances, analysis of similarities (ANOSIM) $P = 0.0361$) was observed for the different treatment groups, with the main distinction consisting of the separation between the control and the diabetic control groups. Distinct bacterial clades dominated different treatment groups, particularly for the control and the diabetic control groups, though several bacterial groups overlapped, demonstrating a core microbiota dominated mainly by *Firmicutes* and Bacteroides. A trend of dysbiosis, characterized by low species richness, was observed in the diabetic rats, albeit not statistically significant. Serum oxidant and anti-oxidant concentrations were not different ($P > 0.05$) among different treatment groups. No significant effects of supplementations of prebiotic, probiotic, and synbiotic were observed on species richness or clustering pattern of the microbiome.

Keywords 16S rRNA sequencing · Gut dysbiosis · Metabolic disorders · Nutritional supplementation · Oxidative stress

Introduction

Type 1 diabetes mellitus (T1DM) is a metabolic disorder that results from a myriad of factors. The disease is usually characterized by oxidative and/or T cell-mediated autoimmune destruction of pancreatic beta cells, leading to partial or complete loss of insulin production [1, 2]. Although limited information is available about the pathogenesis of the disorder, it is generally believed that in genetically susceptible individuals, a chronic inflammatory disease of the gut triggers the primary insult, leading to redox destruction of β-islet cells [3–5]. Furthermore, recent studies have suggested that the risk of development of diabetes is also triggered by the gastrointestinal tract (GIT) microbiome [6] and that the disease is associated with microbial dysbiosis in the GIT [7].

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✉ Haseeb Anwar
drhaseebanwar@gcuf.edu.pk

[1] Department of Physiology, Government College University, Faisalabad 38000, Pakistan

[2] Quality Control Laboratory, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

[3] Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX 77843-4474, USA

[4] Veterinary College, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

The GIT microbiota is considered important for host health, nutrition, and immunity and can be affected by age, stressors, disease, and diet [8]. Several microbes living in the gut are known for their beneficial effects (e.g., anti-allergic, anti-carcinogenic, anti-diabetic, and cholesterol lowering) on host health [3, 9]. Microbes living in the intestine produce numerous unknown metabolites that are absorbed from the gut and influence host health and immunity and alter the gut–brain barrier [10].

Consumption of fermented foods augments gut microbiome and delays the progression of streptozotocin-induced diabetes in a rat model [11]. These functional foods can lower plasma glucose and delay the progression of experimentally induced diabetes in rats [11–13]. Recently, Park, Ahn [14] has elucidated that oral administration of probiotic *Lactobacillus* spp. suppressed insulin resistance, reduced glucose and cholesterol concentrations, reduced reactive oxygen species, and decreased blood pressure. Probiotic *Lactobacillus* can prevent onset of insulin-dependent diabetes mellitus in mice by enhancing the number of beneficial symbionts in the host gut [15]. Also, probiotics are known for reducing predisposing factors for diabetes like obesity, allergies, and autoimmune disorders. Similarly, prebiotics such as xylo-oligosaccharides and fructo-oligosaccharides have also been reported to ameliorate the metabolic abnormalities associated with diabetes such as hyperglycemia, hypercholesterolemia, glucosuria, proteinuria, and diabetic nephropathy [16]. Particularly, supplementation with the prebiotic cell wall extract from *Saccharomyces cerevisiae* for 12 weeks has been shown to decrease blood pressure and improve glycemic indices in patients with T2D [17, 18].

Although some studies [19, 20] have reported that microbial dysbiosis is a contributing factor in the disease pathogenesis, limited literature is available investigating the gut microbiome of diabetic rats fed probiotics and/or prebiotics. Therefore, the present study was designed to study the effects of a prebiotic cell wall extract from *S. cerevisiae* and probiotic *Lactobacillus* spp. on the gut microbiome of diabetic rats using 16S ribosomal RNA (rRNA) gene sequencing.

Materials and methods

Animals, diets, and study design

Thirty adult male Wistar rats of the same weight (200 ±20 g) were divided into five treatment groups. The rats were housed under standard management conditions (two rats/cage, 24±2 °C, 12-h light/12-h dark cycle) with free access to food and water. After 1-week acclimatization period, rats were injected intraperitoneally with 150 mg/kg allaxon monohydrate (Sigma-Aldrich, UK) as a 5 % solution in normal saline (diabetic groups) or the same volume of normal saline (control group). Post injection, 5 % glucose solution was provided for 48 h to prevent initial drug-induced hypoglycemic mortality. Ninety-six hours post injection, blood (Aviva Accu-Chek, Roche Diagnostics) and urine (Benedict's qualitative test) glucose was tested to confirm diabetes in rats injected with allaxon. Only rats with blood glucose level above 180 mg/dL were used in the analysis. The rest of the animals which did not demonstrate the inclusion criteria of hyperglycemia were excluded.

Rats were divided into the following groups: control group fed chow maintenance diet (CMD), diabetic control group fed CMD, diabetic prebiotic group fed CMD supplemented with 1 % *S. cerevisiae* yeast cell wall extract, diabetic probiotic group fed CMD supplemented with a multispecies probiotic of *Lactobacillus* spp. (~10^8 CFU), and diabetic synbiotic group fed CMD supplemented with a combination of both 1 % prebiotic and 10^8 CFU of the probiotic *Lactobacillus* spp. The CMD was composed of 54 % corn starch, 21 % casein, 10 % refined soybean oil, 10 % cane sugar, and 5 % vitamin–mineral premix (National Research Council). Dietary treatments were continued from day 0 to day 30, and blood glucose levels were measured on a weekly basis. At the end of the study period, rats were killed by decapitation, and trunk blood and colon digesta were collected.

Serum oxidant and anti-oxidant analysis

Blood was centrifuged at 1500×*g* at 4 °C for 15 min for serum extraction. Serum total oxidant concentrations (TOC; μm of H_2O_2 equivalent/L) were measured using a colorimetric method based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species [21]. The procedure was calibrated with hydrogen peroxide. Total anti-oxidant concentrations (TAC; mM Eq. of vitamin C/L) were measured using a novel automated colorimetric method using odianisidine dihydrochloride as the substrate as described by Erel [22]. The data obtained for serum TOC and TAC were analyzed using ANOVA to evaluate treatment effects.

Microbiome analysis

Colon digesta was subjected to DNA extraction using the BiOstic® FFPE Tissue DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA), following the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified with primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) at the MR DNA Laboratory (Shallowater, TX, USA). PCR amplification

products were verified on 2 % agarose gels, and samples were purified using calibrated Ampure XP beads. The Illumina TruSeq DNA Library was used to prepare a DNA library and sequenced at MR DNA on an Illumina MiSeq instrument, as described earlier [23]. Raw sequence data were screened, trimmed, denoised, filtered, chimera-depleted, and clustered as operational taxonomic units (OTUs) at 97 % similarity, using QIIME (V1.7) default settings. The sequences obtained in this study were submitted to NCBI Short Read Archive as FASTQ files with accession number SRR1613115.

A total of 1,200,168 sequences were recovered from all samples. To standardize sequence depth, further data analysis was performed on an even sample depth of 57,546. Alpha rarefaction (species richness per sample) and beta diversity (microbiome similarity between samples) were measured and plotted using QIIME. The analysis of similarities (ANOSIM; $P = 0.05$) was performed on unweighted UniFrac distances to compare the microbiome among the different treatment groups. Relative clustering pattern of the microbiome among different treatment groups was analyzed using Pearson's correlation test, and a dendrogram was constructed using default settings in METAGENassist [24]. The Kolmogorov–Smirnov test was used to evaluate the normal distribution of the data. Further, nonparametric Kruskal–Wallis H test was also applied on the data and resulting p values were adjusted for multiple comparisons using Benjamini and Hochberg's false discovery test.

Results

Cecal microbiome

Sequencing of 16S rRNA genes was performed to explore the phylogenetic composition of the microbiome. Approximately, 1,200,168 (minimum 57,546, maximum 153,060, median 71, 981, and standard deviation 29,044) chimera-depleted good-quality 16S rRNA gene sequences were retrieved from all the samples. These sequences corresponded to 23 phyla, 39 classes, 70 orders, 138 families, and 291 genera. Regardless of the high bacterial diversity, only three phyla (*Firmicutes*, *Bacteroidetes*, and *Proteobacteria*) accounted for more than 99 % of all the obtained sequences (Fig. 1).

A cluster hierarchy dendrogram based on the Pearson correlation test using default parameters was constructed in Fig. 2. The vertical axis of the dendrogram represents the dissimilarity between clusters. The horizontal axis represents the samples. The dendrogram revealed that the control and probiotic-supplemented diabetic groups had significantly different (dissimilarity ≥1.0) communities compared to the other treatment groups.

Principal coordinate analysis (PCoA) plots for unweighted UniFrac distances were constructed to evaluate microbiome variation between different treatment groups (Fig. 3). We applied the statistical analysis ANOSIM on the unweighted UniFrac distances and observed a significant clustering pattern in the PCoA plots (ANOSIM with 43,259 permutation, $P = 0.0361$), demonstrating significant differences in microbiomes of different treatment groups. Pairwise

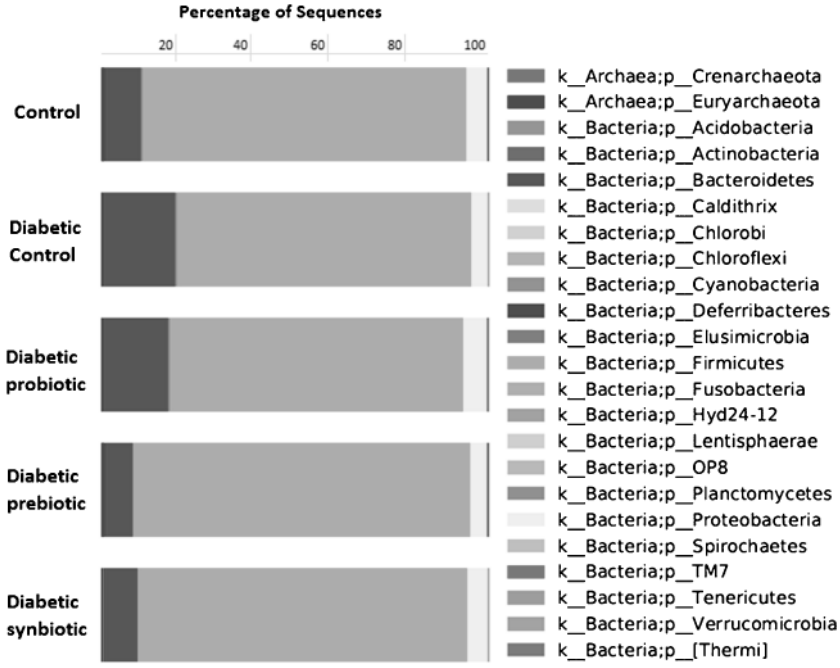

Fig. 1 Composition of colon microbiome of the control, diabetic control, diabetic probiotic, diabetic prebiotic, and diabetic synbiotic rats at the phylum level. The *bars* represent median percentage of sequences

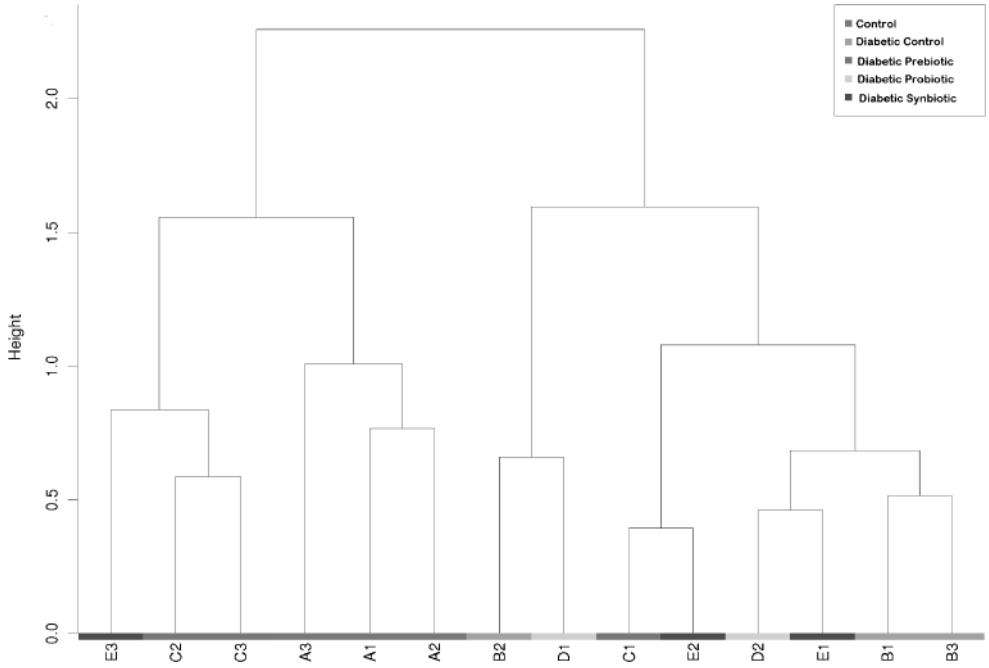

Fig. 2 Pearson correlation hierarchical clustering dendrogram of 16S rRNA-based sequences

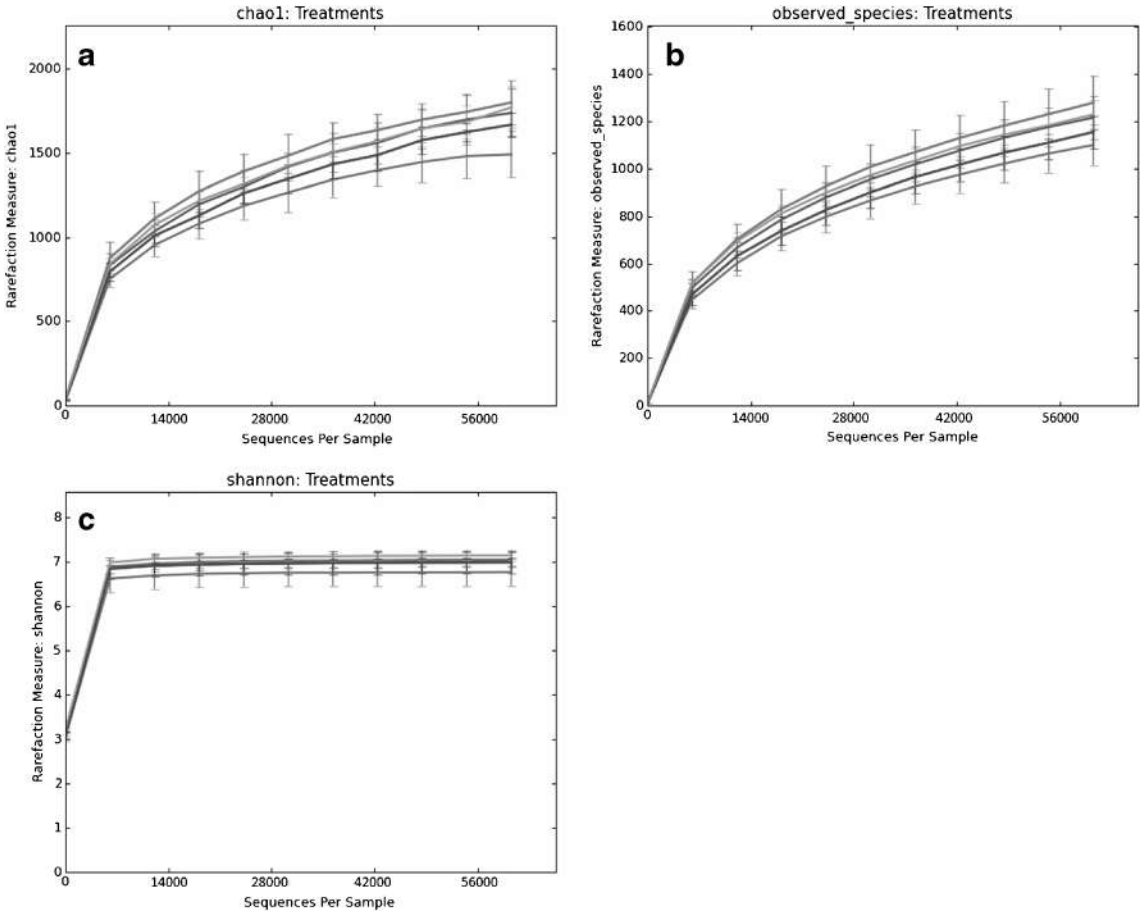

Fig. 3 Alpha diversity measures at 57,546 sequences per sample in the different treatment groups. Bacterial diversity and richness index graphs (Chao 1 (**a**), observed species (**b**), and Shannon–Weaver (**c**)) obtained from colon microbiome samples. Control (*red*), diabetic control (*green*), diabetic probiotic (*blue*), diabetic prebiotic (*purple*), and diabetic synbiotic (*orange*) (Color figure online)

ANOSIM analysis revealed that only the control and the diabetic control groups had significantly different ($P = 0.031$) clustering pattern. All the other treatment groups had non-significantly different ($P > 0.05$) clustering pattern.

The alpha rarefaction analysis (Chao 1, observed species, and Shannon), at an even sample depth of 57,546 sequences per sample, revealed high inter-individual variability in rats from the different treatment groups (Fig. 4). Species richness was highest ($P > 0.05$) in the control group and lowest ($P > 0.05$) in the diabetic control group. However, alpha rarefaction indices were non-significantly different among the different treatment groups.

We further tested our finds by subjecting our data to Kruskal–Wallis one-way analysis of variance and false discovery tests. These tests reveal no significant differences in bacterial taxa on various phylogenetic levels among different treatment groups (Table S1).

Serum biochemistry

We measured serum glucose concentrations on day 30 of the study (Fig. S1). Control group had relatively low ($P > 0.05$) serum glucose concentrations compared with the diabetic rats. The serum oxidant concentrations and anti-oxidant activities were also determined at the termination of the experiment. Perhaps, no significant differences were observed among the different treatment groups (Fig. S2).

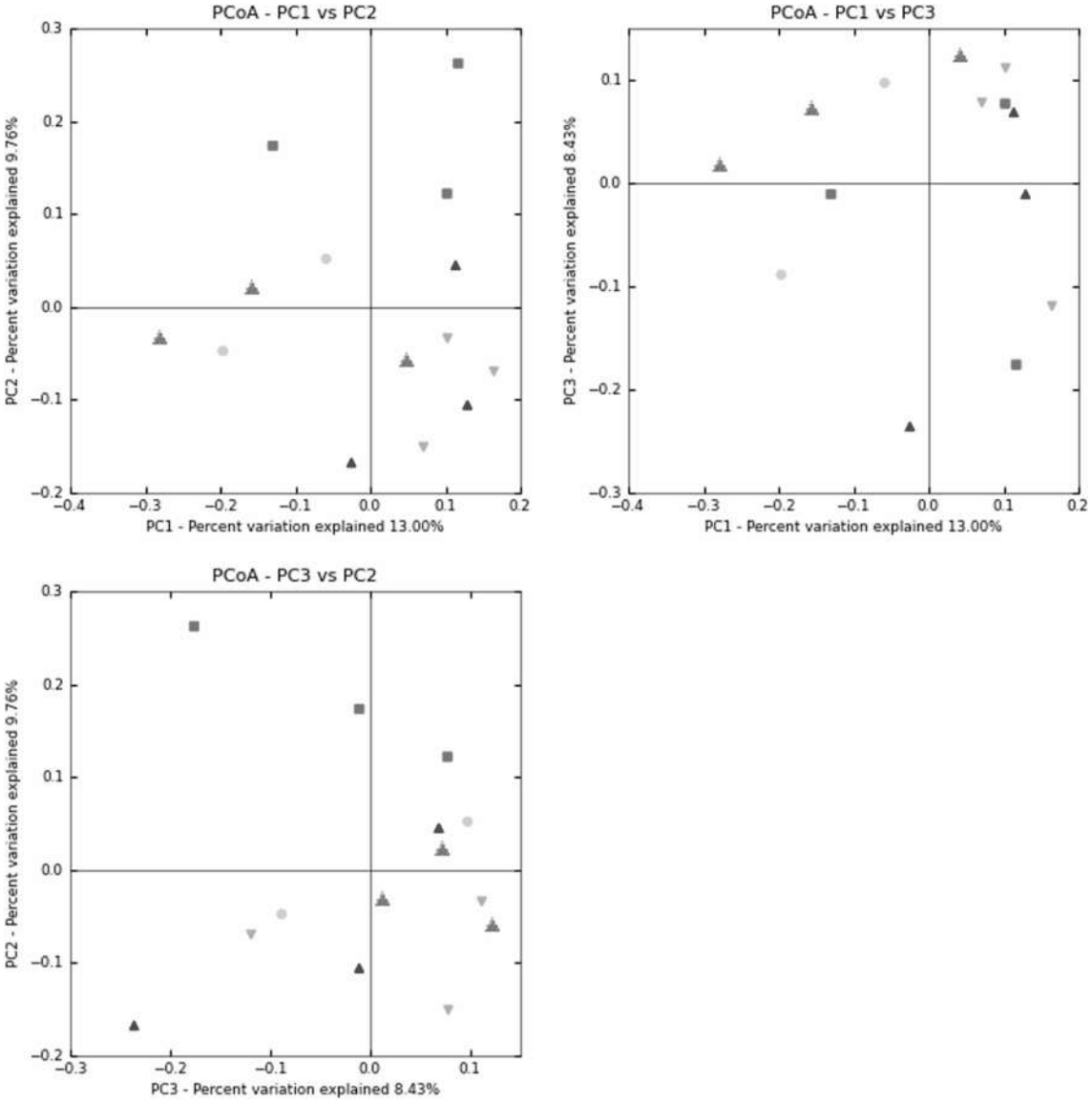

Fig. 4 Principal coordinate analysis (PCoA) plots of the unweighted Unifrac distance matrix. The plots show each combination of the first three principal coordinates. Control (*red, square*), diabetic control (*red, triangular*), diabetic prebiotic (*blue, triangular*), diabetic probiotic (*aqua, circular*), and diabetic synbiotic (*green, triangular*) (Color figure online)

Discussion

Type 1 diabetes is an idiopathic syndrome characterized by destruction of insulin-producing beta cells of the pancreas. Though the exact link is not well established, it is thought that microbial dysbiosis in the gut can aggravate the immune system, thereby bolstering disease pathogenesis [6]. Therefore, present research work is focused on understanding gut microbiome characteristics and its potential role in host health and disease. Similarly, several studies are being conducted to explore the beneficial effects of probiotics and prebiotics for the management of diabetes [11–13]. To the best of our knowledge, only few studies [19, 20] have been conducted so far to investigate the dynamics of the gut microbiome in diabetic rats, supplemented with prebiotics and/or probiotics.

Phylogenetic data presented here were analyzed simultaneously at various taxonomic levels using QIIME 1.7 to classify the microbiomes of control and diabetic rats. Three phyla (*Firmicutes*, *Bacteroidetes*, and *Proteobacteria*) dominated the gut microbiome of all rats, and no significant differences were observed among different treatment groups at the phylum level. Similarly, no significant differences were observed at class- and order-level phylogeny. These findings are in agreement with the earlier work of Qin, Li [7], who found no significant bacterial dysbiosis in diabetic subjects compared to controls. However, our findings differ from some earlier reports as well. Giongo, Gano [20] found a decrease in *Firmicutes* and increase in *Bacteroidetes* in murine T1D model. Similarly, a microbial dysbiosis, characterized by a decline in relative abundance of *Firmicutes* and an increase in proportions of *Bacteroidetes* and *Proteobacteria*, was reported by [25].

In the present study, higher species richness and diversity, as indicated by alpha rarefaction (Fig. 3), were observed in the control group compared to the other treatment groups. We also observed that the first two coordinates of PCoA plots explained 22.76 % variation in the microbiome, suggesting that independent variables (diabetes and supplementations) were responsible for the observed variation between the samples [26]. A significant difference (pairwise ANOSIM $P = 0.031$) in clustering pattern revealed that only diabetes had an influence on the gut microbiome of rats. These findings are in agreement with the previously published Finnish children autoimmune T1D work [20]. In a leptin-resistant obesity model of mice diabetes, Everard, Lazarevic [27] demonstrated that modulation of gut microbiome by prebiotic diet can improve glucose and lipid homeostasis, leptin sensitivity, and activity of targeted enteroendocrine cells in diabetes. The study also showed a positive correlation between prebiotic-induced modulation of gut microbiome and these metabolic parameters, particularly the overrepresentation of *Firmicutes* in prebiotic-fed groups.

It is well known through earlier experiments and clinical studies that oxidative stress plays a major role in the pathogenesis of diabetes [28]. Oral administration of yogurt fortified with *Lactobacillus* spp. has been shown to suppress streptozotocin-induced oxidative damage [11] and improve anti-oxidant status and contribute to better management of T2D [12]. Furthermore, some earlier studies have also reported that the supplementation with *S. cerevisiae* cell wall improved gut microbial diversity [29] and increased the numbers of *Bifidobacterium* spp., *Faecalibacterium* spp., and *Ruminococcus* spp. in the colon [30]. These microbes generally augment production of anti-inflammatory and immune modulatory factors [31]. Further, probiotic and prebiotic supplements route multiple mechanism, which are not yet fully explored, to augment diabetes-associated suffering. Although previous studies have reported that these supplements can reduce plasma triglyceride levels, muscle lipid infiltration, adipose tissue mass, and oxidative stress [27], antithetical to the previous studies, we could not find significant effects of supplementation with either prebiotic, probiotic, or synbiotic on the gut microbiome of diabetic rats. In addition, serum TOC and TAC concentrations were also found unaltered. Though the exact reason for differences in findings is not known, perhaps, it may be proposed that different disease model, dosage (prebiotic 5 vs 100 g/kg, probiotic ~10^8 vs 73^8 CFU/g), duration of supplementations (30 days vs 6 week), microbiome analysis procedure (16S rRNA sequencing vs culture plate), and the number of animals used in the study have attributed to these controversies.

Taken together, these findings suggest a partial role of the gut microbiome in T1D. Though the sample size used here is too low to make a firm conclusion, these data suggest that T1D is associated with decreased diversity of the gut microbiome when compared with non-diabetic subjects. Although review of literature depicts positive role of prebiotic/probiotic supplements on diabetes-associated parameters, perhaps, we failed to modulate oxidative stress in the supplemented groups. Further investigations with higher dosages and longer durations of supplementations are necessary to ascertain better understanding of therapeutic effects of these functional foods. The present study can serve as springboard for future studies to delineate the pathophysiological role of the gut microbiome in diabetes.

Compliance with ethical standards The present study was partially sponsored by the American Association for the Advancement of Science under AAAS–CSTSP International Activity for BMENA Scientific Exchange Program. MU Sohail visited Texas A&M University under AAAS-BMENA Scientific Exchange Program. Other than this, authors declare no conflict of interest.

All procedures and protocols were adopted under the guidelines of the Animal Care and Ethics Committee, Offices of Research, Innovation, and Commercialization, GC University Faisalabad, Pakistan.

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ORIGINAL ARTICLE

Estimation of glycemic carbohydrate and glycemic index/load of commonly consumed cereals, legumes and mixture of cereals and legumes

S. Devindra[1] · Shilpa Chouhan[2] · Charu Katare[2] · Aruna Talari[1] · G.B.K.S. Prasad[3]

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Abstract Aim of the present study is to estimate glycemic carbohydrates and develop data base on glycemic index and glycemic load (GI and GL) of commonly consumed cereals and legumes by using Food and Agriculture Organization (FAO) or World Health Organization (WHO) methods. The results of glycemic carbohydrates in rice was 79.22 %, wheat 63.26 % and pulses in the range from 51.24 % (green gram) to 56.22 %, (chana dhal), mixed dhal 40.09 %, wheat + chana dhal (60:40) 49.94 %, wheat + chana dhal + barley (40:30:30) was 46.89 %, respectively. The results of GI and GL of rice were the highest (GI–78.23, GL–49.38), followed by wheat chapatti (GI–65.66, GL–32.83). The pulses tested were showing lower values ranging from (GI–37.95 to 43.01 and GL–18.97 to 21.50), mixed dhal (GI–43.64, GL–21.82), wheat + chana dhal (60: 40) (GI–32.37, GL–16.18), wheat + chana dhal + barley (40:30:30) GI–39.27, GL–19.63, respectively. The results of the study indicated that pulses have low glycemic indices and glycemic loads, hence, could be safely used in the diet of diabetic patients.

Keywords α-amylase · Anthrone · Amyloglucosidase · Protease · Starch · Glycemic index · Glycemic load

✉ S. Devindra
dr_devindra@rediffmail.com

[1] Food Chemistry Division, National Institute of Nutrition (ICMR), Hyderabad, Telangana State 500007, India

[2] Department of Food & Nutrition, Govt. K.R.G.PG Autonomous College, Gwalior, MP 474001, India

[3] School of Studies in Biochemistry, Jiwaji University, Gwalior, MP 474001, India

Introduction

Carbohydrates play a major role in human diet, comprising about 40–85 % of energy intake. Their most important nutritional property is their easy digestibility in the small intestine. In terms of their physiological and nutritional role, they are often classified as available and unavailable carbohydrates [1]. Numerous studies have shown that carbohydrate rich foods including rice significantly increase the risk of obesity, type 2 diabetes and chronic diseases such as cardiovascular and some cancers [2].

The glycemic carbohydrates provide carbohydrates to body cells, mainly in the form of glucose. In general, only monosaccharides are absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and is continued in the small intestine by pancreatic amylase. The degradation products mainly maltose and oligosaccharides are hydrolyzed further to glucose by a set of enzymes, "disaccharidases," bound to the brush border membrane of the enterocytes. The same enzymes hydrolyse the dietary disaccharides [3].

However, the traditional method of expressing carbohydrate by "difference" is problematic because it includes a number of non-carbohydrate components, such as lignins, organic acids, tannins, waxes, and some malliard products [4] which made an extensive compilation of techniques used for sugar analysis, and Southgate has provided an exhaustive review of the same [5]. Available and accurate methods for the estimation of glycemic carbohydrates in foods is currently gaining a great interest in nutrition research and is essential for computing the correct energy intake and to generate the database of accurate glycemic index of foods.

The current criticisms of GI and why GI is valid are the following : (a) GI methodology is accurate and precise enough for practical use, (b) GI is a property of foods, and (c) GI is biologically meaningful and relevant to virtually everyone. GI is a novel concept from a regulatory point of view and a number

of problems needed to be addressed to successfully translate GI knowledge into practice [6].

The increasing prevalence of diabetes throughout the world is partly related to fast release nature of the staple carbohydrate foods which are more refined [7]. Thus, the dietary management of diabetes requires a sound knowledge of blood glucose as well as insulin responses to meals as the treatment targets reduction of postprandial hyperglycemia and hyperinsulinemia. Using the GI in meal planning can improve diabetes control and other health parameters. Understanding the benefits of the GI and how one can implement it into the diet allows health care practitioners to educate patients about its use [8]. The inclusion of low GI foods in type 2 diabetic meals had shown to reduce both the postprandial and 24 h glucose profiles [9].

In order to calculate the GI, blood glucose concentrations of the samples should be tested for giving 50 g of carbohydrate portion as standard. The portion of food tested should contain 50 g of glycemic (available) carbohydrate. In practice, glycemic carbohydrate is often measured as total carbohydrate minus dietary fiber as determined by the Association of Official Analytical Chemists (AOAC) method. Since this method does not include resistant starch type 1 (RS 1) and resistant starch type 2 (RS 2) and when they are present, they will be mistakenly included as glycemic carbohydrate [1]. The reliability of GI values for individual foods was explained by the 1998 Joint FAO/WHO expert consultation on carbohydrates suggested that for the determination of the GI of a food, six subjects would be required; although the basis for this number was not given [1]. More recently, it has been recommended that a sample of ten should be used, on the grounds that it allows for a "reasonable degree of power and precision for most purposes", although it was acknowledged that more people would be necessary if greater precision was required [10].

Therefore, the present study was carried out for the analysis of glycemic carbohydrates that are digestible in the human upper gastrointestinal tract by using enzymes that mimic the human system under laboratory conditions using modified method of anthrone. The glycemic index/load of commonly consumed Indian foods was developed by using the FAO/WHO method (1998) and attempt has also made to develop the low Glycemic foods using different ratios of cereals and pulses.

Materials and methods

Materials The cereal samples like rice, wheat, and barley; legumes such as red gram, green gram, bengal gram whole, masoor dhal, chana dhal, mixed dhal, and mixed flours of wheat: chana (60:40); wheat: chana: barley (40:30:30) were used in the present study.

Sugars Glucose (>99.5 % purity; Sigma Chemical Co., St. Louis, MO, USA) was used in this study.

Standard glucose Stock solution is 100 mg in 100 mL of distilled water.

Working standard Ten milliliters of stock solution was diluted to 100 mL with distilled water (100 μg/mL).

Anthrone reagent Two hundred milligrams of anthrone was dissolved in 100 ml of ice-cold sulphuric acid.

Enzymes Total Dietary Fiber Kit (Sigma, TDF-100A) was used. This kit includes 10 mL heat-stable α-amylase, 500 mg protease, and 30 mL amyloglucosidase.

Phosphate buffer 0.08 M, pH 6.0. Dissolve 1.400 g anhydrous dibasic sodium (Na_2HPO_4) and 9.68 g monobasic sodium phosphate monohydrate (NaH_2PO_4. H2O) in 1 L water. Check pH level and adjust if necessary.

NaOH 0.275 N. Dissolve 11.00 g NaOH in 1 L water.

HCL 0.325 M. Dilute 325 mL 1 M HCL to 1 L with water.

Glucometer

Glucometer used for the present study was One Touch Ultra 2 Blood Glucose Meter (Life Scam. Inc., CA59035, China). The measuring range was linear between 0.6–33.3 mmol/L (10–600 mg/dL). One Touch Ultra Blue Test Strips, One Touch Ultra pricking device and One Touch Ultra lancet needles were also purchased from Life Scam. Inc., CA59035, China. The glucose powder is used as standard for the present study (Akhil Healthcare Private Limited, India).

Sample preparation and sugar extraction

Duplicate test portions of cereals and legumes were treated with heat-stable α-amylase, protease, and amyloglucosidase in order to hydrolyze proteins and starch under laboratory conditions, as given in the following:

Food samples (100 mg) were taken in to 16 × 125 mm tubes with screw caps in duplicate. Five milliliters of pH 6.0 phosphate buffer (0.08 M) were added to the tubes. The tubes were stored at 4 °C for 12 h for hydration of the matrix. The sample was subjected to enzyme hydrolysis to degrade soluble starch. An α-amylase solution (50 μL) was added, and the tubes were placed in a 95 °C water bath (Daihan Labtech Co., Ltd. Korea). After 30 min, the tubes were removed and cooled to 60 °C and adjusted to pH 7.5 with 1 ml of 0.275 N NaOH. Protease solution (50 μL) was added to the tubes which were incubated at 60 °C for 30 min. Now, 1 mL of 0.325 M HCl was added to the tubes to decrease the pH to

4.5. After adjusting the pH, amyloglucosidase solution (150 μL) was added and then the tubes were incubated at 60 °C for 30 min. The residue was separated by centrifugation. The liquid portion was transformed to 100 ml volumetric flask and made up to the mark with milli Q water.

The amount of available sugars in the supernatant was determined by using anthrone reagent. Different volumes of supernatant, 0.2–1 mL in to a series of test tubes were taken and the volume was made up to 1 mL with distilled water to each tube. Four milliliters of anthrone reagent was added and the tubes were placed in boiling water bath for 8 min and cooled rapidly under running tap water. The optical density of green to dark green was measured at 630 nm against blank and the concentration of available carbohydrate was calculated using standard curve. The standard curve was constructed using glucose as a standard.

Sample processing for glycemic index

Cooking Rice was cooked in an electric automated rice cooker based on manufacturer's instruction using distilled water in the ratio of 1:2 for 30 min. One kilogram of whole legume seeds were soaked in 1:10 ratio of distilled water for 12 h, after which the soaked water was decanted. The soaked seeds were cooked in fresh distilled water for 60 min on a hot plate at 100 °C, keeping the bean to water ratio of 1:10 (*w*/*v*). The cooked seeds were seasoned in little oil with salt, pepper powder, and garlic for sensory attribute.

Preparation of chapatti/roti Wheat flour was sieved through a 20 mm sieve then mixed with water and kneaded. Then dough was divided into equal sized balls and rolled individually in to chapatis. Then roasted on a heated pan on both sides till cooked completely. Mixed flours of wheat and pulses were sieved with little salt and then sufficient water was added to make soft pilable dough. The dough should not be too tight or too loose, and it should be non sticky at this stage. The dough was kneaded and made in to lemon sized balls. Each ball was rolled into thin circles and dusted with flour if required while rolling. Heated tawa was kept in medium flame and baked on both sides for few minutes till golden spots appear.

Subjects and ethical clearance for the study

Healthy, non diabetic individuals (5 males + 5 females for each food) aged 20–30 years and not under medication with a body mass index (BMI) of 21 ± 3 kg/m^2 participated in the study. The study was conducted as a random crossover study. Informed written consent was obtained from each individual. Ethical clearance was obtained from the Institutional Ethics Committee.

Estimation of whole blood glucose concentrations

The fingertips were pricked using the One Touch Ultra lancet device following fasting. The first drop of blood was placed onto the strip and a reading was taken (within 5–10 s) and recorded. The 50 g of standard glucose was given, after 15 min of glucose solution, blood glucose readings at 15, 30, 45, 60, 90, and 120 min intervals after taking the first bite were recorded for 3 days and followed by food for the fourth day.

Calculation of GI

Blood glucose curves were constructed from blood glucose values for each individual at 0–120 min for the control and test foods of each group. The incremental areas under the blood glucose response curve (IAUC) for a 50 g carbohydrate portion of each test food and control food (glucose) were calculated by the trapezoidal rule (modified FAO/WHO, 1998). The GI values were calculated by the method of Jenkins et al. [12]. Values were expressed as mean and standard deviation.

The blood glucose response curve of test foods and standard of each individual were calculated. The GI was calculated as a ratio between IAUC of test to that with the standard of the same individual [1].

Calculation of GL

The GL of food is a number that estimates how much the food will raise a person's blood glucose level after eating it. One unit of glycemic load approximates the effect of consuming one gram of glucose. Glycemic load was calculated by using the following formula: (Grams of carbohydrate in the food (serving size) × GI of the food)/100.

Statistical analysis

The Glycemic carbohydrate results were expressed as mean values ± standard deviations of three separate determinations. The Glycemic index and glycemic load data were subjected to a repeated measure of one way Analysis of Variance (ANOVA), and the significance of difference between means at 5 % was determined by using SPSS (Statistical package for Social Science) version 19.0.

Results and discussion

The results of glycemic carbohydrate content of the tested foods are presented in Table 1 and Fig. 1. From the Table, it is evident that the cereal such as rice, barley, and wheat had the higher levels of glycemic carbohydrate. The percent of

Table 1 Estimation of glycemic carbohydrates from commonly consumed foods

Food sample	Glycemic carbohydrates
Rice	79.22 ± 0.67
Wheat flour	63.26 ± 0.23
Barley	64.99 ± 0.21
Red gram dhal	51.90 ± 1.03
Green gram dhal	51.24 ± 1.72
Bengal gram whole	52.33 ± 1.29
Masoor dhal	52.52 ± 0.83
Chana dhal	56.22 ± 0.62
Wheat + chana (60:40)	49.94 ± 1.27
Wheat + chana + barley (40:30:30)	46.89 ± 0.22
Mixed dhal	40.09 ± 1.56

Each value is the average of triplicate determinations

±, one SD

glycemic carbohydrate showed in rice is 79.22 g, barley 64.99 g, and wheat flour 63.26 g, respectively. The legume cultivars and mixed product used in the present study are showing the lower glycemic carbohydrate concentration when compared with cereals. Among the legumes tested, the percent of glycemic carbohydrates ranged from 51.24 g in green gram to 56.22 g in chana dhal, and mix dhal had the lowest glycemic carbohydrate (40.09 g). The concentration of glycemic carbohydrates in mixed dhal and cereals in different ration is also tested. The percent of glycemic carbohydrate in wheat + chana dhal (60:40) showed 49.94 g and wheat + chana dhal + barley (40:30:30) 46.89 g, respectively. The combination of cereals and pulses resulted in the lower glycemic index and glycemic load values.

To our knowledge, this is the first study to report that, the analysis of glycemic carbohydrates in foods, which are digestible in the human gastrointestinal tract by using enzymes that mimic the human system by using modified Anthrone method. Any analytical procedure for glycemic carbohydrates must of necessity represent a compromise between the "ideal" procedure based on the known properties of the carbohydrates

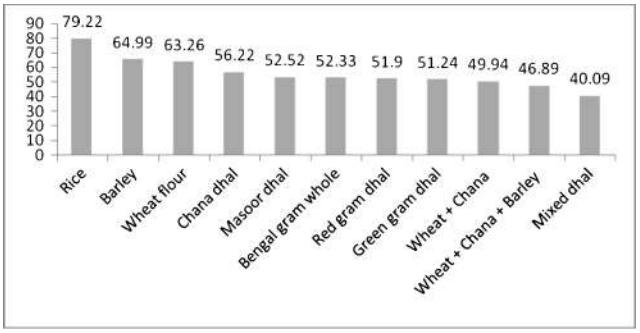

Fig. 1 Glycemic carbohydrates of commonly consumed foods (g/100 g)

and a practical laboratory procedure [11]. Vitaladasa & Belavady [12] have shown the importance of glycemic carbohydrates in normal and therapeutic diets. Babu Jaisingh & Ramesh [13] have shown the determination of fructose in the presence of certain proteins by a modified anthrone-sulfuric acid method. Casterline et al. [14] have reported that the total carbohydrates of 78.4 to 81.4 % in rice cocoa by treating with the same enzymes and analyzed by high performance liquid chromatography (HPLC) using refractive index (RI) detector.

The glycemic carbohydrates and total carbohydrate in different foods of Australia, Belgium, Bulgaria, Germany, Greece, Iceland, Italy, Lithuani, Poland, Portugal, Spain, and Turkey by following the method of by difference [15]. Barreira et al. [16] studied the sugars profile of different chestnut and almond cultivars by HPLC-RI. Miguez Bernardez et al. [17] have reported the HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain). Ellingson et al. [18] have developed a method for the direct determination of glycemic carbohydrates in low-carbohydrate products using high performance anion exchange chromatography. The glycemic carbohydrates in rice varieties ranges from 69.84 to 73.21 %, in vegetables from 1.72 to 9.42 %, and in legumes from 45.82 to 53.12 % which is in accord with the HPLC analysis wherein 74.01 to 80.02 % in rice, 1.24 to 8.24 % in vegetable, and 42.16 to 49.97 % in legumes, respectively [19].

Glucose concentrations obtained were used to calculate incremental area under curves (IAUC) for test foods and standards. GI were calculated as a ratio by using IAUC of test and standard values (Table 2). The processed food of serving sizes contains 50 g of glycemic carbohydrate. The GI and GL are presented in Table 2 and Fig. 2. From the table, it is evident that the cereal such as rice and wheat had the higher levels of GI than that of legume tested. The GI and GL showed in rice are 78.23 and 49.38, and wheat chapati 65.66 and 32.83, respectively. The legume cultivars and mixed product used in

Table 2 Glycemic index and glycemic load of different food samples

Name of foods	GI	GL
Rice	$78.23^{d} \pm 4.24$	$49.38^{d} \pm 2.67$
Wheat chapatti	$65.66^{b} \pm 4.22$	$32.83^{b} \pm 2.11$
Red gram	$43.01^{a} \pm 4.93$	$21.50^{a} \pm 2.46$
Green gram	$42.45^{a} \pm 4.05$	$21.22^{a} \pm 2.02$
Bengal gram	$37.95^{ac} \pm 5.73$	$18.97^{ac} \pm 2.86$
Masoor dhal	$42.15^{a} \pm 3.26$	$21.07^{a} \pm 1.63$
Mixed dhal	$43.64^{a} \pm 6.98$	$21.82^{a} \pm 3.49$
Wheat +chana dhal	$32.37^{c} \pm 9.10$	$16.18^{ce} \pm 5.30$
Wheat + chana dhal +barley	$39.27^{ac} \pm 5.20$	$19.63^{ae} \pm 6.33$

Values are mean and ±SD of three separate determinations. Values in the same row with different letters are significantly different ($P < 0.05$)

GI glycemic index, *GL* glycemic load

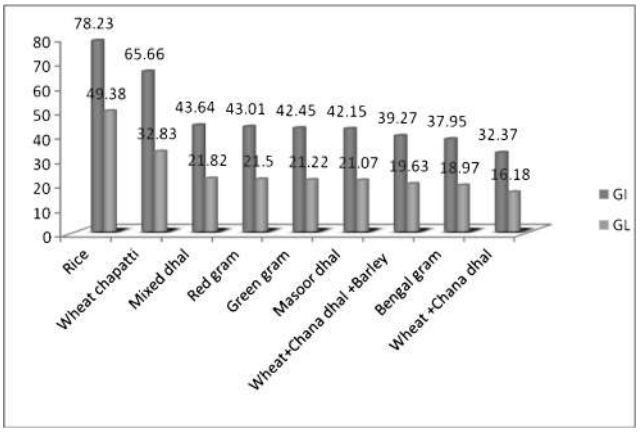

Fig. 2 Glycemic index (GI) and glycemic load (GL) of commonly consumed foods

the present study are showing the lower GI when compared with cereals. Among the legumes tested, the GI and GL are ranging from 43.64 and 21.82 in mix dhal to 37.95 and 18.97 in bengal gram. The wheat and chana dhal (60:40) had shown the lowest GI and GL (32.37 and 16.18). The GI and GL of mixture of wheat + chana dhal + barley meal (40:30:30) are also tested and the results of GI and GL are 39.27 and 19.63, respectively.

GI of the processed legumes such as African yam bean gave the lowest GI value of 17, while cowpea (white variety) gave the highest value of 41. The cowpea (white and black variety and brown variety) gave a value of 30 and 29, respectively, while pigeon pea (cream and brown variety) and groundnut yielded a value of 24 [20]. The mean GI of atta mix roti showed lower 27·3 value than the whole wheat flour roti 45·1. Bengal gram dhal, popularly called chana dhal has the lowest GI value of 16, followed by soy beans 18, kidney beans or rajma 27, chick peas 33, baked beans 48, and black grams 61. [21].

A recent study by the International Rice Research Institute (IRRI) concluded that the most widely consumed varieties of Indian rice have a comparatively low GI as compared to Basmati rice. Additionally, brown rice has only a 10–20 % lower GI value than white rice. A chapati made of wheat flour typically has a GI value of 67. A chick pea flour (besan) chapati scores the lowest—39 on GI scale as compared to maize chapati 89 and barley chapati 61 [22]. In a study, South-Indian snacks, Bisbelle Bhat and Pongal with Sambar elicited the lowest glycemic response, whereas current fast moving South-Indian snacks like Dosai with Podi and Idli with chutney showed the highest glycemic response. Among regional meals, the South- Indian whole meal displayed the lowest GI as compared to the Gujarati meal which showed the highest GI [23].

In another study, the rice has showed a wide range of GI values from as low as 54 to as high as 121. The variation in GI depends on region. The GI of the Indian basmati rice sample in this study was found to be 54.93 thus, placing it in low GI category [24]. Pirasath have reported the GI values of cooked white rice, brown rice, and parboiled rice were 66.61, 60.24, and 55.97, respectively, and also shown the mean glycemic index values of potato and cassava 65.2 and 78.7 [25]. Imran Khan [26] has shown the glycemic indices of pulses. The glycemic responses of conventional leguminous dishes including Mash (*Vigna mungo*), Moong (*Vigna radiata*), Masoor (*Lens esculenta*), Chana dhal (Cicer arietnum) and Biryani incorporated into the mixed meal and served with white boiled rice as conventionally consumed in Pakistan was studied in six normal and six diabetic subjects [27]. Zahra Aslani et al. [28] have studied the effect of lentil sprouts on glycemic control in overweight and obese patients with type 2 diabetes.

Conclusion

The present study demonstrates that modified anthrone method is applicable to determine the glycemic carbohydrates in different varieties of foods. This method can be used for routine analysis of all kinds of foods to generate glycemic carbohydrates content. To our knowledge, this is the first study to report that, the analysis of glycemic carbohydrates in foods, which are digestible in the human gastrointestinal tract by using enzymes mimic the human system. The GI of rice and wheat had the higher levels of GI than that of legume tested. The wheat and chana dhal (60:40) and mixture of wheat + chana dhal + barley meal (40:30:30) had shown the lowest GI and GL. From the results of the study it was concluded that pulses and mixture of pulses and cereals in different ratio have low GI and GL, hence, could be safely used in the diet of diabetic and obese patients. Therefore, when dietary advices are given to diabetic and coronary heart disease patients, not only the basic foods have to be considered but also the slide dishes to be consumed. Consumption of food containing fiber diet will significantly reduce the rise in blood sugar level.

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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.

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ORIGINAL ARTICLE

Seasonal variation of hemoglobin A1c levels in patients with type 2 diabetes

Lulu Hou[1,2] · Mei Li[2,3] · Xianping Huang[1] · Lulu Wang[1] · Pei Sun[1] · Rui Shi[4] · Lin Ding[1,2] · Shuguang Pang[5]

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Abstract The purpose of our study was to retrospectively examine the association between hemoglobin A1c (HbA1c) with season variation in patients with type 2 diabetes from the Chinese mainland. Monthly average of HbA1c values of type 2 diabetes patients in Jinan Central Hospital between 2008 and 2011 were respectively reviewed. The effects of sex, age, and season on HbA1c levels were analyzed. Moreover, a multiple linear regression analysis was performed to discuss the correlations between HbA1c levels and sex, age, and season. The HbA1c levels were significantly higher in winter (December, January, and February) and significantly lower in summer (June, July, and August). Significant difference was found for HbA1c between men and women in the elderly group ($P < 0.01$). The levels of HbA1c were similar in youth and middle groups ($P > 0.05$), but significantly lower in the elderly group ($P < 0.05$). Additionally, negative correlations of HbA1c with season, age, and gender were identified ($P < 0.01$). Our study suggests that cold weather may elevate the levels of HbA1c in type 2 diabetic patients in mainland China. However, more detailed studies are needed to reveal the variation of HbA1c with age and gender.

Keywords Age · Gender · Hemoglobin A1c · Seasonal variation · Type 2 diabetes

Background

Type 2 diabetes is a metabolic disordered disease that is resulted from defects in insulin secretion or insulin action [1]. Hemoglobin A1c (HbA1c) serves as a marker of cumulative glycemic exposure within the proceeding period of 2 to 3 months [2]. The American Diabetes Association has put forward the measurement of HbA1c in all diabetics at least two times each year. The most recently glycemic cutoff point set by the International Diabetes Federation to diagnose diabetes is an HbA1c level of >6.5 % [3].

HbA1c has been shown to be varied with seasons in diabetic patients in many countries. For example, studies from the UK [4], Japan [5, 6], the USA [7], Taiwan [8], and Sweden [9] have found that the HbA1c levels in winter ranged from 0.13 to 0.6 % which is higher than that in summer. Similarly, Tseng et al. [7] detected the HbA1c levels among US diabetic veterans, and found that HbA1c value was higher in winter and lower in summer with a difference of 0.22. Moreover, a previous study suggests that the lowest HbA1c levels are in warmer seasons and the highest levels in cooler seasons in both hemispheres [10]. Although many studies about seasonal variations of HbA1c have been carried out, differences were shown in geographical location, the temperature, and seasonal distribution in all countries and regions and the strength of conclusion was limited by sample size or study design.

Lulu Hou and Mei Li contributed equally to this work.

✉ Shuguang Pang
shuguang_pp@163.com

[1] Jinan Central Hospital Affiliated to Shandong University, Jinan 250013, Shandong Province, People's Republic of China

[2] Medical School of Shandong University, Jinan 250013, Shandong Province, People's Republic of China

[3] Railway Hospital of Yanzhou District, Yanzhou 272100, Shandong Province, People's Republic of China

[4] Department of Medicine, Taishan Medical University, Taishan, Shandong Province, People's Republic of China

[5] Endocrinology Department of Jinan Central Hospital Affiliated to Shandong University, No. 105, Jiefang Road, Jinan 250013, Shandong, People's Republic of China

In 2015, a study in a Taiwan population was designed to explore the impact of temperature on HbA1c in patients with type 2 diabetes [11]. To our knowledge, no study has been designed in the Chinese mainland. Therefore, in order to illuminate the association between HbA1c and season variation, data of HbA1c levels of type 2 diabetes patients from Jinan Central Hospital were enrolled in different seasons and were respectively reviewed.

Materials and methods

Study subjects

The study was approved by the ethic committee of Jinan Central Hospital. Data information of HbA1c levels in these patients selected from the hospital information system, including sex, age, disease, hospitalization date, and administration department, were retrospectively reviewed. Patients aged between 20 and 79 years from China were enrolled in the study.

As a result, HbA1c levels were measured 2188 times for 560 patients in 2008 (3.9 times per person), 2875 times for 723 patients in 2009 (4.0 times per person), 4341 times for 1065 patients in 2010 (4.1 times per person), and 5026 times for 1374 patients in 2011 (3.7 times per person) were selected. Totally, 3722 patients with 14,430 records (3.87 per person) on HbA1c from 2008 to 2011 were included in this study.

According to the age, patients were divided into three subgroups: the youth group (20–39 years), the middle group (40–59 years), and the elderly group (60–79 years). In addition, subgroup analyses were further stratified according to gender. Winter/spring was defined from December to May, and summer/autumn was defined from June to November.

The measurement of HbA1c

As previously reported, HbA1c was measured using high-performance liquid chromatography (HPLC, Bio-RadD10, Hercules, CA, USA) according to the principal of the ion exchange [12]. The HbA1c level that ranged from 4 to 6 % was considered to be normal.

Statistical analysis

Data were recorded as continuous values and presented as mean ± standard deviation (SD). The extracted data were imported to Microsoft Excel 2003 database and were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, USA). One-way ANOVA and univariate analysis were used to analyze the effects of gender, age, and season on HbA1c level. Independent samples *t* test was used to test sexual difference in each age group. Multiple linear regression analysis was used to evaluate the correlations between HbA1c and variables, including gender, age, and season. $P < 0.05$ was considered as significant difference.

Results

Influence of season on HbA1c

As shown in Table 1, the results showed that the HbA1c level was higher in winter from December to February and lower in summer from June to August, suggesting the level of HbA1 has significant seasonal variation ($P < 0.05$). The mean difference between the highest level in winter and the lowest level in summer was 1.17. In 2008, the first

Table 1 The seasonal distribution of HbA1c (x ± SD)

Month	2008		2009		2010		2011	
	HbA1c	No.	HbA1c	No.	HbA1c	No.	HbA1c	No.
1	7.33 ± 1.64	88	7.69 ± 2.15	213	7.24 ± 1.76*	430	7.24 ± 1.81*	381
2	7.47 ± 2.05*	82	8.05 ± 2.03*	304	7.45 ± 1.92*	351	7.85 ± 2.39*	321
3	7.13 ± 1.70	199	7.61 ± 2.06	232	7.12 ± 1.80	480	6.72 ± 2.01	494
4	7.04 ± 1.69	194	7.54 ± 2.30	210	7.16 ± 3.47	279	6.72 ± 1.90	501
5	7.17 ± 1.68	205	7.54 ± 1.99	175	7.20 ± 1.64	414	6.73 ± 1.99	467
6	7.32 ± 2.01	198	7.28 ± 1.91	259	7.09 ± 1.93	332	6.86 ± 2.13	406
7	7.27 ± 1.68	152	7.27 ± 1.81	319	6.83 ± 1.65	243	7.01 ± 1.74	351
8	6.75 ± 1.92*	268	7.09 ± 1.98*	133	6.60 ± 2.17*	439	6.69 ± 2.11*	271
9	7.09 ± 1.72	203	6.88 ± 1.74*	357	6.93 ± 1.69*	301	7.01 ± 1.68	352
10	7.42 ± 1.88	222	7.50 ± 1.87	191	7.08 ± 1.70	341	7.00 ± 1.70	574
11	7.39 ± 1.63	203	7.35 ± 1.97	245	7.07 ± 1.82	409	7.12 ± 1.86	495
12	7.57 ± 2.15*	174	7.67 ± 2.05	237	7.34 ± 1.93	322	7.21 ± 1.82	413
Total	7.21 ± 1.83	2188	7.45 ± 2.00	2875	7.09 ± 1.99	4341	7.10 ± 1.95	5026

* $P < 0.05$ represents a statistical difference concerning monthly averaged HbA1c levels

Table 2 The gender and age distribution of HbA1c ($x \pm SD$)

Age (years)	2008		2009		2010		2011	
	HbA1c	No.	HbA1c	No.	HbA1c	No.	HbA1c	No.
Men								
20–39	7.72 ± 2.34	49	7.85 ± 2.53	76	7.44 ± 2.76	116	7.56 ± 2.51	140
40–59	7.48 ± 1.87	369	7.64 ± 2.21	551	7.30 ± 1.99	748	7.24 ± 2.10	939
60–79	6.99 ± 1.66$^{\#}$	558	7.29 ± 1.94$^{\#}$	712	6.96 ± 1.72	1057	6.83 ± 1.78$^{\#**}$	1194
Total	7.21 ± 1.80	976	7.46 ± 2.10	1339	7.12 ± 1.91	1921	7.04 ± 1.98	2273
Women								
20–39	8.34 ± 2.24	22	7.61 ± 2.77	43	6.73 ± 2.09	58	7.22 ± 2.36	66
40–59	7.48 ± 1.87	328	7.69 ± 2.12	408	7.25 ± 2.05	566	7.29 ± 2.30	716
60–79	7.35 ± 1.67$^{\#}$	509	7.60 ± 1.90$^{\#}$	626	7.33 ± 2.46	895	7.06 ± 1.79$^{\#}$	1053
Total	7.39 ± 1.71*	859	7.63 ± 2.03*	1077	7.28 ± 2.30*	1519	7.06 ± 1.79*	1835

$^{*}P < 0.05$ and $^{**}P < 0.01$ in women compared with the level of HbA1c in men; $^{\#}P < 0.05$ in the elderly group compared with the youth or middle groups both in men and women

two high values were noted in December and February (7.57 ± 2.15 and 7.47 ± 2.05), and the lowest value was observed in August (6.75 ± 1.92). In 2009 and 2010, the highest levels were observed in January (7.69 ± 2.15 vs. 7.24 ± 1.76) and February (8.05 ± 2.03 vs. 7.45 ± 1.92), respectively. In addition, the lowest values were in August (7.09 ± 1.98 vs. 6.60 ± 2.17) and September (6.88 ± 1.74 vs. 6.93 ± 1.69), respectively. In 2011, the first two higher HbA1c values appeared in January and February (7.85 ± 2.39 and 7.24 ± 1.81), and the lowest value was in August (6.69 ± 2.11).

Distribution of HbA1c in subgroups stratified by gender or age

Some characteristics of the gender- and age-specific distributions of HbA1c in the 11,799 subjects were shown in Table 2. There were significant differences in HbA1c level according to gender ($P < 0.05$). In 2008, the total HbA1c levels of men and women were 7.21 ± 1.80 and 7.39 ± 1.71, respectively. In 2009, the levels were 7.46 ± 2.10 in men and 7.63 ± 2.03 in women, 7.12 ± 1.91 in men vs. 7.28 ± 2.30 in women in 2010, and 7.04 ± 1.98 vs. 7.16 ± 2.03 in 2011. However, no significant difference was found on HbA1c level stratified by gender. As for the age of the group >60, higher HbA1c level in women was observed compared with that in men with significant difference ($P < 0.01$). With regard to subgroup analysis stratified by age, the HbA1c level in the elderly group was significantly lower than the level in both the middle and youth groups either in men or women ($P < 0.05$, Table 2). There was no significant difference on HbA1c level between the youth and the middle groups. Although there was no obvious difference among each age group ($P = 0.136$) in 2010, the levels of HbA1c in the youth (7.23 ± 0.16) and the middle group (7.29 ± 0.06) were still slightly higher than that of the elderly group (7.14 ± 0.05).

The association of HbA1c with season, age, and gender

The result of multiple linear regression analysis of HbA1c with gender, age, and season was shown in Table 3. Gender, age, or season were all negatively associated with HbA1c level with standardized regression coefficients −0.050, −0.068, and −0.040, respectively ($P < 0.01$).

Discussion

In the present study, we found that the average levels of HbA1c were higher in winter and lower in summer in patients

Table 3 The association of gender, age, and season with HbA1c (coefficients)

Model	Unstandardized coefficients		Standardized coefficients	*t*	Sig.
	B	Std. error	Beta		
1 (constant)	8.117	0.093		87.307	0.000
Sex	−0.163	0.037	−0.040	−4.362	0.000
Age	−0.233	0.032	−0.068	−7.388	0.000
Time	−0.030	0.006	−0.050	−5.423	0.000

Dependent variable: HbA1c; male = 1. B: progression coefficients; Sig: *P* value <0.01 was considered to be significant

with type 2 diabetes. In patients >60 years, HbA1c levels were significantly lower in men compared with women. Moreover, the level was significantly lower in elderly patients compared with the youth or middle group both in men and women. In addition, the multiple linear regression analysis has suggested negative correlations of HbA1c with gender, age, and season.

Several studies have demonstrated a seasonal variation for HbA1c, which is higher in cooler months and lower in warmer months in many countries [7, 10, 13–15]. In our study, the results indicated the same tendency. The mechanism of HbA1c changes caused by season remains unclear. Several researches have reported that body weight [16–18], fat intake [19, 20], and weight loss [20] have significantly seasonal fluctuations and contribute to HbA1c variation. Moreover, it has been reported that colder temperatures would introduce an increase in dietary intake and decrease in outdoor activity in diabetic patients [21]. In addition, frequency of physical activity was also reported associated with HbA1c level [22]. Bardini et al. [23] have evaluated the seasonal lipid variations in type 2 diabetic patients, and they found that total cholesterol and low density lipoprotein cholesterol are increased during cooler months and associated with higher calorie intake and reduced physical activity. Moreover, the concentration of serum 25-hydroxy vitamin D and serum melatonin may also play roles in diabetes [24, 25]. Thus, the level of HbA1c is higher in summer and lower in winter, which may be related to the multiple possible reasons such as fat intake and physical activity. However, further explorations of these possibilities are still needed.

Furthermore, it is widely accepted that the decline of personal vitality and increased susceptibility to disease are closely associated with age. Thus, age-associated diabetes ranging from asymptomatic to death is considered as immutable and intrinsically human [26]. Previous study in healthy non-diabetic adults has shown that there is no significant sex difference for HbA1c levels except for the group age >60 years, and the distribution is approximately normal at all ages in both sexes [14]. It has been demonstrated that there is an increase of HbA1c with aging in non-diabetic adults aged from 50 to 89 and in subjects with normal glucose tolerance [27, 28]. Previous studies have suggested that low level of testosterone plays a major role in the development of insulin resistance and subsequent type 2 diabetes [29]. The general trend of plasma testosterone is decreased with age, while the trend is inversed in the group with age >60. Recently, Kamezaki et al. [30, 31] have examined the seasonal variations in the prevalence of metabolic syndrome in Japanese subjects, and put forward that increased blood pressure and glucose during winter might be related with elevated metabolic syndrome. Additionally, it has been suggested that the seasonal variation in metabolic syndrome is mildly or moderately related to increased insulin resistance in middle-aged (40–65 years) Japanese men [31]. In accordance with the above studies, no significant sex difference was observed in type 2 diabetes in the middle and youth groups, except for elderly patients (>60 years). Thus, we believe age and gender might not be the main influence factor for HbA1c level variation among middle and youth patients. However, the conclusion should be confirmed by designing the study, taking covariates into consideration such as body weight, severity of diabetes, and types of anti-diabetic therapy.

Conclusions

In conclusion, our study confirms a seasonal variation in HbA1c levels in patients with type 2 diabetes. These effects may be due to the climate because of higher HbA1c levels in winter and lower levels in summer. These results may be valuable for epidemiologic studies and health services research. However, large population studies are still needed to investigate seasonal variation of HbA1c levels in the future.

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Compliance with ethical standards

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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

Prediabetes among Nigerian adolescents: A School-based study of the prevalence, risk factors and pattern of fasting blood glucose in Ibadan, Nigeria

Oluwakemi Arigbede[1] · Ikeola Adeoye[1] · Olumide Jarrett[2] · Oyindamola Yusuf[1]

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Abstract Prediabetes and type 2 diabetes (T2DM) are emerging public health challenges in sub-Saharan Africa which have been given little research focus among adolescents. The behavioural and cardiometabolic factors that drive these conditions have hardly been documented among adolescents in Nigeria. A cross-sectional study was conducted to investigate the prevalence and risk factors of prediabetes among 500 in-school adolescents and their fasting blood glucose pattern in Ibadan, Nigeria. Potential factors including blood pressure, anthropometric measurements and fasting blood glucose (FBG) levels were assessed. Prediabetes was defined as FBG between 100 –125 mg/dl. Data were analyzed using descriptive statistics and bivariate logistic regression at 5 % level of significance. The overall prevalence of prediabetes among the adolescents was 4.0 % 95 % CI (2.2–5.7 %) and the mean FBG of adolescents was 85.3 ± 8.2. Males compared to females had significantly higher levels of FBG—mean difference [1.65:95 % CI (0.017–3.14) $p = 0.03$]. Factors that increased the odds for prediabetes included frequent consumption of carbonated drinks (OR = 1.45; 95 % CI 0.46–3.30; p = 0.48), attending a private school (OR = 2.58; 95 % CI 0.77–9.0; $p = 0.66$) elevated blood pressure (OR = 2.04; 95 % CI 0.57–7.35; $p = 0.57$) and being overweight or obese (OR = 2.91; 95 % CI 0.38–22.3; $p = 0.30$). Correspondingly, while those who skipped breakfast [1.29;95 % CI (−0.23;−2.8) $p = 0.096$] had higher FBG, those who walked daily back from school [−2.07;95 % CI (−3.55; − 0.59) $p = 0.01$] had significantly lower FBG. Prediabetes and risk factors are prevalent among the secondary school adolescents in Ibadan. Surveillance of potential risk factors through school-based screening among adolescents is crucial for prevention and early intervention.

Keywords Prediabetes · Fasting blood glucose · Prevalence · Risk factors · Adolescents · Ibadan

Introduction

Diabetes Mellitus (DM) has been described as a public health challenge of the twenty-first century [1, 2] with a reported global prevalence of 8.3 %, which translates to about 387 million people living with diabetes across the world of which 46.3 % remained undiagnosed [3]. DM used to be uncommon in sub-Saharan Africa few decades ago, but it is now increasing rapidly [4]. An estimated 14.2 million adults aged 20–79 years were reported to have DM in the region in 2015 and this has been projected to increase to 34.2 million by 2040 [3]. Even though, the epidemic is in its early phases in Africa, its drivers include rapid urbanization, ageing, reduced physical activity and unhealthy diet seem prevalent. Unfortunately, Nigeria accounts for the highest number of people with diabetes and impaired glucose tolerance (with approximately 1.6 (1.2–3.8) million and 3.85 million people affected, respectively [2, 5]. Prediabetes or impaired glucose tolerance, defined as the presence of elevated fasting blood glucose within the range of 100–125 mg/dl [6] is the intermediate and reversible stage in the natural history of type 2 diabetes (T2DM) [7, 8].

Increasingly, T2DM has been reported among adolescents especially in the developed countries [6, 9–12] a pattern that

✉ Ikeola Adeoye
adeoyeikeola@yahoo.com

[1] Department of Epidemiology and Medical Statistics, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria

[2] Department of Pediatrics, Faculty of Clinical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria

has begun to emerge in developing countries as well [4, 7, 13, 14]. The increase in prediabetes/T2DM among adolescents has been attributed to the rise of behavioural and cardiometabolic risk factors. For example, in the USA, prediabetes has been associated with childhood obesity [9]. T2DM is a chronic debilitating disease associated with severe complications such as kidney failure, blindness and cardiovascular disorders like coronary heart disease, stroke and peripheral arterial disease which poses severe risks for families, member states and the entire world. [12]. The progression of these complications is worse when the illness starts from childhood or adolescence [15]. More so, the onset of T2DM is insidious and asymptomatic with high prevalence of undiagnosed diabetes (50–85 %) being reported in both developed and developing countries [16]. Therefore routine screening becomes crucial for prevention, early detection and treatment because prediabetes is often a predictor of future development of T2DM [14] and the implication of late diagnosis of prediabetes/T2DM in the adolescent might be grave [17, 18].

The prevalence of DM/prediabetes among the adolescent population has been poorly documented in Nigeria [14]. Bassey and her colleagues while screening in-school adolescents for DM carried out a cross-sectional study among 1008 adolescents aged 10 to 18 years in 12 secondary school in Port Harcourt [19]. Although urine glucose was used as a screening test, fasting blood glucose (FBG) is the recommended test by the World Health Organization (WHO) because of its higher sensitivity and specificity [20]. Screening of adolescents for DM or prediabetes is a cost-effective strategy for early prevention and management [21]. WHO described school health as "a strategic means to prevent important health risks among the youth". Furthermore the WHO global school health initiative stated that "Research in both developed and developing countries demonstrates that school health programmes can simultaneously reduce common health problems, increase the efficiency of the educational system and advance public health education and social and economic development of a nation" (WHO). Schools can create a platform for screening children and adolescents for hyperglycemia, elevated blood pressure risk factors for other chronic disease [5]. Therefore, we conducted a school-based study to determine the pattern of (FBS), the prevalence and risk factors of prediabetes among adolescents in Ibadan, Nigeria.

Materials and methods

Setting and study design

The study was conducted in Ibadan, Oyo State in southwestern Nigeria, the third largest city in Nigeria after Lagos and Kano with 11 local government areas (5 urban and 6 semi-urban). The cross-sectional study was conducted in Ibadan South West Local Government Area (ISWLGA), an urban LGA with 81 government-approved secondary schools: 52 private owned and 29 public secondary schools. The Ethical Review Committee of the Oyo State Ministry of Health approved the study protocol. Permission to carry out the study was obtained from the Ministry of Education, Oyo State and the authorities of each of the selected school. Informed consent as obtained from the parents/guardian while the study participants gave their assent before data collection commenced. The sample size estimation for single proportion was used as estimated proportion of T2DM of 3.6 % among adolescents; the precision level of 2.5 %; $a = 0.05$; and a design effect of 2. A two-stage cluster sampling technique was used to recruit 500 in-school adolescents within the ages of 10 and 19 years. A total of ten schools were randomly selected from a list of all the schools in the LGA. Fifty students were selected from each school. Exclusion criteria for intending participants included students that were severely ill at the time of study and an unwillingness to participate in the study either by students or parents. The study was conducted between October and December 2014.

Diagnosis of type 2 diabetes

The WHO diagnostic criteria for the diagnosis of T2DM was used to assess the glycemic levels of the study participants using the FBG levels. The FBG level was obtained using a accu-check glucometer (active model:GU, serial no:GU03510010). Blood glucose metre reading was expressed as milligrams per deciliter. The study participants were categorized by their blood glucose levels as follows according to the basic training manual for healthcare professionals in developing countries: normal level <126 mg/dl (7 mmol/l), hypoglycemic 60 mg/dl (<3.3 mmol/l), impaired fasting glycemia 100–125 mg/dl and hyperglycemic >126 mg/dl (>7 mmol/l).

Data collection

Research assistants were trained on collection of data using pretested semi-structured questionnaires. Information was collected on seven broad items: sociodemographic characteristics, dietary pattern, physical activities, family history, fasting blood glucose, blood pressure, and anthropometric measurements of weight, height and family history of DM. Dietary habit was accessed using a 7-day food frequency questionnaire. The habit were classified as: Never: Ate the food 0 time in the past 7 days; Rarely: Ate the food 1–2 times in the past 7 days; Occasionally: Ate the food 3–4 times in the past 7 days; Frequently: Ate the food 5–7 times in the past 7 days. Physical activity was assessed by the average time spent on physical activity in the past 7 days. It was classified as: None: 0 days of Physical activity; Insufficient: 1–2 days of at least 60 min of moderate physical activity daily; Sufficient: 4 or more days of at least 60 min of moderate physical activity daily. Sedentary behaviour was

measured by the average time spent on watching television (t.v) and playing video games daily. This was classified as present if participants reported more than 2 h of t.v viewing daily and absent if it was less than 2 h daily.

Anthropometric and clinical measurement

Anthropometric and blood pressure measurements were taken by investigators and research assistants who received adequate training in these procedures.

Weights were measured using a digital weighing scale: (Harson Emperor) after checking for zero error at each measurement and the reading was taken to the nearest 0.1 kg. Heights were measured with an Altimetro tape height measured to the nearest 0.5 cm with the subjects barefoot or with socks, standing erect with heels together and looking straight ahead; two measurements were required in order to reduce error and therefore obtain a more accurate calculation of BMI. The BMI for age was calculated by imputing the necessary information into WHO Anthro plus software version 1.0.4. WHO Anthro plus is a World Health Organization software for personal computers; it was developed to monitor the growth of school-age children and adolescents. Three indicators that are included in Anthro plus are weight-for-age, height-for-age and BMI-for-age. Blood pressure (BP) measurements were taken by using a digital sphygmomanometer (Omron). Three BP readings were obtained at a 5-min interval between readings, and the mean was recorded as the subject's blood pressure reading.

Statistical analysis

The dependent variables were prediabetic status (categorized into two groups) and mean FBS. The explanatory variables were sociodemographic and lifestyle characteristics and the cardiometabolic parameters. Frequency tables were used to present relevant variables. Quantitative variables were summarized with means and standard deviation while categorical variables were summarized with proportions and percentages.

The overall prevalence of prediabetes as well as age and sex-specific prevalences were determined. Univariate logistic regression analyses were used to determine the factors associated with prediabetes odds ratios and 95 % CI were also presented. Mean differences and 95 % CI in the mean FBS by patient characteristics were compared using independent *t* test or one-way ANOVA as appropriate.

Results

Characteristics of the adolescents (Table 1)

The mean age of study participants was 14.6 ± 1.54 years and 46.4 % were males, 63.9 % were Christians and above 60 % of both parents had post-secondary education—father (65.3 %) and mother (61.3 %). Majority were of Yoruba ethnic group (81.0 %) and came from small family size, i.e. having less than four children (81.2 %). Two thirds attended private schools.

According to their lifestyle characteristics, about a third reported frequent consumption of fruits (27.2 %), 20.0 % took carbonated soda drinks more than five times a week, 31.0 % reported insufficient physical activity, 39.4 % walked to school daily and 71.8 % walked back from school daily. On average, the weight (52.4 ± 10.1), height (1.72 ± 0.09), SBP (116.6 ± 13), SBP (63.9 ± 13.2) and mean BMI for age (17.6 ± 2.95).

Prevalence of prediabetes (Table 2)

There was one self-reported case of diabetes which was excluded from the analysis. The overall prevalence of prediabetes among the adolescents was 4.0 % 95 % CI (2.2–5.7) with a higher sex-specific rate among males 4.5 % 95 % CI (1.7–7.2) compared to females 3.5 % 95 % CI (1.2–5.8). The prevalence was also higher in the older age category.

Factors associated with prediabetes

Table 3 presents the unadjusted odds ratios and 95 % confidence intervals of factors associated with prediabetes. Females (OR = 0.77; 95 % CI 0.31–1.93; $p = 0.58$), respondents with sufficient physical activity (OR = 0.97: 95 % CI 0.77–9.0: p = 0.96) and those who reported frequent intake of fruits (OR = 0.8; 95 % CI 0.46–3.30 $p = 0.66$) had a reduced risk of having prediabetes.

On the other hand, adolescents attending private schools were more than 2 times more likely to have prediabetes compared with those in public school. (OR = 2.58; 95 % CI 0.77–9.0; $p = 0.15$). Similarly, those who consumed carbonated drinks frequently had 45 % increased risk compared with those who did not (OR = 1.45; 95 % CI 0.51–4.16; $p = 0.48$). Having elevated blood pressure (OR = 2.04; 95 % CI 0.57–7.35; $p = 0.57$) and being overweight or obese (OR = 2.91; 95 % CI 0.38–22.3: $p = 0.30$) were associated with a higher risk of having prediabetes.

Mean differences in fasting blood glucose by adolescent characteristics (Table 4)

The mean blood glucose of the adolescent was 85.3 ± 8.2. Males had significantly higher mean fasting blood glucose (FBG) compared to females with the mean difference of 1.65:95 % CI (0.017–3.14) $p = 0.03$. Pupils who reported frequent ingestion of carbonated drinks compared with those who did not had higher FBS levels–mean difference of −2.42;95 % CI (−4.3; 5.1) $p = 0.01$.

Conversely, those in public school compared with private schools −2.64;95 % CI (−4.2;−1.06) $p = 0.01$; those who

Table 1 Characteristics of adolescents by sociodemographic and lifestyle factors in Ibadan

Characteristic	Prediabetes		Total
	Yes (19)	No (457)	
Sociodemographic factors			
Age group			
10–14	9 (3.8)	229 (96.2)	238 (50.3)
15–19	10 (4.3)	225 (95.7)	235 (49.7)
Sex			
Male	10 (4.5)	211 (96.5)	221 (46.4)
Female	9 (3.5)	246 (96.5)	255 (53.6)
Fathers' education			
Secondary and below	7 (4.8)	140 (95.2)	147 (34.6)
Post-secondary	10 (3.6)	268 (96.4)	278 (65.4)
Mothers' education			
Secondary and below	5 (2.9)	166 (97.1)	171 (38.7)
Post-secondary	11 (4.1)	260 (95.9)	271 (61.3)
Religion			
Christians	11 (3.6)	292 (96.4)	303 (63.9)
Non-Christians	8 (4.7)	163 (95.3)	171 (36.1)
Ethnicity			
Yorubas	15 (3.9)	369 (96.1)	384 (81.2)
Non-Yorubas	4 (4.5)	85 (96.5)	89 (18.5)
Number of Siblings			
<4	14 (4.1)	329 (95.9)	362 (74.5)
≥4	4 (3.3)	116 (96.7)	124 (25.5)
School type			
Public	3 (2.0)	149 (98.0)	152 (31.9)
Private	16 (4.9)	308 (95.1)	354 (68.1)
Lifestyle characteristics (diet and physical activity)			
Frequent fruits and vegetables			
>5 days per week	6 (4.72)	121 (95.2)	127 (27.2)
≤5 days per week	13 (3.83)	326 (96.1)	339 (72.8)
Frequent carbonated drinks			
>5 days per week	5 (5.5)	86 (94.5)	91 (20.0)
≤5 days per week	14 (3.8)	351 (96.2)	365 (80.0)
Physical Activity			
Insufficient	6 (4.1)	142 (95.9)	148 (31.0)
Sufficient	13 (4.0)	315 (96.0)	328 (69.0)
Walked to school daily			
Yes	5 (2.7)	181 (97.3)	186 (39.4)
No	14 (4.9)	274 (95.1)	288 (60.6)
Walked from school daily			
Yes	14 (3.80)	354 (96.2)	368 (71.8)
No	5 (4.8)	100 (95.2	105 (22.2)
Cardiometabolic parameters			
Weight (kg)	51.6 ± 7.8	52.3 ± 10.2	52.4 ± 10.1
Height (m)	1.69 ± 0.09	1.72 ± 0.10	1.72 ± 0.09
SBP	118.7 ± 10.8	116.6 ± 10.5	116.6 ± 13
DBP	65.6 ± 9.8	64.1 ± 13.2	63.9 ± 13.2
BMI	17.9 ± 2.06	17.6 ± 3.00	17.6 ± 2.95

Table 2 Prevalence and 95 % confidence intervals of prediabetes among Nigerian adolescents by age and sex

	Total	Prediabetes (%)	Normal (%)
All	476	4.0 (2.2–5.7)	96 (94.2–97.7)
Age			
10–14	473	3.8 (1.3–6.2)	96.2 (93.7–98.7)
15–19		4.3 (1.7–6.8)	95.6 (93.1–98.3)
Sex			
Male	476	4.5 (1.7–7.2)	95.4 (92.7–98.2)
Female		3.5 (1.2–5.8)	96.4 (94.1–98.7)

walked back to their homes after school every day −2.07:95 % CI (−3.55; − 0.59) $p = 0.01$ also had a significantly lower FBG.

Discussion

Diabetes mellitus and prediabetes have become an emerging public health problem among children and adolescents in developed and now in developing countries [6, 7]. Although we set out to investigate diabetes among in-school adolescents, the occurrence of diabetes was negligible (0.2 %) among the study population; hence, we investigated the prevalence and risk factors of prediabetes and the FBG pattern among in-school adolescents in Ibadan. An earlier attempt to investigate diabetes mellitus and prediabetes among secondary school students in Nigeria was conducted by Bassey and her co-workers [19] in Port-Harcourt in which students were screened using urine glucose. The prevalence of glycosuria in their study was 0.7 % which was likely to be an underestimate because of its low sensitivity. FBG is the preferred method of screening for DM which was used in our study [22]. To our knowledge, this study is likely the first to determine the prevalence of prediabetes and its risk factors among adolescents in south western, Nigeria using FBG. In spite of the fact that the traditional risk factors associated with abnormal glucose metabolism have been described, within-country studies are crucial for locally targeted public health interventions. This is because recent evidence shows that there are significant within- and across-country variations in both social and behavioural determinants of prediabetes and diabetes justifying the need for locally generated data to inform policy and interventions [23, 24].

Table 3 Risk factors of prediabetes Adolescents (crude odds ratios and 95 % confidence intervals)

Factors	Unadjusted odds ratios	95 % confidence intervals	*p* value
Age			
10–14	1.00	-	0.79
15–19	1.13	(0.44–2.84)	
Sex			
Male	1.00	-	0.58
Female	0.77	(0.31–1.93)	
School type	2.58	(0.77–9.0)	1.49
Private	1.00	-	
Public			
Physical activity			
Insufficient	1.00	-	0.96
Sufficient	0.97	(0.77–9.0)	
Frequent fruits and vegetables			
Yes	1.00	-	
No	1.24	(0.46–3.30)	0.66
Frequent carbonated drinks			
Yes	1.45	(0.51–4.16)	0.48
No	1.00	-	
BMI status			
Underweight	1.00	-	
Normal	2.26	(0.51–4.16)	0.57
Overweight	2.91	(0.38–22.3)	0.30
Blood pressure			
Normal	1.00	-	0.57
Elevated	2.04	(0.57–7.35)	

Table 4 Mean differences and 95 % confidence intervals of fasting blood glucose of adolescent characteristics

	Total	Mean FBS ± SD	Mean difference and 95 % CI	*p* value
All	476	85.3 ± 8.2 95%CI (84.6–86.1)		
Sex				
Male	476	86.2 ± 7.9	1.65 (0.17–3.14)	0.03
Female		84.6 ± 8.5		
Age				
10–14	473	85.4 ± 8.6	−0.047 (−1.45;1.54)	0.95
15–19		85.3 ± 8.0		
School type				
Public	473	83.5 ± 8.2	−2.64 (−4.2; −1.06)	0.01
Private		86.2 ± 8.2		
Physical activity				
Insufficient	476	84.9 ± 8.7	−0.68 (−2.29; 0.92)	0.40
Sufficient		85.5 ± 8.1		
Daily walk to School				
Yes	474	84.4 ± 7.3	−1.35 (−2.87; −0.17)	0.08
No		85.8 ± 8.8		
Daily walk from school				
Yes	470	84.4 ± 8.1	−2.07 (−3.55;-0.59)	0.006
No		86.5 ± 8.3		
Frequent fruits and vegetables				
>5 days per week	466	85.3 ± 8.7	−0.24 (−1.94; 1.45)	0.78
≤5 days per week		85.5 ± 8.1		
Frequent carbonated drinks				
>5 days per week	456	87.4 ± 8.3	−2.42 (−4.3; 5.1)	0.01
≤5 days per week		85.0 ± 8.1		
Skip breakfast				
Never skips	463	84.7 ± 8.6	1.29 (−0.23; −2.8)	0.096
skips		86.0 ± 8.0		
DM in first-degree relatives				
Yes	443	88.2 ± 9.8	2.83 (−1.28;6.96)	0.18
No		85.3 ± 8.2		
BMI				
Normal	463	85.6 ± 7.4		0.89
Overweight		86 ± 6.2		($F = 0.11$)
Underweight		85.3 ± 8.6		
Blood pressure				
Normal	460	84.9 ± 7.8		0.167
Prehypertension		86.4 ± 8.4		($F = 1.8$)
Hypertension		84.5 ± 10.9		

The overall prevalence of prediabetes was 4.0 % 95 % CI (2.2–5.7) with a higher occurrence among males compared to females. Expectedly, studies from developed countries have reported higher prevalence of diabetes and impaired blood glucose compared to developing countries [6, 25]. In the USA, Li and his colleagues [6] using the data of the National Health and Nutrition Examination Survey 2005–2006, estimated a national prevalence of prediabetes as 16 % among the US adolescents. Earlier studies which investigated prediabetes were restricted to obese adolescents because of their increased risk for prediabetes. Shina et al. in the New England Journal [9] reported a 21 % prevalence of impaired glucose tolerance among adolescents attending an obesity clinic in the USA. Generally, there is a paucity of data on prediabetes among adolescents although studies have begun to emerge even in developing countries. For example, an Indian study that estimated the prevalence of prediabetes among school-going children, reported a prevalence of 3.7 % which is similar to our finding [12].

The risk factors of prediabetes investigated in this study were sex, physical activity level, diet which was assessed by the consumption of fruits and vegetables and regular intake of carbonated drinks, BMI status and blood pressure. Notably, none of these risk factors was found to be statistically significant. Likewise, the Indian researchers also did not observe any statistically significant association with major risk factors of diabetes (18). However, this does not preclude the clinical or public health relevance of the findings. The awareness of the relevant risk factors of prediabetes, a reversible condition, for a particular population is crucial for implementing necessary interventions. Gilles et al. [26] in a systematic review that evaluated interventions to delay or prevent T2DM in individuals with prediabetes, noted that lifestyle and drug interventions reduced the rate of progression to T2DM in persons with impaired glucose tolerance. Moreover, lifestyle interventions like weight reduction was as effective as drug treatment [26].

The rise in the occurrence of T2DM and its precursor prediabetes in the paediatric age group has been strongly linked to childhood obesity epidemic in the developed countries. Earlier studies were conducted among obese children who were most at risk [27]. In this study, there was a graduated increase in the odds of having prediabetes from normal (OR = 2.26) and overweight/obese (OR = 2.91) compared with those who were underweight. Researchers have noted that severe obesity has a prominent role in the pathogenesis of T2DM in children and adolescents [15, 28]. Ordinarily, the pathophysiology of DM which leads to abnormalities in glucose metabolism is underlined by changes in the sensitivity and secretion of insulin which changes are increased with obesity. From a practical standpoint, intervention that promote lifestyle changes like healthy diet and physical activity are also crucial in the control of T2DM and prediabetes.

Healthy diet, though a very broad concept, was measured by the frequent consumption of fruits and vegetables in this study, and one in every four adolescents reported the frequent consumption of fruits and vegetables; the practise led to a 20 % reduction in the risk of prediabetes. Carter et al. [28] in a study carried out to investigate the independent effects of intake of fruit and vegetables on incidence of type 2 diabetes reported that the greater intake of green leafy vegetables was associated with a 14 % reduction in risk of type 2 diabetes.

On the other hand, the frequent ingestion of carbonated drinks (CD) was associated with a higher likelihood of having prediabetes (OR = 1.45) and a higher fasting blood glucose levels on average compared with those who took these drinks less frequently. Carbonated drinks commonly called soft drinks or glucose-sweetened beverages are consumed all over the world but probably more commonly among children and adolescents. In our study, 20 % of the adolescents reported a regular consumption of these drinks, a proportion much lower compared to what obtains in the developed world. Harnack et al. [29] reported that 70% of adolescents in the UK consumes soft drinks on a regular basis. Carbonated drinks have high glycaemic index and are energy dense because they have a high content of free glucose, and a regular consumption will result in a positive energy balance. Recently, carbonated drinks have been implicated in a number of health conditions, namely obesity- and weight-related issues (importantly, obesity epidemic among children in the developed countries), tooth decay and osteoporosis. Mozaffarri and her colleagues [30] from the Harvard School of Public Health using data from the 2010 Global Disease burden estimated that 180,000 deaths annually are attributed to the regular consumption of glucose-sweetened beverages which deaths were linked to diabetes, cardiovascular diseases and cancers. Very few studies have investigated their association with diabetes or prediabetes in children or adolescents [31, 32]. WHO recommends that free glucoses should not be more than 10 % of daily energy intake [33]. James et al. [27] in their clustered randomized controlled trial aimed at reducing the consumption of carbonated drinks among 644 children aged 7–11 years in six primary schools in South England reported that school based educational programmes that discouraged CD led to the reduction in overweight and obesity among school children for that year. The implication of this is that the school can provide an effective platform for the control of the risk factors.

In our study, attending a private school was positively associated with prediabetes (OR = 2.58) they also had a higher levels of fasting blood glucose on average compared with adolescents who attended public schools. The plausible explanation for this difference is the variation in their socioeconomic status. In our study, the pupils who attended private secondary schools belonged to a higher socioeconomic class compared to those that attended public schools because a greater proportion of their parents had higher levels of education, had higher paying jobs (civil servants or professional), lived in privately owned apartment, had washing machines and personal cars compared to the children that attended public schools (data not shown). Due to their higher socioeconomic status these children indulge in lifestyles that increase their risk of prediabetes. Importantly sedentary behaviour like being driven to school unlike students in public schools students who do not have the luxury but instead have to walk to and from school. They have access to motorised equipment such as washing machine and spend their leisure time sitting and watching television and playing video games. Several studies have shown that the prevalence of the metabolic syndrome significantly increases with high socioeconomic status in both developing and developed countries [34–36]. Similarly, a cross-sectional study conducted among 693 high school students in Vietnam (Nguyen et al. [37]), reported a positive association between metabolic syndrome and high socioeconomic status. These students came from wealthy families and were reportedly less physically active because their parents usually provide them with “modern” life, which

included recreational facilities such as televisions, computers, an undue reliance on automated household devices like washing machines and the availability of domestic servants, all of which reduced the level of physical activity. Besides, wealthier families are more likely to be able to provide abundant food including energy-dense foods and drinks [37].

Our study had certain limitations, the most important being the low yield of the study outcome among the study population, meaning that a larger sample is required to more accurately examine the relationship between the risk factors of prediabetes among Nigerian in-school adolescents. For the same reason, multivariate analysis could not be done to adjust for co-variates and confounders; hence, the unadjusted odds ratios presented might have underestimated or overestimated the strength of association. Also, the use of a cross section study design precluded the examination of causal relationships. Even though a number of risk factors were explored, they were not exhaustive; for example, the history of gestational diabetes in their mothers and their birth weight were not investigated. Lastly, the self-reported information on dietary intake and physical activity were likely to have been subject to some bias.

But more importantly, our study has important policy implications for the prevention and control of prediabetes and diabetes among adolescents within the formal school setting. The World Health Organization promotes school health programmes as a strategic means to prevent health risk in school-based children particularly the prevention of the factors that lead to premature death, disease and disability. Diabetes being a preventable and chronic debilitating illness is associatedwith life-threatening complications, which have worse outcomes when they begin early. Therefore, our study can inform public health interventions like creating awareness on diabetes, influencing the built environment and promoting physical activities etc.

In conclusion, the prevalence of prediabetes among the study population was low and the sex-specific prevalence rate was higher among males compared to females. Notably, the determinants that drive the occurrence of prediabetes like insufficient physical activity and frequent consumption of carbonated drinks were prevalent among the study population. Pupils attending private schools on average had higher fasting blood glucose levels than those in public schools. Implying that apart from the individual characteristics of the pupils who attend private schools, further research needs to explore the built environment of secondary schools especially private schools and how they can be adjusted to prevent non-communicable diseases among adolescents.

Compliance with ethical standards

Funding This study was self-sponsored, i.e.no form of external funding was received for this study which was conducted by OA under the supervision of IA and OY, in partial fulfilment of the award of a Master's degree in Epidemiology at the College of Medicine University of Ibadan.

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

Ethical standard All procedures performed in the study were approved by the Oyo State Ethical Review Committee of the Oyo State Ministry of Health. Permission to carry out the study was obtained from the Oyo State Ministry of Education and the authorities of each of the selected school. Informed consent was obtained from the parents/guardian while the study participants gave their assent before data collection commenced.

Authors' contributions Conception of research idea was conducted by OA, IA, OJ. OA and IA designed the study. AO conducted the study under the supervision of IA and OY. OA and IA analysed the data and wrote the draught of the manuscript. IA, OJ and OY reviewed and critically revised the manuscript. All authors read and approved the final manuscript. IA finalised the manuscript.

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ORIGINAL ARTICLE

Glycaemic control using different regimens of intermediate- and short-acting insulin in childhood type 1 diabetes mellitus: an experience from a tertiary care centre

Arijit Singha[1] · Beatrice M. Anne[1] · Sumit Kumar Chakrabarti[1] · Sujoy Ghosh[1] · Subhankar Chowdhury[1] · Rana Bhattacharya[1]

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Abstract Multiple-dose insulin and insulin pump therapy have been found to have better glycaemic control in type 1 diabetes compared to other regimens. In developing countries where this may not be an option for all patients, the efficacy of regimens employing intermediate- and short-acting regimens needs to be determined. The objective of the present study is to compare the HbA1c-lowering efficacy of various regimens of short-acting and intermediate-acting human recombinant insulin in children with type 1 diabetes mellitus. Ninety-eight patients with type 1 diabetes were treated with various regimens of intermediate- and short-acting insulin, and their efficacy was compared. The mean HbA1c at enrolment was 11.34, 10.18 and 10.5 % among the different groups. Six months later, there was a significant reduction in HbA1c in patients on the thrice-daily variable mix of short- and intermediate-acting insulin (group A) compared to those on twice-daily premixed insulin (group B) or on the combination of once-daily premixed insulin and twice-daily short-acting insulin ($p = 0.0003$). There was a significant reduction in HbA1c in those who were shifted to the group A regimen after 6 months ($p = 0023$).

✉ Beatrice M. Anne
maglarne@yahoo.com

[1] Department of Endocrinology, IPGMER & SSKM Hospital, AJC Bose Road, Kolkata, West Bengal 700020, India

Keywords Type 1 diabetes · Premixed insulin · Short-acting insulin · Intermediate-acting insulin · Glycaemic control

Introduction

Type 1 diabetes is the form of diabetes which is primarily due to β cell destruction and requires insulin for survival. Type 1 diabetes mellitus can be either autoimmune or idiopathic. In comparison to type 2 diabetes mellitus, type 1 diabetes is associated with loss of first-phase insulin release, and thus, patient becomes dependent on lifelong insulin therapy. The Diabetes Control and Complications Trial (DCCT) clearly showed that intensive insulin therapy (three or more injections per day of insulin) or continuous subcutaneous insulin infusion (CSII) was a key part of improved glycaemia and better outcomes [1]. The study was carried out with short- and intermediate-acting human insulin. The American Diabetes Association (ADA) recommends that type1 diabetes should be treated with multiple-dose insulin (MDI) injections (three to four injections per day of basal and prandial insulin) or CSII therapy. It has been shown in studies that long-acting basal insulin (glargine, detemir) is more efficacious than intermediate-acting insulin in controlling hyperglycaemia [2, 3]. But, in developing countries where a considerable portion of population will not be able to afford them, it is very difficult to prescribe the ideal regimen. For this reason, various regimens of both short-acting and intermediate-acting insulin have been in use. Head-to-head comparison of these various regimens may throw some light on which ones are more efficacious than the others. This may help the clinician choose an optimal regimen for the patients and tailor treatment accordingly. In this retrospective study, we have analyzed three

insulin regimens using regular and neutral protamine Hagedorn (NPH) human insulin in our cohort of type 1 diabetics and compared their efficacy in the form of HbA1c lowering.

Aims and objectives

The primary aim of our study was to compare the HbA1c-lowering efficacy of various regimens of short-acting and intermediate-acting human recombinant insulin in children with type 1 diabetes mellitus.

Materials and methods

A total of 98 patients, who have been enrolled in the type 1 diabetes clinic at our hospital at various times with follow-up over a period of 1 year, have been included in this study. Ethical committee approval was obtained from the institutional ethics committee. Patients have received different combinations of short-acting (regular) and intermediate-acting (NPH) human recombinant insulin during their treatment period at our centre. We have retrospectively analyzed data of these patients. Observing the records of insulin regimen of these patients, we have divided them into three groups.

Group A Short-acting (regular) human insulin before lunch and variable mix dose of short-acting and intermediate-acting (NPH) insulin before breakfast and dinner. The variable mix of short (regular)- and intermediate-acting (NPH) insulin was adjusted according to the post breakfast, post dinner values (with regard to the regular insulin in the mix) and the fasting and predinner blood glucose values (with regard to the NPH insulin in the mix).

Group B Twice-daily premixed 30/70 insulin before breakfast and before dinner. This regimen had been used in patients with inability to take afternoon dose of insulin at school.

Group C Short-acting (regular) human insulin before breakfast and lunch and one premixed 30/70 insulin before dinner. Although not physiological, this regimen was used in the patients who had late-afternoon meals since the morning premixed insulin will not be able to cover the afternoon meal and because of the risk of hypoglycaemia due to the delayed meals if the patients were given premixed insulin in the morning.

In every patient, a baseline HbA1c was done at first visit and every 3-month interval in subsequent follow-up. At 6 months, due to poor glycaemic control, 20 patients (8 of group B and 12 of group C) have been given group A regimen and followed up subsequently (Fig. 1).

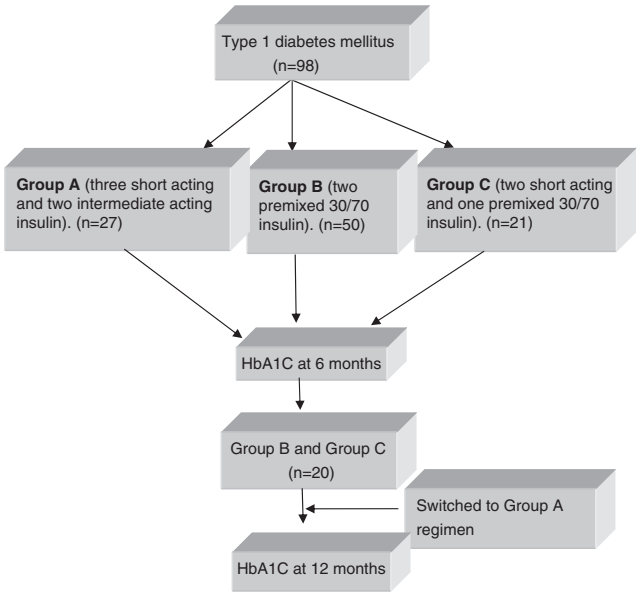

Fig. 1 Overview of the study groups

Thorough history taking (duration of diabetes, osmotic symptoms, micro- and macro-vascular complications, DKA, family history of diabetes, etc.) and clinical examination (general and systemic examination) were done in each patient. Preliminary workup of these patients to establish autoimmunity and insulin dependence has been done at the time of enrolment in our specialty clinic. Dietary advice was provided by a single trained dietician. Five-point self-monitoring of blood glucose (SMBG), with additional measurements as needed, was done by all the patients using Accu-Chek® Active glucometer. Proper injection technique was ensured in all patients. In each visit, patients and parents were counselled regarding the outcome and treatment of diabetes mellitus with special emphasis on glycaemic control and complications.

Results on continuous measurements are presented as mean ± standard deviation. The normality of the study variables was tested through the Anderson–Darling test, Shapiro–Wilk test and QQ plot. The results on categorical measurements are presented in number (%). Significance is assessed at 5 %. $P < 0.05$ was considered as statistically significant. Paired t test (for normally distributed data) was used to find any significant difference in HbA1c at baseline and at follow-up within each cohort of treatment arms. The differences in HbA1c levels between the three groups of patients at baseline and at follow-up were analyzed by ANOVA with post hoc Bonferroni and Tukey's tests. The statistical software namely SAS 9.2 (for Windows) and SPSS Ver. 20 (for Windows) were used for the analysis of the data, and Microsoft Word and Excel were used to generate graphs and tables.

Results

The total number of patients in our study was 98. We have divided these patients on the basis of their treatment regimens into three groups as mentioned above. There were 61 females and 37 males with a mean age group of 10.87 years (SD 4.044). Group A consisted of 27 patients (12 males and 15 females) and mean age was 9.79 ± 4.07SD. Group B consisted of 50 patients (18 males and 32 females) with a mean age of 11.03 years± 5.33SD. Group C consisted of 21 patients (7 males and 14 females) with a mean age of 11.89 years± 5.33SD. The mean height and systolic and diastolic blood pressures did not differ significantly between the study groups at baseline (Table 1). However, mean weight and BMI were significantly higher in group C (p value <0.05).

The mean HbA1C at first visit of group A, group B and group C was 11.34 % (100 mmol/mol), 10.18 % (87 mmol/mol) and 10.5 % (91 mmol/mol) respectively. After 6 months, the mean HbA1c reduced to 8.87 % (73 mmol/mol) in group A, 9.64 % (81 mmol/mol) in group B and 9.63 % (81 mmol/mol) in group C. There was a statistically significant reduction of HbA1c in group A ($p = 0.0003$). However, there was no such statistically significant reduction of HbA1c in group B ($p = 0.213$) or group C ($p = 0.551$) (Table 2, Fig. 2). There was also no significant difference in HbA1c reduction between group A and group C ($p = 0.068$) and group B and group C ($p = 1.00$). But, there was a significant difference in HbA1c reduction between group A and group B ($p = 0.021$). There was no significant difference in the insulin dose in the groups ($p = 0.068$), indicating that the regimen rather than the absolute dose of insulin has contributed to the differences observed in the HbA1c in the different study groups.

Table 2 Change in HbA1c values after 6 months in the different study groups

	Groups		
	Group A Mean ± std. deviation	Group B Mean ± std. deviation	Group C Mean ± std. deviation
HBA1c (baseline)	11.34 ± 3.22	10.18 ± 2.57	10.51 ± 2.04
HBA1c (at 6 months)	8.87 ± 2.53	9.64 ± 2.38	9.63 ± 3.00
p value	0.0003	0.213	0.551

Twenty patients (8 from group B and 12 from group C) were shifted to group A regimen probably due to unsatisfactory glycaemic control. The mean HbA1C at 6 months of these 20 patients before being switched to group A regimen was 10.10 % (87 mmol/mol), and at 12 months (i.e., 6 months after changing to the group A regimen), the mean HbA1C had fallen to 9.11 % (76 mmol/mol) ($p = 0023$) (Fig. 3). Total insulin dose was not changed significantly (p value = 0.6).

Discussion

The results of our study show that there is a significant reduction in HbA1C by using a split mix regimen. There was around 21 % reduction of HbA1C in group A, i.e., in split mix regimen in comparison to ~ 5 and ~ 3 % reduction in group B and group C, respectively, at the end of 6 months. The efficacy of the thrice-daily split mix regimen is also proved by the significant HbA1c reductions ($p < .05$) in the subset of

Table 1 Baseline characteristics of the study groups

Characteristics	Group A ($n = 27$)	Group A ($n = 50$)	Group A ($n = 21$)	p value
Age (years) mean ± SD	9.79 ± 4.07	11.03 ± 3.29	11.87 ± 5.33	0.193
Sex				
Female	5 (55.6 %)	32 (64.0 %)	14 (66.7 %)	
Male	12 (44.4 %)	18 (36.0 %)	7 (33.3 %)	
Total	27	50	21	0.686
Height (cm) mean ± SD	126.42 ± 18.23	136.06 ± 15.53	135.61 ± 23.33	0.074
Weight (kg) mean ± SD	24.34 ± 9.47	31.73 ± 10.12	33.58 ± 12.95	0.004
BMI (kg/m^2) mean ± SD	14.84 ± 2.94	16.73 ± 2.47	17.49 ± 2.44	0.002
Systolic BP (mm of Hg)				
Mean ± SD	99.52 ± 10.78	104.38 ± 12.66	100.57 ± 13.43	0.21
Diastolic BP (mm of Hg)				
Mean ± SD	67.41 ± 9.78	66.48 ± 8.62	67.14 ± 6.22	0.89
Total insulin dose (U)				
Mean ± SD	30.33 ± 21.97	33.08 ± 15.26	42.38 ± 20.26	0.068
HbA1C (%) (baseline)	11.34 ± 3.22	10.18 ± 2.57	10.51 ± 2.04	0.19

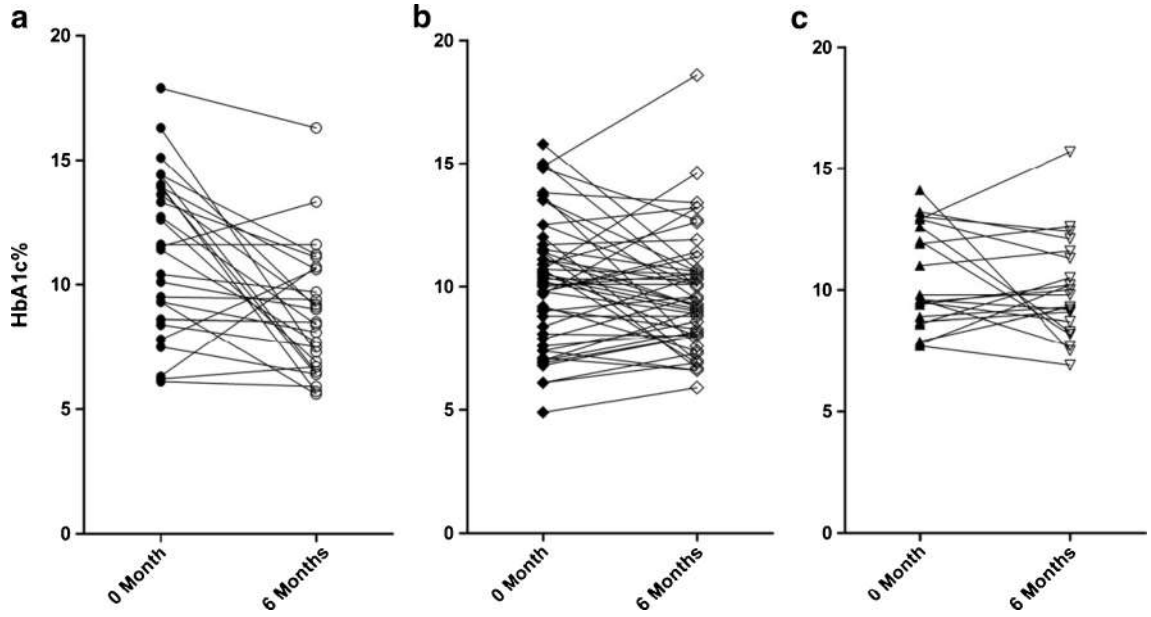

Fig. 2 Change in HbA1c from baseline at 6 months in groups A, B and C

patients from groups A and B who were switched over to group A regimen after 6 months due to poor glycaemic control. Though there is a nonsignificant reduction in HbA1C level in group B at the end of 6 months, it is, to some extent, better than group C regimen in terms of percentage of HbA1C lowering. These findings can be explained by the pathophysiology of type 1 diabetes, which is a state of absolute insulin deficiency, and hence the group C regimen, which had basal insulin only in the nights fared worse than the group B regimen.

The findings of our study are in line with the DIABAUD2 study [4], which was undertaken to assess glycaemic control and factors influencing it in young people with type 1 diabetes from different centres throughout Scotland using prospective measurements of HbA1c. It was seen in this study that in Scotland, the only insulin regimen associated with a lower HbA1c level was one comprising three injections per day. However, for unexplained reasons, the vast majority of patients were treated by twice-daily premixed insulin. However, there are contradictory findings in a few other cross-sectional studies which have failed to show a benefit from multiple-injection regimens in young people [5].

In a study by Mortensen et al. [6], the glycaemic control in their preadolescent children on twice-daily premixed insulin regimen appeared comparable to those on a variable combination of short- and long-acting insulin. However, there was a divergence in the adolescent age group who seemed to have better glycaemic control with individual combinations rather than premixed insulin. Most of the patients in our study were in the preadolescence group with a mean age of 10.87 years, and the spilt mix regimen was found to be better than the premixed regimens. The patients on split mix regimen (group A) had more flexibility of insulin dosing according to their blood glucose as compared to those in the premixed regimens. This is probably one of the contributors to the better glycaemic control seen in this group.

Diverse opinions exist regarding the findings of the DCCT study which propounded intensive insulin regimen as the major factor in achieving glycaemic control in type 1 diabetes. The counterargument to this proposition was supported by the Hvidore Study Group [7] who used a single cross-sectional centralized HbA1c and showed that control deteriorated significantly throughout adolescence, despite increase in insulin dose, and that the type of insulin regimen appeared to make no impact on this deterioration. Moreover, it was suggested that other factors like intensive medical follow-up, additional nursing and dietetic input and frequent office visits were the major contributors to this improved glycaemic control. In our study, the children received this "clinical support package" uniformly. Hence, we were able to assess the efficacy of the different insulin regimens without this confounding factor.

Although split mix regimen seems to score over the premixed regimens, it has some practical difficulties. Our clinic experience has shown us that school-going children in developing countries may find it a challenge to adapt to the split mix regimen. There are various reasons behind this. The children may not be able to take the afternoon dose of insulin at school because of the lack of facilities for proper transport and storage of insulin. Moreover, as diabetes education and awareness are at the nascent stage in our population, there is the stigma attached to diabetes, especially in children. The school-going children may have social constraints like peer pressure and peer ridicule. All these factors hinder the prescription of the ideal split mix regimen in our clinic. Hence,

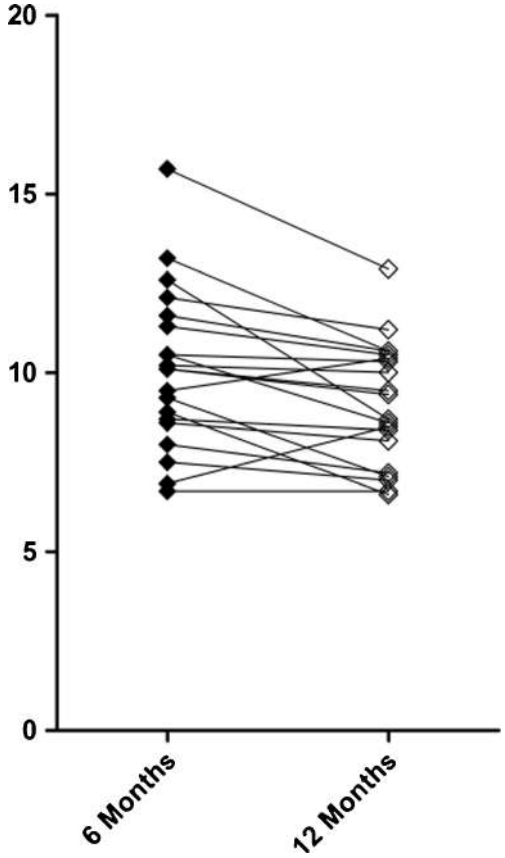

Fig. 3 Reduction in HbA1c in the patients from groups B and C switched over to group A regimen after 6 months

in these situations, we recommend twice-daily split mix regimen in these patients in keeping with the findings of our present study, which showed that twice-daily split mix regimen (group B) was better than the combination of regular insulin and premixed insulin once daily (group C). This difference in the HbA1c lowering between the group B and group C is probably because the patients in group B with the twice-daily premixed insulin had better coverage of their basal insulin requirements as compared to those on only night-time premixed insulin whose daytime basal insulin requirements were not met, considering the pharmacotherapeutics of the different insulin formulations [8].

Some studies suggest that use of CSII may confer an advantage over multiple daily injections in achieving better glycaemic control others not [9–14]. CSII is more expensive than MDI administration and requires a highly motivated patient without psychological problems and an experienced diabetes team who can provide regular and frequent input into the ongoing care of the patient. Hence, the scope of this treatment is still limited in a developing economy.

Our study does have a few limitations. One is that the sample size was small, and larger patient population has to be studied in this regard. The number of clinic visits was not taken into account, as some studies have shown that this is an important factor affecting glycaemic control. In a study by Aljabri et al. [15], pharmacologically treated patients missing more than 30 % of scheduled visits had an HbA1c value 0.7 point higher relative to those with perfect attendance, a difference that was found to be clinically relevant. Missing appointments could have a direct effect on clinical outcomes by reducing continuity of care, missing opportunities to measure clinical variables or adjust medications, delaying the appropriate timing of interventions and screenings and hindering the development of a trusting provider–patient relationship. The number of appointments necessary per year for optimal health care and diabetes control varies greatly, and there is no recommended minimum. But, the general assumption is that office visits are medically necessary and that a missed appointment is potentially detrimental to care management.

Although our patients were on regular follow-up, compliance and adherence to treatment could be a potential confounder as the patients were continuing treatment at home. Changes in physical activity and dietary patterns were not taken into account, but all our patients received the same education regarding lifestyle. The number of hypoglycaemic episodes in the patients should have been recorded to assess whether the better glycaemic control was achieved at the expense of hypoglycaemia. These are the few limitations of our study.

However, our study also has its strengths. The yardstick that we used to measure glycaemic control namely HbA1c was done from a single lab using the same method (HPLC), avoiding any confusion in comparison of the cohorts. Previous studies have highlighted this important facet in the follow-up of diabetic patients [16]. Since the same patients were given the different regimens, the comparison of the different regimens is better due to elimination of intrinsic factors as potential confounders.

Conclusion

To conclude, although the efficacy of newer insulins has been proven time and again in various studies, these ideal regimens still are out of reach of many diabetic patients, especially in developing countries. From our study, we conclude that split mix regimen using thrice-daily short-acting regular insulin before each meal and twice-daily intermediate-acting NPH insulin is better than twice-daily premixed insulin and the twice-daily short-acting regular and one predinner premixed insulin in type 1 diabetes mellitus children. In situations where this ideal regimen may not be feasible, the twice-daily premixed insulin may be a better regimen than the twice-daily regular and predinner premixed insulin.

Compliance with ethical standards

Funding No funding received for this study from any source. The insulin, glucometers and the glucose test strips are sponsored by Novo Nordisk as part of their programme "changing diabetes in children (CDIC)".

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

Sport participation in pediatric age affects modifications in diabetes markers in adulthood

Manoel C. S. Lima[1] · Bruna C. Turi[1,2] · Danilo R. P. Silva[3] · Raphael M. Ritti-Dias[4] · Gabriel G. Cucato[4] · Jamile S. Codogno[1,2] · Rômulo A Fernandes[1,2]

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Abstract The purpose of this study was to analyze the effect of early sport participation on diabetes markers among adults. This longitudinal study analyzed 107 participants during 12 months of follow-up. Diabetes markers were measured by fasting insulin, fasting glucose, insulin resistance, and glycated hemoglobin. Sports participation during childhood and adolescence was self-reported. Current physical activity was measured by pedometer. Adults with no engagement in sports during early life showed positive relationship between current physical inactivity and higher modification in glucose ($\beta = 1.045$ [95%CI 0.267; 1.823]), insulin ($\beta = 0.763$ [95%CI 0.121; 1.405]), and insulin resistance ($\beta = 0.295$ [95%CI 0.062; 0.529]). Adults engaged in sports during early life had lower values of glucose (p value = 0.029; Eta-squared = 0.049). Glucose levels decreased through the follow-up among adults with early sports participation (p value = 0.005; Eta-squared = 0.074). There was association between lack of early engagement in sports and higher occurrence of altered values during the follow-up for insulin resistance (OR = 8.37 [2.10; 33.3]) and insulin (OR = 7.61 [2.27; 25.4]). Engagement in sport activities during early life affects glycemic variables in adulthood, as well as longitudinal relationship between physical activity in adulthood, and glycemic control also seems affected by early sport participation.

Keywords Physical activity and health · Adolescence · Childhood · Insulin resistance · Glycemic control

✉ Bruna C. Turi
brunaturi@hotmail.com

[1] Laboratory of InVestigation in Exercise (LIVE), Department of Physical Education, São Paulo State University (UNESP), 305 Roberto Simonsen St., Presidente Prudente, SP 19060-900, Brazil

[2] Post-Graduation Program in Kinesiology, Institute of Biosciences, São Paulo State University (UNESP), Rio Claro, SP, Brazil

[3] Post-Graduation Program in Physical Education, Center of Physical Education and Sports, Londrina State University (UEL), Londrina, PR, Brazil

[4] Albert Einstein Israelite Hospital, São Paulo, SP, Brazil

Introduction

Type 2 diabetes mellitus (T2DM) constitutes a chronic disease highly prevalent in adults [1], which is associated with elevated health care costs and early mortality [2–4]. Although T2DM constitutes a public health problem mainly observed among adults, alterations observed at adulthood are initiated during early life [5–7]. In fact, the events during early life seem relevant in the programing of organs of the human body and their functions at adulthood and epigenetic pathways have been used to support these theories [8, 9].

Physical inactivity is a relevant risk factor related to the development of T2DM and its complications in adulthood [10, 11]. Interestingly, physical activity during childhood and adolescence constitutes a stressful agent with potential to affect DNA global methylation in adulthood [12], but its protective effects on prevention of diseases are not completely clear [7]. Previous studies reported that early sport participation was related to lower arterial intima-media thickness, better lipid profile, and lower prevalence of arterial hypertension and T2DM in adulthood [11, 13–17].

On the other hand, the impact of exercise routinely performed during early life on diabetes markers in adulthood remains unclear. In fact, the few studies that analyzed T2DM as outcome had cross-sectional design and assessed current physical activity with questionnaire. Therefore, the purpose of this study was to analyze the effect of early sport

participation on diabetes markers among adults, as well as to identify if early sport participation affects the relationship between current physical activity and diabetes markers in adulthood.

Methods

Study design, participants and follow-up

This was a prospective longitudinal study analyzing physical activity and diabetes markers at baseline and after 12 months of follow-up. The study was carried out in a Brazilian city (Presidente Prudente, located at western region of Sao Paulo State; ~200,000 inhabitants) from 2013 to 2014. All procedures performed in this longitudinal study were approved by the ethical research group of the Sao Paulo State University (UNESP), Presidente Prudente, Sao Paulo State, Brazil.

The study was presented to four fitness clubs located at different geographical regions of the metropolitan area of the city, as well as all department and centers of research of the university. The inclusion criteria were as follows: (i) aged between 30 and 50 years old; (ii) no previous history of stroke or myocardial infarction; (iii) no amputation or visual problems related to diabetes mellitus; (iv) no physical limitation that affects physical activity, and (v) either to be classified as persistently active (sport participation in both childhood and adolescence) or persistently inactive (no sport participation in both childhood and adolescence). At baseline, 122 participants fulfilled the inclusion criteria and were enrolled in the study. After 12 months of follow-up, there were 15 dropouts (14 participants declared no interest to continue participating in the study and one death [stroke; participant indicated no sport participation in early life]).

Diabetes markers

All blood sample collections and biochemical analyses were performed in a private laboratory, which meets the standardization criteria of quality control adopted by the Brazilian Health Ministry. A 12-h fasting blood sample collection was taken to measure fasting glucose, insulin and glycated hemoglobin (HbA_{1C}). Fasting values of glucose and insulin were used to calculate homeostatic model assessment-estimated insulin resistance (HOMA-IR). Numerical variables were categorized as normal or altered (fasting glucose ≥100 mg/dL; HOMA-IR ≥ 2.0 [18]; fasting insulin ≥ Percentile 75 [P75]; HbA_{1C} ≥ P75) in both moments of the study (baseline and follow-up). Therefore, for each diabetes markers, the participants were classified as follows: "no alteration in both moments", "alteration in just one moment," or "alteration in both moments". For statistical analysis, all diabetes markers were treated as normal (category "none alteration in both moments") and altered values in at least one moment of the study (categories "alteration in just one moment" and "alteration in both moments").

Early sport participation

Early sport participation was analyzed using two questions [7, 13, 19]: (i) "Outside school, have you ever been engaged in any organized/supervised sport activities for at least 1 year from 7 to 10 years old?" and (ii) "Outside school, have you ever been engaged in any organized/supervised sport activities for at least 1 year from 11 to 17 years old?" Other physical activities such as dance (e.g., ballet) were also included. Participants were classified as persistently active (sport participation in both childhood and adolescence) or persistently sedentary (no sport participation in both childhood and adolescence). Adequate levels of reproducibility for early sport participation have been previously reported [13].

Potential confounders

Chronological age, sex, skipping breakfast (outcome used in the multivariate model: skip breakfast at least 1 day per week at baseline), smoking habit (outcome used in the multivariate model: current smoker, former smoker, and new smoker during the follow-up), and alcohol consumption (outcome used in the multivariate model: the consumption of alcohol at least once week in both moments of the follow-up) were assessed through face-to-face interview. Resting systolic and diastolic blood pressure (SBP and DBP, respectively) were measured in a seated position after 10 min of rest (baseline measures was included in the multivariate models).

Whole body fatness was assessed using a Dual-Energy X-ray Absorptiometry (DEXA) scanner (Lunar DPX-NT; General Electric Healthcare, Little Chalfont, Buckinghamshire), with software version 4.7. The scanner quality was tested by a trained researcher prior to each day of measurement, following the manufacturer's recommendations. All measurements were performed at the university laboratory in a controlled temperature room. The participants wore light clothing, without shoes and remained in the supine position on the machine for approximately 15 min. The whole body fatness was expressed in percentage values, and baseline results were used as numerical variable in the multivariate models.

Current physical activity was objectively measured by pedometer (Digi-Walker Yamax, SW200) during a consecutive 7-day period. The device was fixed laterally at the hip and taken off only during periods of sleep, shower, and activities in pool. At the end of each day, the participants recorded the number of steps performed throughout the day. Every morning, to begin collecting data, the "reset" button was pushed to zero out the device. The mean values of steps in the week were assigned as the level of current physical activity, and the

Table 1 General characteristics of the sample involved in the longitudinal study (Brazil, $n = 107$)

Variables	Categories	Descriptive statistic ($n = 107$)	
		n (%)	(95%CI)
Sex	Male	64 (59.8)	(50.5–69.1)
	Female	43 (41.2)	(30.9–49.4)
Age (years)	<35	34 (31.8)	(22.9–40.6)
	35–44.9	49 (45.8)	(36.5–55.2)
	≥45	24 (22.4)	(14.5–30.3)
Early sport participation	No	55 (51.4)	(41.9–60.8)
	Yes	52 (48.6)	(39.1–58.1)
Skin color	White	88 (82.2)	(75.1–89.4)
	Black	08 (7.5)	(2.4–12.4)
	Others	11 (10.3)	(4.5–16.1)
Skipping breakfast	No	66 (61.7)	(52.4–70.8)
	Yes	41 (38.3)	(29.1–47.5)
Alcohol consumption	No in both moments	19 (17.2)	(10.5–25.1)
	At least once during follow-up	88 (82.8)	(75.1–89.4)
Smoking habit	No in both moments	82 (76.6)	(68.6–84.6)
	Former/at least once during follow-up	25 (23.4)	(15.3–31.3)
Glucose (≥100 mg/dL)	Normal in both moments	87 (81.3)	(73.9–88.7)
	Altered at least once during follow-up	20 (18.7)	(11.3–26.1)
HbA_{1c} (≥P75)	Normal in both moments	61 (57)	(47.6–66.3)
	Altered at least once during follow-up	46 (43)	(33.6–52.3)
HOMA-IR (≥2.0)	Normal in both moments	79 (73.8)	(65.5–82.1)
	Altered at least once during follow-up	28 (26.2)	(17.8–34.5)
Insulin (≥P75)	Normal in both moments	71 (66.4)	(57.4–75.3)
	Altered at least once during follow-up	36 (33.6)	(24.6–42.6)

sample was stratified as physically active (≥10,000 steps/day) or physically inactive (<10,000 steps/day) [14] in both moments of the follow-up. Finally, two numerical variables were created taking into account the number of days in which participants met cutoffs for physical activity (≥10,000 steps/day) and physical inactivity (≤5000 steps/day) at both baseline and follow-up. Therefore, two numerical variables ranging from zero (no days) to 14 (all days analyzed) were created, denoting physical activity (≥10,000 steps/day) and inactivity (≤5000 steps/day) during the follow-up period.

Statistical analysis

Numerical variables were presented as mean values and 95 % confidence intervals (95%CI), while categorical variables were presented as prevalence and 95%CI. Analysis of variance (ANOVA) for repeated measures compared numerical variables and sport participation in early life at baseline and end of follow-up. ANOVA for repeated measures was adjusted by potential confounders, while measures of effect size for each ANOVA parameter (time, early sport participation, and time × early sport participation) were identified by Eta-Squared values. Associations between categorical variables were expressed by the chi-square test (Yates' correction was applied in 2 × 2 contingence tables), while binary logistic regression provided measures of effect size (odds ratio [OR] and its 95%CI) of those associations. Hosmer-Lemeshow test was used to identify how fit was the multivariate models created by the binary logistic regression (p values >0.05 denotes adequate fit of the multivariate models). Pearson correlation and linear regression (adjusted by sex, age, and body fatness) denoted the relationship among numerical variables. Statistical significance (p value) was set at p value <0.05, and the statistical software BioEstat (release 5.0) performed all the analysis.

Results

One hundred and seven adults were followed during 12 months (11.7 ± 1.1 months [64 men and 43 women]). The sample was composed predominantly of participants with white skin (82.2 % [75.1–89.4 %]). Skipping breakfast was a habit reported by 38.3 % (29.1–47.5 %) of adults, while

alcohol and smoking were habits reported (at least once during the follow-up period) by 82.8 and 23.4 % of the sample, respectively (Table 1).

Table 2 shows the relationship between current physical activity and diabetes markers according to early sports participation. Among adults with no engagement in sports during early life, there was a positive relationship between current physical inactivity and higher modification in glucose (β = 1.045 [95%CI, 0.267; 1.823]), insulin (β = 0.763 [95%CI, 0.121; 1.405]), and insulin resistance (β = 0.295 [95%CI, 0.062; 0.529]) independently of sex, age, and body fatness.

Figure 1 shows the estimated means of glycemic variables after 12 months of follow-up among adults stratified by early sport participation. Adults engaged in sports during early life had lower values of glucose in both moments of the study (p value = 0.029; Eta-squared = 0.049, small effect size). Compared to baseline values, glucose in follow-up decreased in adults with early sport participation (p value = 0.005; Eta-squared = 0.074, moderate effect size). HbA_{1c} slightly increased in both groups, but in both moments, adults with early sport participation presented lower values of HbA_{1c} (p value = 0.009; Eta-squared = 0.066, moderate effect size). Insulin resistance and fasting insulin values did not change during the follow-up period, but the values were lower in both moments among adults with early sports participation (Fig. 1).

There was an association between the lack of engagement in sports during early life and higher occurrence of altered values during the follow-up for insulin resistance (OR = 8.37 [2.10–33.3]) and insulin (OR = 7.61 [2.27–25.4] even when adjusted by potential confounders (Fig. 2). No association between early sport participation and altered values of glucose (p value = 0.110) and HbA_{1c} (p value = 0.265) were observed.

Discussion

This longitudinal study identified that adults who were engaged in sports during childhood and adolescence presented lower values of diabetes markers at baseline and during follow-up in comparison with adults with no history of sports participation. Additionally, the lack of sport participation in early life also affected the association between physical activity at adulthood and diabetes markers.

Our findings showed that participants who were engaged in sports during childhood and adolescence presented lower levels of diabetes markers (glucose, insulin resistance, and insulin) at baseline and after 12 months of follow-up in comparison with participants without history of sports participation. Similarly, Finnish former athletes when compared to their controls have better results in the impaired glucose tolerance test, as well as lower prevalence of diabetes mellitus and consumption of diabetes medicines [11].

Since diabetes is closely related with body fatness and physical inactivity, participants engaged in sports during early life are more likely to maintain this behavior throughout life [11, 20]. Moreover, sport participation has been associated with higher energy expenditure in other domains of physical activity, such as leisure-time physical activity (moderate-to-vigorous intensity) and less sedentary behavior in youth [21]. All these aspects could help to preserve lower body fatness values throughout life (mainly during the growth spurt in adolescence) and normal function of glucose and insulin (no imbalance) [11].

The increasing prevalence of physical inactivity in pediatric populations constitutes a worldwide concern and it is not restricted to developed nations [22]. Our findings show relevant effect of physical inactivity in early life on harmful health outcomes in adulthood. These results reiterate the necessity of public policies promoting physical activity among children and adolescents, since being physically active only in

Table 2 Relationship between current physical activity and glycemic variables according to early sport participation (Brazil, n = 107)

Sport participation in childhood and adolescence	Glucose (mean difference) r (95%CI)	HbA_{1c} (mean difference) r (95%CI)	HOMA-IR (mean difference) r (95%CI)	Insulin (mean difference) r (95%CI)
No (n = 55)				
<5000 steps/day**	0.295 (0.035; 520)	0.031 (−0.236; 0.294)	0.293 (0.030; 0.518)	0.295 (0.032; 0.520)
Linear regression	β = 1.045 (0.267; 1.823)*	–	β = 0.295 (0.062; 0.529)*	β = 0.763 (0.121; 1.405)*
≥10,000 steps/day** linear regression	−0.119 (−0.373; 0.151)	−0.007 (−0.272; 0.259)	−0.120 (−0.373; 0.150)	−0.112 (−0.366; 0.158)
Yes (n = 52)				
<5000 steps/day** linear regression	−0.050 (−0.319; 0.226)	0.087 (−0.190; 0.352)	−0.188 (−0.438; 0.089)	−0.202 (−0.450; 0.075)
≥10,000 steps/day** linear regression	−0.134 (−0.393; 0.144)	−0.067 (−0.334; 0.210)	−0.106 (−0.368; 0.172)	−0.101 (−0.364; 0.177)

95%CI = 95 % confidence interval

*linear regression adjusted by sex, age, body fatness percentage and changes in body fatness percentage

**sum of days meeting that cut-off in both moments of analysis (baseline and follow-up)

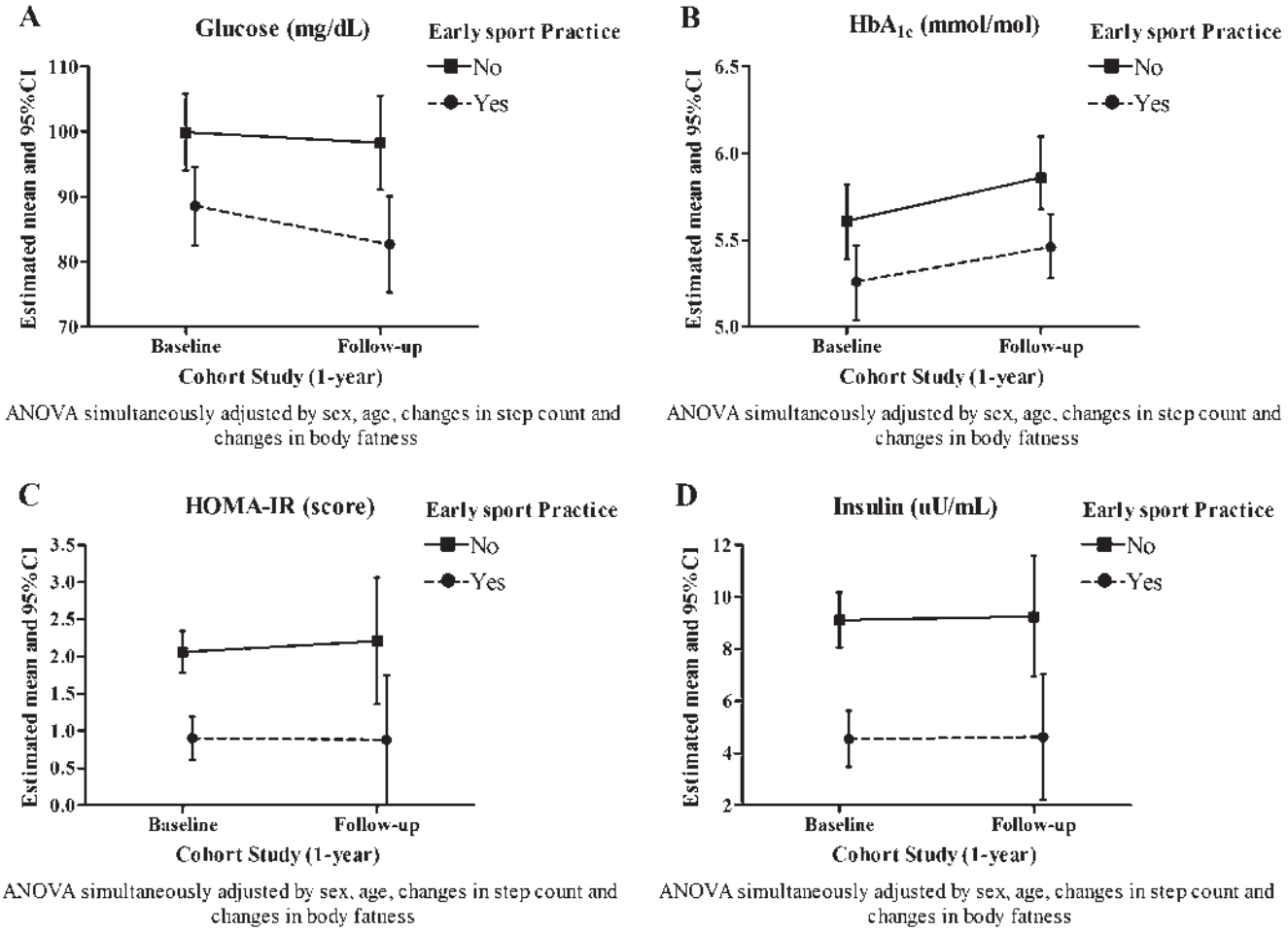

Fig. 1 Estimated means (ANOVA for repeated measures) of glycemic variables after 12 months of follow-up stratified by early sport participation (Brazil, $n = 107$)

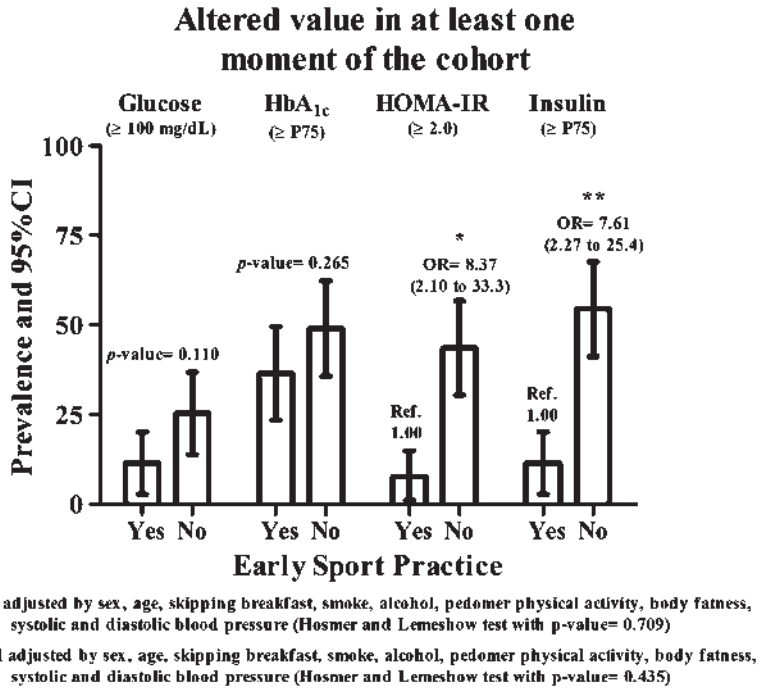

Fig. 2 Associations between altered values of glycemic parameters and sport participation in early life during the follow-up (Brazil, $n = 107$)

adulthood is apparently not enough to guarantee good metabolic health.

Additionally, sports participation in childhood and adolescence affected the relationship between current physical activity and diabetes markers, in which lower levels of physical activity in adulthood were related to diabetes markers only in participants with no engagement in sports during early life. Epigenetic pathways have been proposed to support these findings [7, 23]. During critical periods of human life, behavioral and environmental agents can induce epigenetic variations and thereby permanently affect metabolism, increasing the risk of development of diseases [23]. It is known that physical activity in early life promotes good health not only during childhood and adolescence, but also in the subsequent years (adulthood), and it happens because these two periods of life are characterized by higher sensitivity to epigenetic modifications [7, 8, 19, 23]. In fact, sport participation during childhood and adolescence affects gene polymorphisms/expression, as well as DNA methylation in adulthood [12, 24]. Under these circumstances, it is possible to consider that adults who were active during early life are less likely to experience harmful metabolic events preceding the development of diabetes.

Limitations should be highlighted. Considering that the maintenance of physical activity is not constant, its instability should be taken into account [25]. Moreover, participants recalled sport participation in childhood (7–10 years old) and adolescence (11–17 years old) and recall bias cannot be completely excluded. About the longitudinal analyses, longer periods of follow-up would be more appropriate to analyze the interaction between glycemic variables and early sport participation. Finally, the absence of worldwide-accepted cutoffs to HOMA-IR is a limitation, which deserves to be mentioned due to its possible impact on the observed associations.

In summary, it is possible to conclude that the engagement in sport activities during early life affects glycemic variables in adulthood, as well as longitudinal relationship between physical activity in adulthood and glycemic control, seems affected by early sport participation as well.

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Compliance with ethical standards The authors would like to declare the following: (i) this study received no funding resources; (ii) there is no conflict of interest in the realization of this study; and (iii) all ethical procedures related to human research were strictly followed by the research team in all steps of the study.

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

Ethical approval The authors declare that 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.

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ORIGINAL ARTICLE

Zymogram profiling of myeloperoxidase in association with increased risk of infection susceptibility in diabetic foot ulcer

Saumya Mary Mathew[1] · **T. V. Suchithra**[1]

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Abstract An increase in blood glucose level can lead to altered immunological response of the body and increased infection susceptibility in diabetic condition. This condition along with the variation in the oxidant-antioxidant status can further lead to impaired wound healing. Myeloperoxidase (MPO) is one of the key enzymes involved in oxidative stress and phagocytosis. The main focus of this study was on the prevalence of isozymes of MPO in diabetic foot ulcer condition. Prior to the zymogram studies, the variations in leukocyte MPO activity in diabetic patients with and without foot infection were analysed. The MPO activity in diabetic foot ulcer patients was elevated than that of diabetic patients without foot ulcer complications, but the levels were not up to the MPO levels of the controls. The difference in banding pattern was observed in native PAGE analysis of MPO enzyme isolated from diabetic foot ulcer patients compared to diabetic and non-diabetic control groups. The multiple isozymic bands observed in diabetic foot ulcer condition were unique from that of diabetic patients without complication and normal healthy individuals.

Keywords Myeloperoxidase · Diabetic foot ulcer · Isozyme · Foot infection · Impaired wound healing

Introduction

Myeloperoxidase (MPO) is one of the important enzymes of the immune host defence system. It is a heme containing [1] glycoprotein of MW 150 kDa secreted by azurophilic granules of polymorphonuclear neutrophils [2]. MPO being an important enzyme for free radical production and oxidative stress, it has been widely studied for its role in the phagocytic killing. A deficiency of MPO production causes a defect in the phagocytic system leading to decreased ability to kill the invading microorganisms. The MPO deficiencies are of two types: acquired and hereditary deficiency. The acquired MPO deficiency may occur during the multi-step process of MPO synthesis. The initial translational product of MPO is subjected to proteolytic cleavage, N-linked glycosylation and heme addition to form proMPO (90 kDa) in the endoplasmic reticulum. During its transport to azurophilic granules through Golgi-network, the proMPO is again subjected to proteolytic cleavage to make α and β chains and linked by disulfide bonds to form monomer of MPO having MW 73 kDa. Then, it dimerises to form the mature MPO (150 kDa) via cysteine residues. Any error in post-synthetic processing or packaging into azurophilic granules can cause acquired MPO deficiency [3]. Diabetes mellitus, lead intoxication, iron deficiency, chronic inflammatory processes and malignant tumours can also lead to acquired MPO deficiency [4]. MPO deficiency at the cellular level is common in patients with diabetes mellitus [5].

It can also elicit some other physiological effects like reduced host defences and increased infection susceptibility [6]. For instance, they seem to have a reduced capacity in overcoming infections by *Staphylococcus aureus* [7] and *Candida albicans* [8]. In response to infections, the MPO levels will shoot up in the normal non-diabetic individuals [9]. When infection rate is increased, neutrophils are recruited to the site of infection and they release elevated levels of a variety of oxidants by the MPO system. MPO produces reactive oxidant species like hypochlorous acid (HOCl) and it contributes to both bacterial killing and oxidative injury of host tissue at the sites of inflammation [10]. This study has specifically been

✉ T. V. Suchithra
drsuchithratv@nitc.ac.in

[1] School of Biotechnology, National Institute of Technology Calicut, Calicut, Kerala 673601, India

designed to evaluate the isozyme pattern of MPO enzyme in accordance with MPO activity in diabetics without infection and in infection-complicated diabetes.

Materials and methods

Sample collection

The subjects of investigation were grouped into three, namely group 1: non-diabetic controls without any wound healing impairment, group 2: diabetes mellitus patients without foot ulcer problems and group 3: diabetes mellitus patients with foot ulcer and impaired wound healing. Clinically, infected ulcers that come under the University of Texas wound classification system (UT 1B, 1D, 2B, 2D, 3B, 3D) were included in group 3 [11]. According to Wagner classification, these patients were included in grade 2 to 5. In each group, 30 individuals were included.

Blood samples were selected from Medical Trust Hospital and Diabetes Care Centre, Kulanada, Pandalam, Kerala, India. The study was approved by the Ethics Review committee of Medical Trust Hospital and Diabetes Care Centre, Pandalam. Informed written consent was obtained from the patients and controls before including them in this study. Patients were screened for the presence of diabetes according to the international standards for the diagnosis of diabetes mellitus [12]. The medical history, haematological and biochemical profiling were done for the selection and recorded in proforma. Care was taken to exclude the cases suffering from eosinophilia and those receiving antioxidant therapies.

Extraction of MPO

Four millilitres of venous, blood was collected in a collection tube containing acid-citrate-dextrose solution. MPO was then extracted from neutrophils and its activity was estimated by 4-amino antipyrine method [2].

Estimation of myeloperoxidase activity

MPO activity was estimated using the method of Matheson et al. [2] and expressed in unit activity. One unit was defined as that amount of enzyme that catalyses the conversion of 1 μmol of substrate per minute. The chemicals used for this study were of the highest quality of commercially available from Merk.

Statistical analysis

Statistical analysis of the parameters was done using SPSS package for windows version 22.0. Data are expressed as mean ± standard error of mean. Values of $p < 0.05$ were considered statistically significant.

Zymogram studies

Native polyacrylamide gel electrophoresis was done for separation of purified MPO enzyme. The MPO activity was visualised by incubating the gel in specific peroxidase stain containing 50 mM Phosphate buffer, pH 7.0, 0.3 mg/ml nitro blue tetrazolium (NBT), 2.0 mg/ml NADH, 0.4 mg/ml Phenol and 0.02 % H_2O_2at room temperature in the dark until dark blue band appeared. The gel was then fixed in 25 % ethanol [13]. 4-Amino antipyrine was obtained from Merk. NBT and NADH were purchased from Sisco Research Laboratories. Other reagents used were of the highest quality of commercially available.

Results

The Biochemical analysis of MPO activity in the different study groups of the present study explains totally different levels of MPO activity (Fig. 1). Among all study groups, normal controls showed the highest activity of $1 \times 10^{-5} \pm$ SE 1.94×10^{-6} and it possessed statistically significant difference from the other groups ($p < 0.01$). In uncomplicated diabetic condition, the MPO activity decreased to a value of $5.13 \times 10^{-6} \pm$ SE 8.67×10^{-7}. In diabetic patients having foot ulcer, the MPO activity was increased than that of the diabetic patients without infection complications ($p < 0.01$). Their mean MPO activity ($8.95 \times 10^{-6} \pm$ SE 1.99×10^{-6}) was nearer but lower than that of the normal healthy individuals.

Further, in zymogram analysis, the MPO bands of the complicated diabetic group were analysed in comparison with that of uncomplicated diabetic and normal groups (Fig. 2). The prevalence of different isozymic forms of human MPO was observed in healthy individuals of the non-diabetic control. Two bands of MPO (A and B) with MW ~200 and ~150 kDa, respectively, were observed in native gels of normal healthy individuals. In the uncomplicated diabetic group, in addition to band A and B, one more band C was observed with MW ~132 kDa. The diabetic patients with foot ulcer (complicated diabetes) showed a total of eight bands (A–H) in the zymogram analysis. Among the eight bands, A and B were normal forms of MPO, because they found

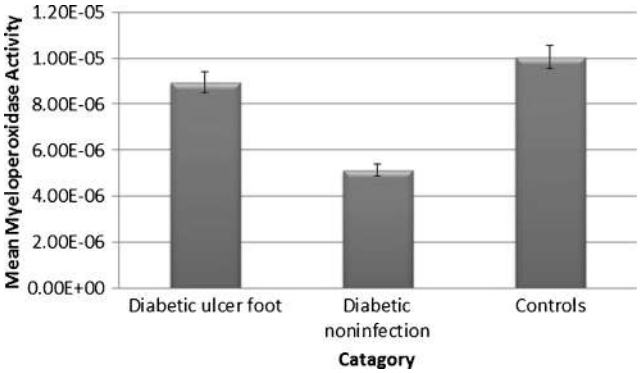

Fig. 1 Myeloperoxidase activity in different study groups

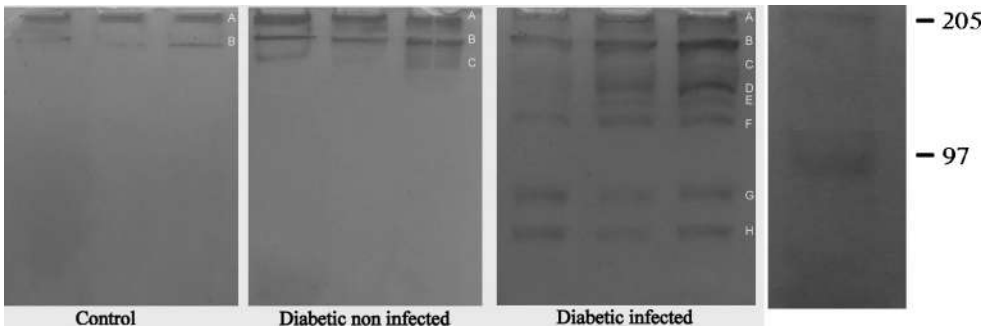

Fig. 2 Isozyme pattern of MPO in diabetic ulcer foot patients and control

in normal healthy individuals also. The band C was the same as that of the diabetic patients without infection. The other five bands visualised were the bands (D–H) of MW ~120, 118, 100, 90 and 73 kDa, respectively. Those 5 bands were obtained only in diabetic patients with foot ulcers compared to other groups.

Discussion

This study mainly focuses on the different isozymic expression of MPO in diabetic patients with infected foot ulcer in comparison with diabetic and non-diabetic controls using native PAGE analysis and specific staining for peroxidase. Even though normal MPO isozymes have been reported [14], no studies are so far reported in diabetic patients especially with infected foot ulcer. Since non-healing foot infections and amputation rise to be a life-threatening problem of diabetic individuals with foot ulcer, this results carries high relevance in the present medical scenario. Two bands of MPO (A and B) were observed in all the study groups. The band B of MW ~150 kDa corresponds to mature enzyme of MPO [15]. Previous studies by Strauven et al. [14] and Park et al. [16] support the zymogram result of non-diabetic control group of this study. Even though the non-diabetic controls possess only two isoforms, they showed significantly highest MPO activity among the study groups ($P < 0.05$). Their mean MPO activity can be considered as normal level as they are free from disease. In normal healthy individuals, MPO activity can be raised during infection to facilitate the phagocytic killing of the invading microorganisms and then later it returns to the normal level of MPO activity [17]. The MPO activity in group 2 revealed that the diabetic condition possesses the least activity among the study groups. Previous studies have reported the decrease in MPO activity in diabetic patients [18, 19]. In group 2, the presence of band C revealed the presence of another form of enzymatic expression with the incidence of diabetes mellitus and this band correlated with MW of homodimer that has lost one light chain (131.5 kDa) [20].

The group 3 comprised of diabetic patients having foot ulcer infection at the level of University of Texas wound classification system (UT 1B, 1D, 2B, 2D, 3B, 3D). Their level of MPO activity was seen to be nearer to the group 1, but significantly elevated than that of the diabetic patients without infection complications ($P < 0.05$). The same relationship was observed previously in another study also [19]. The bands of 90 and 73 KDa seen in diabetic foot ulcer condition are catalytically active intermediate forms of MPO produced during MPO synthesis. The 90 kDa band is of proMPO [21] formed in endoplasplasmic reticulum before the transport to azurophilic granules. Whereas, the band of 73 kDa corresponds to the catalytically active monomer of mature MPO formed during its transport to the trans-Golgi-network and storage in azurophilic granule [22]. This indicates that the defective maturation of MPO has lead to the accumulation of enzymatically active precursor (proMPO) and monomer of the enzyme in complicated diabetic condition. The absence of 73 and 90 kDa bands in the native gel of normal as well as uncomplicated diabetes and its presence in complicated diabetes implies more precursor forms of MPO to be present in diabetic foot ulcer condition. All forms of MPO obtained are enzymatically active and this has been proved by the appearance of bands in native gels in response to specific peroxidase stain. The presence of monomers and proMPO in complicated diabetes may be due to change in amino acid sequence and defects in maturation, thus leading to reduced MPO activity and in turn reduced phagocytic killing in diabetic foot ulcer patients.

Thus, a highly significant reduction observed in MPO activity in diabetic control groups. The MPO levels of complicated diabetes group were placed between those of normal and the uncomplicated group. In uncomplicated diabetes group, even though the MPO activity was very less and insufficient for the phagocytosis of the microorganisms, they were not affected by infections due to proper disease management and extreme care of injuries and infections. But in diabetic patients with infected foot ulcer, there was a hike in MPO activity than diabetic control group and that is not up to normal levels. Even though the MPO activity in diabetic foot ulcer patients was near to that of the healthy individuals, they are unable to have effective phagocytic killing to prevent bacterial infections [19]. In diabetic individuals, some other biochemical situations alter the phagocytosis. The immune system is weakened by hyperglycemia and it leads to a reduced phagocytic activity in them [23]. This reduced phagocytosis is

caused by the glycosylation of complement activation proteins needed for opsonization of invading pathogens in diabetic condition [24]. Such defects in opsonization fail the phagocytosis in diabetic patients. Thus, the reduction in MPO activity leads to the diminished intracellular killing of microorganisms in diabetic condition [8].

Precisely, this study reveals the occurrence of multiple isozymes in diabetic patients (group 2) and in infected condition (group 3). They have exhibited differences in both isozyme pattern and activity of MPO enzyme from that of the control group. Here, the complicated group exhibited multiple bands and low MPO activity, while normal and uncomplicated groups showed lesser number of bands and high MPO activity. The reduction of MPO activity in the infected condition may be due to mutation in the gene, difference in the amino-acid sequence of the protein or defective maturation of enzyme [3].

Conclusion

In the three groups of study we have considered, the non-diabetic normal individuals without infection gave normal MPO activity with two different types of isozymes. The diabetic patients without infection gave least MPO activity with three isozymic bands for MPO. Here, the diabetic individuals without infection gave similar band as that of normal individuals with an additional band of 90 kDa as that present in the diabetic patients with foot infection. The patients with diabetic infection gave a total of eight isozymic bands in which four bands are unique for diabetic infection. From our study, we could thus conclude that the MPO activity shoots up in diabetic infected condition but are unable to reach the activity of the normal healthy individuals; because in this condition, more precursor MPO is formed which are unable to convert to mature MPO. These precursors are visualised as multiple bands in diabetic foot ulcer condition.

Compliance with ethical standards

Funding This study was funded by the Faculty research grant of NITC Calicut (Sanction Order No. NITC/Dean (C&SR)/FRG10/0113).

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

Comparative evaluation of clinical and inflammatory factors in response to the pharmacological managements in metabolic syndrome

Arpit Jain[1] · Suraj S. Yadav[1] · Pradeep Dwivedi[2] · Devendra K. Katiyar[1] · Kauser Usman[3] · Sanjay Khattri[1]

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Abstract Our study focused to assess the effect of various potential treatments on components of metabolic syndrome (MetS). This is a prospective, open-label, parallel group and randomized control trial of 90-day duration in which patients (n = 159) were randomly assigned in four groups to receive "diet and lifestyle modification" (n = 40), "metformin 500 mg twice daily" (n = 39), "pioglitazone 15 mg once daily" (n = 39), and "rosuvastatin 10 mg once daily" (n = 41). Clinical, biochemical, and inflammatory markers of each participant were evaluated. Fasting plasma glucose (FPG) level was lower in all except rosuvastatin group while HDL-cholesterol level increased in rosuvastatin group only ($p < 0.05$). Serum triglyceride was significantly and comparably decreased in both pioglitazone and rosuvastatin group ($p < 0.05$). Significant decrease in hsCRP and increase in serum adiponectin ($p < 0.05$) were reported in all the groups from baseline. The study can add a step forward in devising standard pharmacotherapeutic regimen beyond the current treatment modalities.

Keywords Metabolic syndrome · Pharmacotherapy · Metformin · Pioglitazone · Rosuvastatin · Adiponectin · C-reactive protein

Suraj S. Yadav has Equal contribution as first author

✉ Sanjay Khattri
drsanjaykhattri@gmail.com

[1] Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, UP 226003, India

[2] Department of Pharmacology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India

[3] Department of Medicine, King George's Medical University, Lucknow, UP 226003, India

Introduction

Metabolic syndrome (MetS) is a complex constellation of disorders that increase the risk of cardiovascular events [1]. The developing nations are witnessing an increasing trend in MetS incidence compare than global incidence. MetS is a state of chronic low-grade inflammation as a consequence of complex interplay between genetic and environmental factors [2]. Although diet and lifestyle modifications primarily weight loss, diet, and exercise have been a proven mode of management for the patients of MetS, drug therapy is often needed to manage the individual components of MetS in high risk patients. However, pharmacotherapy addressing MetS as single entity is lacking. Screening of the disorder is also posed with a major challenge of initiating appropriate interventions for individuals who are non-compliant to diet and lifestyle modifications. The Diabetes Prevention Program (DPP) reported that metformin therapy in subjects with prediabetes delays the development of diabetes (T2D) and cardiovascular complications. Thiazolidinediones also have demonstrated efficacy in delaying type 2 diabetes in people with impaired glucose tolerance and insulin resistance [3, 4]. Statins are widely studied in cardiovascular risk prevention. The statin family inhibits that rate-determining step in cholesterol biosynthesis and proved to be efficient in lipid profile correction [5, 6]. At present, there is no specific medication therapy recommended for the treatment of the pro-inflammatory state characterized by elevated C-reactive protein, tumor necrosis factor-alpha, and interleukin-6. To our knowledge, however, there is no report published in Indian population comparing the efficacy of metformin, pioglitazone, and rosuvastatin together with the various components of MetS as well as inflammatory markers (high sensitive C-reactive protein) and endogenous insulin sensitizers (adiponectin) thought to play a major role in its root causation. The results of the study may significantly

add to our knowledge and go a long way in devising a standard therapy for MetS.

Materials and methods

Study design and ethical aspects

The study was prospective, open-label, randomized and active controlled trial of 12-week treatment duration. It is a proof of concept study in which sample size was calculated using level of significance ($\alpha = 0.05$, two-tailed), power of the study ($1\text{-}\beta = 80\ \%$), and effect size was determined using Cohen's predetermined criteria [7]. The study was approved by the institutional ethics committee (approval ref code. 64 ECM II-B/P5) and has been registered with clinical trial registry of India (CTRI201410005139).

Informed consent

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

Enrollment of patients

A total of 215 obese patients attending the OPD of Medicine were examined for waist circumference (WC) and blood pressure (BP) measurement following which they were called for biochemical analysis on the next day in fasting state. Patients ($n = 159$) who satisfied the selection criteria were randomly assigned to receive diet and lifestyle modification ($n = 40$), metformin 500 mg twice daily ($n = 39$), pioglitazone 15 mg once daily ($n = 39$), and rosuvastatin 10 mg once daily ($n = 41$) for 12 weeks after a 2-week washout period. Group allocation of participants represented in Fig. 1 is in accordance to the recommendation of CONSORT (Consolidated Standards of Reporting Trials).

Cases on diet and lifestyle modification were managed according to the American Heart Association guidelines for cardiovascular disease prevention [8]. Cases of this group were kept on a low-calorie, low-fat, and fiber-rich diet along with physical activity of moderate intensity such as brisk walk for 30–60 min for not less than 4 days per week, and with no more than two consecutive days without exercise [8, 9]. Patients were also encouraged to increase their daily lifestyle activities, such as taking walking breaks during workday, gardening, and doing household work. All participants were followed up and regularly counseled telephonically or hospital visit during study period.

Randomization was computer aided. On the day of randomization, participants were asked to report in fasting state and venous blood sample was drawn. Subjects were evaluated every 4 weeks for clinical assessment and to ensure compliance. At 12 weeks, all baseline parameters were repeated. Patients who did not complete the follow-up were excluded from the study.

Inclusion and exclusion criteria

Inclusion criteria included the patients with 18–60 years of age and either sex, diagnosed with MetS as per the IDF consensus worldwide definition, 2006. Exclusion criteria were unwillingness for giving consent, type 1 and type 2 diabetes mellitus, severely deranged lipid profile (TG >500 mg/dL, LDL-C > 250 mg /dL), liver enzymes >2 times of upper limit and serum creatinine >2 mg/dL, history of heart disease, familial dyslipidemia, hyper or hypothyroidism, major medical/surgical illness, hypersensitivity to any of the used drugs, pregnant and lactating females, patient on any other long-term concomitant drugs known to affect glucose tolerance or lipid profile.

Clinical variables

Waist circumference was measured midway between the margin of the lowest ribs and the iliac crest at the point of minimal inspiration. All biochemical measurements were carried out with an appropriate kit according to the manufacturers' instructions. FPG, TG, and HDL-C were measured using Cobas C111 auto analyzer (Roche's Diagnostics, USA) and related kits.

Immunoassays

Estimation of serum adiponectin and high-sensitive C-reactive protein (hsCRP) was done by sandwich ELISA method using Raybiotech (USA) and Biotron (USA) kits, respectively.

Statistical analysis

All analyses were performed on SPSS (Version 20.0 for Windows, Chicago, IL, USA). Data were summarized as Mean ± SD. Intragroup comparisons were made by paired t test while intergroup comparison was done by using analysis of variance followed by post hoc Tukey's HSD test. Two-tailed $p < 0.05$ was considered statistically significant.

Results

A total of 159 patients diagnosed with MetS were randomized out of which 143 patients were finally analyzed due to drop out of 16 cases. The reason for dropout in metformin and pioglitazone group was due to change of contact details and relocation from given address. No serious adverse event was reported in any of the groups during the treatment. The end

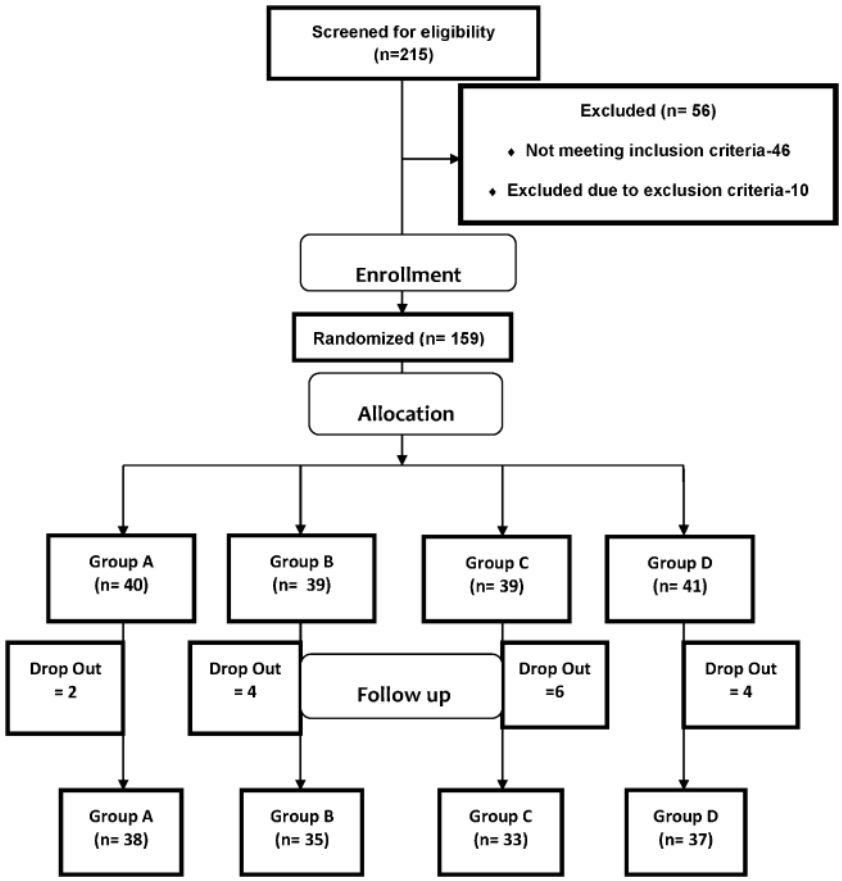

Fig. 1 Study design indicating group wise allocation of participants

point of study mainly considerd as lipid profile correction and improvement in dysglycemia The patients' baseline and demographic characteristics are summarized in Table 1. There was no significant difference in demographic and baseline characteristics between groups at the time of randomization.

Anthropometric variable

No significant alteration was observed in waist circumference in any of the groups between baseline and 12 weeks of follow-up (Table 2). Although, waist circumference showed a downward trend with diet and lifestyle and metformin, this decrease was not significant ($p \geq 0.05$).

Glycemic status

Fasting plasma glucose levels were significantly lower at the end of 12 weeks in all the groups ($p < 0.05$) except rosuvastatin ($p \geq 0.05$). Also, the decrease was comparable in these groups (Table 2).

Lipid profile

Only rosuvastatin significantly increased HDL cholesterol level from baseline (41.04 ± 0.34 to 46.29 ± 11.29; $p < 0.05$). Serum levels of TG were significantly decreased at 12 weeks with both pioglitazone and rosuvastatin (decrease from 184.35 ± 62.05 to 136.52 ± 32.92; $p < 0.05$ and 181.17 ± 62.78 to 141.96 ± 44.67; $p < 0.05$, respectively). Also, there was no significant difference in TG reduction between pioglitazone and rosuvastatin ($p \geq 0.05$) as shown in Table 2.

Inflammatory markers

Significant decrease was seen in hsCRP levels in all the groups ($p < 0.05$, Table 2). Pioglitazone and rosuvastatin were found to be significantly better than diet and lifestyle modification as well as metformin ($p < 0.05$) and comparable to each other after 12 weeks of respective treatment ($p \geq 0.05$). Serum adiponectin levels were found to be significantly higher in all the groups after 12 weeks of follow-up ($p < 0.05$, Table 2). Both pioglitazone and rosuvastatin were found to be significantly better than diet and lifestyle modification and metformin ($p < 0.05$). Also, no significant difference was seen between pioglitazone and rosuvastatin at the end of 12 weeks ($p \geq 0.05$).

Table 1 Demographic and baseline characteristics of studied subjects

Variables (Mean ± SD)	Diet and Lifestyle modification (n = 38)	Metformin (n = 35)	Pioglitazone (n = 33)	Rosuvastatin (n = 37)	p value
Age (years)	40.44 ± 9.44	42.37 ± 7.81	39.86 ± 8.91	43.01 ± 10.12	NS
Gender (M/F)	17/21	15/20	15/18	17/20	NS
Weight (kg)	69.78 ± 18.39	71.16 ± 12.45	73.24 ± 15.61	68.35 ± 13.10	NS
Height (cm)	156.91 ± 7.24	157.43 ± 8.18	155.13 ± 7.65	154.58 ± 7.99	NS
BMI (kg/m^2)	28.13 ± 6.16	28.95 ± 6.23	29.21 ± 6.11	28.70 ± 5.71	NS
WC (cm)	99.28 ± 7.29	102.66 ± 10.93	100.09 ± 7.81	101.97 ± 9.02	NS
SBP (mm/Hg)	135.6 ± 14.47	132.21 ± 14.13	134.87 ± 15.04	134.78 ± 14.43	NS
DBP (mm/Hg)	89.15 ± 9.81	88.83 ± 9.20	89.54 ± 8.85	88.79 ± 8.68	NS
FPG (mg/dl)	106.31 ± 21.20	105.06 ± 20.68	104.78 ± 21.33	103.93 ± 21.15	NS
TG (mg/dl)	171.95 ± 68.18	179.89 ± 65.01	184.35 ± 62.05	181.17 ± 62.78	NS
HDL (mg/dl)	41.75 ± 9.25	39.64 ± 9.38	41.14 ± 11.65	41.04 ± 10.34	NS
CRP (mg/l)	4.62 ± 1.44	4.19 ± 1.55	4.50 ± 1.64	4.37 ± 1.60	NS
Adiponectin(μg/ml)	5.51 ± 2.05	4.77 ± 2.41	4.33 ± 1.86	5.05 ± 2.28	NS

NS not significant (p value >0.05)

Discussion

In this 12-week duration study, we evaluated and compared the effectiveness of metformin, pioglitazone, and rosuvastatin in the correction of clinical and laboratory parameters associate with MetS. The selection of drugs for trial was based on effectiveness of therapies in clinical practices in different components of metabolic syndrome to assess their overall effect.

Table 2 Assessment of clinical, biochemical, and inflammatory variables before and after therapy

Parameter	Groups	0 week	12 week	p value
WC(cm)	Diet and lifestyle modification	99.28 ± 7.29	96.94 ± 9.77	NS
	Metformin	102.66 ± 10.93	99.75 ± 10.25	NS
	Pioglitazone	100.09 ± 7.81	101.53 ± 7.47	NS
	Rosuvastatin	101.97 ± 9.02	100.31 ± 8.96	NS
FPG(mg/dl)	Diet and lifestyle modification	106.31 ± 21.20	93.25 ± 12.95	<0.05
	Metformin	105.06 ± 20.68	88.69 ± 15.36	<0.05
	Pioglitazone	104.78 ± 21.33	88.08 ± 11.44	<0.05
	Rosuvastatin	103.93 ± 21.15	100.14 ± 19.04	NS
HDL(mg/dl)	Diet and lifestyle modification	41.75 ± 9.25	42.84 ± 9.73	NS
	Metformin	39.64 ± 9.38	40.52 ± 9.80	NS
	Pioglitazone	41.14 ± 11.65	42.35 ± 7.70	NS
	Rosuvastatin	41.04 ± 10.34	46.29 ± 11.29	<0.05
TG(mg/dl)	Diet and lifestyle modification	171.95 ± 68.18	165.08 ± 45.54	NS
	Metformin	179.89 ± 65.01	168.63 ± 56.40	NS
	Pioglitazone	184.35 ± 62.05	136.52 ± 32.92	<0.05
	Rosuvastatin	181.17 ± 62.78	141.96 ± 44.67	<0.05
CRP(mg/l)	Diet and lifestyle modification	4.62 ± 1.44	4.12 ± 1.45	<0.05
	Metformin	4.19 ± 1.55	3.76 ± 1.49	<0.05
	Pioglitazone	4.50 ± 1.64	2.80 ± 1.16	<0.05
	Rosuvastatin	4.37 ± 1.60	3.51 ± 1.38	<0.05
Adiponectin (μg/ml)	Diet and lifestyle modification	5.51 ± 2.05	6.30 ± 2.04	<0.05
	Metformin	4.77 ± 2.41	5.58 ± 2.80	<0.05
	Pioglitazone	4.33 ± 1.86	11.10 ± 3.44	<0.05
	Rosuvastatin	5.05 ± 2.28	11.12 ± 3.54	<0.05

NS not significant (p value >0.05)

Despite of other statins, Rosuvastatin is now reported to be one of the potent statins due to higher efficacy in reducing LDL cholesterol and having higher interactions with HMG-CoA reductase with longer half-time than other statins [10]. In our study, we have evaluated effectiveness of emerging and promising statin rosuvastatin in patients with metabolic syndrome, so as to guide the present treatment strategies in the management of metabolic syndrome in our studied north Indian population. We have found out the efficacy of all three drugs with each other and with the standard treatment, i.e., diet and lifestyle modification in patients with MetS.

None of the interventions caused significant reduction in waist circumference. These results are in agreement with previous findings that did not show significant difference in anthropometric parameters by various treatments [9, 11–14]. However, diabetes prevention program reported significant decrease in waist circumference with both lifestyle modification and metformin. This difference is probably due to longer duration, stricter compliance of lifestyle intervention, as well as usage of higher dose of metformin (850 mg BD) in the study [14]. Our study outcomes suggested improvement in glycemic control in all the groups except rosuvastatin. Also, the decrease in FPG was comparable with diet and lifestyle modification, metformin and pioglitazone. Our findings were also supported by previous studies which demonstrated similar improvement in glycemic control by these modalities [2, 8–10]. Previous studies also showed that rosuvastatin could not significantly decrease the fasting levels of glucose [15, 16]. We have observed significant increase in HDL cholesterol with only rosuvastatin. Similar results were reported by various studies in patients with hypercholesterolemia [17] and MetS [15, 18]. Statins cause modest increases in HDL-C and apo A-I probably mediated by reductions in Cholesteryl ester transfer protein (CETP) activity. Although, mechanism (reverse cholesterol transport) by which rosuvastatin increases serum HDL cholesterol [19] is not clearly known, it has been suggested in animal study that rosuvastatin activates ATP-Binding Cassette Transporter A1-dependent efflux and promotes reverse cholesterol transport in macrophage cells [20].

In contrast to our finding, Szapary et.al. 2006 reported significant increase in serum HDL with pioglitazone [21]. This difference may be attributed to higher dose used (30 mg/day for 6 weeks followed by 45 mg/day for further 6 weeks) or due to genetic variation between populations. Fasting serum TG was significantly reduced by both pioglitazone and rosuvastatin [11, 15, 22, 23]. The likely mechanism of lowering of serum TG by rosuvastatin can be explained by reduction in hepatic VLDL production due to reduced synthesis of cholesterol [22]. The hsCRP is one of the robust and independent predictors of future cardiovascular events [24, 25]. Povel et al. 2013 suggested extension of traditional MetS features with hsCRP improves predictive ability for type 2 diabetes and CVD [26]. There was significant reduction noticed in our study in CRP in all the groups with pioglitazone and rosuvastatin being superior to diet and lifestyle modification and metformin and comparable to each other. Stalenhoef et al. 2005 have even repoted 28–29 % reduction in serum hsCRP [15]. Although detailed mechanism through which these modalities decrease inflammation is yet to be known, this may give clinically important prognostic information concerning future cardiovascular risk in MetS patients.

Adiponectin is an endogenous insulin sensitizer and exerts its effect by binding to its receptors such as (AdipoR) leading to activation of AMPK, PPARα, and other unidentified signaling pathways. The lower level is associated with an increased risk of type 2 diabetes mellitus and chronic heart disease. Significant increase in the levels of serum adiponectin was seen in all the groups, with increment being significantly greater with pioglitazone and rosuvastatin than diet and lifestyle modification and metformin. The increase in serum adiponectin by pioglitazone is probably related to enhanced production by smaller adipocytes as preadipocytes are differentiated into smaller and metabolically active ones [27]. Alternatively, direct stimulation of adipocytes by pioglitazone through PPAR-γ may increase the m-RNA expression of adiponectin. According to Kodowaki et al. 2006, a possible therapeutic strategy for the treatment of the metabolic syndrome and associated complications disease may include the upregulation of plasma adiponectin levels, the upregulation of adiponectin receptors, or the development of adiponectin receptor agonists [28].

Considering the escalating prevalence of MetS throughout the world, there is an urgent need to meet the challenges of MetS and its consequences. In our study, pioglitazone and rosuvastatin were found to be superior to current standard therapy "diet and lifestyle modifications." To establish that the above corrections are sustained and translated into reduction in relevant clinical events, further long-term studies with larger sample size and varied populations are warranted. However, the results are encouraging and are a step forward in devising standard pharmacotherapeutic regimen beyond the current treatment modalities.

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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.

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On association between diabetes status and stature of individual in Bangladesh: an ordinal regression analysis

Ashis Talukder[1] · **Taslim S. Mallick**[1]

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Abstract Contravening findings are found in the literature about the association between individuals stature and diabetes status. This paper attempts to find evidence regarding the nature and strength of this association by analyzing a nationally representative data of Bangladesh. Under ordinal regression setup, a partial proportional odds model, a variant of the proportional odds model, has been found to fit better compared to the proportional odds model for analyzing diabetes status in Bangladesh. A strong evidence of inverse relationship is found between diabetes and height. Gender, education level, wealth index, place of residence, division and type of work are also found to be significantly associated with diabetes.

Keywords Diabetes status · Non-communicable disease · Ordinal regression · Proportional odds model

Introduction

Considerable amount of work has been done for identifying possible risk factors of diabetes. It has been found that obesity, poor diet, physical inactivity, family history, and ethnicity are risk factors for type 2 diabetes [1–5]. Recent studies consider height of respondent as a potential risk factor of diabetes [4, 6–12]. The nature of association between height and type 2 diabetes was found positive monotone (as the level of height increases, responses on diabetes status tend to increase toward higher levels) in [7], negative monotone in [6, 12, 13], negative monotone only in women [8], negative monotone only in men [9], and independent in [4]. On the other hand, height was also found to be inversely associated with type 1 and gestational diabetes [14, 15]. In view of the contradictory reports, we studied the association between diabetes and individual stature employing an ordinal regression analysis method in a large national level survey from Bangladesh.

Since prevalence of diabetes is high in South East Asia (SEA) region, special attention has been paid for identifying other possible causes and risk factors of diabetes. Meta analysis of studies between 1995 and 2010 shows an upward trend in the prevalence of diabetes in Bangladesh (a developing country in SEA) [16]. Recent studies on Bangladesh have used different logistic regression models and identified that age, educational level, body mass index (BMI), presence of hypertension, household economic status, and region of residence are significantly associated with individuals diabetes or prediabetes status [2, 3, 6, 17]. There also exists a Bangladeshi diabetes hospital-based study [3] which models three ordinal states of diabetes by using a proportional odds assumption based ordinal logistic regression model. This study [3], however, cannot be generalized since it is not based on nationally representative survey. Moreover, proportional odds is a strong assumption (also known as parallel line assumption) which often does not hold by statistical tests and hence reliability of the results obtained under this assumption, when violated, is questionable.

This paper attempts to analyze Bangladesh Demographic and Health Survey (BDHS) 2011 data, a nationally representative sample survey, in order to summarize the recent status of diabetes and its correlates in Bangladesh, where prevalence of diabetes is higher as compared to the global one. Our main objective is to explore the relationship,

✉ Taslim S. Mallick
tsmallick@yahoo.com

[1] Department of Statistics, Biostatistics and Informatics, University of Dhaka, Dhaka, Bangladesh

adjusted by selected socio-economic and demographic variables, between individuals height with diabetes status measured in three ordinal states by using appropriate ordinal regression model.

Data and variables

BDHS 2011 is a nationally representative sample survey conducted by the National Institute of Population Research and Training (Bangladesh) in collaboration with ICF international (USA) and Mitra and Associates (Bangladesh). This survey used a two-stage stratified cluster sampling of households and obtained a random sample of total 83,731 household members from 17,141 households. This is the first national level survey where information on biomarker component such as systolic blood pressure (SBP), fasting plasma glucose (FPG), and height and weight measurements were collected from a subsample of 8835 individuals. FPG were measured from 3831 females and 3734 males of age 35 years or older. To find the determinants of diabetes and prediabetes status, we categorize the ordinal response variable diabetes status as a function of FPG value following WHO guidelines [18].

$$\text{Diabetes status} = \begin{cases} 1, \text{ Diabetic } (\text{FPG} \geq 7.0 \text{ mmol/l}) \\ 2, \text{ Prediabetic } (6.1 \leq \text{FPG} \leq 6.9 \text{ mmol/l}) \\ 3, \text{ Normal } (3.9 \leq \text{FPG} \leq 6.0 \text{ mmol/l}) \end{cases} \quad (1)$$

A mixture of nominal and ordinal categorical explanatory variables are considered. These are height with four ordered categories based on quartiles of continuous height (in meter), gender (male, female), education level (no education, primary, secondary, above secondary), wealth index (poor, middle, rich) based on percentiles of wealth scores, place of residence (urban, rural), division of residence (Barisal, Chittagong, Dhaka, Khulna, Rajshahi, Rangpur and Sylhet), hypertension (present, if SBP >140 mmHg and absent, otherwise), type of work (desk-work based, physical activity based). Type of work has been used as proxy for physical activity. Respondents are assumed to be involved in physical activities if his/her work responsibility involves physical work. This group includes farmer, agricultural worker, fisherman, poultry raising, cattle raising, rickshaw driver, brick breaking, road building, construction worker, boatman, domestic servant, factory worker, and beggar. On the other hand, physically inactive are those belonging to either of the categories unemployed/student, land owner, home-based manufacturing, carpenter, construction supervisor, tailor, doctor, lawyer, dentist, accountant, teacher, nurse, family welfare visitor, mid and high level services, big businessman, small business/trader, retired, housewife, and imam/religious leader.

Since height and wealth index categories are based on quartiles and terciles, respectively, each of the height categories contains 25 % respondents and each of wealth index categories contains 33.3 % respondents. The distributions of the other selected variables are given in Table 1. Males and females are almost equal in numbers. Percent belonging to educational level decreases as the level goes up. 67 % of the respondents are from the rural area. Division-wise the highest number 17.4 % live in Dhaka and the least 11.4 % live in Barisal division. The prevalence of hypertension is 21.4 %, and almost 25 % of the respondents occupation is physical work is related. The prevalence of diabetes and prediabetes are 10.2 and 26.3 %, respectively.

Methods

The relationship between selected explanatory variables and diabetes status has been assessed in bivariate and

Table 1 Frequency distribution of the selected variables

Characteristics	Measurement scale	Frequency	Percentage
Gender	Nominal		
Female		3831	50.6
Male		3734	49.4
Education level	Ordinal		
No education		3431	45.4
Primary		2083	27.5
Secondary		1404	18.6
Above secondary		647	8.6
Place of residence	Nominal		
Rural		5076	67.1
Urban		2489	32.9
Division	Nominal		
Dhaka		1316	17.4
Barisal		866	11.4
Chittagong		1119	14.8
Khulna		1205	15.9
Rajshahi		1068	14.1
Rangpur		1068	14.1
Sylhet		923	12.2
Hypertension	Nominal		
Yes		1613	21.4
No		5931	78.6
Physical activity	Nominal		
Yes		1862	24.8
No		5654	75.2
Diabetes status	Ordinal		
Normal		4806	63.5
Prediabetic		1986	26.3
Diabetic		773	10.2

multivariate setup. Recall that the dependent variable diabetes status is measured in ordinal scale. In the bivariate setup, we consider gamma measure based on concordant and discordant pairs [19] to explain the nature and strength of association with diabetes status when the explanatory variable is also measured in ordinal scale. On the other hand, the association is assessed by comparing percentages of response categories conditional on the nominal explanatory variables. For testing statistical significance of association in the bivariate setup, asymptotic normal test based on concordant and discordant pairs and Chi-squared test of independence are used for ordinal and nominal explanatory variables, respectively. Bivariate analysis results are reported in Table 2.

Under multivariate setup, we consider three competitive models: (i) proportional odds (PO) model, (ii) partial proportional odds (PPO) model, and (iii) polytomous logistic regression (PLR) model. Among these three models, PO and PPO treats the categorical response as ordinal while the other ignores the ordinal nature of the responses. Note that ordinal models such as PO and PPO generally have higher power for detecting effect of an explanatory variable as compared to models ignoring order, such as PLR [20].

Table 2 Association between selected variables and diabetes status using gamma measure and chi-square test

Characteristics	Measurement scale	Diabetes status			$\hat{\gamma}$	χ^2
		Normal %	Prediabetic %	Diabetic %	(p value)	(p value)
Height (m)	Ordinal	–	–	–	–	–
< 1.51		60.5	28.3	11.2	−0.036	–
1.51-1.58		62.3	27.4	10.3	(0.068)	
1.58-1.64		64.2	26.9	8.8		
> 1.64		64.0	24.5	11.5		
Gender	Nominal	–	–	–	–	–
Female		63.2	26.5	10.3	–	0.439
Male		63.9	26.0	10.1		(0.803)
Education level	Ordinal	–	–	–	–	–
No education		66.1	26.2	7.7	0.110	–
Primary		62.9	26.8	10.2	(0.000)	
Secondary		62.7	25.0	12.3		
Above secondary		53.6	27.2	19.2		
Wealth index	Ordinal	–	–	–	–	–
Poor		65.1	28.2	6.7	0.101	
Middle		65.4	26.4	8.2	(0.000)	
Rich		60.1	24.2	15.8		
Place of residence	Nominal	–	–	–	–	–
Urban		61.9	24.4	13.7		(0.000)
Division	Nominal	–	–	–	–	–
Dhaka		67.0	23.4	9.6	–	127.804
Barisal		53.2	34.9	11.9		(0.000)
Chittagong		56.5	30.3	13.2		
Khulna		70.7	21.2	8.0		
Rajshahi		64.6	25.7	9.7		
Rangpur		69.8	21.9	8.3		
Sylhet		58.9	29.6	11.5		
Hypertension	Nominal	–	–	–	–	–
Yes		66.3	21.8	11.9	–	24.28
No		62.7	27.5	9.8		(0.000)
Physical activity	Nominal	–	–	–	–	–
Yes		66.0	27.0	7.0	–	26.99
No		62.8	26.0	11.2		(0.000)

For a subject i ($i = 1, 2, \cdots, n$), let Y_i denote the k-category response with ordinal categories $1, 2, \cdots, j, \cdots, k$. Also, let $x_i = (x_{i1}, x_{i2}, \cdots, x_{ip})'$ be the vector of k explanatory variables associated with Y_i. The proportional odds model simultaneously uses all $k-1$ cumulative logits which is written as

$$logit\,[P(Y_i \leq j)] = \alpha_j + \beta' x_i \;\; for \;\; j = 1, \cdots, k-1, \quad (2)$$

where $\beta = (\beta_1, \beta_2, \cdots, \beta_p)'$ is the vector of regression parameters associated with x_i, and α_j is the intercept for the jth cumulative logit. In (2), $logit[P(Y_i \leq j)] = log[P(Y_i \leq j)/P(Y_i > j)]$. Note that under PO, it is assumed that the effect of each explanatory variable is the same for any cumulative logit. Although interpretation of the results under proportional odds is convenient, this assumption is very restrictive and often violated by statistical test such as score test [21] or test based on deviance. If proportional odds assumption is violated, partial proportional odds (PPO) model [21] is an alternative. Under this setup, some of the explanatory variables in x_i have a PO structure, but others do not. Deviance test can be used to select covariates that requires separate effect parameters. For details see [20].

Since PLR model is widely used for analyzing categorical data with more than two categories, we also consider this model for our analysis. After fitting these models to the data, we compare AIC values and select the model with minimum AIC as our final model.

Results

The results of the bivariate analyses are reported in Table 2. From the table, it is clear that height, education level, and wealth index have significant monotone relation with diabetes status. Gamma estimates show that there exist significant weak negative association with height categories and significant weak positive association with education level and wealth index. Therefore, with the increase in height, people tend to fall in 'normal' category, while with the increase in education level and wealth index, people tend to fall in 'diabetic' category. Place of residence, division, hypertension, and physical activity status are significantly associated with diabetes status, while no strong evidence of association is observed for gender. The prevalence of diabetes is highest in Chittagong and least in Khulna division. On the other hand, prevalence of prediabetes is highest in Barisal and least in Khulna division. The prevalence of diabetes is 2.1 % higher among the respondents who have hypertension and 4.2 % lower among those whose occupation is physical activity related.

Under multivariate setup, we first fit PO model. The estimated effects of the selected covariates are shown in Table 3. Strong evidence of inverse association between height and diabetes status is observed. That is, as height increases, the risk of transition from normal to diabetic state decreases. Note that the deviance-based chi-squared test reveals strong evidence that the proportional odds assumption has been violated (χ^2 statistic = 117.9, df = 18, $p < 0.01$). That is, a single effect parameter for each of the selected explanatory variables cannot be used to model separate logits of cumulative probabilities. This suggests that a better ordinal regression model can be constructed by assuming PO for a subset of covariates, and non PO for the subset of remaining covariates. We next attempt to fit such a model, known as PPO model.

To fit an appropriate PPO model, a series of tests have been performed for each of the selected covariates. The test results are given in Appendix. Comparing the p values, we select a PPO model, where height and division having PO structure and the remaining covariates having non PO structure. The parameter estimates of this PPO model are also shown in Table 3.

Significant inverse relationship between height and diabetes status is also evident from the PPO model. The strength of association is similar to that observed in the PO model. To be specific, the odds of diabetes is [1-exp(−0.144)] = 13 % lower for those with height 1.51–1.58 m ($p = 0.076$), 23 % lower for those with height 1.58–1.64 m ($p < 0.01$) and 26 % lower for those with height >1.64 m ($p < 0.01$) as compared to those whose height is <1.51 m. The estimated odds ratio (OR) along with 95 % confidence interval (CI) are shown in Table 4. The rate of occurrence of diabetes is significantly lower for the males as compared to females($p < 0.01$). The odds of diabetes is 31 % lower for the males. On the other hand, the odds of diabetes significantly increases with education level. The odds of switching status from normal state is 32 % higher for rich people as compared to poor. The prevalence of diabetes is significantly higher in urban area ($p < 0.01$). People residing in Barisal, Chittagong, and Sylhet division have higher odds of diabetes as compared to Dhaka ($p < 0.01$), where this odds is 18 % lower in Khulna division ($p = 0.056$). It is surprising to see that the prevalence of hypertension has no significant impact on the occurrence of diabetes, rather it increases the risk of prediabetes ($p < 0.05$). Strong evidence is found that physical activity-related jobs increases the chance of staying normal. The odds of diabetes is 26 % lower for those whose jobs are physical activity related ($p < 0.01$).

Since PLR is routinely used to model categorical response with more than two categories, we also fit this model. The PLR-based estimates are shown in Table 3 in the

Table 3 Proportional odds, partial proportional odds, and polytomous logistic regression model based estimated effects of selected covariates

Characteristics	PO model	PPO model		PLR model	
		Diabetic	Diabetic and prediabetic	Diabetic	Prediabetic
	Estimate (p value)	vs. (prediabetic and normal)	vs. (Normal)	vs. Normal	vs. Normal
		Estimate (p value)	Estimate (p value)	Estimate (p value)	Estimate (p value)
Intercept (α_1)	−2.392 (0.000)	−2.577 (0.000)	–	−2.106 (0.000)	–
Intercept (α_2)	−0.718 (0.000)	–	−0.711 (0.000)	–	−1.079 (0.000)
Height (m)	–	–	–	–	–
< 1.51 (ref)		–	–	–	–
1.51-1.58	−0.150 (0.063)	−0.144 (0.076)	−0.144 (0.076)	−0.311 (0.023)	−0.069 (0.453)
1.58-1.64	−0.265 (0.001)	−0.257 (0.002)	−0.257 (0.002)	−0.604 (0.000)	−0.088 (0.354)
> 1.64	−0.300 (0.000)	−0.296 (0.000)	−0.296 (0.000)	−0.514 (0.000)	−0.197 (0.039)
Gender	–	–	–	–	–
Female (ref)	–	–	–		
Male	−0.191 (0.001)	−0.369 (0.000)	−0.151 (0.011)	−0.432 (0.000)	−0.051 (0.444)
Education level	–	–	–	–	–
No education (ref)	–	–	–	–	–
Primary	0.110 (0.119)	0.279 (0.016)	0.087 (0.227)	0.326 (0.008)	−0.003 (0.965)
Secondary	0.090 (0.280)	0.370 (0.004)	0.038 (0.652)	0.404 (0.004)	−0.111 (0.247)
Above secondary	0.642 (0.000)	0.642 (0.000)	0.544 (0.000)	1.114 (0.000)	0.257 (0.042)
Wealth index	–	–	–	–	–
Poor (<−0.056) (ref)	–	–	–	–	–
Middle (−0.056−0.023)	−0.020 (0.767)	0.094 (0.459)	−0.037 (0.598)	0.095 (0.473)	−0.073 (0.351)
Rich (≥ 0.023)	0.384 (0.000)	0.384 (0.000)	0.279 (0.000)	0.901 (0.000)	0.032 (0.686)
Place of residence	–	–	–	–	–
Rural (ref)	–	–	–	–	–
Urban	0.080 (0.197)	0.358 (0.000)	0.022 (0.730)	0.353 (0.000)	−0.106 (0.143)
Division	–	–	–	–	–
Dhaka (ref)	–	–	–	–	–
Barisal	0.512 (0.000)	0.513 (0.000)	0.513 (0.000)	0.464 (0.010)	0.606 (0.000)
Chittagong	0.458 (0.000)	0.458 (0.000)	0.458 (0.000)	0.576 (0.000)	0.417 (0.000)
Khulna	−0.196 (0.056)	−0.201 (0.056)	−0.201 (0.056)	−0.269 (0.133)	−0.158 (0.177)
Rajshahi	0.094 (0.367)	0.094 (0.367)	0.094 (0.367)	0.064 (0.717)	0.124 (0.296)
Rangpur	−0.126 (0.226)	−0.120 (0.250)	−0.120 (0.250)	−0.164 (0.367)	−0.106 (0.372)
Sylhet	0.422 (0.000)	0.418 (0.000)	0.418 (0.000)	0.499 (0.003)	0.395 (0.000)
Hypertension	–	–	–	–	–
No (ref)	–	–	–	–	–
Yes	0.120 (0.083)	−0.160 (0.128)	0.172 (0.015)	−0.098 (0.374)	0.294 (0.000)
Physical activity	–	–	–	–	–
No (ref)	–	–	–	–	–
Yes	−0.146 (0.027)	−0.300 (0.008)	−0.120 (0.075)	−0.313 (0.008)	−0.061 (0.405)
AIC	9075.288	8895.625		8988.375	

last two columns. Among these three fitted models, the PPO model fits the data with the smallest AIC value. Therefore, PPO model fits the data best. Note that, although the difference in AIC between PPO and PLR model is large, results obtained by these two models are very similar.

Discussion

We analyzed the relationship between height and diabetes status in a recent nationally representative survey from Bangladesh. From the bivariate analysis of BDHS, 2011

Table 4 Estimated OR and 95 % CI based on PPO model

Characteristics	PPO model	
	Diabetic vs. (prediabetic and normal) OR (95 % CI)	Diabetic and prediabetic vs. (normal) OR (95 % CI)
Height (m)	–	– –
< 1.51 (ref)		–
1.51–1.58	0.865 (0.738, 1.015)	0.865 (0.738, 1.015)
1.58–1.64	0.772 (0.655, 0.912)	0.772 (0.655, 0.912)
>1.64	0.744 (0.626, 0.883)	0.744 (0.626, 0.883)
Gender	–	–
Female (ref)	–	–
Male	0.691 (0.575, 0.831)	0.859 (0.764, 0.966)
Education level	–	–
No education (ref)	–	–
Primary	1.323 (1.153, 1.662)	1.092 (0.947, 1.259)
Secondary	1.448 (1.122, 1.868)	1.039 (0.879, 1.228)
Above secondary	2.548 (1.914, 3.393)	1.723 (1.391, 2.136)
Wealth index	–	–
Poor (<−0.056) (ref)	–	–
Middle (−0.056−0.023)	1.099 (0.856, 1.411)	0.963 (0.836, 1.108)
Rich (≥ 0.023)	2.379 (1.907, 2.968)	1.323(1.152, 1.519)
Place of residence	–	–
Rural (ref)	–	–
Urban	1.431 (1.185, 1.728)	1.022 (0.902, 1.159)
Division	–	– –
Dhaka (ref)	–	–
Barisal	1.671 (1.357, 2.057)	1.671 (1.357, 2.057)
Chittagong	1.581 (1.303, 1.919)	1.581 (1.303, 1.919)
Khulna	0.817 (0.668, 1.00)	0.817 (0.668, 1.00)
Rajshahi	1.099 (0.896, 1.349)	1.099 (0.896, 1.349)
Rangpur	0.887 (0.723, 1.088)	0.887 (0.723, 1.088)
Sylhet	1.519 (1.238, 1.865)	1.519 (1.238, 1.865)
Hypertension	–	– –
No (ref)	–	–
Yes	0.852 (0.693, 1.048)	1.188 (1.133, 1.367)
Physical activity	–	–
No (ref)	–	–
Yes	0.740 (0.591, 0.927)	0.887 (0.776, 1.013)

data, we found that height, education level, wealth index, place of residence, division, hypertension, and type of work are significantly associated with the occurrence of diabetes and prediabetes. On the other hand, adjusted effects are estimated using PO, PPO, and PLR models. Since PPO model has higher power for detecting effect of an explanatory variable as compared to PLR [20], we consider PPO model as better fitted model to our data. The AIC values also confirm that PPO model fits better for the selected data.

Based on the PPO model, we found strong evidence that as height increases, the odds of diabetes decreases. Females have higher odds of diabetes. Education level is significantly

associated with diabetes—the proportion of diabetes among individuals having education levels more than 'No education,' are significantly higher as compared to those with 'No education.' Individuals from urban area have higher likelihood of having diabetes. Individuals from Barisal, Chittagong, and Sylhet have higher odds of diabetes. We also found evidence that the prevalence of hypertension forces people to switch from 'normal' state. However, it has no significant impact on the occurrence of diabetes. A strong evidence has been found that the odds of diabetes is higher among the group of people who works in physical activity related jobs.

Unlike other risk factors, increased stature and cognition were positively selected during evolution of humans [22]. Besides diabetes, height was inversely related with cardiovascular disease [23], cardiorespiratory diseases [24], and with increased risk of different forms of cancer [25] as well as death due to cancer [26]. A number of postulates were suggested to explain the stature-cancer link: that height does not by itself cause cancer, but could only be a biomarker by the influence of genetic or environmental factors causing cancer [27]. Alternatively, height could serve as a biomarker for biologic mediators of risk' such as the number of cells in the body and the levels of circulating hormones such as sex hormones, growth hormone, and its mediators [27]. A later study provided further evidence for IGF-1 acting as a mediator between height and the risk of developing cancer [28].

While the association between obesity, insulin resistance, and some forms of malignancies have been recognized, the role of stature mediating this, could at present, be considered speculative. However, clinical relation between stature and diabetes and its complications were reported. A cross-sectional study published in 1988 described that type 2 diabetes mellitus was associated with short stature, which was seen along with high waist-hip ratio, independent of body fat content [29]. Similarly, an analysis of the relation between height of the mother and her risk of developing gestational diabetes showed that those in the shortest height quartile had more than 50 % increase in the odds of having gestational diabetes [14]. In addition, tall stature is also a risk factor for the development of diabetic sensory neuropathy [30].

Differential effect of height was reported among children with type 1 diabetes mellitus. In a large cohort of more than 450 children with diabetes, except in those presenting during infancy or early adolescence, taller children had greater risk of developing diabetes mellitus [15].

In conclusion, our analysis from a large nationally representative population from Bangladesh provides strong evidence for an inverse relationship between diabetes and height. Although data from the current study cannot provide mechanistic links, it buttresses earlier hypotheses, and suggests further work in unraveling the link between stature and risk of diabetes in order to both understand the cause and suggest appropriate interventions.

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Compliance with ethical standards This study used a secondary data collected by NIPORT, Bangladesh and MEASURE DHS. All procedures performed in this study involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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

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

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Appendix

Table 5 Deviance tests results for testing PO assumption for each covariate

Covariate	Deviance	Df	*p* value
Height	4.740	3	0.191
Gender	6.897	1	0.008
Education level	15.733	3	0.001
Wealth Index	37.578	2	0.000
Place of residence	13.098	1	0.000
Division	5.892	6	0.435
Hypertension	12.860	1	0.000
Physical activity	3.058	1	0.080

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ORIGINAL ARTICLE

Effect of community management of diabetes mellitus on patients with type 2 diabetes mellitus concomitant with depression

Ke Xiaoyun[1] · **Zhai Hailong**[1] · **Wang Qingzhi**[2]

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Abstract The objective of the study was to explore the effect of management of type 2 diabetes mellitus (T2DM) concomitant with depression in a community setting. Ninety-one cases of patients with T2DM concomitant with depression were selected as study subjects and were randomly divided into two groups, 45 cases as the control group treated with comprehensive treatment for diabetes and 46 cases as the observation group received management of T2DM concomitant with depression besides the above treatments the control group received. After 3 months, body mass index (BMI), fasting plasma glucose (FPG), postprandial two-hour plasma glucose (2hPG), glycosylated hemoglobin (HbA1c), and Hamilton depression scale (HAMD scoring) were compared between the two groups. While BMI in the observation group had no significant difference compared with the control group, FPG, 2hPG, HbA1c, and HAMD scoring in the observation group were significantly higher than those in the control group ($P<0.05$). Standardized management of T2DM concomitant with depression in a community setting can improve the treatment effect for patients with T2DM concomitant with depression.

Keywords Type 2 diabetes mellitus · Depression · Disease management · Psychotherapy · Health education · Community health services

✉ Zhai Hailong
zhl135246@sohu.com

[1] Hubei Polytechnic University, Medical School, Huangshi, Hubei 435003, China

[2] Tongji Medical School, Huazhong University of Science and Technology, Wuhan, Hubei 430032, China

Introduction

Diabetes mellitus (DM) is a common chronic metabolic disease. According to the World Health Organization, it is estimated that 422 million people had diabetes in 2014, representing 8.5 % of adults worldwide [1]. Type 2 diabetes mellitus (T2DM), the most common form of diabetes, typically occurs in those who are overweight or obese. In this disease, the beta cells initially continue to secrete insulin, but the body has become resistant to it, requiring higher levels to restore glucose homeostasis. Individuals with T2DM are significantly more likely than age-matched controls to be disabled, incapacitated, or unemployed [2].

Depression is a clinical syndrome such as depressed mood or loss of interest or pleasure. The clinical symptom must be present for most of the day, every day for at least 2 weeks, represent a change from the individual's usual self, and cause significant distress or impairment [3]. Depression is also a common concomitant psychological disorder in patients with T2DM, resulted from metabolic disturbance [4]. The prevalence rate of depression is nearly twice as high in people with T2DM compared to those without [5].

There is a bidirectional relationship between T2DM and depression. Individuals with newly diagnosed T2DM were 30 % more likely to have had an episode of depression in the past 3 years than were controls without diabetes [6]. Several prospective studies have shown an increased risk of incident T2DM in subjects with previous depression [7, 8]. Furthermore, glycemic control in patients with T2DM concomitant with depression was not satisfied for their poor treatment compliance. On the other hand, due to too many complications and long-term comprehensive treatment, patients with diabetes were prone to negative emotions, which could aggravate depression. Diabetes and depression, both of which have some common pathogenesis such as increased cortisol

[9], influence each other and promote each other, which make patients' condition worse. The impact of comorbid T2DM and depression appears to be additive; such patients are more likely to experience disability-related work loss and have an increased risk of mortality than patients with T2DM or MDD alone [10].

Up to date, there are some management approaches for depression in T2DM. Two randomized controlled depression treatment trials in patients with T2DM concluded that psychotherapy and antidepressant medication (ADM) were each moderately effective for depression in T2DM and that cognitive behavior therapy (CBT) had beneficial effects on glycemic control [11, 12]. Integrated care management, a simple, brief intervention integrating treatment of T2DM and depression, in which the integrated care manager collaborated with physicians to offer education, guideline-based treatment recommendations to patients and to monitor adherence and clinical status, was successful in improving outcomes including lower HbA 1c levels, lower remission of depression, and better adherence to oral hypoglycemic agents and ADM in primary care [13].

Having summarized the management approaches in the above studies and our clinical experiences, we draw up a management of T2DM concomitant with depression including treatment of T2DM and psychological intervention, behavioral interventions, health education, and peer education besides health record, assessment, and home visits. The study aimed to investigate the effect of management of T2DM concomitant with depression.

Subjects and methods

Diagnostic criteria

Diabetes: diagnostic criteria for T2DM recommended by WHO in 1999, i.e., fasting glucose ≥ 7.0 mmol/L or 2hPG ≥ 11.1 mmol/L [14].

Depression: the international classification of diseases, 10th Edition (ICD-10), a depressive episode (F32) or recurrent depressive disorder (F33), as well as the Chinese classification and diagnostic criteria for mental disorders, 3rd Edition (CCMD-3). Hamilton depression scale (HAMD scoring): 17–35.

Subjects

Ninety-six cases of inpatient and outpatient patients with T2DM concomitant with depression in the afflicted hospital to our university from September 2011 to February 2014 were selected. Five of them were reluctant to sign test consent forms, and the remaining 91 patients, who filled patient consent forms which were in compliance with the Helsinki Convention and were approved by the ethics committee, were selected as objects. All subjects were excluded from serious complications of diabetes, history of mental illness, and history of taking antidepressant medication within 2 months. They were randomly divided into two groups by drawing lots, 45 cases as the control group and 46 cases as the observation group. Age, gender, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), duration of diabetes, and HAMD scoring were not statistically significant between the two groups ($P > 0.05$), as shown in Table 1.

Methods

While the control group were treated with the comprehensive therapy for diabetes including diabetes education, glycemia monitoring, appropriate exercises, diet control, control of blood pressure, blood lipid, and glycemia, the observation group received management of T2DM concomitant with depression, including psychological intervention, behavioral interventions, health education, and peer education besides health record, assessment, and home visits, plus the comprehensive therapy for diabetes. Both groups were treated for 3 months.

Psychological interventions Psychological intervention was performed by a professional psychologist with cognitive behavioral therapy and supportive psychotherapy once a week. By talking with patients, the psychologist learned about their psychological social factors such as life experience, work stress, and family status, eliminated their anxiety, tension, and even pessimistic mood, encouraged them by creating environments supporting the treatment of their disorders, made them have ability of recognition and control of negative mood and self-psychological regulation skills, and improved their ability of self-correction and adaption to their changes of disorder condition.

Behavioral interventions After learning about their unhealthy habits and behaviors, patients' bad addictions and habits were corrected based on their different situations. We help them draw up their personalized and comprehensive program, especially in diet, exercise, glycemia monitoring, personal interests, and hobbies.

Health education Concentrated education and individual education were combined. Concentrated education mainly comprised preaching diabetes-associated knowledge 2–3 times a month, supplemented with distribution of popular scientific information on diabetes and playing of videos on knowledge associated with diabetes. Individual education required the patients to come to the Department of Endocrinology, Department of Psychiatry, and Department of Psychology twice a month and discussed with physicians and nurses there,

Table 1 Comparison of general characteristic between the observation group (n = 46) and the control group (n = 45)

Group	Age (years)	Gender (males/females)	Duration of diabetes (years)	FPG (mmol/L)	HbA1c (%)	HAMD scoring
Observation	55.3 ± 5.2	27/19	10.4 ± 3.9	8.3 ± 0.9	7.9 ± 1.6	26.9 ± 3.4
Control	54.9 ± 6.1	25/20	11.9 ± 3.4	8.7 ± 0.6	8.2 ± 1.2	26.3 ± 3.1

Data are means ± SD. A Student's t test was used to test differences between the observation and control groups. $P < 0.05$ was considered significantly different

FPG fasting plasma glucose, *HbA1c* glycosylated hemoglobin, *HAMD* Hamilton depression scale

who would provide individual health guidance for patients according to their individual conditions, face to face for 1–2 h. Main contents of health education included basic knowledge related to diabetes, diabetes symptoms and its risk factors, individual treatment goal, basic treatment methods, drug adverse drug reaction, healthy lifestyle, reasonable diet arrangements, appropriate exercises, correct medication method and medication attentions, method and meaning of self-glycemia monitoring, recognition and control of complications, correct selection and using of insulin, psychological education for diabetes, and self-psychological regulation.

Peer education Communications among patients were the main contents. In other words, patients encouraged each other, introduced mutual experience and feeling, and drew on lessons during the concentrated teaching interval.

Detection

BMI, FPG, 2hPG, HbA1c, and HAMD scoring were compared between the two groups. FPG and 2hPG were detected with the glucose oxidase method and HbA1c with the micro-column chromatography method.

Statistics

SPSS12.0 was applied for statistical analysis. Quantitative data were described with x ±s. Averages between the two groups were compared with Student' s test after all the data were confirmed to be distributed randomly. $P < 0.05$ was regarded as significant difference.

Results

While BMI in the observation group had no significant difference compared with the control group, FPG, 2hPG, HbA1c, and HAMD scoring in the observation group were significantly higher than those in the control group (P<0.05), as shown in Table 2.

Discussion

In our study, males/females in the observation group and the control group were similar. Age, FPG, HbA1c, duration of diabetes, HAMD scoring before treatment, and interventions in the two groups were not statistically significant between the two groups ($P > 0.05$). That is to say, the two groups did not differ statistically on baseline measures. However, after treatment and interventions, our study found that management of T2DM concomitant with depression, mainly including psychological intervention, behavioral interventions, health education, and peer education, plus comprehensive therapy for diabetes could significantly lower FPG, 2hPG, HbA1c, and HAMD scoring in the observation group than the control group which was treated with comprehensive therapy for diabetes alone. In another word, management of T2DM concomitant with depression could improve treatment effect of T2DM concomitant with depression. The efficacy of management of T2DM concomitant with depression on glucose control and depressive symptoms was consistent with previous study, in which percentages of patients achieved greater glucose control in the integrated care intervention group compared with patients in the usual care group as well as fewer depressive

Table 2 BMI, FPG, 2hPG, HbA1c, and HAMD scoring in the observation group (n = 46) and control group (n = 45)

Group	BMI (kg/m^2)	FPG (mmol/L)	2hPG (mmol/L)	HbA1c (%)	HAMD scoring
Observation	24.3 ± 3.2	6.6 ± 0.5*	8.6 ± 1.9*	7.8 ± 1.3*	10.5 ± 3.2*
Control	24.1 ± 3.3	7.2 ± 0.8	9.7 ± 2.3	8.5 ± 1.2	24.3 ± 4.6

Data are means ± SD. A Student's t test was used to test differences between the observation and control group

BMI body mass index, *FPG* fasting plasma glucose, *HbA1c* glycosylated hemoglobin, *HAMD* Hamilton depression scale

*$P < 0.05$ was considered significantly different

symptoms [13]. Our study indicated depression in T2DM can be treated with moderate success by various psychological and pharmacological interventions, which are often implemented through collaborative care and stepped-care approaches [15].

T2DM and depression might have a common pathogenesis. Common epigenetic factors may contribute to T2DM and depression. One important factor is a low socioeconomic status that increases the odds for T2DM [16] but also appears to be a cause for depression [17]. The other common causes for T2DM and depression are poor sleep, lack of physical exercises, and diet. Taking these factors into account, a key candidate for a common pathway could be the activation and disturbance of the stress system. Chronic stress activates the hypothalamus–pituitary–adrenal axis (HPA axis) and the sympathetic nervous system (SNS), increasing the production of cortisol in the adrenal cortex and the production of adrenalin and noradrenalin in the adrenal medulla [18]. Chronic hypercortisolemia and prolonged SNS activation promote insulin resistance and visceral obesity and lead to metabolic syndrome and T2DM. On the other hand, chronic stress has behavioral consequences: noradrenalin, cortisol, and other hormones activate the fear system determining anxiety, anorexia, or hyperphagia; the same mediators cause tachyphylaxis of the reward system, which produces depression and cravings for food, other substances, or stress [19]. Excess cortisol disturbs neurogenesis in the hippocampus [20], a region involved in depression as well as in T2DM [21]. Moreover, chronic stress induces immune dysfunction directly or through the HPA axis or SNS, increasing the production of inflammatory cytokines. High amounts of inflammatory cytokines interact with the normal functioning of the pancreatic β cells, induce insulin resistance, and, thus, promote the appearance of T2DM [22]. Many new studies suggest that inflammatory responses are also involved in the pathophysiology of depression. Proinflammatory cytokines have been found to interact with many of the pathophysiological domains that characterize depression, including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and behavior. Fifty percent of the patients treated with interferon alfa develop depression, and patients with depression had statistically higher blood levels of cytokines like tumor necrosis factor and interleukin 6 than those without depression [23]. These correlations suggested that stress and inflammation both promote depression and T2DM.

So, the following factors could contribute to the better effect in our study: (1) Patients's cognitive abilities on complications of T2DM and impact of negative mood due to depression were improved, since cognitive disorder is one of main factors which affect treatment effect and prognosis of T2DM concomitant with depression [24], and their stress, a common cause of T2DM and depression [19], was alleviated by psychological intervention and health education in management of T2DM concomitant with depression. (2) Behavior intervention in management of T2DM concomitant with depression could effectively control glycemia (HbA1c) [12] and reduced inflammation factor level, which could mitigate depression [23], due to high glycemia [25]. (3) By standard management, patients' glycemia was well controlled. In particular, after health education, patients stuck to exercises, which could adjust to adaptation of HPA axis and reduce excess cortisol release. (4) Psychotherapy and behavioral intervention can relieve depression and help glycemic control, which ameliorated the HPA axis dysfunction.

Giving improved glycemic control in the treatment of depression by the use of selective serotonin reuptake inhibitors or psychological approaches is conflicting [15]; ADM were mot administered in our management of T2DM concomitant with depression.

In short, standardized management of T2DM concomitant with depression can improve the treatment effect for patients with type 2 diabetes mellitus concomitant with depression. However, a study with larger sample size and longer observation time is needed.

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Compliance with ethical standards

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

Ethical approval Ninety-one subjects filled patient consent forms which were in compliance with the Helsinki Convention and were approved by the ethics committee.

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

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ORIGINAL ARTICLE

Assessment of prescription adherence to the AACE guidelines and risk factors for type 2 diabetes in a South Indian tertiary care hospital

Ravindrababu Pingili[1,2] · Dilip Arumekala[1] · Yamini Gorrepati[1] · Ramya Sri Gottam[1] · Chandrika Konathala[1] · Siva Reddy Challa[1,2] · Naveenbabu Kilaru[1,2]

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Abstract Genetic, environmental, and metabolic risk factors are interrelated and contributed to the development of type 2 diabetes mellitus (T2DM). The American Association of Clinical Endocrinologists (AACE) guidelines are gold standard for the management of T2DM. The objective of the study was to assess the prescription adherence to AACE treatment guidelines and risk factors for T2DM in a South Indian tertiary care hospital. A cross-sectional study was carried out in 950 subjects (with or without T2DM), and prescription adherence was assessed by comparing with the AACE guidelines. Odds ratios were calculated in univariate logistic regression analysis for risk factors. T2DM was significantly higher in the subjects of age above 40 years, females, married, BMI, urban residence, higher socioeconomic class, hypertension as comorbidity, physical inactivity, smokers, alcoholics, and stress. Metformin was the most widely prescribed drug followed by α-glucosidase inhibitors in monotherapy. The overall rate of prescription adherence was 88.0 % (when HbA1c <7.5 %, $P < 0.01$); 86.63 % (when HbA1c 7.5–9 %, $P < 0.001$), and 80 % (when HbA1c >9 %, $P < 0.001$). Univariate regression analysis showed that the age (above 40 years), female gender, married, BMI (>25 kg/m2), urban residence, body weight (above 50 kg), high economic class, hypertension, past alcoholic, physical inactivity, and stress are the significantly risk factors for T2DM. The present study results suggested that prescription adherence to AACE guidelines was optimal. Female gender, age (above 50 years), hypertension, stress, physical inactivity, urban residence, body weight, BMI, and high socioeconomic status are the major risk factors for T2DM.

Keywords Diabetes mellitus · Risk factors · Medication adherence · Stress · Physical inactivity

✉ Ravindrababu Pingili
ravindrapingili@gmail.com

[1] Department of Pharmacy Practice, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh 520010, India

[2] Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh 520010, India

Introduction

Prevalence of type 2 diabetes mellitus (T2DM) is increasing globally and has reached epidemic proportions in many countries. The recent estimates by the International Diabetes Federation (IDF) showed that the number of adults affected by the disease in 2011 was 366 million which was projected to increase to 552 million by 2030 [1]. Nearly 80 % of the affected people live in middle- and low-income countries [2]. Genetic, environmental, and metabolic risk factors are interrelated and contribute to the development of T2DM. A family history of diabetes, an increase in body mass index (BMI), and impaired insulin secretion and action, hypertension physical inactivity, urbanization, industrialization, and globalization are the major risk factors for T2DM [3].

T2DM complications are divided into macrovascular and microvascular. Microvascular complications (nephropathy, retinopathy, and neuropathy) may develop early and are strongly related to glycated hemoglobin (HbA1c), but microvascular complications do not correlate linearly with HbA1c [4]. The American Association of Clinical Endocrinalists (AACE) guidelines are "gold standard" for the management of T2DM (Fig. 1) [5]. AACE guidelines were designed based on the HbA1c, a gold standard biomarker in T2DM [6]. It is well known that the treatment outcome is far from the standard

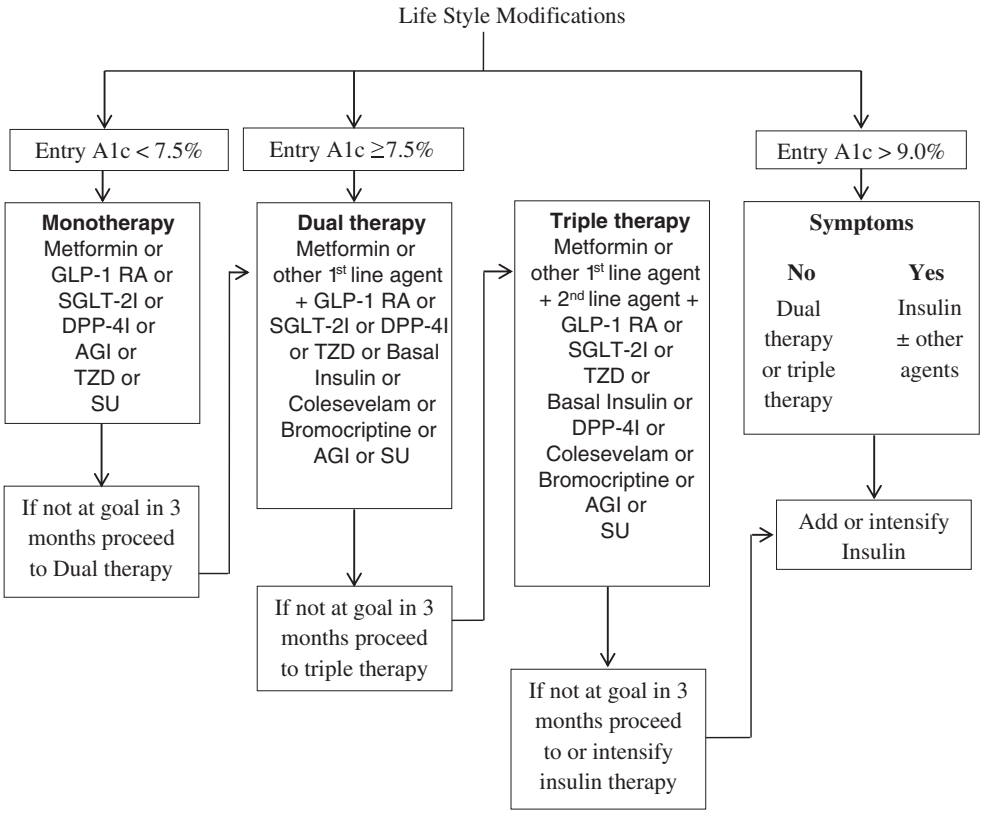

Fig. 1 Algorithm for treatment of diabetes mellitus according to the AACE/ACE guidelines-2015. *GLP-1 RA* glucagon-like peptide receptor agonists, *SGLT-2I* sodium glucose cotransporter 2 inhibitors, *DPP-4I* dipeptidyl peptidase 4 inhibitors, *AGI* alpha-glucosidase inhibitors, *TZD* thiazolidinediones, *SUs* sulfonylureas

guidelines among diabetes patients even in developed countries. Studies in India indicate that clinical outcome is very poor among majority of the patients even in cities where facilities for ideal diabetes treatment are available [7].

The challenges for diabetes care in developed and developing countries include improved education to alert the population about the risk factors for diabetes and to train the patients to manage their disease more effectively by following standard guidelines like the AACE. Therefore, the present study was planned to identify the major risk factors and to evaluate the prescription adherence to the AACE guidelines in a South Indian tertiary care hospital.

Materials and methods

For this purpose, a cross-sectional study was carried out at outpatient's department of Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Vijayawada, Andhra Pradesh, South India. The study was initiated after approval by the Institutes Ethical Review Committee, KVSR Siddhartha College of Pharmaceutical Sciences (SCOPS), Vijayawada, India. KVSR SCOPS was recognized by All India Council of Technical Education (AICTE) and Pharmacy Council of India (PCI), New Delhi, Govt. of India. The protocol approval number was KVSRSCOPS/IEC/2015/003.

Selection of participants

Patients of either sex diagnosed with or without T2DM of any duration (as per AACE guidelines) and willing to participate were included in the study. A total of 950 patients (600 patients with T2DM and 350 patients without T2DM) were enrolled in the study.

Inclusion criteria

Prescriptions from all diabetic patients of either gender are included.

Exclusion criteria

We excluded newly diagnosed diabetic patients, type 1 diabetic patients, diabetic patient on antidiabetic therapy for <1 year, diabetic coma patients, repeat attendance, patients not willing for informed consent, and blood glucose investigation.

Data collection

Physicians were requested to report the clinical and biochemical data not exceeding 6 months before the observation. Biochemical parameters were derived from the latest laboratory investigation reports documented in the

clinical records. A total of 480 prescriptions were collected from diabetic patients. Each prescription includes the drug, quantity, duration, and date of dispensing. Each antidiabetic medication was categorized into one of the following classes: Biguanides (Metformin), sulfonylureas, α-glucosidase inhibitors, thiazolidiones, dipeptidyl peptidase 4 inhibitors, glucagon-like peptide-incretin receptor agonist, and sodium glucose cotransporter inhibitors. The information regarding sociodemographics (age, sex, marital status, education, family history of known hypertension, BMI, monthly income, and comorbid conditions) and lifestyle characteristics (residential area, alcohol consumption, smoking status, food habits, stress at work place, and physical activity) was collected by interviewing the participant to identify the possible risk factor. All the relevant data were collected in a predesigned paper case record form with prior consent of the participant. Prescription adherence was assessed by comparing with the AACE guidelines (Fig. 1), and odds ratios were calculated in univariate logistic regression analysis for risk factors.

Statistical analysis

Statistical analyses were performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA) and Graph Pad Prism 5.0 software (San Diego, CA). Estimates were expressed as mean ± SD. One-way analysis of variance or Student's *t* test was used to compare groups for continuous variables, and χ^2 test was used to compare proportions between the two groups. Univariate logistic regression analysis was used to examine the association between various exposures (age, gender, place of residence, generalized obesity, cigarette smoking, alcohol consumption, income status, and literacy level) and outcome (T2DM). *P* value < 0.05 was considered significant.

Table 1 Biochemical and clinical characteristics of patients with T2DM

Variable	Patients with T2DM *n* (%)
Age (years)	
0–20 years	2 (0.33)
21–40 years	85 (14.11)
41–60 years	360 (60.00)
Above 60 years	153 (25.50)
Body weight (Kg)	
< 50	16 (2.00)
50–70	247 (42.00)
> 70	337 (56.00)
HbA_{1C}	
< 7.5	218 (36.33)
7.5–9	178 (29.66)
> 9	204 (34.00)
Fasting blood glucose (mg/dl)	
70–80	9 (1.50)
80–120	223 (37.16)
121–160	215 (35.83)
161–200	86 (14.30)
> 200	70 (11.61)
Lipid profile (mg/dl)	
HDL	35 (5.50)
TG	55 (9.00)
Normal	510 (85.00)
Duration of T2DM (years)	
< 5	181 (30.12)
5–10	224 (37.42)
> 10	195 (32.50)
Following T2DM education	
Yes	400 (66.66)
No	200 (33.33)

T2DM type2 diabetes mellitus, *HbA1c* glycated hemoglobin, *HDL* high-density lipoproteins, *TG* triglycerides

Results

A total of 950 subjects (600 with T2DM and 350 without T2DM) were included in the study, and the clinical characteristics of T2DM were presented in Table 1. T2DM was significantly higher in females (52.15 %, $P = 0.034$) than in males (47.92 %). About 60 % diabetic patients are in the age group of 41–60 years and 56.00 % have body weight > 70 kg. Tables 2 and 3 show the sociodemographics and life style characteristics of subjects with and without T2DM, respectively. The prevalence of T2DM was significantly higher in females (52.15 %, $P = 0.037$) than in males. Diabetic patients were older (mean ± SD age was 54.04 ± 11.18 years) and had a greater BMI (>25 kg/m^2, $P < 0.0009$). T2DM was significantly higher in the subjects of age (41–60 years, 60.07 %, $P = 0.013$; above 61 years, 25.52 %, $P = 0.025$), married (99.20 %, $P < 0.0001$), body weight (above 70 kg, 56.00 %, $P < 0.0001$), housewives (37.65 %, $P = 0.018$), urban residence (38.02 %, P 0.024), upper class of socioeconomic status (21.9 %, $P < 0.0001$), hypertension (27.85 %, $P < 0.0001$), physical inactivity (58.23 %, $P < 0.0001$), past alcoholic history (3.45 %, $P = 0.032$), and stress (75.13 %, $P = 0.004$) for nondiabetic subjects. Age (21–40 years, $P = 0.466$), education ($P = 0.281$), mixed (vegetarian and nonvegetarian) food style, smoking, current alcoholic, junk foods, fruits, soft drinks, tea, and coffee are not significantly associated with the T2DM.

Table 2 Sociodemographic characteristics of patients with or without T2DM

Variable	Without T2DM *n* (%)	With T2DM *n* (%)	*P* value[a]
Gender			
Male	193 (55.10)	288 (47.90)	Ref
Female	157 (44.90)	312 (52.10)	0.037
Age			
Mean ± SD	48.375 ± 16.841	54.041 ± 11.180	<0.0001[b]
0–20 years	6.0 (2.00)	2 (0.33)	Ref
21–40 years	104 (30.00)	85 (14.11)	0.466
41–60 years	160 (45.00)	360 (60.07)	0.013
Above 60 years	80 (23.00)	153 (25.50)	0.025
Marital status			
Unmarried	37 (10.60)	5 (0.80)	Ref
Married	313 (89.40)	595 (99.20)	<0.0001
Education			
Uneducated	182 (52.00)	289 (48.10)	Ref
Educated	168 (48.00)	311 (51.90)	0.281
BMI (kg/m^2)			
< 25 kg/m^2	255 (73.00)	270 (45.00)	Ref
> 25 kg/m^2	95 (27.00)	330 (55.00)	<0.0001
Body weight (Kg)			
< 50	42 (12.00)	16 (2.00)	Ref
50–70	225 (64.00)	247 (42.00)	0.0004
> 70	83 (24.00)	337 (56.00)	<0.0001
Nature of work			
Not working anywhere	71 (20.30)	106 (17.60)	Ref
Private job	89 (25.40)	174 (29.17)	0.190
Govt. job	34 (9.70)	11 (1.83)	<0.0001
Daily labor	59 (16.90)	83 (13.80)	<0.0001
Housewife	97 (27.70)	226 (37.60)	0.018
Locality			
Rural	243 (69.40)	372 (62.00)	Ref
Urban	107 (30.60)	228 (38.00)	0.024
Socioeconomic status			
Lower class	311 (88.80)	469 (78.16)	Ref
Upper class	39 (11.20)	131 (21.94)	<0.0001
Comorbidities			
No	312 (89.00)	370 (61.70)	Ref
Hypertension	25 (7.10)	167 (27.80)	<0.0001
History of CVDs	2 (0.57)	28 (4.67)	0.546
Endocrine diseases[c]	5 (1.42)	24 (4.30)	0.254
Other diseases	6 (1.71)	11 (1.83)	0.282

T2DM type 2 diabetes mellitus, *BMI* body mass index, *CVDs* cardio vascular diseases

[a] Chi-square test

[b] Unpaired *t* test

[c] Hypothyroidism and hyperthyroidism

Drug utilization pattern was assessed and presented the results in Table 4. Metformin (79.35 %) was most widely prescribed drug followed by voglibose (5.04 %) in monotherapy (88.00 %). The combination of metformin with sulfonylureas was most commonly prescribed (38.88 %) in two-drug combination therapy (69.97 %) followed by metformin with DPP4-inhibitors (13.88 %). In triple combination (16.66 %) therapy, the most prescribed combination was metformin +

Table 3 Food and life style characteristics of patients with or without T2DM

Variable	Without T2DM *n* (%)	With T2DM *n* (%)	*P* value[a]
Food habits			
Vegetarian	19 (5.43)	43 (7.20)	Ref
Mixed	331 (94.57)	557 (92.80)	0.341
Physical activity			
Regular exercise	102 (29.10)	251 (41.80)	Ref
No physical activity	248 (70.90)	349 (58.20)	<0.0001
Smoking			
No	292 (83.40)	522 (87.00)	Ref
Yes	44 (12.60)	48 (8.00)	0.030
Past smoker	14 (4.00)	30 (5.00)	0.0960
Alcohol consumption			
No	292 (83.40)	545 (90.80)	Ref
Yes	48 (13.70)	35 (5.80)	<0.0001
Past alcoholic	10 (2.90)	20 (3.40)	0.032
Junk foods			
No	62 (17.80)	163 (27.10)	Ref
Weekly once	128 (36.70)	213 (35.50)	0.014
Weekly twice	68 (19.50)	47 (7.83)	<0.0001
Weekly thrice and more	8 (2.30)	12 (2.00)	0.300
Occasionally	84 (23.80)	165 (27.50)	0.163
Fruits/fruit juices			
No	42 (12.00)	56 (9.35)	Ref
Weekly once	147 (42.00)	317 (52.83)	0.045
Weekly twice	58 (16.60)	83 (13.83)	0.791
Weekly thrice and more	26 (7.40)	10 (1.66)	0.003
Occasionally	77 (22.00)	134 (22.33)	0.315
Soft drinks			
No	118 (33.80)	411 (68.80)	Ref
Weekly once	12 (3.40)	50 (8.40)	0.745
Weekly twice	3 (0.90)	14 (2.30)	0.775
Weekly thrice and more	2 (0.30)	10 (1.20)	1.000
Occasionally	215 (61.60)	115 (19.30)	<0.0001
Tea/coffee			
No	54 (15.60)	0	Ref
Daily once without sugar	0	305 (50.90)	<0.0001
Daily twice without sugar	0	220 (36.50)	<0.0001
Daily thrice without sugar	9 (2.57)	35 (5.90)	<0.0001
Daily once with sugar	129 (37.20)	40 (6.70)	<0.0001
Daily twice with sugar	132 (38.00)	0	
Daily thrice with sugar	26 (7.30)	0	
Stress			
No	116 (33.50)	146 (24.90)	Ref
Stress	234 (66.50)	454 (75.10)	0.004

[a] Chi-square test

sulfonylureas + thiazolidinediones (13.33 %) followed by metformin + sulfonylureas + α-glucosidase inhibitors (3.33 %). Antidiabetic prescriptions were compared with the AACE guidelines for its optimal adherence and the results present in the Table 4. The overall rate of prescription adherence was 88.0 % (monotherapy, $HbA_{1c} < 7.5$ %; $P < 0.01$); 86.63 % (dual and triple therapy, HbA_{1c} 7.5–9 %; $P < 0.001$), and 80 % (multiple drug therapy, $HbA_{1c} > 9$ %; $P < 0.001$).

Table 4 Prescription adherence to the AACE management guidelines

HbA_{1c} level	Recommendations	Adherence rate (%)	Nonadherence rate (%)	*P* value
HbA_{1c} < 7.5 %	Monotherapy (*N = 218*)	*88.00*	*12.00*	<0.0001
	1. Metformin	79.30	0	
	2. AG inhibitors (voglibose)	5.04		
	3. Sulfonylureas	2.29		
	4.TZD (pioglitazone)	1.37		
	5. DPP4-inhibitors	0		
	6. GLP-IRA	0		
	7. SGLT-2i	0		
HbA_{1c} 7.5–9 %	Dual therapy (*N = 178*)	*86.63*	*13.37*	<0.0001
	1. Metformin + DPP4-inhibitors	13.88	0	
	2. Metformin + sulfonylureas	38.88		
	3. Metformin + AG inhibitors	8.88		
	4. Metformin + thiazolidinedione	8.33		
	Triple therapy			
	Metformin + sulfonylureas + thiazolidinediones	13.33	0	
	Metformin + sulfonylureas + AG inhibitors	3.33		
HbA_{1c} > 9 %	Multiple therapy (*N = 204*)	*80*	*20*	<0.0001
	Metformin + first line therapy + second line therapy + Insulin	80	20	

Tha values in the italics indicated that how many prescriptions followed monotherapy, dual therapy, triple therapy and multiple therapies

AACE American Association of Clinical Endocrinologists, *T2DM* type 2 diabetes mellitus, *AG* alpha-glucosidase, *TZD* thiazolidiones, *DPP* dipeptidyl peptidase, *GLP-IRA* glucogon-like peptide-incretin receptor agonist, *SGLT* sodium glucose cotransporter

Univariate regression analysis was performed to determine the odds ratios for the modifiable and nonmodifiable risk factors for T2DM (Table 5). The analysis showed that age (between 41 and 60) years (odds ratio 6.750, 95 % confidence interval 1.347–33.82, $P = 0.013$), age (above 61 years, odds ratio 5.738, 95 % confidence interval 1.132–29.09, $P = 0.025$), female gender (odds ratio 1.341, 95 % confidence interval 1.029–1.747, $P = 0.031$), married (odds ratio 14.07, 95 % confidence interval 5.473–36.16, $P < 0.0001$), BMI (>25 kg/m^2, odds ratio 3.281, 95 % confidence interval 2.466–4.364, $P < 0.0001$), urban residence (odds ratio 1.401, 95 % confidence interval 1.057–1.856, $P = 0.020$), body weight (50–70 kg, odds ratio 2.882, 95 % confidence interval 1.576–5.270, $P = 0.0004$; >70 kg, odds ratio 10.660, 95 % confidence interval 5.710–19.89, $P < 0.0001$), daily labor (odds ratio 4.348, 95 % confidence interval 2.039–9.275, $P < 0.0001$), housewives (odds ratio 1.656, 95 % confidence interval 1.099–2.496, $P = 0.018$), upper class of socioeconomic status (odds ratio 2.860, 95 % confidence interval 1.861–4.393, $P < 0.0001$), history of hypertension (odds ratio 5.633, 95 % confidence interval 3.604–8.804, $P < 0.0001$), past alcoholic (odds ratio 2.743, 95 % confidence interval 1.143–6.583, $P = 0.032$), physical inactivity (odds ratio 1.749, 95 % confidence interval 1.319–2.318, $P < 0.0001$), and stress (odds ratio 3.300, 95 % confidence interval 1.718–6.340, $P = 0.0001$) are significant risk factors for T2DM.

Discussion

The present study's results suggested that prescription adherence to the AACE guidelines were optimal. Female gender, age (above 50 years), hypertension, stress, physical inactivity, urban residence, body weight, BMI, and high socioeconomic status are the major risk factors for T2DM. India is largely a rural nation and the recent available reports indicate rising prevalence of the disease in the rural areas also [8, 9]. In another 20 years, nearly one fifth of the world's diabetic population will be in India. India faces several major challenges in the management and prevention of T2DM due to rising prevalence in urban and rural areas, genetic, environmental risk factors, and suboptimal diabetes control.

Gender

Gender is one of the risk factors for T2DM. Vashitha et al. [10] conducted a study on the prevalence and predictors of T2DM and concluded that the prevalence was significantly higher in female gender (51.66 %, $P < 0.001$), and females are more at risk of T2DM [10]. In another study conducted on the prevalence and risk factors among people with T2DM revealed that the female gender (58.30 %, $P = 0.05$) was a significant risk factor (odds ratio 1.54, 95 % confidence interval 1.00–2.38, $P = 0.05$) of T2DM [11]. The present study's results also suggested that the

Table 5 Univariate regression analysis of modifiable and nonmodifiable risk factors for type 2 diabetes mellitus

Variable	OR (95 % CI)	*P* value
Age		
21–40 years	2.452 (0.482–12.47)	0.469
41–60 years	6.750 (1.347–33.82)	0.013
Above 60 years	5.738 (1.132–29.09)	0.025
Female gender	1.341 (1.029–1.747)	0.031
Married	14.07 (5.473–36.16)	<0.0001
Educated	1.166 (0.895–1.518)	0.281
BMI (>25 kg/m^2)	3.281 (2.466–4.364)	<0.0001
Urban residence	1.401 (1.057–1.856)	0.020
Body weight (Kg)		
50–70	2.882 (1.576–5.270)	0.0004
> 70	10.660 (5.710–19.89)	<0.0001
Nature of work		
Not working anywhere	Ref	Ref
Private job	1.310 (0.882–1.943)	0.190
Govt job	0.165 (0.080–0.342)	<0.0001
Daily labor	4.348 (2.039–9.275)	<0.0001
Housewife	1.656 (1.099–2.496)	0.018
Socioeconomic status		
Upper class	2.860 (1.861–4.393)	<0.0001
Comorbidities		
No	Ref	Ref
HTN	5.633 (3.604–8.804)	<0.0001
History of CVDs	2.096 (0.469–9.348)	0.546
Endocrine diseases	0.342 (0.060–1.931)	0.254
Other diseases	0.381 (0.095–1.526)	0.282
Food and other habits		
Mixed (vegetarian and nonvegetarian)	0.743 (0.426–1.298)	0.341
Smoking	0.610 (0.395–0.941)	0.030
Past smoking	1.964 (0.923–4.179)	0.096
Alcohol consumption	0.390 (0.247–0.617)	<0.0001
Past alcoholic	2.743 (1.143–6.583)	0.032
Physical inactivity	1.749 (1.319–2.318)	<0.0001
Stress	3.300 (1.718–6.340)	0.0001
Junk foods		
No	Ref	Ref
Weekly once	0.633 (0.439–0.912)	0.633
Weekly twice	0.262 (0.163–0.422)	0.262
Weekly thrice and more	0.570 (0.222–1.463)	0.570
Occasionally	0.747 (0.504–1.107)	0.747
Fruits/fruit juices		
No	Ref	Ref
Weekly once	1.167 (1.036–2.525)	0.045
Weekly twice	1.073 (0.636–1.809)	0.791
Weekly thrice and more	0.288 (0.125–0.662)	0.003
Occasionally	1.305 (0.800–2.128)	0.315
Soft drinks		
No	Ref	Ref
Weekly once	1.196 (0.616–2.32)	0.745
Weekly twice	1.340 (0.378–4.742)	0.775
Weekly thrice and more	1.436 (0.310–6.645)	1.000
Occasionally	0.153 (1.113–0.208)	<0.0001
Tea/coffee		
No	Ref	Ref
Daily once without sugar	0.665 (1.30–3.395)	<0.0001
Daily twice without sugar	0.008 (0.0004–0.148)	<0.0001
Daily thrice without sugar	0.079 (0.035–0.180)	<0.0001
Daily once with sugar	0.012 (0.0007–0.198)	<0.0001

female gender (52.10 %, P = 0.037) was significantly associated with T2DM and was a significant risk factor for T2DM (odds ratio 1.341, 95 % confidence interval 1.029–1.747, P = 0.031).

Age

Increasing age is significantly associated and most significant risk factor for T2DM. In the present study conducted

in southern India, subjects of age 40–60 years (60.07 %, $P = 0.013$) and above 60 years (25.50 %, $P = 0.025$) are at more risk of T2DM (age 40–60 years, odds ratio 6.750, 95 % confidence interval 1.347–33.82, $P = 0.031$; above 60 years, odds ratio 5.738, 95 % confidence interval 1.132–29.09, $P = 0.025$). Shivananda et al. [12] conducted a study and reported that there was a significant association of age 40–60 years ($n = 147$, 24.2 %) and above 60 years (25.60 %) to T2DM [12]. Another study was conducted by Ravikumar et al. [13] on the prevalence and risk factors of T2DM and reported that the increasing age ($P < 0.001$) was significantly associated and risk factor (odds ratio 4.5, 95 % confidence interval 3.6–5.8, $P < 0.001$) of T2DM [13].

Marital status

The present study's results revealed that marital status (99.2 %, $P < 0.0001$) was significantly associated and was the major risk factor for T2DM (odds ratio 14.07, 95 % confidence interval 5.473–36.16, $P < 0.0001$). The present study results were supported by the study conducted by Christy et al. (2014) on the prevalence, correlate, and management of T2DM which concluded that marital status ($n = 136$, 9.10 %, $P < 0.001$) was associated with T2DM [14]. Another study also agreed that marital status ($n = 208$, 61.20 %) was associated with T2DM [11].

Education

A study conducted by Ravikumar et al. [13] on the prevalence and risk factors of T2DM concluded that educational qualification ($P < 0.001$) was significantly related to the presence of T2DM [13]. Another study conducted by Fahad et al. [11] on uncontrolled T2DM prevalence and risk factors among people with T2DM reported that education qualification ($n = 208$, 61.25 %; $P = 0.34$) was not significantly related to T2DM [11]. The present study's results suggested that educational status was not significantly associated with (51.90 %, $P = 0.281$) and not a risk factor of T2DM (odds ratio 1.166, 95 % confidence interval 0.896–1.518, $P = 0.281$).

Obesity

Obesity is one of the major risk factors for T2DM. Chao et al. (2015) conducted a study on T2DM incidence in Chinese contributions of overweight and obesity ($P < 0.001$) and indicated that T2DM is more attributable to overweight and obesity in china [15]. Another study revealed that BMI ≥ 25 kg/m^2 ($P < 0.001$) was a significant risk factor of T2DM [14]. Present study's results also agreed that obese patients ($P < 0.0001$) are at more risk of T2DM (odds ratio 3.281, 95 % confidence interval: 2.466–4.364, $P < 0.0001$).

Housewives and unemployed

Fahad et al. [11] conducted a study on uncontrolled T2DM prevalence and risk factors among people with T2DM and concluded that housewives and unemployed are at risk for T2DM [11]. In the present study, housewives (odds ratio 1.656, 95 % confidence interval 1.099–2.496, $P = 0.018$) and daily labors (odds ratio 4.348, 95 % confidence interval 2.039–9.275, $P < 0.0001$) are at more risk for T2DM.

Urban residence

Urban people are at more risk of T2DM. Ivan et al. [16] conducted a study on the prevalence of T2DM and concluded that urban population ($P < 0.001$) is more prone to T2DM than rural population [16]. The present study also indicated that the urban population is at more risk of developing T2DM than the rural population (odds ratio 1.401, 95 % confidence interval 1.057–1.856, $P = 0.020$).

Socioeconomic status

Low socioeconomic status (SES) is associated with T2DM. Lee et al. [17] conducted a study on sex differences in the association between socioeconomic status and T2DM and reported that lower SES and T2DM are significantly associated to each other ($n = 132$, 28.40 %; $P < 0.001$) [17]. The present study was conducted in developing country (India) and identified that T2DM was more prevalent in low SES subjects (24.66 %, $P = 0.0001$).

Comorbidities

Hypertension ($P < 0.001$) was positively associated with T2DM [13]. Shivananda et al. [12] conducted a study on the association of age to T2DM and reported that hypertension ($n = 147$, 28.40 %) was more significant risk factor for T2DM [12]. The present study's results are also supported that hypertension (27.80 %, $P < 0.0001$) was a risk factor for T2DM (odds ratio 5.633, 95 % confidence interval 3.604–8.804, $P < 0.0001$). Another study conducted by Christy et al. [14] on the prevalence, correlate, and management of T2DM in Lebanon, findings from a national population-based study, had concluded that hypertension ($n = 121$, 17.30 %, $P < 0.001$) is significantly associated with T2DM [14].

Life style and food habits

Life style habits and diet plays a major role in T2DM. Tonstad et al. (2013) conducted a study on vegetarian diets and

incidence of T2DM and identified that vegetarian diets were associated with substantial and independent reduction in T2DM incidence when compared with nonvegetarians ($n = 616$, 61.60 %, $P < 0.0001$) [18]. But, in the present study (92.80 %, $P = 0.341$), there may be bias because questions have not been answered correctly by the patients.

Physical inactivity

Physical inactivity is one of the risk factors for the development of obesity which results in increased risk for T2DM. A study conducted by Sanz et al. [19] on physical exercise for the prevention and treatment of T2DM reported that physical exercise can delay progression to T2DM [19]. In the present study, it was significant that physical inactivity (58.20 %) was a risk factor to T2DM. Another study on physical activity and T2DM conducted by Duclos et al. [20] concluded that less physical activity was a risk factor for T2DM ($P < 0.001$) [20].

Alcohol

Alcohol is a risk factor for T2DM, but alcohol consumption ($n = 120$, 9.30 %, $P = 0.14$) was not associated with increased incidence of T2DM [14]. The current study's results also suggested that alcohol consumption was not a risk factor for T2DM. Therefore, further studies are needed to evaluate the exact impact of alcohol consumption on risk for T2DM (odds ratio 0.390, 95 % confidence interval 0.247–0.617, $P < 0.0001$).

Smoking

When compared to people who never smoked, people who are currently smoking are at no risk and people who are past smokers and not smoking in the present ($n = 91$, 7.50 %, $P < 0.001$) are at risk for incidence of T2DM [14]. The present study's results also suggested that smoking (odds ratio 0.610, 95 % confidence interval 0.395–0.941, $P = 0.030$) was not a risk factor for T2DM. There are some studies which showed the association between smoking and T2DM. Therefore, the impact of smoking on incidence of T2DM should be studied in the future.

Junk foods

A study conducted by Jacqueline et al. [21] on dietary patterns and T2DM reported that consumption of unhealthy marketed foods increase the risk of T2DM [21]. In the present study, consumption of junk foods is associated with increased incidence of T2DM but statistically not significant.

Fruit juices and soft drinks

In the present study, people taking fruit juices and soft drinks are associated with increased risk of acquiring T2DM (52.83 %, $P = 0.045$ and 19.30 %, $P < 0.0001$) when compared to people not taking them. In this study, it is also concluded that there is association between the frequency of consuming soft drinks, fruit juices, and acquiring T2DM. Ehab et al. [22] conducted a study on soft drinks, fruit juice, and vegetable juice intake and risk of diabetes mellitus and concluded that increased consumption of fruits and soft drinks around the world was associated with parallel increase in incidence of T2DM ($P < 0.001$) [22].

Stress

The present study's results suggested that when compared to persons with no stress, the persons with stress either may be from job/working stress or family stress (75.15 %, $P = 0.004$) are at more risk for developing T2DM. A study published by Fahad et al. [11] reported that patients with stress either from anxiety/depression are at increased risk for developing T2DM when compared to stable patients [11]. Another relevant study conducted by Ding et al. [23] also revealed that patients with stress had high odds of acquiring T2DM ($n = 56{,}787$, 1.60 %, $P < 0.01$) when compared to no-stress patients [23]. Therefore, a person may decrease his/her risk of acquiring T2DM by lowering his/her stress levels.

Tea and coffee

Consumption of coffee or both tea and coffee are associated with increased risk for acquiring T2DM. It has also shown that the frequency of consuming tea or coffee or both is contributing to the extent of risk for T2DM (50.92 %, $P < 0.0001$). The results from current study showed that increasing the frequency of coffee intake or both coffee and tea increased the risk for T2DM. Also, there is a most recent study that concluded that drinking tea or coffee cups had an increased risk of acquiring T2DM. Rob et al. [24] conducted a study on coffee consumption and risk of T2DM and concluded that coffee consumption of ≤ 2 cups/day are at more risk for T2DM ($n = 125{,}774$, 95 %, $P = 0.0002$) [24]. Therefore, there is a need of further studies to identify and evaluate the exact impact of tea or coffee on incidence of T2DM.

Prescription adherence

Rajesh et al. [25] conducted a study on a retrospective observational analysis of clinical outcomes before and

after the publication of the AACE/ACE guidelines, and it has been suggested that the adherence of the prescribers to the standard guidelines for prescription of antidiabetic agents was optimal [25]. The overall rate of prescription adherence was 88.0 % (when HbA1c <7.5 %, $P < 0.01$); 86.63 % (when HbA1c 7.5–9 %, $P < 0.001$), and 80 % (when HbA1c >9 %, $P < 0.001$). In the current study, also physician adherence to AACE guidelines for prescribing drugs to T2DM treatment was optimal and physicians adhered to AACE guidelines.

Conclusion

The present study's results indicated that the prescription adherence was optimal and physicians are strictly following the AACE guidelines to prescribe antidiabetic agents. Increased age, female gender, BMI (>25 kg/m^2), urban residence, body weight (>70 kg), daily labors, housewives, high economic status, hypertension, physical inactivity, and stress are the major risk factors for T2DM.

Key finding

1. T2DM prevalence was significantly higher in females compared to males ($P = 0.037$).
2. Hypertension (27.8 %, $P < 0.0001$) was the major comorbidity of T2DM.
3. Age, BMI, socioeconomic status, marital status, body weight, urban residence, physical inactivity, and stress are significantly associated and identified to be the major risk factors for T2DM.
4. Educational status, mixed (vegetarian and nonvegetarian) food habits, smoking, alcohol, junk foods, fruits/fruit juices, soft drinks, and tea and coffee are not significantly associated with T2DM.
5. Though alcohol, smoking, and junk foods are well-known risk factors for T2DM, the risk was insignificant in the present study. Further studies are needed to evaluate the impact of these factors on the prevalence of T2DM.
6. Metformin (79.3 %) was the most widely used drug in monotherapy (88.0 %) followed by voglibose (5.04 %).
7. In combination therapy (86.63), the most commonly prescribed combination was metformin + sulfonylureas (38.88 %).
8. According to the AACE guidelines, the overall rate of prescription adherence was 88.0 % (in monotherapy, $P < 0.0001$), 86.63 % (in combination therapy, $P < 0.001$), and 80 % (in multiple therapy, $P < 0.0001$).

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Compliance with ethical standards

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ORIGINAL ARTICLE

Increased risk of type 2 diabetes mellitus in the Maru Raika community of Rajasthan: a cross-sectional study

Kush Purohit[1] · **Hanwant Singh Rathore**[2] · **Ilse Köhler-Rollefson**[2,3]

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Abstract Type 2 diabetes mellitus (DM) is a chronic metabolic disorder affecting increasing numbers of the global population. Understanding dietary and lifestyle factors that influence the risk of DM will allow us to develop better management strategies for this disease. Here, we aimed to assess the prevalence of PDM in the Raika pastoralist community of Rajasthan, India. This indigenous group has previously shown a 0% prevalence of DM, but we aimed to assess current prevalence due to various lifestyle changes within the community. Three hundred fifteen adult Raika community members from different villages of the Jodhpur, Pali, and Sirohi districts of Rajasthan with no previous diagnosis of DM were selected for participation. Demographic and clinical profiles were obtained. Fasting glucose (FG) and glucose tolerance (GT) tests were performed to diagnose prediabetes (PDM) and DM. Data was assessed using logistic regression in Stata/IC 14. We found prevalence of PDM and DM in the Raika community to be 15.87 and 1.27%, respectively. In the Maru Raika subcaste, we found significantly increased BMI (20.39 kg/m^2 vs 20.26%, $p = 0.002$) and PDM prevalence (19.05 kg/m^2 vs 11.73%, $p = 0.038$, respectively) when compared to the Godwar Raika subcaste. PDM prevalence has significantly increased in the Raika camel-herding community, and demographic, dietary, and lifestyle changes in the traditional Raika camel herders may affect DM prevalence within this rural community.

Keywords Diabetes mellitus · Prediabetes · Prevalence · Raika · Rajasthan · India

✉ Kush Purohit
KushPurohitPitt@gmail.com

[1] 152 Dillon Dr., Pittsburgh, PA 15243, USA

[2] Lokhit Pashu-Palak Sansthan, PO Box 1, District Pali, Sadri, Rajasthan 306702, India

[3] League for Pastoral Peoples and Endogenous Livestock, Pragelatostrasse 20, 64372 Ober-Ramstadt, Germany

Introduction

Type 2 diabetes mellitus (DM) is a chronic metabolic disease affecting approximately 8.8% of the global adult population (415 million individuals) and is associated with a $673 billion global economic burden [1]. DM is characterized by hyperglycemia and insulin resistance, and can lead to severe cardiovascular, metabolic, and renal dysfunction. For instance, diabetic retinopathy, caused by chronic vascular damage due to hyperglycemia, afflicts approximately 21.7% of known diabetics in some parts of urban India, contributing to India's epidemic of blindness [2]. Since 1992, DM rates in India have been rising, with some studies documenting an alarming tripling of prevalence over the last 20 years in rural areas of the country as well [3–5]. Interestingly, diasporic Indians have been shown to have DM rates roughly 4–5 times greater than that of the native population, suggesting that the genetics of the Asian Indian population (such as a "thrifty phenotype") may be contributing to DM's rise in India [6].

To prepare and localize the public health policies necessary to combat this rise in DM, characterizing the prevalence of prediabetes (PDM), a condition involving blood glucose levels slightly lower than those of DM patients, may be a useful tool. This is because studies have found that 25–30% of those with PDM, as diagnosed by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), are likely to develop DM in the next 11 years [7]. Despite the utility of PDM prevalence, there is a scarcity of published research on

PDM prevalence rates in both urban and rural areas of India. This dearth of information underscores the urgency for additional research, especially as DM rates have been steadily climbing in the rural parts of India with increased illiteracy and decreased access to health-care services. For example, low educational status was found to be significantly correlated to microvascular complications of DM in Rajasthan, placing rural DM patients at a greater risk for preventable complications and adverse outcomes due to reduced awareness [8].

In 2004, the Raika community of Rajasthan, a group of indigenous pastoralists composed of the endogamous Maru Raika and Godwar Raika subcastes, was identified as a population with a 0% prevalence of DM [9–11]. The Raika community is a particularly unique group of pastoralists due to their traditional lifestyles as camel herders localized near the Thar Desert of Rajasthan and Gujarat. The community's lifestyle and dietary habits, including camel milk consumption, have been hypothesized to contribute to their paradoxically low rates of DM and PDM prevalence using fasting blood glucose (FG) and oral glucose tolerance (OGTT) tests [9–12].

In this study, we aimed to determine the current state of PDM prevalence in the Raika community, as no additional studies on Raika DM prevalence have been conducted over the past decade. Another goal of this study was to determine differences in prevalence between the Maru and Godwar Raika subcastes. We used questionnaires, FG, and modified glucose tolerance (GT) tests to collect cross-sectional demographic and epidemiological data on this community.

Materials and methods

Data collection Our study aimed to collect cross-sectional epidemiological and demographic data from Maru and Godwar Raika community members in south-central Rajasthan, including the Pali, Sirohi, and Jodhpur districts of Rajasthan. Participants included both male and female community members at least 18 years of age with no previous diagnosis of DM or hypertension, and informed consent was obtained from all individual participants included in the study. All data was collected between August 2014 and April 2015. This project was conducted through the Fulbright US Student program and was approved by the Fulbright National Screening Committee in the USA and the Fulbright Commission in India.

Participant demographic data, including age, occupation, and dietary/exercise habits, were collected using a questionnaire. Additional data such as height, weight, and blood pressure (BP) were collected for each participant using standardized procedures before FG and modified GT tests were performed. Blood pressure was measured using the Omron Healthcare M2 digital blood pressure monitor, while blood glucose levels were measured using the Bayer Contour USB glucose meter. Body mass index (BMI) was calculated using the height and weight of each participant.

Research participants underwent either FG or modified GT testing. Fasted participants underwent FG testing, while non-fasted participants underwent GT testing 2 h following the last meal. Both tests were not conducted for each participant because of the participants' limited availability and time constraints.

Diagnostic criteria Hypertension was diagnosed if BP exceeded >140 mmHg (systolic) and/or >85 mmHg (diastolic). PDM was diagnosed with FG values between 110 and 139 mg/dL or GT values between 140 and 199 mg/dL, while DM was diagnosed with values of ≥140 mg/dL and ≥200 mg/dL, respectively. These criteria were made according to the World Health Organization (WHO) diagnostic values [13, 14].

Sample size The study sample size was calculated using previously published data on PDM and DM prevalence in the Raika community [9]. Using this prevalence data as a proportion, the required sample size to produce data with a confidence level of 95% and margin of error of 5% for our study was calculated to be approximately $n = 116$ individuals. Our study included $n = 315$ research participants.

Statistical analysis The data were assessed by using Stata/IC 14. Prevalence rate was reported in number and percentage. Numerical variables were reported as mean ± standard deviation and ordinal variables in percentage. Intergroup comparisons for numerical variables and proportions were performed using Student's t test. Odds ratios were calculated using binary logistic regression to estimate the association between PDM, age, BMI, and activity level. p values <0.05 were considered statistically significant.

Results

Demographic profile of participants The demographic data collected via questionnaire is summarized in Table 1. The mean age of all participants ($n = 315$) was 49 years ± 16.17, ranging from 18 to 94 years old. The mean BMI of all participants was determined to be 19.56 kg/m^2 ± 3.72, ranging from 13 to 33. Self-reporting also found that all of the research participants followed a strict vegetarian diet, and 43.8% reported an active lifestyle, as opposed to a moderate or sedentary lifestyle (56.2%), as defined by WHO criteria [15]. According to questionnaires, sheep and goat herding (22.0%), manual labor (20.4%), camel herding (11.2%), agriculture (10.4%), and unemployment (18.4%) were the most frequently reported occupations.

Table 1 Demographic profile of study population

Demographic feature	Total (n = 315)
Mean age (years)	49.00
±SD	±16.17
Mean BMI (kg/m^2)	19.56
±SD	±3.72
Diet	
Vegetarian	315/315
(%)	(100%)
Non-vegetarian	0/315
(%)	(0%)
Activity level	
Highly-active	138/315
(%)	(43.81%)
Moderately active/sedentary	177/315
(%)	(56.19%)

Table summarizing demographic data of Raika community research participants (n = 315) collected via questionnaire, including mean age, mean body mass index (BMI), diet, and activity level

Table 2 Epidemiological profile of study population

Clinical feature	Total (n = 315)
Mean fasting glucose (mg/dL)	95.36
±SD	±13.65
IFG prevalence	25/129
(%)	(19.38%)
Mean glucose tolerance (mg/dL)	117.41
±SD	±21.71
IGT prevalence	25/186
(%)	(13.44%)
DM prevalence	4/315
(%)	(1.27%)
PDM prevalence	50/315
(%)	(15.87%)
Blood pressure	
Mean systolic (mmHg)	119.58
±SD	±23.61
Mean diastolic (mmHg)	77.72
±SD	±17.22

Table summarizing epidemiological data of Raika community research participants (n = 315) collected via fasting glucose (FG) test, glucose tolerance (GT) test, and blood pressure (BP) measurement, including mean FG, impaired fasting glucose (IFG) prevalence, mean GT, impaired glucose tolerance (IGT) prevalence, type 2 diabetes mellitus (DM) prevalence, prediabetes (PDM) prevalence, mean systolic BP, and mean diastolic BP

DM prevalence in the Raika community The epidemiological data collected via blood glucose and blood pressure testing is summarized in Table 2. The mean fasting blood glucose level for all participants that underwent FG testing (n = 129) was found to be 95.36 mg/dL ± 13.65. The mean glucose tolerance level for all participants that underwent GT testing 2 h postprandially (n = 186) was found to be 117.41 mg/dL ± 21.71. Using the WHO guidelines to diagnose DM, 1.27% of Raika individuals tested presented with fasting or a 2-h postprandial blood glucose level characteristic of DM.

PDM prevalence in the Raika community The IFG prevalence of all participants that underwent FG testing (n = 129) was found to be approximately 19.38%, while the IGT prevalence of all surveyed individuals that underwent GT testing (n = 186) was found to be approximately 13.44% (Table 2). In total, approximately 15.87% of individuals presented with blood glucose level characteristic of PDM through either IFG or IGT.

Blood pressure of participants The BP data collected via digital blood pressure monitor is summarized in Table 2. The mean systolic BP of all participants (n = 315) was found to be 119.58 mmHg ± 23.61, while the mean diastolic BP was found to be 77.72 mmHg ± 17.22. Approximately 4.44% of all participants presented with BPs greater than 140 mmHg/85 mmHg. According to WHO diagnostic criteria, these patients have hypertension.

Logistic regression analysis for PDM in the Raika community In order to understand clinical factors most strongly associated with developing DM or PDM within the Raika community, we performed logistic regression analysis with reported odds ratios, summarized in Table 3. Analysis was completed for PDM, but not DM due to the low number of DM observations (n < 5). We found that BMI and activity level were most strongly associated with PDM in the Raika community, with reported odds ratios of 1.18 (95% CI 1.18–1.07, p = 0.001) and 0.41 (95% CI 0.19–0.88, p = 0.021), respectively. Age was not found to be significantly associated with Raika community PDM prevalence.

Maru and Godwar Raika subcastes The comparison of the epidemiological profiles of Maru and Godwar Raika community members is summarized in Table 4. The mean age for participants from the Godwar and Maru Raika subcastes was found to be 47.90 years ± 16.68 and 50.18 years ± 15.59, respectively, while the mean BMI was found to be 19.05 kg/m^2 ± 3.48 and 20.39 kg/m^2 ± 3.97, respectively. We found that DM prevalence in the Godwar Raika community was 1.23%, and 1.31% in the Maru Raika community, while PDM prevalence was determined to be 11.73 and 20.26% in the Godwar and Maru Raika communities, respectively. Mean systolic and mean diastolic BP were 118.04 mmHg ± 21.89 and 76.02 mmHg ± 18.12 in the Godwar Raika participants, and 121.22 mmHg ± 25.44 and 79.51 mmHg ± 16.27 in the Maru

Table 3 Logistic regression analysis of PDM with odds ratios

Factor	Odds ratio	95% confidence interval		*p* value
		Lower	Upper	
Age	1.018527	0.9933824	1.044307	0.150
BMI	1.178101	1.06983	1.29733	0.001
Activity level	0.4099748	0.1917707	0.8764603	0.021

Table summarizing results from logistic regression analysis of prediabetes (PDM) in the study group ($n = 315$) with reported odds ratios. Body mass index (BMI) and reduced activity level were significantly associated with PDM prevalence ($p = 0.001$ and $p = 0.021$, respectively). Age was not found to be significantly associated with PDM prevalence ($p = 0.150$)

Table 5 Logistic regression analysis of PDM in Maru Raika community with odds ratios

PDM	Odds ratio	95% confidence interval		*p* value
		Lower	Upper	
Age	1.018554	0.9988858	1.03861	0.065
Maru Raika	1.848398	0.9904581	3.44949	0.050

Table summarizing results from logistic regression analysis of prediabetes (PDM) within the study group ($n = 315$) with reported odds ratios. Maru Raika subcaste membership, but not age, showed trends toward significance in its association with PDM prevalence ($p = 0.050$ and $p = 0.065$, respectively)

Raika participants, respectively. Differences in mean age, DM prevalence, systolic, and diastolic BP were not found to be statistically significant. However, BMI and PDM prevalence were found to be significantly increased in the Maru Raika subcaste ($p = 0.002$ and $p = 0.038$, respectively). Additionally, when age-corrected comparisons between the Godwar and Raika group were made using binary logistic regression analysis (summarized in Table 5), it was found that Maru Raika membership is significantly associated with developing PDM with a reported odds ratio of 1.85 (95% CI 0.99–3.45, $p = 0.050$).

Discussion

India is home to the second largest population of diabetics in the world after China, and the increase in DM over the next 15 years is projected to affect low-income and developing countries disproportionately [1]. This increase will leave rural Indians, such as those in Rajasthan, particularly vulnerable to developing DM due to reduced awareness and access to health-care services. Additionally, rural poverty will undoubtedly increase DM complications as the economic burden and expenditure due to DM in India increases [16]. PDM serves as a useful tool to predict later DM clinical onset, and may provide insight into the changing glycemic profiles of communities as we prepare to combat the upsurge of this lifestyle disease [7].

In 2006, the Raika camel-herding community of Rajasthan was found to have significantly reduced DM prevalence (0.38% DM prevalence, 8.15% PDM prevalence) when compared to their non-Raika counterparts [9]. Here, we aimed to assess current Raika DM prevalence and develop a greater understanding of its distribution within the endogamous

Table 4 Comparison of Godwar and Maru Raika epidemiological profiles

Clinical feature	Godwar Raika ($n = 162$)	Maru Raika ($n = 153$)
Mean age (years)	47.90	50.18
±SD	±16.68	±15.59
Mean BMI (kg/m^2)[a]	19.05	20.39
±SD	±3.48	±3.97
DM prevalence	2/162	2/153
(%)	(1.23%)	(1.31%)
PDM prevalence	19/162	31/153
(%)*	(11.73%)	(20.26%)
Blood pressure		
Mean systolic (mmHg)	118.04	121.22
±SD	±21.86	±25.44
Mean diastolic (mmHg)	76.02	79.51
±SD	±18.12	±16.27

Table comparing the clinical features of the Godwar Raika participants ($n = 162$) with the Maru Raika participants (153), including mean age, mean body mass index (BMI), type 2 diabetes mellitus (DM) prevalence, prediabetes (PDM) prevalence, mean systolic blood pressure (BP), and mean diastolic BP.

[a] Mean BMI and PDM prevalence were found to be significantly increased in the Maru Raika participants ($p = 0.002$ and $p = 0.038$, respectively). Mean age, DM prevalence, systolic BP, and diastolic BP were not found to be significantly different between the two groups

Godwar and Maru subcastes, as there exists a well-maintained dichotomy within the community. Given their endogamous marriage rituals, it is possible that family histories and genetic factors may differentially affect one group. Therefore, in addition to characterizing the current prevalence of diabetes within the Raika, we also attempted to distinguish prevalence rates between the two groups.

Increased PDM prevalence in Raika community since 2006

Our study found that DM prevalence was 1.27% in the total Raika study population, and PDM prevalence was found to be 15.87%, indicating that a number of Raika individuals are at an increased risk for the development of DM within the next decade. As various studies in Rajasthan have reported DM prevalence rates of 5.5–8.6%, our study supports previous findings identifying the Raika community as exhibiting significantly decreased rates of DM when compared to control groups [4, 5, 9–11].

However, when comparing current prevalence data with those collected by Agrawal et al., we found a significantly increased prevalence of PDM in the 2015 Raika population ($p < 0.001$), but not DM prevalence (Table 6, Fig. 1). Our findings indicate an almost doubling of PDM prevalence in the Raika community over the last 10 years, and a statistically significant increase in both fasting blood glucose values, from 86.27 to 95.36 mg/dL ($p < 0.0001$), and 2 h postprandial glucose tolerance, from 113.78 to 117.41 mg/dL ($p = 0.006$). These results suggest that although Raika DM prevalence is still significantly lower than that of the general population, these individuals now have a greater risk to develop DM in the future than they did before. As further analysis using logistic regression revealed that BMI and activity level were the strongest predictors of PDM, lifestyle intervention in this community may be beneficial in reducing DM risk and in preventing DM onset. This supports previous research showing that a "package" of lifestyle changes, including increased physical activity and reduced adiposity, is highly effective in preventing PDM progression to DM [15, 17].

Increased PDM prevalence in Maru Raika community

DM prevalence was distributed as 1.23 and 1.31% in the Godwar and Maru Raika communities, respectively, and the difference was not found to be statistically significant. However, PDM prevalence, distributed as 11.73 and 20.26% in the Godwar and Maru groups, respectively, was significantly increased in Maru Raika individuals when compared to Godwar Raika individuals ($p = 0.038$). Further statistical analysis via logistic regression showed that membership in the Maru Raika clan showed trends of significant association with developing PDM ($p = 0.050$) when prevalence was controlled for age. Because the lower limit confidence interval is less than one, however, we cannot irrefutably conclude that Maru Raika clan membership is associated with PDM risk. These findings suggest that although DM prevalence is still relatively uniformly distributed among the Raika community, Maru Raika members have a higher propensity for developing PDM, a key indicator for future DM prevalence. Therefore, there is a possibility for individuals from the Maru Raika subcaste to exhibit increased DM prevalence within the next decade, when compared to the Godwar Raika individuals.

Causes of increased PDM prevalence

Although it was hypothesized that the Raika community of Rajasthan exhibited DM resistance, our data demonstrate that DM prevalence in this community may be increasing along with the rest of the Indian population. Previous studies describing a trend of increasing DM prevalence in India due to genetic factors, lifestyle changes, and urbanization may provide insight into why the Raika community is experiencing these changes as well [5].

Initially, camel milk consumption and an active nomadic lifestyle were identified as the most protective factors against DM in the Raika community [9–11]. Since 2004, however, a series of agricultural policies and forest closures in Rajasthan (such as the Kumbalgarh National Park) have severely restricted available land for camel, sheep, and goat grazing [18]. Advances in transportation and agriculture have reduced demand for camels and camel dung fertilizer. This has rendered camel herding an increasingly unviable source of income, as evidenced by a drastic estimated 60% reduction of Rajasthan's camel population over the last 20 years [12]. Ultimately, these changes have profoundly affected the traditional Raika community by (1) halting their previously active and nomadic lifestyles, (2) increasing urbanization, and (3) reducing camel milk consumption. Indeed, whereas approximately 65% of respondents reported active lifestyles in 2005–2006, only 44% of participants in the current study did the same [9]. Additionally, an array of biochemical properties of camel milk has been identified as agents promoting hypoglycemic states by attenuating diabetes-mediated increases of inflammation and the incretin hormones TNF-α and TGF-β1 [19].

These factors may also account for the difference in PDM prevalence between the Godwar and Maru subcastes. Traditionally, the Maru Raika community has been described as camel herders, while the Godwar Raika pastoralists are sheep and goat herders [12]. Reduced camel demand and limited grazing land may have therefore disproportionately affected the Maru Raika clan, as camel

Table 6 Comparison of 2006 and 2015 Raika community DM prevalence

Clinical feature	2006 (n = 1055) [9]	2015 (n = 315)
Mean age (years)	47.48	49.00
±SD	±12.47	±16.17
Mean BMI (kg/m^2)	20.85	19.56
±SD	±2.88	±3.72
Mean fasting glucose (mg/dL)*	86.27	95.36
±SD	±11.85	±13.65
Mean glucose tolerance (mg/dL)*	113.78	117.41
±SD	±15.66	±21.71
PDM prevalence	86/1055	50/315
(%)*	(8.15%)	(15.87%)
DM prevalence	4/1055	4/315
(%)	(0.38%)	(1.27%)
Blood pressure		
Mean systolic BP (mmHg)	119.08	119.58
±SD	±14.49	±23.61
Mean diastolic BP (mmHg)	75.89	77.72
±SD	±11.55	±17.22

Table comparing the clinical features from results of the Agrawal et al., 2007 study (n = 1055) [9] and the present study (n = 315), including mean age, mean body mass index (BMI), mean fasting glucose (FG), mean glucose tolerance (GT), prediabetes (PDM) prevalence, type 2 diabetes mellitus (DM) prevalence, mean systolic blood pressure (BP), and mean diastolic BP

*Mean FG, mean GT, and PDM prevalence were found to be significantly increased in the 2015 study ($p < 0.0001$, $p = 0.006$, $p < 0.001$, respectively). Mean age, mean BMI, DM prevalence, systolic BP, and diastolic BP were not found to be significantly different between the two groups

herders require increased grazing land and pay higher grazing fees than do sheep or goat herders. More importantly, the Maru Raika population is localized in the more urbanized Jodhpur district of Rajasthan, whereas the Godwar Raika population lives in the more rural Pali and Sirohi districts [12]. This discrepancy may explain the increased BMI and PDM prevalence when compared to the Godwar Raika clan, as an urban lifestyle, including highly processed and less nutritious foods and reduced physical activity, and may contribute to the glycemic profile of the Maru Raika community.

Limitations

The limitations to our study include sample size, as a greater number of DM observations ($n > 5$) would allow increased statistical power. Future studies should include a larger sample size for increased reliability and accuracy. Additionally, direct

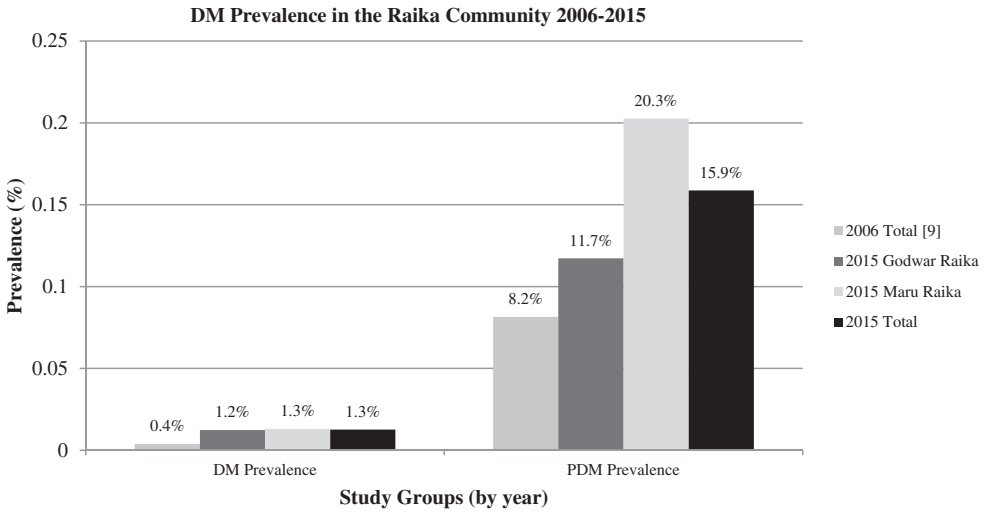

Fig 1 PDM Prevalence in the Raika community from 2006 to 2015. Figure depicting Raika community type 2 diabetes mellitus (*DM*) and prediabetes (*PDM*) prevalence from 2006 [9] to 2015, including differences between the Godwar and Maru Raika subcastes in 2015. Comparisons illustrate a statistically significant increase in PDM prevalence in 2015 when compared to PDM prevalence rates in 2006

comparisons between our findings and Agrawal et al. should be approached with some degree of caution, as it is important to keep in mind the geographical and demographic differences between these two studies. The 2005–2006 study included participants from the Bikaner and Jaisalmer districts of Rajasthan, while our research was completed primarily in the Jodhpur and Pali districts [9]. Furthermore, Agrawal et al. used an oral glucose tolerance test (OGTT), in which participants' blood sugar levels are recorded 2 h following a 75 g oral glucose load. Our study used a modified glucose tolerance (GT) test to record blood glucose levels 2 h following the last meal. Variations in patients' last meals may have confounding effects on our GT test results. In contrast to the 2006 study, our study used the FG or GT test for each patient, but not both. As some prediabetic individuals may present with a normal value for one test but not the other, it is possible that our findings may in fact underestimate the prevalence of PDM [13]. This is also true for the prevalence of DM, as our study excluded previously diagnosed individuals. For this reason, future studies examining PDM prevalence should use both FG and GT (or OGTT) tests for greater accuracy. Additionally, HbA1c testing is widely accepted to be a more accurate measurement of hyperglycemia, whereas single blood glucose tests may be more unreliable [20]. Lastly, glucose tests were conducted at different times of day, allowing possible confounding effects of stress and circadian rhythms on circulating blood glucose levels.

Conclusions

Our study has found evidence that the Raika community of Rajasthan has a 1.27% prevalence of DM and 15.87% prevalence of PDM. It has been found that the PDM rates and mean BMI values in the Maru Raika subcaste are significantly higher than those of the Godwar subcaste. Lastly, the PDM prevalence, mean FG, and mean GT values of the Raika community were found to be significantly increased since previous studies conducted in 2005–2006, and we speculate that this increase is possibly due to a variety of outlined factors. Ultimately, it is our hope that this work will contribute to efforts to prevent and treat DM in all ethnic groups.

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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

Type 1 diabetes mellitus and eating disorders

Mini Joseph[1] · **Asha Hesarghatta Shyamasunder**[1] · **Priya Mammen**[2] · **Nihal Thomas**[1]

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Abstract The choice of type and quantity of food is vital to achieving glycaemic control in diabetes, more so in type 1 diabetes mellitus. The attention to detail could however reach a level of obsession of an eating disorder and thereby have a negative impact on glycaemic control. We conducted a study to see if there was a risk of developing eating disorders among adolescent, young and middle-aged adults with type 1 diabetes mellitus and whether it has an association with HbA_1C levels. A cross-sectional study was conducted on 113 type 1 diabetes mellitus patients and age-gender-matched healthy controls. The two groups were screened using the Eating Attitude Test-26 (EAT-26) questionnaire. EAT-26 identified type 1 diabetes as having a high risk for developing eating disorder when compared to those without diabetes (OR = 38.5 with 95% CI 8.7, 170.7; $p < 0.001$). The risk of developing eating disorder increased with the duration of diabetes. There was no significant difference in the risk between males and females. The risk of developing eating disorder did not correlate with glycaemic control. EAT-26 identified subjects with type 1 diabetes as high risk for developing eating disorder in comparison to those without diabetes. In our setting, this did not reflect on poor glycaemic control.

Keywords Eating disorders · Type 1 diabetes mellitus · EAT-26

✉ Mini Joseph
minijoseph66@yahoo.in

[1] Department of Endocrinology, Diabetes and Metabolism, Christian Medical College & Hospital, Vellore, Tamil Nadu, India

[2] Department of Child & Adolescent Psychiatry Unit, Mental Health Centre, Bagayam, Christian Medical College & Hospital, Vellore, Tamil Nadu, India

Introduction

Patients with type 1 diabetes mellitus (T1DM) are prone to significant changes in their blood glucose levels with various types and amount of food. Hence, they often have to make careful choice of foods to obtain appropriate proportion of macronutrients (carbohydrates, proteins and fats) and also reduce blood glucose fluctuations. This intense diet consciousness is imbibed at an early point in life and increases the patient's risk for disturbances in eating behaviours. Since both the entities T1DM and eating disorder affect largely the adolescent and young adult population, these disorders tend to coexist in this age group [1–3].

The Diagnostic and Statistical Manual of Mental Disorders (DSM-V, 5th edition) defines eating disorder as a serious mental illness characterised by eating, exercise and body weight or shape becoming an unhealthy preoccupation of someone's life. This includes anorexia nervosa, bulimia nervosa, binge eating disorders (BED) or the other eating and feeding disorders [4].

Previously, eating disorders were mostly reported from the Western society; it now pervades the Asian and African continents due to large-scale globalisation. However, the lifetime prevalence of bulimia nervosa (DSM-V/CIDI) varied between countries. The World Mental Health Surveys report that in a low-income country like Colombia, the prevalence was 0.4%; it was higher in middle-income countries like Brazil (2.0%), and in the high-income European countries, it ranged from 0.7 to 0.9% [2]. The prevalence of eating disorders among young North American adolescents (1–3%) was comparable to their Japanese counterparts (0.025–2.9%) [5]. In the study by Mammen et al., using International Classification of Diseases (ICD 10) coding of diagnoses from a tertiary centre in south India, the prevalence of eating disorder among adolescents and children was 1.25% [6].

Subjects with diabetes and eating disorders may omit or reduce insulin to lose weight. Diabulimia is the term used to define the condition when prescribed insulin doses are omitted or altered with the sole goal of achieving weight loss [7, 8].

Western literature has indicated a higher prevalence of eating disorder in diabetes patients as against those without diabetes [8, 9]. Faulty eating habits which may be seen as relatively mild in a person without diabetes can have life-threatening consequences in a patient with diabetes. It can cause poor glycaemic control, dehydration, fatigue, muscle wasting and increased risks of developing infections. If eating disorders are not recognised and treated early, the resulting poor metabolic control can lead to progression of diabetes-related vascular complications. A disordered eating status was more predictive of diabetic retinopathy than the duration of diabetes [1, 8].

Thus, early screening, diagnosis and correction of eating disorders can potentially improve the quality of life of patients. The main objectives of this study were to screen for the risk of developing eating disorders among young adults with and without T1DM and study its association with metabolic control in T1DM patients.

Methods

This cross-sectional study was conducted at the Young Adults Diabetes Clinic, Department of Endocrinology, Diabetes and Metabolism of Christian Medical College, Vellore, India, over a 3 month period. Adolescents, young and middle-aged adults with T1DM (n = 113; 15–43 years of age) were recruited as cases after obtaining informed consent. During their routine hospital visit to the out-patient Diabetes Clinic, they were administered the Eating Attitude Test (EAT-26) questionnaire [11] in English or their mother tongue (Tamil) to detect the risk of developing eating disorders. The Tamil version was translated and back translated to check its accuracy. The control group consisted of age-gender-matched healthy volunteers who visited the hospital as accompanying relatives (n = 61; 16–42 years of age). A face-to-face interview of 30-min duration between the subject and investigator was conducted to collect the data.

The EAT-26 questionnaire is a validated economical screening tool which has been widely used to detect the risk of eating disorders in clinical and non-clinical samples [11]. It consists of 26 questions which are related to eating attitudes or behaviours. The questions pertain to dieting, bulimia, preoccupation and oral control. The patient responds to each question by indicating as to whether the question applies to him/her always, usually, often, sometimes, rarely or never (scoring 5, 4, 3, 2, 1 and 0, respectively). An overall score greater than 20 indicates a risk of developing eating disorders.

Height and weight of the subjects were determined using standard procedures. Insulin dose and glycosylated haemoglobin (HbA_1C) levels were collected from patient medical records. HbA_1C was measured by high-performance liquid chromatography (HPLC). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and approval was obtained from the Institutional Review Board of Christian Medical College, Vellore, India (IRB Min. No. 9547 dated 22.07.2015).

Statistical analysis The data was analysed using SPSS version 18. Chi-square analysis, odds ratio and logistic regression (univariate and multivariate) were done to study the association between eating disorder in diabetes and non-diabetes subjects. p values <0.05 were considered significant.

Results

A total of 113 patients with T1DM and 61 control subjects completed the EAT-26 questionnaire, with approximately equal number of males and females. The basal characteristics of the cases and controls are presented in Table 1. Cases and controls did not differ significantly with respect to gender and age. Controls had a significantly higher body mass index (BMI) than T1DM subjects (23.7 vs. 20.9 kg/m^2, $p < 0.001$) (the BMIs of four non-ambulatory T1DM patients were not

Table 1 Comparison of basal characteristics of subjects with and without Diabetes mellitus

Profile	With T1DM n (%)	Without T1DM n (%)	p value
Gender			
Male	58 (51)	31 (51)	–
Female	55 (49)	30 (49)	
Age[a]	24.6 (6.3)	26.7 (5.3)	$p = 0.288$
BMI[a]	20.9 (3.0)	23.7 (4.7)	*$p < 0.001$
BMI category[b]			
Underweight	23 (21.1)	6 (10)	
Normal	56 (51.4)	24 (40)	–
Overweight	19 (17.4)	10 (16.7)	
Obese	11 (10.1)	20 (33.3)	
EAT-26 score	29.8 (8.6)	16.8 (11.9)	*$p < 0.001$
Risk for eating disorder			
Yes	111 (98.2)	36 (59)	–
No	2 (1.8)	25 (41)	

*Significant at 1% level

[a] Reported in mean and standard deviation. BMI: TIDM n = 109; controls n = 61

[b] The Asia-Pacific perspective: redefining obesity and its treatment. Melb Int Diabetes Inst. 2000;11–2

Table 2 Clinical characteristics of type 1 diabetes mellitus patients (n = 113)

Variables	Males (n = 58)	Females (n = 55)	p value
Mean HbA1C (SD) %	9.0 (2.2)	9.2 (3.1)	0.93
Duration of diabetes(SD) years	8.8 (7.2)	9.3 (7.2)	0.71
Regularity in insulin injections			
Yes	94.8%	87%	0.15
No	5.2%	13%	
Insulin regimes			
Split mix	75.4%	68.5%	0.42
Basal bolus	24.6%	31.5%	

recorded. In the control group, the BMI of one subject was excluded due to heavy clothing (n = 60).

Table 2 depicts the characteristics of T1DM patients. The mean duration of diabetes in the entire group was 9.1 years (SD 7.2 years; range 1–31 years) and their overall mean HbA_1C level was 9.1% (SD 2.7%; range 5.9 to 19.7). There were no significant differences between male and female T1DM patients with respect to their BMI, duration of diabetes, insulin regimens, regularity of insulin administration and HbA_1C levels. The TIDM group had a significantly higher EAT-26 score indicating that they were at higher risk of developing eating disorder ($p < 0.001$). No significant association was found between BMI and the risk of developing eating disorders in the two groups ($p = 0.855$). Multivariate logistic regression after adjusting for BMI indicated that T1DM subjects were 33 times more likely at risk for developing eating disorders (OR = 33, 95% CI 7.4, 152.0; Table 3) compared to the non-diabetes counterparts ($p < 0.001$). In the TIDM group, gender, age, BMI, HbA_1C and insulin regimen were not significantly associated with risk of developing eating disorder. Duration of diabetes was significantly associated with risk of developing an eating disorder ($p = 0.013$, OR = 11.03, 95% CI = 1.4, 87.1).

Discussion

This study showed that T1DM patients were at 33 times greater risk for developing eating disorders when compared to subjects without diabetes. This is in line with a meta-analysis of 13 studies comparing the risk of developing eating disorders among T1DM and subjects without diabetes [12]. A Canadian study using the DSM-IV criteria found that subjects with diabetes had a higher prevalence of eating disorders (10%) when compared to non-diabetes controls (4%) (odds ratio 2.4, 95% CI 1.5 to 3.7; $p < 0.001$) [10]. Similar reports have been published from different TIDM populations across the world [13, 14]. T1DM patients constitute a vulnerable population who have to consciously make the right choices at each meal to attain an acceptable degree of glycaemic control. Hence, it is not surprising to find that eating behaviours tend to get overstated. Nutritionists and medical personnel should maintain a high index of suspicion when managing T1DM patients. The EAT-26 is a quick and easy tool to screen patients for risk of developing eating disorders. Timely intervention with the help of a mental health professional during routine out-patient visits will ensure appropriate treatment.

In our study, there was no difference in the risk of developing eating disorders between male and female patients. The only factor that increased the risk of developing eating disorders was the duration of diabetes. Whether the risk of eating disorders escalates over the years or is initiated at a specific point in time necessitates long-term follow-up studies.

In our study, BMI was not linked to the risk of developing an eating disorder. This is in line with studies by Neumark-Sztainer et al., who found inconsistent associations between BMI and eating disorders [9]. Similar findings were reported by Kaminsky and Dewey who also found no significant differences in the prevalence of eating disorder between adolescents with type 1 diabetes and healthy subjects [15]. In contrast, Colton et al., in a study of adolescent subjects with T1DM, found a higher prevalence of eating disorders in those

Table 3 Risk factor analysis

Variable	Univariate			Multivariate		
	OR	95% CI	p value	OR	95% CI	p value
EAT-26 score	38.5	8.7, 170.7	<0.001	33.5	7.4, 152.0	*$p < 0.001$
BMI	0.88	0.79, 0.97	0.013	0.99	0.88, 1.1	$p = 0.671$

*Significant at 1% level

with higher BMI using the Children's Eating Disorders Examination score. He rationalised that this could be due to a greater dissatisfaction about body image [16].

The metabolic control (measured as glycosylated haemoglobin level) was not associated with eating behaviour in our study population. Similar findings were reported by Colton et al. [16]. In contrast, Jones et al. found that the mean HbA_1C was higher in diabetes subjects with eating disorders in comparison to those without diabetes (9.4 vs. 8.6%; $p = 0.04$) [10]. Pinhas-Hamiel et al. had also reported that eating disorders compromised glycaemic control of the patients [17].

Nash and Skinner [18] in their extensive review of eating disorders among T1DM patients proposed studying the insulin regimens and the risk of developing eating disorders. In our patients, the "split mix" and "basal-bolus" insulin regimens were largely practised [19]. There was no significant association between the insulin regimen and the risk of developing eating disorders.

Some patients with T1DM may omit insulin injections to control body weight. However, most of our patients took their insulin injections regularly. In the few cases where some insulin injections were missed, the major reason for non-compliance was the non-availability of insulin due to lack of resources. No patient was admitted to the in-patient facility due to omission of insulin to control weight. However, Western literature has reported that 11% of the patients with diabetes resorted to underdosing of insulin and 42% misused their insulin to control weight [10]. Neumark-Sztainer et al. studied young adolescents with T1DM using the AHEAD survey (Assessing Health and Eating among Adolescents with Diabetes) and found a higher prevalence of unhealthy eating practices among females (37.9%) as against their male counterparts (15.9%) [9]. Deliberate underdosing or omission of insulin was resorted to as a weight loss strategy among the females. About 10.3% skipped insulin doses, and 7.4% of the subjects reported taking lower doses of insulin to control their weight. Only one male subject in that study practised either of these behaviours [9]. Similar findings were reported by Ackard et al. who found that 1.4% male and 7.4% female youth resorted to insulin dosage reduction as a means of weight control [20].

To our knowledge, this is the first study from southern India that looked at the risk of developing eating disorders in subjects with and without T1DM. The EAT-26 questionnaire identified subjects with type 1 diabetes as high risk for developing eating disorder in comparison to those without diabetes. In our setting, this did not reflect on poor glycaemic control.

The limitation of our study was that we do not have long-term data on the eating behaviour of our T1DM patients. Longitudinal studies are required to understand the factors triggering the initiation and progress of eating disorders in this group of patients.

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Compliance with ethical standards The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and approval was obtained from the Institutional Review Board of Christian Medical College, Vellore, India (IRB Min. No. 9547 dated 22.07.2015).

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

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ORIGINAL ARTICLE

Mortality and natural progression of type 1 diabetes patients enrolled in the Rwanda LFAC program from 2004 to 2012

Sara L. Marshall[1] · Deborah V. Edidin[2] · Vincent C. Arena[1] · Dorothy J. Becker[3] · Clareann H. Bunker[1] · Crispin Gishoma[4] · Francois Gishoma[4] · Ronald E. LaPorte[1] · Vedaste Kaberuka[4] · Graham Ogle[5] · Wilson Rubanzana[6] · Laurien Sibomana[1] · Trevor J. Orchard[1]

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Abstract The natural history and mortality of type 1 diabetes in adolescents in Africa is not well characterized. Our aim is, therefore, to describe these characteristics for cases in the Rwanda Life For a Child (LFAC) program. Participants (≤25 years old) were the first 500 children and youth enrolled in the Rwanda LFAC program from 2004 to 2012. Clinical and demographic data were extracted from LFAC forms, and vital status was evaluated as of November 1, 2011. For the first 500 participants, 5-year survival was 93.8% while crude mortality was 13.9/1000 (95% CI 9.0–20.6/1000) person years of diabetes. However, since vital status is unknown for 134 participants, mortality could be as high as 40.2/1000 person years of diabetes if all missing cases died. Mortality was directly associated with age at diagnosis, and inversely to calendar year of first visit, BMI, and monitoring frequency. Hypertension prevalence reached 46% by 2012. Mortality rates associated with type 1 diabetes in Rwanda are similar to those in other African countries, but higher than rates in developed countries. Delayed diagnosis may contribute to excess mortality risk, but recent improvements in survival suggest that advancements are being made. Hypertension and loss to follow-up need to be addressed.

Keywords Type 1 diabetes · Youth · Children · Natural history · Rwanda · Africa

Introduction

Diabetes is a disease of growing concern in the developing world. An estimated 18.7 million people in Africa will be affected with diabetes by 2025 [1], posing a large problem for a population that already has limited access to healthcare and insulin. Help for such countries usually comes in the form of external support. One such program is the Life For a Child (LFAC) program, which is managed by the International Diabetes Federation in conjunction with Australian Diabetes Council and HOPE *worldwide*. LFAC's mission is to support the provision of the best possible diabetes healthcare, given local circumstances, by supplying children and adolescents (≤25 years) in developing countries with the necessary insulin and glucose testing supplies and with HbA1c testing capability. The program also offers diabetes education, and advanced training and advice to both patients and local healthcare providers.

The Association Rwandaise des Diabetiques (ARD) is a diabetes association located in Kigali City, Rwanda, that receives support from the LFAC program. The program there was initiated in 2004 with 25 children, and has expanded over the years and now provides support for over 630 children. Participants in this program are provided insulin on a monthly basis and in turn are required to undergo an annual clinic evaluation with suggested quarterly visit follow-up (as of 2010). The short-term effects of this program have been previously reported [2], but little is known concerning the

✉ Trevor J. Orchard
tjo@pitt.edu

[1] University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA 15213, USA

[2] Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

[3] University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

[4] Association Rwandaise des Diabetiques, Kigali, Rwanda

[5] International Diabetes Federation Life for a Child Program and Australian Diabetes Council, Sydney, NSW, Australia

[6] National University of Rwanda, Kigali, Rwanda

mortality and natural progression of the disease in the members of the Rwanda LFAC program who have been enrolled since its inception.

The objective of this report is, therefore, to describe the status of the first 500 cases registered with the ARD since 2004. We will report on the utilization of the LFAC program (number and frequency of visits), losses to follow-up, clinical measures, and mortality as of November 2011.

Research design and methods

The University of Pittsburgh's IRB determined that this project was exempt from review under the "Existing Data" category.

Study population

This report focuses on the first 500 children and youth enrolled in the Rwanda LFAC program from 2004 to 2012. To be enrolled, participants must be living in Rwanda, ≤25 years old, and needing assistance with obtaining insulin and other diabetes supplies.

Data collection

Although LFAC registration initiated in 2004, vital status and clinic visit results were first recorded beginning in 2009, and thus, this report comprises clinical data from 2009 to 2012. Clinical and demographic data [sex, province, date of birth, diagnosis date, insulin regimen, glucose monitoring frequency, height, weight, systolic and diastolic blood pressure (BP), tuning fork vibratory sensation and monofilament response tests, HbA1c, and albumin creatinine ratios (A/C ratios)] were extracted from LFAC clinical forms.

Laboratory data

Blood (finger prick) and urine (spot samples) were collected from each patient and processed on the Siemens DCA Vantage™ (which reports DCCT-related values). Data for HbA1c and A/C ratio were collected from these samples. The maximum HbA1c value for this machine is ">14% (>130 mmol/mol)," so for data analysis purposes, these results were reported as "14.1% (131 mmol/mol)." The inter-assay CV range for the HbA1c measures was from 2.1 to 3.8% during the data collection.

Complication assessment

Neuropathy was defined as failure to feel a 10g monofilament (less than seven of ten correct responses) on the dorsum of the great toe and/or failure to feel vibration from a 128-Hz turning fork placed on the dorsum of the great toe for 10 s [3]. Microalbuminuria (MA) was defined as an albumin/creatinine (A/C) ratio of 30–299 mg/g in a spot urine sample and overt nephropathy as an A/C ratio ≥300 mg/g. Hypertension was defined as having either SBP or DBP over the 95th percentile for those <18 years, or systolic BP ≥130 mmHg or diastolic BP ≥80 mmHg or a history of BP medication for those ≥18 years. The percentage of those using BP medication, however, is low due to limited availability.

Vital status assessment

Vital status is reported as of November 1, 2011 through clinic attendance, deaths reported to the ARD, hospital surveys, and additional tracing by a forensic epidemiologist (R.W.). A participant was considered to be alive as of this date if he/she attended clinic after November 1, 2011, or he/she was known to be alive by contact with hospital staff or other participants, or through investigation. Autopsies are not customary in Rwanda and death certificate data is likewise sparse. Thus, cause of death was determined through contact with local hospitals and families, but is limited in both quality and completeness.

Data analysis

Descriptive statistics were calculated for clinical and utilization data. ANOVA, two-sample, and paired *t* tests were used for comparisons of continuous variables, while χ^2 tests and Fisher's exact tests were for comparisons of categorical variables. Tukey's HSD test was used for any post hoc pairwise comparisons. A p value of 0.05 with appropriate Bonferroni corrections was used to assess significance for multiple comparisons. Time between visits was calculated assuming that a year had 365.25 days and a month had 30.44 days.

Person years of diabetes were calculated as of November 1, 2011 (n = 361; total known person years of diabetes = 1792 years). Ninety-five percent confidence intervals were computed assuming a Poisson distribution. Kaplan-Meier curves were constructed to estimate cumulative survival and log-rank tests were used to test for significant differences in survival between sub-groups. Survival curves were censored at 10 years as less than 20% of the original cohort remained after this duration. Cox regression models were used to examine differences in survival for all continuous variables as well as simultaneously adjusting for multiple variables (model 1). The proportional hazards assumptions of the models were confirmed by testing time-dependent interaction variables. All hazard ratios (HRs) are reported per increase of 1 unit of measure.

All statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC) statistical software.

Results

Of the first 500 registered LFAC participants, a total of 488 (97.6%) participants have at least one recorded clinic visit, while 12 were registered by name only (received insulin, but have no demographic or clinical data). Seven participants had unrecorded sex. Mean duration of diabetes was 4.9 ± 3.2 years and only 18 (3.8%) participants were diagnosed before age 5 years, while 186 (39.3%) were diagnosed between the ages of 15 and 19 years (Figs. 1 and 2). Seventy-five of the original 500 registered participants (15%) are 26 years or older and are no longer eligible for support from LFAC.

Utilization

Seventy-seven (15.4%) participants were registered only with no clinical visit (including 12 with no demographic or clinical data) and 53 (10.6%) had only one visit in 2009–2012. Those who had only one visit from 2009 to 2012 were significantly older than those who had multiple visits and were diagnosed at an older age than participants with no visit (Table 1).

Three hundred seventy participants (74%) had multiple visits from 2009 to 2012 [mean number of visits of 5.1 ± 2.8 (range 1–12 visits)], and their mean HbA1c decreased significantly from 11.1 ± 2.7% to 9.5 ± 2.5% (98 ± 30 mmol/mol to 80 ± 28 mmol/mol) between their first and last visit (Table 2). At the most recent visit, 3.5% (n = 2/57) had neuropathy, 10.1% (n = 10/99) had overt nephropathy, and 18.2% (n = 18/99) had MA. These rates were not significantly different from those seen at the first visit (neuropathy 2.2%, n = 5; overt nephropathy 5.3%, n = 8; MA 18.5%, n = 28; Table 2). The prevalence of hypertension, however, increased significantly over time (35.1 to 46.1%).

Within the last year (since November 1, 2011), 319 (63.8%) of the first 500 registered participants have been seen. Of the 181 (36.2%) who have not been seen, 33 are known to have died, 5 were found to be duplicate IDs, and 17 are otherwise known to be alive (Fig. 3). Forty of those not seen in the last year have aged out of the LFAC program. The most common reported reasons for non-attendance were being away at boarding school (n = 4), lack of transport (n = 3), sick on day of visit (n = 3), did not hear radio announcement (n = 2), were pregnant (n = 2), or no longer believed they had diabetes (n = 2).

Vital status

A total of 25 participants [n = 15 (60.0%) females; n = 10 (40.0%) males] were known to have died as of November 1, 2011. Thus, the crude mortality for this cohort was 6.9% (25/361; 95% CI, 4.5–10.2%) or 13.9/1000 person years of diabetes (95% CI 9.0–20.6/1000). [Note: an additional eight participants died in 2012; therefore, a more accurate estimate of mortality is 9.1% (33/361; 95% CI 6.3–12.8) or 18.1/1000 person years of diabetes.] Since vital status was unknown for 134 (26.8%) participants, in a worst-case scenario (assuming all had died), the mortality rate could be as high as 32.1% (159/495; 95% CI 27.0–37.1%) or 40.2/1000 (95% CI 32.0–49.9/1000) person years of diabetes; the most optimistic (assuming all are alive) could be as low as 5.0% (25/495; 95% CI 3.3–7.4) or 12.3/1000 (95% CI 7.9–18.1/1000) person years of diabetes.

For those who died as of November 1, 2011, cause of death was unknown for 16 (64.0%), with hypoglycemia being the most common known cause (n = 4, 16.0%), and renal failure accounting for 2 (8.0%) deaths. Single deaths resulted from gastroenteritis, pneumonia, pulmonary embolism, rectal hemorrhage/hepatitis, and hyperglycemia. Unfortunately, none of the reported deaths had an official autopsy. Mean age at time of death was 19.4 ± 4.0 years (range 12–25 years), mean age at

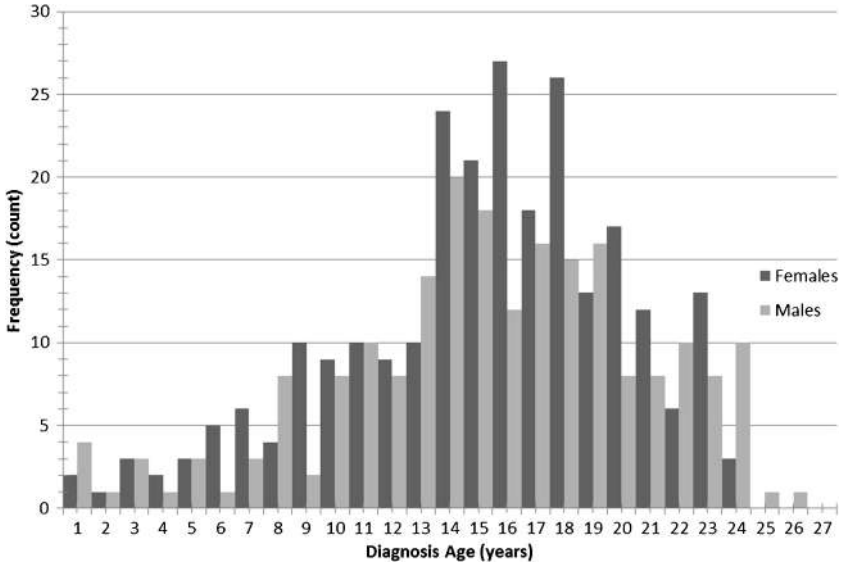

Fig. 1 Histogram of diagnosis age for the first 500 LFAC program participants, by sex

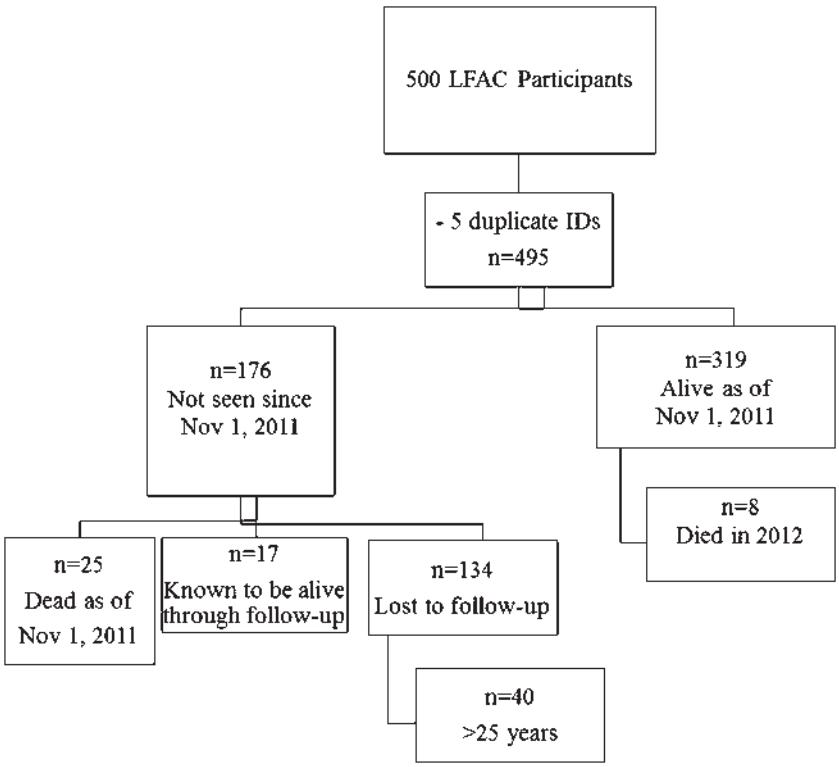

Fig. 2 Flow chart outlining participant follow-up and vital status

diagnosis for the deceased was 14.1 ± 5.0 years (range 1–23 years), and mean diabetes duration was 4.5 ± 3.5 years (range 0–11 years). Twenty-two of the deceased (88.0%) reported having no glucose meter at home versus 33% (n = 99) of those who were seen in the last year ($p < 0.0001$). Of those who died, 11 had a recorded HbA1c value (mean 9.8 ± 2.0%; 83 ± 22 mmol/mol), with a mean number of HbA1c measures of 2.1 ± 2.0 per person. This was significantly fewer than those alive ($p = 0.002$). There were no significant associations between province and mortality, though the Western Province had the highest number of deaths (n = 9).

Five-year (post-diagnosis) survival for this cohort was 93.8% (85.1% worst-case scenario) and 10-year survival was 82.5% (66.2% worst-case scenario). Survival did not differ significantly by sex (Fig. 3a), province, year of first visit, year of diagnosis, year of birth, diagnosis before or after 15 years of age, or complication status. However, those whose first visit was in 2009 had significantly higher mortality than the subsequent years combined ($p = 0.03$) (Fig. 3b), suggesting that survival is improving over time.

In Cox regression age at diagnosis, BMI, monitoring frequency, number of injections, and weight at last clinic visit were univariately (all negatively except for age at diagnosis) related to survival, as was weight at baseline (Table 3). In a multivariable model, each additional year in age at diabetes diagnosis resulted in a 15% higher mortality risk (HR 1.15, 95% CI 1.03–1.29), each additional unit of BMI decreased the risk of death by 22% (HR 0.78, 95% CI 0.67–0.91), and each additional monitoring per week decreased risk by 7% (HR 0.93, 95% CI 0.86–0.99).

Table 1 Characteristics of participants with multiple clinic visits from 2009 to 2012, those who only had one clinic visit, and those who have no recorded clinic visits

	Visit status		
	Multiple visits	One visit	No visit[b]
N	370	53	65
Age at diagnosis (years)	15.4 ± 4.9	16.8 ± 5.1	12.7 ± 5.8[a]
Male (column % (*n*))	43.0 (156)	43.4 (23)	44.6 (29)
Age in 2012 (years)	20.8 ± 4.6	22.3 ± 5.0	20.7 ± 4.9
Province (row % (*n*))			
East	73.1 (38)	7.7 (4)	19.2 (10)
Kigali City	71.5 (88)	9.7 (12)	18.7 (23)
North	84.2 (48)	8.8 (5)	7.0 (4)
West	76.2 (64)	15.5 (13)	8.3 (7)
South	72.2 (130)	10.6 (19)	17.2 (31)
Year of first visit % (*n*)			
2009	159 (87.4)	23 (12.6)	–
2010	126 (87.5)	18 (12.5)	–
2011	82 (88.2)	11 (11.8)	–
2012	3 (75.0)	1 (25.0)	–

[a] Significantly different from those with multi-visits and one visit

[b] Excludes an additional 12 with no visit, but with no demographic or clinical data reported

Table 2 Clinic data from the first and most recent clinic visit for participants who had multiple visits between 2009 and 2012 ($N = 370$)

	First clinic visit	Most recent clinic visit
Time between visits (months)		26.6 ± 10.8
Age (years)	18.6 ± 4.5	20.6 ± 4.5*
Male % (*n*)	43.0 (156)	
Age at diagnosis (years)	15.4 ± 4.9	
Duration of diabetes (years)	3.0 ± 2.9	5.0 ± 3.1*
Glucose monitoring (per week)	1.3 ± 3.9	8.0 ± 8.0*
HbA1c (mmol/mol)	98 ± 30	80 ± 28*
HbA1c (%)	11.1 ± 2.7	9.5 ± 2.5*
<8.0%	17.0 (55)	28.9 (96)*
8–11.9%	40.9 (132)	51.5 (171)*
12–14%	14.6 (47)	9.6 (32)*
>14%	27.6 (89)	9.6 (32)*
Insulin injections (per day)	1.8 ± 0.6	2.0 ± 0.5*
Units of insulin (per day)	32.4 ± 15.7	37.8 ± 15.4*
Units of insulin per kilogram	0.70 ± 0.37	0.75 ± 0.30
Weight (kg)	47.6 ± 12.6	51.6 ± 11.6*
Height (cm)	154.5 ± 14.3	156.7 ± 12.8*
BMI (kg/m^2)	19.8 ± 3.5	20.7 ± 3.2*
Systolic BP (mmHg)	113 ± 16	120 ± 17*
Diastolic BP (mmHg)	73 ± 12	79 ± 12*
Neuropathy % (*n*)	2.2 (5)[a]	3.5 (2)[b]
Microalbuminuria % (*n*)	18.5 (28)[c]	18.2 (18)[d]
Nephropathy % (*n*)	5.3 (8)[c]	10.1 (10)[d]
Hypertension % (*n*)	35.1 (127)[f]	46.1 (165)[g]*

*Significantly different from first clinic visit ($p < 0.05$)

[a] *n* tested = 230

[b] *n* tested = 57

[c] *n* tested = 151

[d] *n* tested = 99

[f] *n* tested = 362

[g] *n* tested = 358

Discussion

In this study of the natural history of the first 500 participants registered in the IDF's LFAC program in Rwanda, we estimated the crude mortality to be 13.9/1000 person years of diabetes and determined that mortality was directly associated with age at diabetes diagnosis and inversely to year of first visit, weight at baseline, and monitoring frequency. However, since vital status is unknown for 134 participants, mortality could be as high as 40.2/1000 person years of diabetes. For the 310 participants with multiple visits since 2009, we saw a significant decrease in HbA1c (11.1 ± 2.7% to 9.5 ± 2.5%; 98 ± 30 mmol/mol to 80 ± 28 mmol/mol) and consistent rates of complications except for hypertension, which rose significantly.

Our estimated mortality and survival rates (5-year survival = 93.8–85.1%; 10-year survival = 82.5–66.2%) are consistent with previous studies in African youth, e.g., Ethiopia (mortality = 15.5/1000 person years) [4] and South Africa (10-year survival = 84%) [5], though 5-year survival in Tanzania was significantly poorer (71–60%) [6]. Mortality in youth with type 1 diabetes from developed countries are much lower rates than Rwanda, ranging from 0.06% in the UK [7] to 6.1/1000 person years of diabetes in Lithuania (Table 4) [8–11], though it is consistently lower in African Americans than Caucasian Americans [12].

The association we found between mortality and diagnosis age is supported by results from Mozambique and Zambia [13], and Estonia, Lithuania, and Finland [8]. Each additional year of age at diagnosis conferred a higher risk of mortality, and we believe this may partially be due to the effects of surviving for several years with undiagnosed diabetes. We hypothesize that by the time some of these participants are formally diagnosed, they are in especially poor condition and therefore at a higher risk of complications and death.

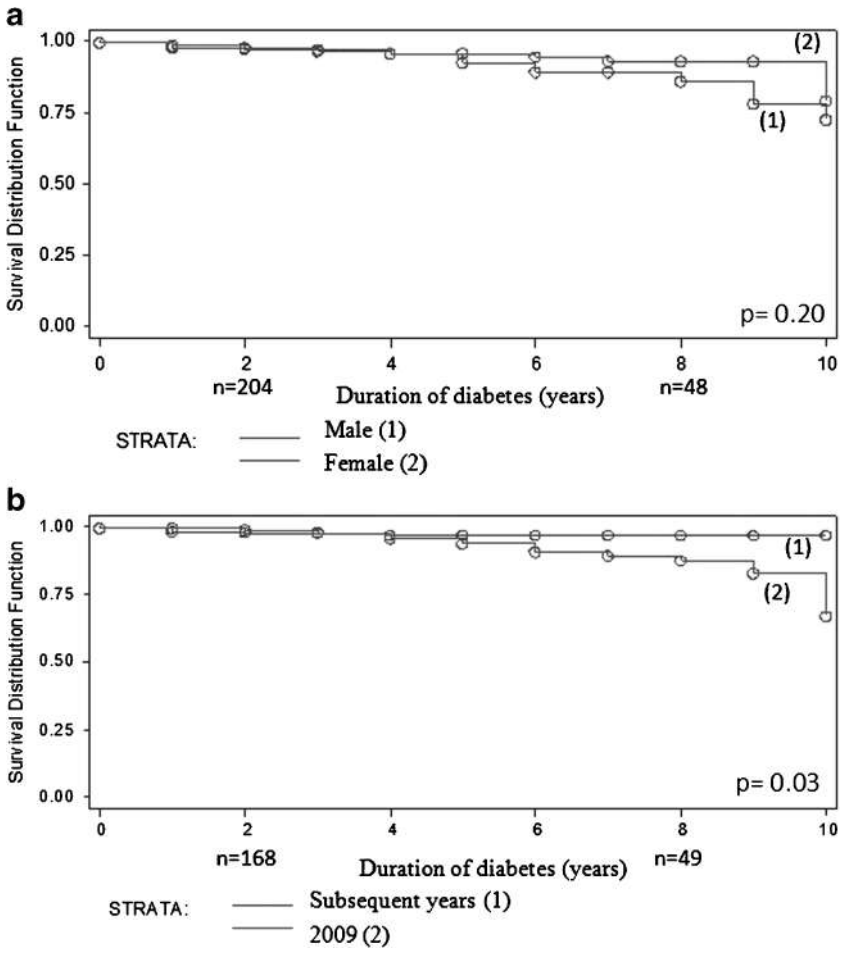

Fig. 3 Survival by sex (**a**) and year of first clinic visit (2009 vs. all subsequent years) (**b**)

This ability to survive with apparent type 1 diabetes for years without insulin is consistent with the presence of a different type of diabetes in this community as discussed later. Our findings that higher BMI and weight at baseline is protective also support this hypothesis as we have previously found that those in worse control have lower weight consistent with lack of insulin and dehydration/hypovolemia [2].

The majority of those who were deceased reported not having a meter at home. Compounding this, adequate emergency services are often distant or unavailable at certain times (e.g., at night), and glucagon was not available due to its cost. Thus, it is not unexpected that a common known cause of death was hypoglycemia. This finding is also consistent with others showing that within the first 10 years, cause of death is primarily due to acute causes [4, 11, 14] and underscores the importance of ensuring that all LFAC members have access to glucose meters and strips and appropriate education, especially in light of the high rate of food insecurity in this population.

Table 3 Cox regression models for mortality (HRs and their associated 95% CIs are reported)

	Variables that were univariately significant		Model 1	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age at diagnosis (years)	1.12 (1.01–1.2)	0.01	1.15 (1.03–1.29)	0.01
BMI at most recent visit	0.80 (0.70–0.92)	0.002	0.78 (0.67–0.91)	0.001
Monitoring frequency at most recent visit (times per week)	0.91 (0.85–0.97)	0.006	0.93 (0.86–0.99)	0.046
Number of injections at most recent visit (per day)	0.50 (0.26–0.98)	0.03	N/S	–
Weight at baseline (kg)	0.96 (0.93–0.99)	0.02	N/S	–
Weight at most recent visit (kg)	0.94 (0.91–0.97)	0.002	N/S	–

Table 4 Mortality comparisons for Rwanda and other studies

Country	Crude mortality (%) (95% CI)	Mortality per 1000 person years (95% CI)	5-year survival (%)	10-year survival (%)
Rwanda	6.9 (4.5–10.2)	13.9 (9.0–20.6)	93.8	82.5
Rwanda (worst-case scenario)	32.1 (27.0–37.1)	40.2 (32.0–49.9)	85.1	66.2
Rwanda (best-case scenario)	5.0 (3.3–7.4)	12.3 (7.9–18.1)	–	–
Ethiopia	–	15.5	–	–
South Africa	–	–	–	84
Tanzania	–	–	71–60	–
United Kingdom	0.6	–	–	–
Lithuania	0.04	6.1	97.3	94.0
Estonia	0.02	3.7	99.0	94.3
Finland	0.006	0.8	99.8	99.1
US African Americans (30 years)	–	15.8	98	96

It is, however, very likely that several of the deaths due to unknown causes were from DKA, but were not properly identified as such. Therefore, we cannot say that hypoglycemia is the most common cause of death, as over half were due to unknown causes.

Although 18.8% of our initial cohort has been lost to follow-up, these results are similar to other mortality studies in Africa [5, 14, 15]. The high turnover rate of the nursing (and medical) staff at the district hospitals has also limited our follow-up of LFAC participants, and many of the new nurses have never seen the patients we are trying to locate. We are currently working on developing awareness programs within schools, working with local nurses to help provide for transport, and developing local support groups of children that provide phone chains and social incentives to attend visits.

Over the course of the LFAC program, several individuals have come back to the clinic years after their last insulin dose (n = 21 after 2 years; n = 2 after 3 years) without apparently maintaining insulin therapy. This phenomenon has also been recorded in children thought to have type 1 diabetes in Ethiopia who had interrupted insulin supplies (for 9 ± 12 weeks; range 1–78 weeks), of whom only 4% developed any DKA [16]. While these individuals were in poor condition when they returned, the fact that they survived so long without any exogenous insulin suggests that there may be a different type of diabetes (with some significant residual beta cell function) present in this population. This would be consistent with results from other studies from Ethiopia, suggesting the previously recognized "Malnutrition-related Diabetes" (MRDM) should be re-considered in Africa [17–19]. Unfortunately, due to the lack of access to typology testing at this time, we were unable to officially determine diabetes type for participants.

The prevalence of hypertension increased over time and was also elevated in this population in comparison to rates reported in US African Americans (AfAm = 9.8%, Rwanda 46.1%) [20]. We have previously postulated that this is in part due to poorer glucose control in Rwanda, as those whose control worsened over time saw more dramatic increases in BP than those who were in good control or who saw improved control [21]. This increase in hypertension prevalence, and the positive association between hypertension and MA, highlights the need for improved BP control in this cohort for complication and mortality prevention.

The key limitations of this study are the large proportion lost to follow-up and the fact that mortality data was limited by lack of autopsy data and a formal national death index.

The major strengths of this study, however, are that our cohort of 500 participants is the largest cohort for whom mortality of type 1 diabetes in sub-Saharan Africa has been reported, and this is the first follow-up and mortality report for diabetic youth and adolescents in Rwanda.

Conclusion

In summary, these data demonstrate that mortality rates for those with type 1 diabetes in Rwanda are similar to other African type 1 diabetes populations, but higher than those in developed countries. Delayed diagnosis of type 1 diabetes may contribute to the increased risk of mortality, highlighting the importance for increased awareness and timely diagnosis. However, the improvements in survival since 2009 are encouraging and reflect global trends [22]. Utilization of the

LFAC program in Rwanda has increased significantly since its inception in 2004, and HbA1c has decreased considerably as a cumulative result of its efforts. The relationship of improved survival with increased frequency of self-monitoring of blood glucose, and more frequent injections provide clear guidelines to improve care. Although several participants have died and many more have been lost to follow-up, we hope that by recognizing and addressing the identified barriers, more extensive and long-term care will be available in the future.

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Compliance with ethical standards

Funding No funding was received for this study. Insulin, syringes, glucose meters, and strips were received free from the LFAC program. DCA machines, HbA1C, and A/C reagents were provided by the University of Pittsburgh.

Conflict of interest Sara L. Marshall, Deborah V. Edidin, Vincent C. Arena, Dorothy J. Becker, Clareann H. Bunker, Crispin Gishoma, Francois Gishoma, Ronald E. LaPorte, Vedaste Kaberuka, Graham Ogle, Wilson Rubanzana, and Laurien Sibomana have no conflict-of-interest to declare. Dr. Trevor Orchard serves on Eli Lilly and Company Advisory Panel.

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.

Authors' contributions Marshall SL designed the study, performed research and analysis and wrote the paper; Orchard TJ designed the study, reviewed analysis, revised the manuscript; Edidin DV, Gishoma C, Gishoma F, Kaberuka V, Sibomana L, and Rubanzana W performed research, collected data, and revised the manuscript; Arena VC reviewed analysis and revised the manuscript; Becker DJ, Bunker CH, Ogle G, and LaPorte RE provided oversight and revised the manuscript.

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ORIGINAL ARTICLE

Sausage toe: an upsetting symptom in diabetic patients

Tolga Dinc[1] · **Hakan Kocaoglu**[2] · **Selami Ilgaz Kayilioglu**[1] · **Arife Polat Duzgun**[1] · **Faruk Coskun**[1]

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Abstract With diabetes reaching almost epidemic proportions, foot problems have become increasingly prevalent worldwide, resulting in major economic consequences for both diabetic patients and society. Although some detailed studies regarding this condition are present in the existing literature, no clear consensus has been established regarding the diagnosis and treatment of diabetic foot infection and osteomyelitis. Thus, any sign that may be used to predict osteomyelitis of the foot in diabetic patients is valuable. We believe that the presence of a "sausage toe" could be a reliable sign of osteomyelitis. This cohort study was carried out at the Chronic Wound Clinic of a major institute over a period of 3 years. All diabetic patients who had a sausage toe were identified and followed up prospectively. A swollen toe that had lost its normal contours and resembled a sausage was defined as a sausage toe. All 16 patients included in this study had type II diabetes (min 5-year duration). The mean HbA_{1c} level among the patients was 9.28% (4–5.6% nondiabetic range). All patients had reduced pinprick and vibration sensation but good peripheral pulsation in their affected foot. Overall, six patients were diagnosed with underlying bone infections. Additionally, six patients received hyperbaric oxygen therapy and a total of ten patients required surgical intervention. Four patients required surgical debridement of ulcers that developed during the follow-up. Five patients who were diagnosed with osteomyelitis required local amputations. Complete resolution of six sausage toes was achieved using nonsurgical treatment modalities. The definition of a sausage toe is rather subjective. Although its definition has relatively low specificity, a sausage toe is an easily identifiable and cost-free clinical sign that can be used as an indicator of underlying bone infection.

Keywords Sausage toe · Osteomyelitis · Diabetic foot · Diabetic complications

Introduction

Diabetes is a disease with various manifestations that occur secondary to hyperglycemia [1]. Peripheral arterial disease, neuropathy, nephropathy, and immunopathy are among the major comorbidities associated with diabetes [2]. In addition to these comorbidities, many patients may face serious and even life-threatening foot problems [3]. Approximately 15% of diabetic patients have been reported to develop foot ulcerations during the course of their lifetime [2].

Soft tissue and bone infections of the foot are among the leading causes of both hospital admission and lower extremity amputation in diabetic patients [4]. Unfortunately, these infections hinder diabetes control and uncontrolled diabetes adversely affects the host's immunologic responses, thereby resulting in a downward spiral [5]. Thus, the early detection of any symptoms associated with serious foot infections is very important.

Although diabetic foot infections are very common, at present, sufficient literature addressing the diagnostic signs for which physicians should be vigilant of is not available.

✉ Tolga Dinc
tolga_dr@hotmail.com

[1] Department of General Surgery, Ankara Numune Training and Research Hospital, Ankara, Turkey

[2] Department of Orthopedics, Ankara University Faculty of Medicine, Ankara, Turkey

This paper describes the characteristics and follow-up of 16 diabetic patients who had a "sausage toe." With this case series, we aimed to evaluate the effectiveness of using the presence of a sausage toe as a sign of serious infection and/or osteomyelitis in diabetic patients.

Patients and method

This cohort study aimed to investigate the effectiveness of using the presence of a sausage toe as an important clinical sign of underlying serious infections and/or osteomyelitis in a diabetic population. This study was carried out at the Chronic Wound Clinic of our institute over a period of 3 years. All diabetic patients who had a sausage toe were identified and followed up prospectively. A swollen toe that had lost its normal contours and resembled a sausage was defined as a sausage toe. Identification of sausage toes was performed by two of the authors, one of whom is the senior author. Additionally, the senior author and the second author, who is an orthopedist, diagnosed underlying osteomyelitis using clinical and radiographic criteria.

Medical and epidemiological data for the patients were collected during their first referral visit. In addition, the whole blood counts, erythrocyte sedimentation rates (ESR), and C-reactive protein (CRP) and glycated hemoglobin (HbA_{1c}) levels of the patients were evaluated. Plain radiographs were performed using both the anterio-posterior and oblique views. If an ulcer with an exposed bone was identified at initial presentation, a swab culture (probe-to-bone test) was performed. All patients were followed up until either complete resolution of the sausage digit or amputation of the involved toe.

Results

The clinical details of the patients and their sausage toes (involved toe, ulcers if found, location, and size) are summarized in Table 1. All 16 patients had type II diabetes (min 5-year duration). The mean HbA_{1c} level among the patients was 9.28% (4–5.6% nondiabetic range). All patients had reduced pinprick and vibration sensation but good peripheral pulsation in their affected foot. Three patients had a history of coronary artery disease; one patient had a history of hyperthyroidism, and one patient had Parkinson's disease. Two patients had a history of lower extremity amputation, both of which were infection-related. One patient had undergone a below-the-knee amputation and was referred for treatment of their contralateral foot, and one patient had undergone a first digit amputation and was referred for treatment of their ipsilateral foot.

There were no patients with any clinical infections other than the evaluated foot infection. The patients' mean white blood cell count was 8540 cells/mL (range of 6400–15,900 cells/mL, and only one patient had a white blood cell

Table 1 Clinical details of patients

No.	Age (years)	Sex	Toe	Ulceration	Location	Hyperbaric oxygen therapy	Underlying osteomyelitis	Surgical intervention
1	69	M	R 2	Pin-point	Dorsum	+	−	Debridement
2	58	F	L 2	Pin-point	Medial	−	−	−
3	46	M	R 1	−	−	−	−	−
4	60	F	L 1	<1 cm	Plantar	+	+	Toe amputation
5	63	F	L 5	−	−	−	−	−
6	53	M	R 1	−	−	−	−	Debridement
7	61	F	R 1	Pin-point	Plantar	+	−	Debridement
8	54	M	R 1	Bone-exposed	Lateral	+	+	Toe amputation
9	63	F	R 5	Fistulae	Lateral	−	+	Toe amputation
10	66	M	L 1	Pin-point	Medial	−	+	Toe amputation
11	71	F	L 1	Pin-point	Medial	−	−	−
12	60	M	R 2	Pin-point	Dorsum	+	+	Toe amputation
13	68	M	R 2	Pin-point	Dorsum	−	−	−
14	57	M	R 5	Pin-point	Lateral	−	−	Debridement
15	60	M	R 2	−	−	−	−	−
16	58	M	L 2	Bone-exposed	Dorsum	+	+	Debridement

Patient 14 had a previous toe amputation and patient 16 had a below-the-knee amputation on the right side before presentation

M male, *F* female, *R* right foot, *L* left foot

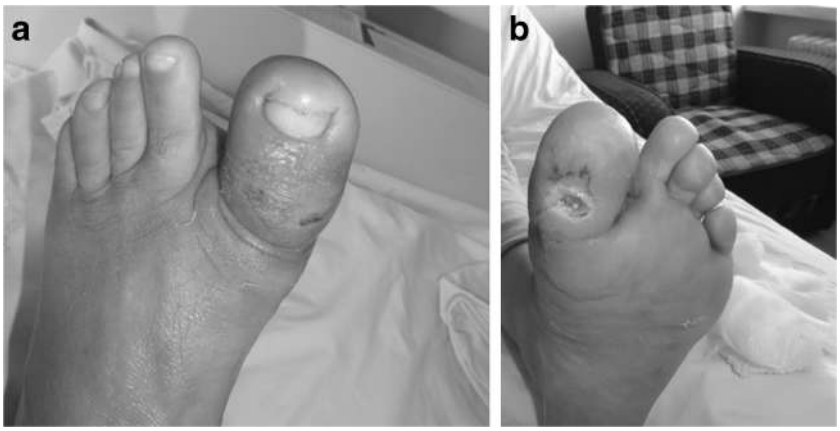

Fig. 1 (**a**) Sausage toe in a 60-year-old female patient (case 4). The swollen first toe of the left foot can easily be differentiated from the other toes. (**b**) Patient presented at initial consultation with a small ulcer on the plantar side of the foot

count above the normal value of 10,500 cells/mL). The mean erythrocyte sedimentation rate among the patients was 41.2 mm/h, and the mean CRP level was 25.7 mg/L. Two patients had an ulcer identified during their first appointment. Swab cultures showed that the infectious agents associated with these ulcers were methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Proteus mirabilis.*

All of the patients received educational information regarding daily foot care and wound care practices that could be performed at home. All patients were referred to infectious disease specialists throughout the follow-up period for appropriate oral or intravenous antibiotic medication. Most of the patients received oral antibiotics as initial treatment and were followed up as outpatient cases. Two of the 16 patients with bone-exposed ulcers and one patient with drainage of the fistula were hospitalized at initial presentation to administer intravenous antibiotics and hyperbaric oxygen therapy at our clinic. Additionally, during follow-up, 7 of the 13 patients who were not initially admitted for these therapies subsequently required hospitalization for treatment administration. Additional hyperbaric oxygen therapy and intravenous antibiotics were administered to three patients when their infections were exacerbated. Thus, overall, six patients received hyperbaric oxygen therapy and a total of ten patients required surgical intervention. One of the patients who was diagnosed with osteomyelitis required surgical debridement of an ulcer that developed during the follow-up. The remaining five patients required local amputations upon clinical osteomyelitis diagnosis (Fig. 1). The duration of antibiotic treatment varied from 4 to 6 weeks depending upon the responses of the ulcers and the amputation sites. All surgically treated patients healed with complete closure of the skin and without any signs of inflammation (Fig. 2). Among the 16 evaluated patients, complete resolution was achieved for six sausage toes.

Discussion

With diabetes reaching almost epidemic proportions, foot problems have become increasingly prevalent worldwide, resulting in major economic consequences for both diabetic patients and society [6]. Although some detailed studies are present in the existing literature, no clear consensus has been established regarding the diagnosis and treatment of diabetic foot infection and osteomyelitis [1, 2, 5]. Thus, any sign that may be used to predict osteomyelitis of the foot in diabetic patients is valuable. In our cohort, 6 of the 16 patients were diagnosed with osteomyelitis. Furthermore, surgical treatment was required for four patients with diabetic foot infection. We believe that the presence of a sausage toe could serve as a reliable sign of serious infection and/or osteomyelitis.

In practice, dactylitis, or sausage finger, is a condition that is considered to be indicative of several very different diseases [7]. The term was first utilized in rheumatology literature because of its close association with psoriatic arthritis [8]. Nevertheless, a sausage digit has been identified as a feature of many other diseases such as sarcoidosis, sickle cell anemia, and tuberculosis [9, 10]. To the best of our knowledge, there

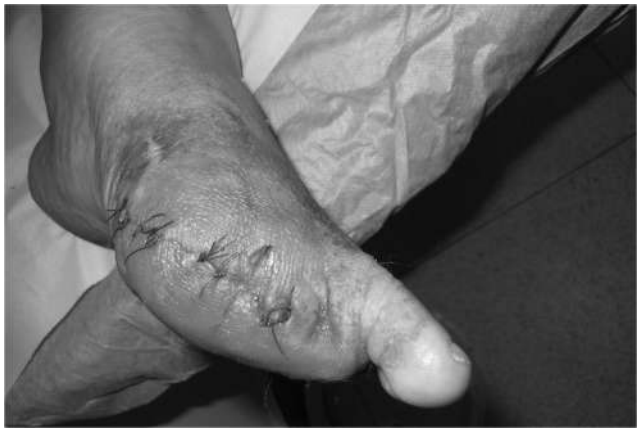

Fig. 2 Early postoperative appearance of the affected foot

has been only one paper published so far that has indicated a link between osteomyelitis and sausage toes [11].

The diagnosis of osteomyelitis in areas other than the foot can be achieved by other diagnostic means. Conversely, plain radiography of the foot has been reported to only detect underlying osteomyelitis of the foot with a sensitivity of 0.54 and specificity of 0.68 [12], whereas, the exposed bone or probe-to-bone test have demonstrated a sensitivity of 0.60 and specificity of 0.91 [12], and MR imaging has been reported to have a sensitivity of 0.82 and specificity of 0.90 [13]. On the other hand, leukocyte bone scans have been reported to have a sensitivity of 0.74 and specificity of 0.68 [12]. The diagnostic odds ratios previously observed in association with clinical examination, radiography, MRI, bone scans, and leukocyte scans were 49.45, 2.84, 24.36, 2.10, and 10.07, respectively [12]. Thus, the need for distinct diagnostic criteria for osteomyelitis of the foot is clear.

This cohort demonstrated that diabetic patients with sausage toes required prolonged hospitalization time and long-term antibiotherapy, and almost 60% of the evaluated patients required surgical intervention. This unique group of diabetic patients needed more specialized and meticulous treatment and follow-up when compared to patients without sausage toes.

Conclusion

The definition of a sausage toe is rather subjective. Although its definition has a relatively low specificity, a sausage toe is an easily identifiable and cost-free clinical sign that can be used as an indicator of underlying tissue/bone infection.

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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.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(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 years 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 16 centres across India at present and more centers are being inspected for accredition. 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

S.N.	Institute Name	Institute Location
1.	Diacon Hospital	Bengaluru, Karnataka
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
15.	Aegle Clinic	Pune, Maharashtra
16.	Tulip Hospital	Sonipat, Haryana

COURSE DETAILS

Name of the Course: Advanced Certiicate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

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.

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

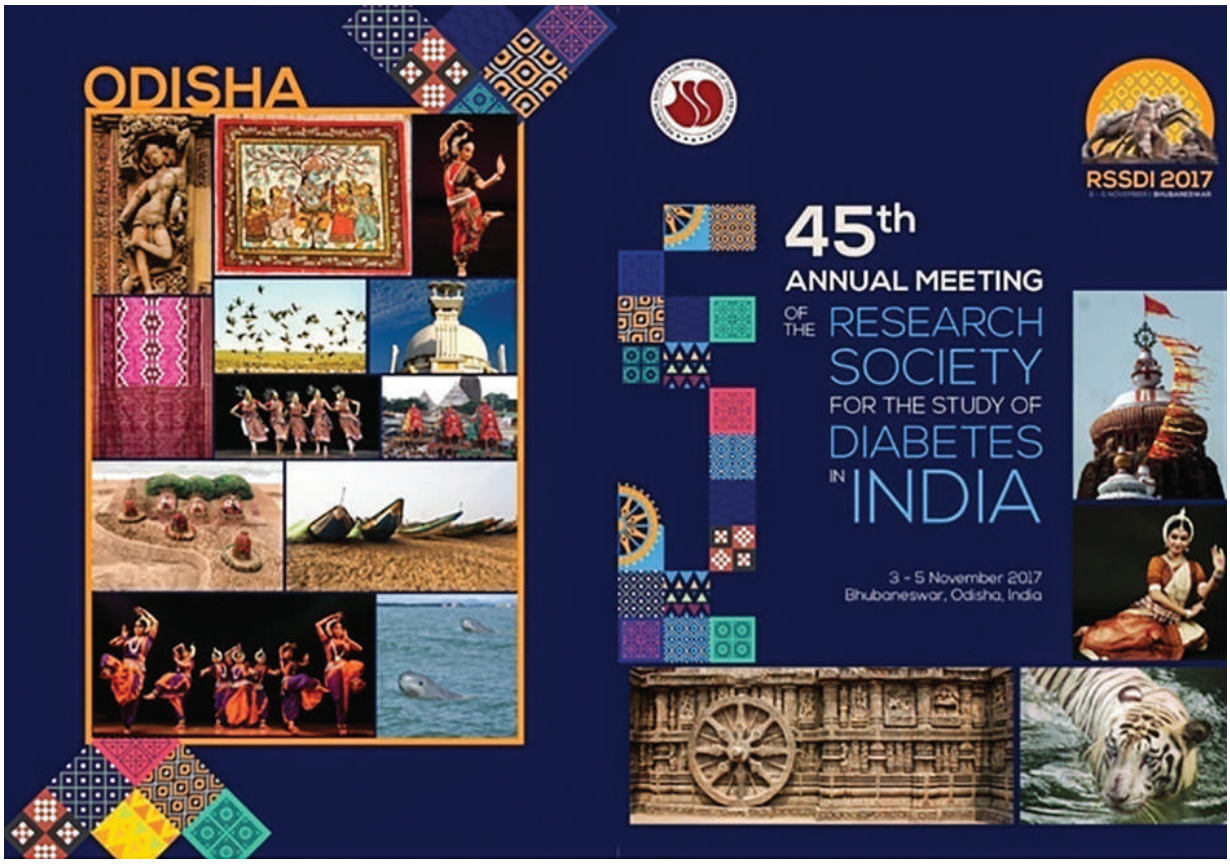
Session: 1st January & 1st July

Announcements

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

Invitation to the RSSDI,2017 Conference



Dear Friends,

We have great pleasure in inviting you to the 45th Annual Conference of Research Society for the Study of Diabetes in India (RSSDI) to be held in the smart city of Bhubaneswar from 3rd to 5th November 2017.

Bhubaneswar is the capital city of scenic Odisha. the soul of India which is naturally beautiful with forests. wildlife, sea beaches, heritage temples and many historical monuments. It is also known for its flavor of hospitality and varieties of delicious foods. We assure your convenient travel and comfortable stay with a relaxing time and a complete updated academic exposure.

The organizing committee will leave no stone unturned to make the event memorable for you and your family. please block your dates and register early to be a part of the event.

Wishing you a very happy. bright and prosperous new year ahead.

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Bhubaneswar, Odisha

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