ISSN 0973-3930

Volume 38 | Issue 2 | April – June 2018

International Journal of Diabetes in Developing Countries

Official Publication of
Research Society for the
Study of Diabetes in India

Springer

International Journal of Diabetes in Developing Countries
Incorporating Diabetes Bulletin

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International Journal of Diabetes in Developing Countries targets a readership consisting of clinicians, research workers, paramedical personnel, nutritionists and health care personnel working in the field of diabetes. Original research work and reviews of interest to the above group of readers is considered for publication in the journal.

The journal has a goal of serving as an important resource material in diabetes for its readers, mainly in the developing world.

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International Journal of Diabetes in Developing Countries is published 4 times a year. Volume 38 (4 issues) of will be published in 2018.

ISSN 0973-3930 print version
ISSN 1998-3832 electronic version

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International Journal of Diabetes in Developing Countries

Volume 38 · Number 2 · April–June 2018

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):131–132
https://doi.org/10.1007/s13410-018-0638-x

EDITORIAL

Fibrocalculous pancreatic diabetes—current scenario in developing countries

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Published online: 13 April 2018

Fibrocalculous pancreatitis diabetes (FCPD), a secondary form of diabetes due to tropical chronic pancreatitis (TCP), is seen exclusively in developing countries of the tropical world. From a worldwide perspective, alcoholism is the most common cause of chronic pancreatitis. However, in many tropical countries like India and Bangladesh, the commonest cause of chronic pancreatitis is TCP [1].

TCP is characterised by recurrent attacks of pain abdomen usually in the first or second decade of life along with steatorrhea and diabetes which usually sets in by the third decade. Some of its distinctive features are presence of large intraductal calculi, accelerated course of the disease and high susceptibility to pancreatic cancer [2].

Geevarghese, one of the pioneers in the field, documented one of the largest series of FCPD cases in the world from Kerala state [3]. Indeed, in Kerala in the 1960s, FCPD constituted 29.3% of the total diabetes registered at a medical college [4]. However, this figure dramatically reduced during subsequent years and a prevalence of 0.36% was reported during the periods 2001–03 and 0.2% in the periods 2006–10 in one hospital-based series [5].

Balakrishnan et al. [6], in a nation-wide prospective study in India on chronic pancreatitis based on clinical and radiological criteria, reported a prevalence of 3.2% for FCPD, 38.7% for alcoholic pancreatitis and 60.2% for 'idiopathic pancreatitis', although the latter could include TCP as well.

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Changing disease spectrum

Over the last two decades, the prevalence and clinical spectrum of FCPD and TCP have been showing a subtle change, perhaps due to changes in nutritional status and lifestyle changes. The disease now occurs in older age groups, with the mean age at presentation being nearly a decade later, than in the previous reports. The presentation of the disease has also become more heterogeneous, with only about 10–15% of patients presenting with the classical picture of FCPD. While the frequency of classical TCP is decreasing, pancreatitis due to alcohol and probably other environmental toxins is on the rise [7, 8].

The etiopathogenesis of FCPD is still poorly understood. Earlier hypotheses attributed the disease to protein calorie, malnutrition and cassava (tapioca) intake [9]. However, these hypotheses have never been proven either in well-designed case control studies or in experimental models [10].

Familial clustering of FCPD has been described by several authors and the role of genetic predisposition has been actively pursued. Recent studies have shown serum protease inhibitor Kazal type 1 (SPINK1), cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2) and chymotrypsinogen C to be associated with FCPD [10]. The N34S mutation of the SPINK-1 has been reported to be strongly associated with idiopathic and familial pancreatitis [11].

Recently, Mahurkar et al. [12] have proposed a two hit model for the pathogenesis of diabetes in TCP. The first hit includes the mutation of one or more genes, resulting in the formation of supertrypsin in the acinar cell of the pancreas. The second hit probably involves certain unidentified genes which may lead to one or more phenotypes such as stone formation, fibrosis and/or diabetes mellitus.

Patients with FCPD are known to have severe diabetes but there is a wide spectrum of clinical presentation, with some patients initially requiring only oral antidiabetic drugs but progressing to insulin requirement in the later stages, along with pancreatic supplements for the exocrine insufficiency. A

conspicuous clinical feature of FCPD is the absence of ketosis, in spite of high plasma glucose levels. This is due to partial preservation of pancreatic beta cell function [13] and possibly other mechanisms involved in ketone body synthesis and counter regulatory hormones [14]. Although insulin secretory defect is the major cause of diabetes, growing evidence for a possible role for insulin resistance, role of glucagon and body composition abnormalities, has added new dimensions to the pathogenesis of FCPD [15, 16].

Complications in FCPD can be due to long-standing diabetes which can cause both microvascular as well as macrovascular complications. Advanced retinopathy has been reported in FCPD patients [17]. Nephropathy, peripheral neuropathy and autonomic neuropathy also have been reported, but overall, macrovascular complications are less common in FCPD patients [18]. Pancreatic cancer is the most sinister complication of chronic pancreatitis and several prospective and retrospective studies have described FCPD as a premalignant condition. FCPD patients should therefore be monitored with CA-19.9 measurements and imaging (USG or CT scan) for the early diagnosis of pancreatic cancer. They should also be regularly monitored for all fat soluble vitamins especially vitamin D, whose deficiency can cause pancreatic osteodystrophy.

In this issue of IJDDC, Zabeen et al. [19] report a series of FCPD patients among Bangladesh children and adolescents. They report that 106 (25%) of a series of 429 children and adolescents with diabetes diagnosed below 18 years of age had FCPD. They report clinical features which have been frequently associated with FCPD. For example, compared to type 1 diabetes (T1D), FCPD patients had older age at onset, less common DKA and atypical symptoms at presentation.

Interestingly, 9% of FCPD patients and 3% of T1D had cataract. While cataract has been described earlier in children with both disorders, this high prevalence of cataract is rather surprising. The high A1c levels at diagnosis suggest a long period of uncontrolled diabetes leading to sorbitol-induced changes in the lens [20]. The authors are to be congratulated for picking up so many cases of FCPD among children. This is, thanks to routine screening with abdominal X-rays and ultrasound abdomen to rule out FCPD, something which is not commonly done nowadays, in most diabetes clinics.

The prognosis of FCPD patients has improved in the last two to three decades and of its natural history is also better understood but its etiopathogenesis continues to be elusive. Despite its relatively low prevalence, a differential diagnosis of FCPD must be borne in mind during evaluation of a young diabetic patient in a tropical country, especially if patient is lean and there is a history of abdominal pain or steatorrhea or if there is absence of ketosis.

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):133–137
https://doi.org/10.1007/s13410-018-0643-0

POSITION STATEMENT

Diabetes and Employment

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Published online: 3 May 2018

Abstract

Uncomplicated diabetes does not require any adjustment regarding employment, but those with complications should undergo detailed assessment to determine safety and effectiveness to perform duties. Though there is no such guidance in India, the American Diabetes Association (ADA) adopted a position statement that any person with diabetes, whether insulin treated or non-insulin treated, should be eligible for any employment for which he/she is otherwise qualified. Patients suffering from diabetes should be assessed individually, and after reviewing medical and treatment history, medical fitness to the job should be assigned. Maintenance of proper medical documentation and use of screening guidelines are a must. For this to be implemented properly, one needs state and national laws. A health care professional (HCP) treating the employee though preferred should have expertise in treating diabetes. If there is a disagreement between the opinions of physicians, an independent opinion from a HCP with clinical expertise in diabetes should be taken. An employer should not enquire about an employee's condition till a job has been offered and can only do so if it is not safe or poses a threat to his health and also if expert opinion and medical documentation suggest so. Screening guidelines, though not used in India but regularly used in Western countries, can be used in evaluation. Safety risks should be assessed individually—recurrent hypoglycemia, not a single episode, may pose a risk whereas hyperglycemia and chronic complications though may not pose immediate risk should be assessed separately. Periodic safety assessments should be done. Jobs requiring operating firearms or running dangerous machinery may have safety concerns with patients having severe hypoglycemia or those on insulin or secretagogues. Hypoglycemia usually can be effectively prevented or self-treated by ingestion of glucose. Severe hypoglycemia requiring assistance may pose a risk; even if it is a single incidence, one has to be followed up properly and investigated to find out its cause. In recurrent severe hypoglycemia, as episodes cannot be explained, it is a risk for the employee himself as well as the public. Hyperglycemia

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leading to chronic complications is only relevant when it interferes with the performance in actual jobs. Self-monitored blood glucose measurements over a period of time give actual information and should be evaluated by a HCP with expertise in diabetes. Multiple incidents of severe hypoglycemia may pose a problem in high-risk occupations, and the factors responsible should be properly evaluated. Hypoglycemia unawareness increases the risk of a sudden episode of severe hypoglycemia and should be treated with changes in diabetes management. Chronic complications may pose a risk in some jobs, but if not present, possible future development should not be taken into consideration. The tools that do not accurately reflect the current state of diabetes like urine glucose and HbA1C should not be used. The term uncontrolled diabetes should also not be used as it is not well defined. A few accommodations are required at a job for diabetes patients to be able to perform their work responsibilities effectively and safely: testing blood glucose at regular intervals, administering insulin as and when required with proper storage facilities, access to snacks, and a flexible work schedule to accommodate needs and modifications if required for chronic complications.

Keywords Diabetes · Employment

As of 2015, more than 69 million Indians have type 2 diabetes (T2D) [1]. Thus, a larger number of people with diabetes will have to cope with the complications of this chronic disease. Uncomplicated diabetes, in general, usually has little or no impact on an individual's ability to do a job. Hence for most of the professions, employment decisions should not be based on the fact that a person is affected with diabetes. When questions arise about the medical fitness of a person with diabetes for any job, a health care professional (HCP) with expertise in treating diabetes should perform an individual assessment to determine whether that person can safely and effectively perform the duties of the job in question [2].

In 1984, the American Diabetes Association adopted the following position on employment:

> Any person with diabetes, whether insulin treated or non–insulin treated, should be eligible for any employment for which he/she is otherwise qualified [2].

There is a lack of any such guidance in the Indian setting.

This document aims to provide some general guidelines for assessing individuals with diabetes for employment, including how to perform an assessment and what changes in the workplace may be needed to make the working environment conducive, for an individual with diabetes.

Evaluating Individuals with Diabetes for Employment

Recommendations

- People with diabetes should be individually considered for employment based on the requirements of the specific job and the individual's medical condition, treatment regimen, and medical history.
- A HCP with expertise in treating diabetes should perform an assessment, when questions arise about the medical fitness of a person with diabetes for a job
- Employment evaluations should be based on sufficient and appropriate medical data
- Screening guidelines and protocols can prove to be useful tools in making decisions about employment if they are used objectively and based on the latest scientific knowledge about diabetes and its management.

Restricting individuals with diabetes from certain jobs because of the diagnosis of diabetes or the use of insulin, without assessing the individual's abilities or circumstances is inappropriate. Such "blanket bans" are unwarranted.

Employment decisions should not be based on generalizations or stereotypes regarding the effects of diabetes since the impact of diabetes and its management varies widely among individuals. Therefore, a proper assessment of individual candidates for employment or current employees must take this variability into account.

In addition, state and national laws should be drawn up and enforced which will require employers to make decisions that are based on assessment of the circumstances and capabilities of the individual with diabetes for the particular job in question. This will also allow persons with diabetes to be protected from discrimination in employment and other areas.

Role of Health Care Professionals (HCPs)

An opinion from a HCP should be sought before any adverse employment decision, such as failure to recruit or promote or terminate.

Questions arising about the medical fitness of a person with diabetes for any job should be referred to a HCP who should perform an individualized assessment. The individual's treating physician is generally the HCP with the best knowledge of his/her diabetes. If the employer decides to use its own physician to perform the evaluation, it should be a HCP who has expertise in treating diabetes. Moreover, it is important to seek the opinions of the individual's treating physician and carefully consider it.

If there is a disagreement between the opinion of the employee's treating physician and that of the employer's

physician, the evaluation of the individual should be done by an independent HCP with significant clinical expertise in diabetes.

Individual Assessment

Employers should not enquire about an individual's diabetes status directly or indirectly before making a job offer. If required, a medical examination may be asked for, once an offer of employment has been made and before the person starts the job.

An employer may withdraw an offer from an individual with diabetes only if it is established that he/she cannot do the essential functions of the job or would pose a direct threat to his/her own health or safety.

An employer should not rely on a medical evaluation to deny an employment opportunity to an individual with diabetes unless it is conducted by a HCP with expertise in diabetes and based on sufficient and appropriate medical data.

An evaluation should never be made based only on one data, such as a single blood glucose result or A1C result.

Screening Guidelines

Various screening guidelines for evaluating individuals with diabetes in many high-risk jobs have been developed in Western countries, in recent years, for example; the US. Marshall Service and Federal Occupational Health Law Enforcement Program Diabetes Protocol.

Though these are not India-specific, these guidelines/protocols can be useful tools in making decisions about individual candidates if they are used objectively and based on the latest scientific knowledge about diabetes and its management.

These available guidelines and protocols are listed below in Appendix.

Evaluating The Safety Risk Of Employees With Diabetes

Employers who deny jobs they perceive all people with diabetes to be a safety risk based on misconceptions and lack of current knowledge about diabetes.

Recommendations

- Determine whether the concerns for safety risk are reasonable in the perspective the job duties the individual must perform.
- A single episode of severe hypoglycemia should not disqualify an individual from employment, but an individual with recurrent episodes of unexplained severe hypoglycemia may be unable to safely perform certain high risk jobs particularly jobs or tasks involving significant risk of harm to employees or the public and must be evaluated appropriately.
- Hyperglycemia does not pose an immediate risk of sudden debility on the job.
- Long-term complications e.g. retinopathy, neuropathy, nephropathy or macrovascular heart problems should have a role in employment decisions only when they are established and may interfere with the performance of the actual job being considered.
- Proper safety assessments should include review of blood glucose test results, history of severe hypoglycemia, presence of hypoglycemia unawareness, and presence of diabetes-related complications.

Safety Concerns

While evaluating safety concerns, it is important to determine whether the concerns are reasonable in the perspective of the job duties the individual is expected to perform.

For most jobs (such as jobs in an office or retail) there is no reason to believe that the individual's diabetes will put other employees or the public at risk.

In other types of jobs (such as those where the individual must carry a firearm or operate dangerous machinery) the safety concern is whether the employee will become suddenly disoriented, which happens usually due to low blood glucose (hypoglycemia). These occur more commonly in people receiving certain types of treatment such as insulin or secretagogues like sulfonylureas either alone or in combinations.

Hypoglycemia

The recent American Diabetes Association guidelines define level [1] hypoglycemia as a blood glucose level < 70 mg/dL[3]. It is a potential side effect of some diabetes treatments, like insulin and sulfonylureas. It can usually be effectively self-treated by ingestion of glucose and is not often associated with loss of consciousness.

Severe hypoglycemia (level 3), that requires the assistance of another person, is a medical emergency [3]. Symptoms may include confusion, severe cognitive impairment or rarely loss of consciousness. Most individuals with diabetes can recognize the early warning signs and quickly self-treat the problem by drinking or eating. Also, with self-monitoring of blood glucose levels, it is easier to detect mildly low glucose levels which can then be self-treated [4].

A single episode of severe hypoglycemia should not disqualify an individual from employment. In the case of a single episode of severe hypoglycemia, detailed history and appropriate evaluation should be undertaken by HCP with expertise in diabetes to determine whether it was an isolated incident, what could be the cause of the low blood glucose, whether

adjustment to the OHA or insulin regimen may remove this risk etc. Also, the likelihood of such an episode happening again is to be assessed meticulously. Some episodes of severe hypoglycemia can be explained and corrected with the assistance of a diabetes health care professional.

On the contrary, recurrent episodes of severe hypoglycemia indicate that the individual may not be able to safely perform a job, particularly jobs or tasks involving significant risk of harm to employees or the public, especially when these episodes cannot be explained.

Hyperglycemia

High blood glucose levels (hyperglycemia) can cause long-term complications over years or decades but does not normally lead to any adverse effect on job performance. The symptoms of hyperglycemia generally develop over days and do not occur suddenly.

Though high blood glucose may cause long-term complications like neuropathy, retinopathy, nephropathy or cardiovascular disease, not all individuals with diabetes develop these complications. They only become relevant in employment decisions only when they are established and interfere with the performance of the actual job being considered, e.g. visual impairment, due to retinopathy, that interferes with performance of the job.

Aspects of a Safety Assessment

- Blood Glucose Test Results

A single blood glucose test result only gives information about an individual's blood glucose level at one particular point of time. Because blood glucose levels fluctuate throughout the day, one test result will not help in assessing the overall health of a person with diabetes. The results of a series of self-monitored blood glucose measurements over a period of time; however, can give valuable information about an individual's diabetes health. Blood glucose records should be assessed by a HCP with expertise indiabetes [4].

- History of Severe Hypoglycemia

An individual having diabetes over an extended period of time, and has not experienced severe hypoglycemia is unlikely to experience one in the future. Conversely, multiple incidents of severe hypoglycemia may pose a problem in high-risk occupations.

It is important to examine the circumstances of each incident, as some incidents can be explained due to changes in therapy regimen, intercurrent illness, or other factors and thus may be unlikely to recur.

- Hypoglycemia Unawareness

Over time, some individuals lose the ability to recognize the early warning signs of hypoglycemia. They are at increased risk for a sudden episode of severe hypoglycemia. However, there is a chance to lessen this risk with careful changes to the diabetes management regimen i.e., more frequent blood glucose testing or frequent meals.

- Presence of Diabetes-Related Complications

Chronic complications that may result from long-term diabetes involve the blood vessels and nerves. These problems can lead to amputation, blindness or other vision problems, including loss of vision, deteriorating kidney function, heart attack or stroke. These complications could affect job performance and safety; hence they should be evaluated by a specialist.

If complications are not present, their possible future development should not be taken into consideration, since with medical monitoring and therapies, these complications can often be avoided or delayed. Thus, many people with diabetes never develop any of these complications, and those who do generally develop them over a period of years.

- Inappropriate Assessments

The following tools do not accurately reflect the current state of diabetes and should not be used in an assessment of employment.

oUrine Glucose Tests: Not an appropriate and accurate method for assessing diabetes control. It is not a reliable or accurate indicator of blood glucose levels. Blood glucose monitoring is more accurate to measure glycemic control [5].

oHemoglobin A1C (HbA1C): Reflects average glycemia over several months and correlate with mean plasma glucose levels. It provides HCPs with information about the effectiveness of the individual's treatment regimen, but is often misused in assessing whether an individual can safely perform a job. Because it is an identification of average glucose control, it is of low value in predicting short-term complications and shouldn't be used to evaluate employment situations. It doesn't provide information on whether the individual is at significant risk for hypoglycemia or suboptimal job performance.

oUncontrolled Diabetes: Sometimes an individual's diabetes is described as "uncontrolled," or "poorly controlled". These terms are not well defined and are not relevant to job evaluations.

III. Accommodating Employees With Diabetes

Recommendations

- Individuals with diabetes may need certain changes or accommodations at the job to be able to perform their work responsibilities effectively and safely.
- These include taking into consideration daily diabetes needs and, when present, the complications of diabetes. All such changes must be tailored to the individual and effective in helping the individual perform his or her job.

Accommodating Daily Diabetes Management Needs

It is impossible to provide an exhaustive list of potential changes an employer needs to make while accommodating an employee with diabetes. However, the key message in accommodating such an employee is to ensure that changes are tailored to the individual and effective in helping him/her perform their job.

Many of the requirements that employees with diabetes need daily are those that allow them to manage their diabetes in the workplace. They are usually simple and can be provided with little or no disruption in the workplace.

- Testing Blood Glucose Breaks: to allow an individual to test blood glucose levels when needed.
- Administering Insulin: to allow an individual to administer insulin when it is prescribed.
- Storing Insulin: to allow an individual to store insulin and other supplies if work conditions (such as extreme temperatures or long distance of travel) prevent the supplies from being carried by the person daily. A refrigerator is the basic requirement for storage of insulin [6].
- Food and Drink: Individuals with diabetes will need access to food and/or beverages during the day. This is especially important if the employee needs to respond to low blood glucose levels or maintain hydration if glucose levels are high. They should also be permitted to consume food or beverages as needed at their desk or work station.
- Flexible Work Schedule: Employees may need leave or a flexible work schedule to accommodate medical appointments or other diabetes care needs. Certain types of work schedules, e.g. shift workers, can make it difficult to manage diabetes effectively.
- Complications of Diabetes: For some individuals, it is also necessary to make modifications for long-term complications. For example, an employee with visual impairments owing to retinopathy may benefit from using a larger computer screen or other visual aids.

Most of the above changes can be made without putting other employees at risk, or disrupting the workplace.

Conclusion

Individuals with diabetes can and do serve as productive members of the workforce.

Employers should assume that people with diabetes have the same career goals and aspirations as any other employee. A person's diabetes should play no part in decisions about transfers and promotions. It is prudent to concentrate only on the appropriateness of the person's skills for a new position and determine if reasonable accommodations are needed. In order to gain greatest productivity from the employee, it is important to capitalize on the person's strengths and accommodate limitations.

The therapies for, and effects of, diabetes vary between individuals, so employers must consider each person's capacities and needs on an individual basis.

Appendix

1. American College of Occupational and Environmental Medicine's National Consensus Guideline for the Medical Evaluation of Law Enforcement Officers
2. National Fire Protection Association's Standard on Comprehensive Occupational Medical Program for Fire Departments
3. The U.S. Department of Transportation's Federal Motor Carrier Safety Administration's Diabetes Exemption Program
4. The U.S. Marshall Serviceand Federal Occupational Health Law Enforcement Program Diabetes Protocol

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):138–145
DOI 10.1007/s13410-017-0582-1

REVIEW ARTICLE

Brief report of the effects of the aerobic, resistance, and high-intensity interval training in type 2 diabetes mellitus individuals

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Received: 16 February 2017 / Accepted: 6 September 2017 / Published online: 25 September 2017

Abstract Insulin resistance is the main feature in type 2 diabetes mellitus (T2DM). Insulin resistance occurs when there is a failure or reduced activation of phosphoidilinositol-3-kinase pathway. Physical exercise (PE) can prevent and fight insulin resistance. Resistance training (RT), aerobic training (AT), and high-intensity interval training (HIIT) are different types of PE and have the ability to produce increased phosphorylation of insulin receptors favoring the increasing of the activity of insulin pathways during and after physical activity. The objective of this study was to check through a literature review the benefits of AT, RT, and HIIT over insulin resistance in T2DM. It was made a research in PubMed using the words PE, insulin resistance, diabetes, AT, RT, and HIIT. The most relevant papers with results over glycemia were selected. There was better glycemia management in moderate AT and RT, and during HIIT sessions. HIIT presented increased cardiovascular risks to T2DM individuals. Lower risks with more benefits were seen at moderate AT. All three types of PE can be performed as a tool to prevent and fight insulin resistance in T2DM. When evaluating safety and benefits to diabetics, moderate AT provides best benefits than RT and HIIT to T2DM individuals.

Keywords Insulin resistance · Physical exercise · Diabetes mellitus

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Introduction

Diabetes mellitus (DM) is a world health problem. It is a metabolic disorder which in 2002 reached over 173 millions of people all over the world, and this number is increasing [1], although there might be about 400 millions of people with DM in the early thirties of this century [2]. These epidemic features are developing in many countries such as USA where 64.5% of the population are overweight [3]. A cross-sectional survey done in Rajasthan, India's biggest state, found out that the subjects had a sedentary lifestyle and there still is an urgent need to change bad daily habits to prevent chronic morbidities, such as type 2 diabetes mellitus (T2DM) [4]. T2DM reaches over 90% of the DM diagnosed cases [5, 6] and its main characteristic is the insulin resistance [7]. Insulin is the main metabolic hormone of the human body produced by the pancreatic beta (β) cells. It controls the glucose metabolism and its effects happen mainly after the meals [8]. Reduction at insulin hormone sensibilization conducts β-pancreatic cells to produce higher insulin concentrations in order to maintain the glycemia level normalization. Thus, this leads to hyperglycemia and hyperinsulinemia that are the clinical features of T2DM [9]. The insulin molecular pathway which controls most of this metabolic process is named phosphoidilinositol-3-kinase (PI3K) [5]. Physical exercise (PE) has been proved as an important tool to fight T2DM and also to prevent the changes in the molecular mechanisms of PI3K pathway [10].

The aim of this study was to elucidate the effects of three different types of PE over insulin resistance in T2DM. This review summarizes the effects of aerobic training (AT), resistance training (RT), and high-intensity interval training (HIIT) over insulin resistance in T2DM. A research was made in PubMed using the words insulin resistance, diabetes, PE, AT, RT, and HIIT. The most relevant papers according to citations and with results towards to glycemia management were selected.

Diabetes mellitus

The etiology of the word diabetes comes originally from a Greek word which means siphon because doctors from the old Greece observed that diabetics drink a lot of water, and as a consequence, they tend to use the toilet a lot [11]. The etiology of the word mellitus comes from the Latin version of honey because there is a taste of sugar in the urine of the diabetic patient [12]. There are different types of diabetes but the most common are types 1 and 2. Type 1 DM is related to the destruction or absence of β-pancreatic cells. On the other hand, T2DM is related with the resistance to the insulin hormone, which causes the increasing of blood glucose levels and many different complications, such as DNA damage to β-pancreatic cells leading to a failure on its functionality [5, 13]. There are other diabetes types, such as gestational diabetes, but T2DM has the higher incidence and prevalence [2].

Insulin resistance in type 2 diabetes mellitus

Insulin resistance involves molecular alterations in different points of glucose captured by the adipose or muscle tissue. Modifications in intracellular signalization of the PI3K pathway such as the reduction of kinase activity of the insulin receptor (IR) and the consequently lower phosphorylation of substrates 1 and 2 of the IR (IRS-1 and IRS-2) lead to a minor translocation of glucose transporters to peripheral tissues [5, 14]. There are different types of glucose transporters, but GLUT4 is the most important found in adipose and muscle tissues. GLUT4 is the only one of its family that is not located in the cell membrane. It remains in the vesicles into the cells. It is necessary to exist a signalization through the PI3K pathway in order to translocate the vesicle until the membrane to GLUT4 be set free to capture and take glucose to muscle and adipose tissues (Fig. 1) [5, 15, 16].

Intracellular signalization begins with the connection between insulin and its specific membrane receptor. This IR is compounded of four subunits where two of them are considered to be alpha (α) and two of them are β. The α subunit inhibits the tyrosine kinase activity of the β subunit. When insulin is connected to IR, the α subunit is inhibited and β is allowed to have a kinase activity. This leads to a conformational alteration of the molecule and auto phosphorylation of its receptor [13]. IR activates many protein substrates and six of them belong to insulin receptor substrate (IRS) family. When IRS are activated makes to come up sites of connection between molecules with Src 2 domain where the most important activity will be developed by PI3K. Studies in knockout mice identified some of the functions of IRS [17–19]. Failure in activating IRS-1 does not cause hyperglycemia but leads to insulin resistance and growth retardation [5]. IRS-2 can compensate for the absence of IRS-1 activity for a while, which explains the absence of hyperglycemia attached to the insulin resistance [18]. IRS-3 and IRS-4 have no influence in glycemic metabolism and they are more related with protein synthesis [17]. IRS-5 and IRS-6 are considered poor substrates for the IR because of their weak phosphorylation levels [20]. The PI3K pathway is associated to T2DM development through the inhibition of IRS-1 and IRS-2 activities which will lead to a reduced expression of activated kinase tyrosine (AKT) and GLUT4 [5, 17, 18].

The PI3K pathway is vital for glucose transport mediated by insulin signalization and also acts on mitogenesis and

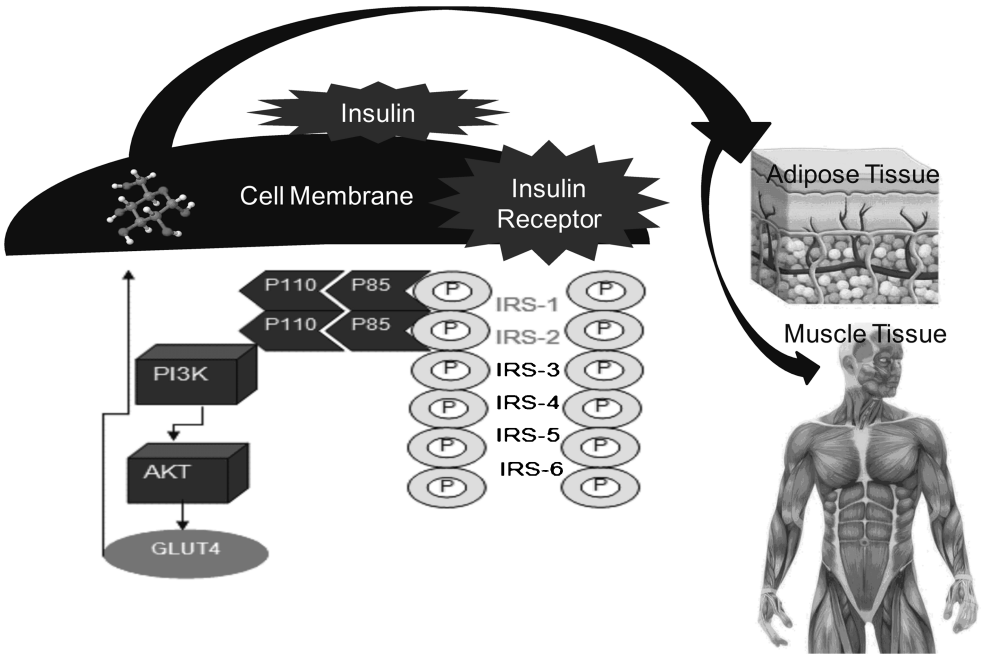


Fig. 1 PI3K pathway. Signalization route starts with a binding between the hormone insulin and its receptor. The binding promotes a conformational alteration in the insulin receptor. IRS is activated and phosphorylates p85 which is associated to p110. PI3K is a dimmer compound by p85 and p110. Thus, PI3K phosphorylates activated kinase tyrosine (AKT) which signalizes vesicles where there are glucose transporters. GLUT4 is the glucose transporter found inside these vesicles, and it is translocated to the cell membrane by these vesicles and catches and takes glucose to the adipose and muscle tissues. (Adapted from Folli et al. 1992)

cellular differentiation [5, 19, 21, 22]. PI3K is compounded by two proteins: p85 and p110, where p85 acts regulating p110 activity which has catalytic properties [23]. The activation of p110 is done by IRS and leads to a phosphorylation in site 3 of the inositol ring from the phosphatidylinositol producing phosphatidylinositol-3-phosphate and/or 3,4-bi-phosphate and/or 3,4,5-tri-phosphate. AKT is one of the targets of PI3K. AKT activity allows the vesicle containing GLUT4 to translocate until the membrane captures glucose [23, 24]. Phosphatidylinositol-3,4,5-tri-phosphate regulates the activity of pyruvate dehydrogenase (PDK) isoforms which phosphorylates C kinase protein (PKC) isoforms. PKC activity is related to gain of fats and obesity. Fatty mass gain is associated with T2DM and can induct to central obesity [6, 25]. Insulin resistance which originated from obesity happens because of PKC activity. Most of the individuals with T2DM present gain of mass and are overweight [26], and PE is an essential tool in order to avoid the developing of obesity and T2DM [7, 27].

Insulin resistance and physical exercise

Intervention through a healthy diet and PE can prevent or act as a non-pharmacological tool to fight T2DM [9]. Glucose uptake is induced by PE at skeletal muscle. This glucose uptake is sustained up to 48 h after the exercise. The increase of the sensibility of the receptors and its substrates to insulin on skeletal muscle cell and greater GLUT4 expression are positive changes induced by PE [28, 29]. The effect of PE is different between healthy and diabetic individuals towards insulin action. In diabetics, the PI3K pathway and glucose capture can be improved until 40% more through PE while healthy individuals do not get such improvement. Thus, there are a few facts to be considered that might help glycemia control such as the exercise type, the duration, and the intensity of it. These variables may modify the result and higher glucose capture will be related to them [30]. There are several combinations that can be done between the different styles of exercises, but the three main types of exercises evaluated in this study were RT, AT, and HIIT.

Insulin resistance and resistance training

RT is a type of PE based on weight lifting. RT consists of muscle contractions done against an external resistance (such as weights), and those contractions lead to increases in muscular mass, strength, endurance, and tone [31]. It is considered to be moderate RT if the individual lifts between 60 and 80% of the maximum load, and intense if he/she lifts more than 80% of the maximum weight that can be lifted for just one repetition. Intense RT could be done at least twice a week and with a constant supervision of the blood glucose level by anyone [6]. It is more common to use the moderate and light resistance training for diabetics, but intense RT is also recommended to promote greater reduction in blood glucose than moderate [32–34]. Nevertheless, when comparing moderate and intense RT, it was revealed both intensities to be capable to reduce the blood glucose level [10].

It is suggested by a large number of authors that an immediate consequence of a single bout of intense RT reveals to elevate the GLUT4 transport [35–37]. This will lead to a higher glucose capture and will help to normalize and manage the blood glucose level [38]. On the other hand, long-term RT can increase the number of mitochondria and the expression of fatty and glucose transporters benefiting the oxidation process in the skeletal muscle [39]. Blood glucose control will favor the body weight reduction [38, 39]. Both short- and long-term RT improve the glucose capture but the results are quite better on long-term trainings [40]. Despite all of this, acute RT may be more effective than aerobic exercise depending on duration and intensity of it in order to reduce blood pressure [34].

A recent study revealed a single bout of RT or AT is able to reduce equally blood glucose levels [10]. RT and AT provide benefits over insulin resistance and can be performed using a vast combination of duration, frequency, and intensity between them [6], but just the usage of RT at low-intensity level does not lead to significant physiological changes. Due to the difficulty of having volunteers to do a tissue removal, it is needed to use experimental models with animals to get to evaluate more accurately changes into cell molecular signalization. A recent study analyzed if RT would enhance components of the insulin-signaling cascade in normal and high-fat-fed rodent skeletal muscle [32]. The authors revealed RT increased insulin-stimulated carbohydrate metabolism in normal skeletal muscle and reversed the effects of high-fat diet-induced skeletal muscle insulin resistance by modifying components of the PI3K pathway and glucose transporter system There are a lot of studies about RT and diabetes and lipid metabolism [29, 31, 41], but it is still not completely clear of the molecular changes related to PI3K activation and PKC in PE at T2DM individuals.

Insulin resistance and aerobic training

AT is also called endurance and it is based on having type 1 muscle fibers worked out more than type 2 muscle fibers. Type 1 muscle fibers are referred to as slow twitch oxidative characterized by high endurance. Type 2 muscle fibers or fast glycolytic fibers are recruited for a very short period of high-intensity busts of power, such as sprints. AT activities recommended to diabetics are in the moderate zone because they are sufficiently handled by aerobic metabolism and may be performed for extended and usually continuous periods of time [42, 43]. The intensity of the PE is determined by the maximum oxygen consumption (VO_{2max}) in 1 min, and if the AT is

performed between 60 and 80% of the VO_{2max}, it is considered to be a moderate exercise to healthy individuals and intense if above 80% [43], but these values change agreeing to gender, and age. It reflects the individual physical fitness level and is an important determinant of their endurance capacity. If the person has a pathological condition such as T2DM, these values to the moderate zone may change and there also are other ways to calculate the zone intensity to anyone, like heart rate for example.

Endurance training at 60% of the VO_{2max} has been proved to be effective for burning fat tissue and fighting insulin resistance [44]. A study of 12 weeks walking at 60% of VO_{2max} [45] lowered pro-inflammation proteins such as tumor necrosis factor-α and interleukin-6 which are significantly higher in insulin resistance and obese patients and got to reduce total body weight by lowering body fat. This is important because it has been shown in several studies overseas [7, 45, 46] that excessive storage of body fat plays an important role in inflammation and insulin resistance by taking to an imbalance between the hormone action and insulin-resistant cytokines expressed specifically in fat cells. Fatty acid oxidation improves insulin action, and physical training induces higher β-oxidation at adipose tissue reducing the number of adipose cells and contributing to a body weight reduction and consequently to control the blood glucose level [47, 48]. A single week of endurance training improves insulin sensibility in T2DM individuals, but despite this, long-term AT is more effective than short-term in individuals with insulin resistance [6].

The chronic endurance exercise can contribute to a greater GLUT4 expression and a greater glycogen synthase enzyme activity helping to fight insulin resistance. It has been assumed that, when talking about chronic PE, AT provides better results to glucose metabolism than RT through a higher activation of red muscle fibers or type 1 muscle fibers helping to improve mitochondrial biogenesis [49, 50]. This happens because the process in AT is the oxidative phosphorylation which leads to multiply the number of mitochondria as much as necessary to support the following training sessions. Basically, if you do long and frequent sessions of AT, you will have more mitochondria, and there will be an improvement of the $VO_{2max.}$, less oxidative stress, and inflammation.

It is recommended to do moderate AT at least 30 min 5 days a week or 25 min of intense AT 3 days a week for healthy individuals [6]. Due to the T2DM pathology and the several risks associated, like hypoglycemia and cardiovascular risks, the recommendations for diabetics are towards moderate AT. In spite of the risks, intense AT can be done, but one should have a personal orientation and supervision [6, 30]. Finally, lower levels of AT are not capable of significant reduction on body weight and fat. Combining moderate and intense AT can help to achieve excellent results to T2DM individuals over blood glucose management and body weight reduction.

Insulin resistance and high-intensity interval training

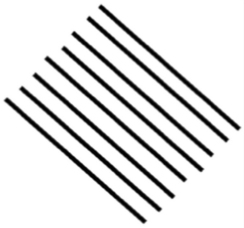
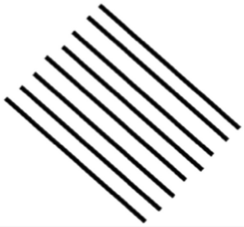
Moderate AT can achieve blood glucose reduction but bouts of intense exercise can give better results to healthy individuals [51]. Interval training refers to a basic concept of mixing periods of intense exercise with other periods of lower intensity effort or even complete rest for recovery [52]. It has been proven that it does not matter the period of the rest interval during HIIT because longer or shorter breaks do not cause different muscle or performance adaptations [53]. The workout periods of HIIT may range from 5 s to 8 min long and must be performed at 80 to 95% of a person's estimated maximal heart rate, which is the maximum number of times your heart will beat in a minute without overexerting yourself, according to the American College of Sports Medicine [54].

HIIT interventions improve cardiovascular health, yet the acute effects on circulating and functional biomarkers of cardiovascular function are not clear in individuals with T2DM. A recent scientific article [55] investigated blood pressure, endothelial-dependent dilation, and circulating measures of endothelial activation in T2DM subjects. The participants were diabetics who did not have cardiac problems and were subdivided in two different groups who are unaccustomed (study 1) and accustomed to HIIT (study 2). In study 1, endothelial markers did not differ from baseline between T2DM and control participants within the first 2 h after HIIT, except at 30 min after HIIT for glucose, which was reduced more in T2DM individuals than in controls. Study 2 saw no significant difference in any circulating markers. These two studies of this article suggest that acute HIIT does not alter circulating and functional markers of cardio(vascular) health in individuals with T2DM.

When comparing HIIT with moderate AT, both were effective in fighting high levels of hypertriglyceridemia and hyperinsulinemia contributing to lowering insulin resistance [56]. Thus, to T2DM individuals, HIIT can be dangerous depending on the stage of their condition and the presence or development of comorbidities related to diabetes such as cardiovascular disease which happens to be the most common cause of death in populations with diabetes [6]. Considering HIIT an exercise that causes an extreme metabolic stress, moderate AT should be chosen preferentially to be performed by T2DM subjects for safety reasons (Table 1).

Individuals with T2DM usually have a deficit on oxygen consumption and because of it, there is a higher risk of fainting, and of having cardiovascular problems [52]. This oxygen deficit starts when you develop insulin resistance which can make you achieve extreme metabolic stress easier than healthy individuals due to an endothelial damage that will favor the development of cardiovascular diseases [6]. HIIT benefits the exercise posterior oxygen consumption (EPOC) [53, 57]. EPOC will favor higher activity of the carnitine palmitoyltransferase complex which is responsible for a huge

Table 1 Main physiological benefits and cardiovascular risk of AT, RT, and HIIT at different intensities to T2DM individuals. Results shown were made based on many published studies that evaluated glycemia management and cardiovascular risks [2, 29–31, 35–37, 39, 40, 51]

T2DM individuals	Low intensity	Medium intensity	High intensity
 Aerobic Training	No significant blood glucose reduction, loss of body mass, or blood pressure No cardiovascular risk	Reduction of the blood glucose level Body weight reduction Management of blood pressure Low cardiovascular risk	Reduction of glycemia Body weight reduction Better control of blood pressure Higher cardiovascular risk
 Resistance Training	No significant blood glucose reduction, loss of body weight, or better blood pressure control No cardiovascular risk	Reduction of glycemia Improvement of blood pressure control Low cardiovascular risk	Reduction of the blood glucose level Improvement of blood pressure control Higher cardiovascular risk
			Reduction of glycemia Body weight reduction Better control of blood pressure Higher cardiovascular risk

part of the fatty acid oxidation process contributing on this way to body mass reduction and diminishing oxidative stress, and the action of inflammatory proteins. HIIT activates an enzyme called activated protein kinase (AMPK), which plays an important role in cellular energy homeostasis, and increases the expression of peroxisome proliferator-activated receptor-coactivator-1α (PGC-1α), which works as a regulator of mitochondrial biogenesis [27, 58, 59] helping to increase aerobic capacity.

PGC-1α role in type 2 diabetes mellitus and brain insulin resistance

T2DM and Alzheimer's disease (AD) appear to share similar pathogenic mechanisms [60] such as their main feature which is insulin resistance. T2DM individuals are at increased risk of developing cognitive decline, dementia, or AD because of the insulin resistance. AD brains exhibit defective insulin signaling with altered levels and cellular distribution of insulin receptors [61]. CNS is an important target for the actions of peripheral hormones, including insulin, and others [62]. Insulin stimulates neuronal survival and synaptic plasticity and contributes to higher brain functions, including cognition [63]. Failure of insulin-initiated signaling pathways in the CNS has been associated with brain disorders [62, 63]. Recent evidence suggests that PE reduces the risk of cognitive decline and the development of dementia and neurodegenerative disorders such as AD. Physical exercise stimulates the activation of several signaling factors that contribute to maintaining neuronal homeostasis, such as those mediated by PGC-1α [27] besides stimulating neurogenesis in the adult brain [64].

A recent review [64] showed that PGC-1α causes production of the FNDC5 protein which is cleaved to give a new product irisin (FNDC5/irisin) in response to the PE. FNDC5/irisin is an exercise-induced myokine reported to modulate peripheral metabolism and found to stimulate brain-derived neurotrophic factor (BDNF) expression in the hippocampus, the key memory center in the brain. PE boosts hippocampal FNDC5/irisin levels and protects mice of memory deficits, offering novel insight into the beneficial effects of exercise in the brain. Through similar mechanisms, the elevation of the expression of PGC-1α/FNDC5/irisin in response to PE helps to control glycemia in T2DM and to prevent cognitive decline due to brain insulin resistance. Description of neuroprotection by FNDC5/irisin contributes for the explanation of the cognitive benefits of exercise in patients with AD or even in those with age-related cognitive dysfunction related or not to T2DM. Understanding the molecular mechanisms of how irisin reduces and prevents systemic and brain insulin resistance could help to develop therapies to manage and prevent T2DM, and cognitive decline in any neurodegenerative disorder. Irisin is a recent discovery and has been proven that its production happens in response to PE with the concomitant increasing expression of PGC-1α which is more stimulated during AT according to the literature [27, 65]. On the other

hand, irisin production has not been demonstrated in RT. Physical activity should be encouraged all over the world to avoid the progress of epidemic T2DM and the comorbidities and consequences related to this condition.

A recent study in India [4] observed that nearly half of the women were physically inactive. In a country where most of the women are housewives and less educated, it is extremely important to emphasize the benefits of physical activity to the body and brain. Indian dietary guidelines include physical activity; however, public awareness of the benefits of regular exercise to improve health is a major public health challenge. This is very important in view of high burden of T2DM in India.

Conclusions

RT, AT, and HIIT are able to prevent and fight insulin resistance present in T2DM. All exercise types contribute to a better functioning of PI3K pathway. They can be performed in order to achieve different benefits as lowering blood glucose level, managing blood pressure, and reducing body fat. All these contributions given by PE help to improve the quality of life of T2DM individuals. Frequency of PE to diabetics should be no less than three times a week and always training at least in moderate intensity. When evaluating safety and best PE benefits for diabetics, moderate AT provides best benefits than RT and HIIT. Moderate AT is recommended as the best and safest PE to T2DM subjects.

Acknowledgements I would like to thank CAPES.

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):146–152
https://doi.org/10.1007/s13410-017-0599-5

REVIEW ARTICLE

Diabetes and tuberculosis in Mexico: results from epidemiological studies

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Received: 4 September 2017 / Accepted: 8 December 2017 / Published online: 5 January 2018

Abstract
The association between tuberculosis (TB) and type 2 diabetes mellitus (DM2) has been recognized during centuries, and recently, a vast number of studies have evidenced their relationship. Uncontrolled diabetes and a poor glycemic control increase the risk for TB infection. Both diseases are considered chronic diseases; constituting important worldwide health problems. In Mexico, the prevalence of DM2 among TB patients varies, rising levels up to 36%. Several epidemiological studies have been conducted in the country and in the Border States, giving an estimate of the real situation of this comorbidity and in consequence, contributing to improve the diagnosis and follow-up of patients. In this review, we updated the research data on DM2 by means of observational and experimental studies conducted in Mexico in the last 17 years. Results show the continuous increase of TB-DM2 incidence and the need for the establishment of proper control methods acting over this dual axis.

Keywords Tuberculosis · Diabetes · Mexico · Epidemiological studies · Comorbidity

Introduction

The International Diabetes Federation (IDF) reported that 415 million people worldwide suffer from DM (90% of all cases are type 2 diabetic patients) [1], with a devastating effect on public health and economy. In 2030, it is estimated that 12.6% (95% CI 9.2–17.3%) of new TB cases in the 10 countries with the highest TB burden will be attributable to TB [2]. According to Mexican Diabetes Federation, in 2015 Mexico occupied the sixth place in prevalence of diabetes in the world [3]. On the other hand, the World Health Organization (WHO) estimates that TB is a re-emergent health problem in Mexico, reporting an incidence of 16.8 cases per 100,000 habitants in 2012 [4]. It is well known that DM2 increase three times (in average) the susceptibility to TB. In fact, DM2 is considered one of the most important risk factors for the progression of TB, besides patients with TB and DM2 have a deficient response to conventional treatment against TB worsening the prognosis of these patients. Considering the high incidence and prevalence of DM2 in Mexican population (even more than human immunodeficiency virus/acquired immunodeficiency syndrome) [5], an opportune diagnosis and treatment for DM2 in TB patients could have a better prognosis for both diseases. Pan-American Health Organization (PAHO) and WHO have proposed the implementation of a strategy for the simultaneous treatment for TB and DM2 in health programs, which mainly cover the detection, treatment of DM2 in TB patients and vice-versa. However, several associated risk factors such as alcoholism, smoking, obesity, and under nutrition together with environmental factors make a blur scenery. In this work, we provide an analysis of the situation of TB-DM2 comorbidity in Mexico according to the collected data in the last 17 years and we analyze the future outcome for both diseases.

Mexico and the double burden of tuberculosis/diabetes

In 2010, Mexico reported 18,122 new TB cases, of which 20% were associated with DM2. Forty-three percent of these cases were women, with a rate man/woman of 1.3. The states more affected by TB-DM2 comorbidity were Baja California, Chiapas, Guerrero, Nuevo León, Oaxaca, Tamaulipas, and Veracruz [6], reporting 52% of the total of TB/DM2 cases in the

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country during the same period of time. The situation has worsened for both diseases in the last years. Only 2 years after, in 2012, the number of TB patients diagnosed with DM2 increased 134.2%, and the incidence rate increased to 82.64% [7]. The last data available, for the year 2013, show a tendency to increase the rate of TB and DM2 at least for 12 federative entities with respect to the national average (Fig. 1).

It is very important to understand the mechanisms that make a diabetic patient susceptible to TB infection. In Mexico, according to National Health Survey data from 2012, more than half of diabetic patients live in poverty, most of them without access to health services [4]. These conditions are in great extent related to toxic habits, such as smoking, alcoholism or other drugs use/abuse, besides overcrowding and an immune system deficient

Fig. 1 TB-DM2 prevalence in Mexico. Data were collected from Plataforma Única de información /SUIVE/DGE/SS 2013. *represents Border States of Mexico

STATE	Rate 2003-2007-2012	Rate 2013	Trend
AGUASCALIENTES	0.6	2.1	⇑
BAJA CALIFORNIA *	6.4	2.6	⇓
BAJA CALIFORNIA SUR	3.3	2.5	⇓
CAMPECHE *	3.3	3.2	⇓
COAHUILA*	4.4	6.3	⇑
COLIMA	4.1	3.3	⇓
CHIAPAS*	4.6	5.4	⇑
CHIHUAHUA*	3.4	2.2	⇓
DISTRITO FEDERAL	1.8	1.7	⇓
DURANGO	2.8	4.7	⇑
GUANAJUATO	1.5	2.3	⇑
GUERRERO	8.9	13	⇑
HIDALGO	1.8	2.9	⇑
JALISCO	2.2	2.1	⇓
MEXICO	1.3	0.8	⇓
MICHOACAN	1.5	2.6	⇑
MORELOS	2.5	2.9	⇑
NAYARIT	3.2	5.2	⇑
NUEVO LEON *	7.1	5.5	⇓
OAXACA	3.6	5.9	⇑
PUEBLA	2.5	3.4	⇑
QUERETARO	2.7	2.8	⇓
QUINTANA ROO*	3.9	0.5	⇓
SAN LUIS POTOSI	3.1	5.7	⇑
SINALOA	3.6	5.2	⇑
SONORA *	4.2	3.1	⇓
TABASCO*	5.5	7	⇑
TAMAULIPAS *	9.2	7.2	⇓
TLAXCALA	1.1	2.2	⇑
VERACRUZ	6.3	8.1	⇑
YUCATAN	2.2	2.8	⇑
ZACATECAS	1.4	3.6	⇑
NATIONAL	**3.4**	**3.8**	⇑

* Border States

Source: Plataforma Única de Información/SUIVE/DGE/SS. 2013.

TB-Diabetes Mellitus prevalence Mexico, 2013

mainly by undernutrition, which are considered as risk factors for TB infection, [8]. Although the molecular mechanisms that predispose to TB infection in diabetic patients are not completely understood, it is known that existing alterations in innate and adaptive immune response, including: alterations in the complement pathway, decrease phagocytic activity of alveolar macrophages, decrease in subpopulations of Th1 cytokines, among others, factors that enhance TB infection [9]. Such alterations explain the increased susceptibility to TB and have been well addressed in different animal models (guinea pig, rat, mice) in which both diseases have been reproduced [10–12].

Epidemiological studies conducted in Mexican population

Different research groups in Mexico have conducted important studies directed to elucidate the molecular mechanisms of susceptibility, to identify risk factors, to determine the prevalence of both diseases, etc. We analyzed some of the most relevant studies conducted in the period of 2000–2017, based mainly in epidemiologic approaches. Most of the studies have been conducted in the border USA-Mexico, or in south Mexico, due to the high incidence of both diseases in these regions.

Experimental studies

In vitro studies

Arce-Mendoza and colleagues reported the differential expression of three membrane receptors of blood mononuclear cells in DM2 patients, TB patients, and TB-DM2 patients. Mononuclear cells were isolated and cultured to obtain adherent cells, which were stimulated with *M. tuberculosis* H37Rv lipids. In this study, it was demonstrated that DM2 affects the expression of CD64, CD206 (which are receptors with an important role in *M. tuberculosis* internalization in macrophages) and Receptor for Advanced Glycation End products (RAGE), a predisposing factor for TB, which captures glycosylated proteins. The double condition decreases the expression of CD64 and CD2016 receptors, compared to pulmonary TB patients alone, while RAGE expression increases. The latter suggests a role of this receptor as *M. tuberculosis* receptor [13].

In another study conducted by the same research group, the vitamin D serum levels, CYP27B1-hydrolase enzyme, vitamin D receptor and antimicrobial peptides gene expression of healthy donors, DM2 and TB patients were evaluated in monocytes-derived macrophages (MDM). Treatment of MDM from DM2 patients infected with *M. tuberculosis*, with Vitamin D, resulted in the efficient elimination of the bacteria [14]. These results evidence the positive use of Vitamin D in the prophylaxis against TB and the importance of vitamin D receptors for an efficient anti-mycobacterial immune response.

Alveolar macrophages and cathelicidins play a very important role in the containment of *M. tuberculosis* infections. Studies performed by Montoya-Rosales and colleagues evaluated the effect of high glucose levels in macrophage viability, phagocytosis index and LL-37 gene expression levels. They demonstrated that high glucose levels lead to LL-37 down-regulation, reduced the percentage of phagocytosis, which enhance the bacilli replication. However, when macrophage is infected with *M. tuberculosis*, LL-37 levels increase as glucose concentrations increase, promoting an anti-inflammatory response with low mycobacterial elimination, giving the opportunity to establish progressive infection, as it has been previously demonstrated in diabetic patients with pulmonary TB [15, 16].

In another experiment performed in southern Texas and north-eastern Mexico, blood from patients with TB and with TB and DM2 was stimulated in vitro with purified protein derivative (PPD) from *M. tuberculosis*. It was demonstrated that patients with TB and DM2 had higher levels of innate and type 1 cytokine responses compared to non-diabetic subjects [17]. Although this result seems to be contradictory, authors explain that, possibly as result of an increase in advanced glycation end products that bind and modify protein function, diabetic patients present suppression of downstream signal transduction of Th1 and innate immune response cytokines, and accumulation of dysfunctional cytokines in plasma due to advanced glycation end products modification. Besides, diabetic patients may produce higher levels of innate and type 1 cytokines because of higher bacterial load at the time of tuberculosis diagnosis.

The studies described in this section in conjunct allow to conclude that DM2 increase susceptibility to TB infection in the context of Mexican population. The role of membrane receptors, antimicrobial peptides expression, phagocytosis index in macrophages as well as cytokines response have been assessed in different ex vivo models, giving evidences about the involvement of DM2 in the increased susceptibility to TB disease.

Observational studies

Case-control studies

Comparison of chest X-rays from 192 patients with pulmonary TB and DM2 and 130 patients with only pulmonary TB registered in the database in the period 1990–1994, was performed by the National Institute for Respiratory Diseases (Mexico City). The study showed that in diabetic patients, lower lung lesions (atypical radiological images) are common at all ages, suggesting that DM and aging predispose to similar radiological changes in patients with TB [18].

For evaluating the role of DM2 as a risk factor for TB development, a case-control study with cross-sectional data

using the database from the Texas hospital obtained from 1999 to 2001 was performed. This study confirmed that the risk for diabetic patients to develop TB was twice higher than the risk of people without DM2 [19].

Restrepo and colleagues. retrospectively analyzed six years of data of diagnosed TB patients, obtained from the South of Texas and the north-eastern of Mexico. From data collected, it was possible to conclude that patients with TB and DM2 were more likely to have hemoptysis, pulmonary cavitations, be smear positive at diagnosis and remain positive at the end of the second month of treatment [20]. This work reported for the first time, the effect of DM2 in a population diagnosed with TB at the USA border. Later, Abdelbary and colleagues reassessed prevalence of DM2 and its associated factors among 8431 TB patients using surveillance data from 2006 to 2013 for the Mexican state of Tamaulipas. The authors found that lower education and higher unemployment (factors related with a poor control of DM2) are significantly associated with TB-DM2 comorbidity and not only with DM2. In this descriptive analysis, the prevalence of DM2 reported in the TB patients was 25.2%, one of the highest rates in Mexico and in the world [21].

A pilot case-control study performed with Mexican mestizo population in Juarez City (Chihuahua), demonstrated that, following multivariate logistic regression analysis, TB was associated with poor nutrition, DM2, family history of TB and independence of birth-place [22].

Delgado-Sanchez and colleagues reported a case-control study with retrospective data from the National Tuberculosis Registry (2000–2012). In this large population-based study (over a decade), of 191,923 registered patients, 181,378 had information about previous DM2 diagnosis. The study showed that the incidence rates of pulmonary TB associated with DM2 increased by 82.64% and increased too the probability to treatment failure [7].

To evaluate the contribution of DM2 in the context of multidrug resistant strains, a retrospective case-control study was performed. The study analyzed 36 pulmonary multidrug-resistant (MDR) TB patients reported during the period 1998–2013 in the state of San Luis Potosi. The authors found that MDR-TB and DM2 are associated in 47.2% of MDR-TB patients [23].

Another retrospective study carried out in the Texas-Mexico border analyzed 1436 TB patients recruited during the period 1998–2003. From the total number of Mexican patients (1436 patients), 112 patients were MDR-TB and from these 112 patients, 33 patients (29.5%) reported DM2. The study concluded that MDR-TB in Mexico was significantly associated with DM2 (OR 1.8 95% CI 1.1–2.9) [24].

Perez-Navarro and colleagues reported a double design study (case-control and retrospective cohort) in Veracruz with 67 patients with comorbidity TB-DM2 and 109 with TB only. The authors showed an increased risk of 2.8 (95% CI 2.2–3.4) in development of drug resistance against TB in patients living with this comorbidity [25]. The same research group reported a case-control study to determine the factors associated with the presence of pulmonary TB in patients with DM2 and the effect of development of drug resistance and multi-drug resistance, in the Mexican state of Veracruz, from January 2011 to October 2013. As results, they concluded that patients with TB-DM2 presented a 4.7-fold (CI 1.4–11.3) higher risk of developing drug-resistant TB and 3.5-fold (CI 1.1–11.1) higher risk of developing MDR-TB. Individuals with TB-DM2 had a 2.3-fold (CI 1.5–4.1) greater chance of persisting as TB-positive by the second month of treatment [26].

The influence of DM2 in the stage of TB infection (LTB or pulmonary TB) has also been evaluated by different authors. From January 2010 to February 2011 in a Public Hospital in Nuevo Leon, Monterrey (north-eastern Mexico), 97 TB patients were compared to LTB individuals ($n = 97$) and it was found that marital state, diabetes and smoking were independently predictive of TB [27]. From the analysis of single nucleotide polymorphisms, it was demonstrated that genetic differences may contribute to variation in disease susceptibility in the region.

The expression of cathelicidin (LL-37), human neutrophil peptide (HNP-1), and human beta defensins 2 and 3 (HBD-2, HBD-3) was analyzed during LTB and active pulmonary TB, in presence or absence of DM2, in patients recruited at Mexican Institute for Social Security (IMSS) and the General Hospital #1 of Zacatecas. The study showed that patients with DM2 had lower antimicrobial peptides gene expression, enhancing the risk for TB reactivation [28]. This study is very important due to the recognized role of AMPs in the defense against *M. tuberculosis* infection [29].

Cross-sectional studies

In the border region of Tamaulipas, Hernández-Guerrero et al. reported by an observational, descriptive, retrospective and cross sectional study, that DM2 was the most frequent comorbidity in TB patients [30].

A prospective study was conducted in Texas-Mexico border, where TB patients were tested for DM2. The study showed a prevalence of 36% in Mexico for DM2 in TB patients [31]. With the same collected data, the expression of monocyte surface markers (CCR2, RAGE, etc.) was evaluated. DM2 was associated with increased CCR2 expression. The up-regulation of this marker may limit the migration of monocytes from diabetic patients to the lungs or other infected tissues. The authors did not find differences in RAGE expression, at least under the experimental conditions assessed [32].

Martinez Aguilar and colleagues performed a cross-sectional study to elucidate the prevalence of LTB and its associated factors in TB patients with DM2, showing that the prevalence of LTB in those patients was 51.3%, being environmental conditions and the poor control of DM2 the most important risk factors for the development of TB. This work was the first report of the prevalence of LTB among

Mexicans living with DM2 [33] and was conducted in patients affiliated with the Mexican Social Security Institute (IMSS) in Durango and Zacatecas states located in northern Mexico, during October 2006 and June 2007. These results correlate with the cross-sectional study conducted in St Louis-Mexico border with 109 recruited participants, which showed that diabetes with high blood sugar was associated with LTBI positivity (OR 0.7, 95%CI 0.51–0.98) [34].

Cohort studies

In a population-based cohort study enrolling 581 TB patients from 12 municipalities in Orizaba Health Jurisdiction in the Mexican state of Veracruz, it was found that the rate of TB increased 6.8 times (95% CI, 5.7–8.2) in patients with DM2. Also, the proportion of TB attributable to DM2 was higher for the 45–64 year age group [35]. This study was based on compiled data from the period 1995 to 2003. The prevalence of DM among 1262 patients with pulmonary TB in a study conducted in the same region but in the period from 1995 to 2010 was 29.63%. The study also showed that patients with DM2 and TB had more clinical manifestations, delayed sputum conversion, higher probability of treatment failure, recurrence and relapse [36].

An open cohort study from March 2006–March 2014 in Veracruz, was the first cohort study of newly diagnosed Mexicans that were followed from the time of TB diagnosis until completion of TB treatment. From the sample analyzed (507 patients), 324 had TB and 183 had TB-DM, giving a value of 36% of coexistence of both diseases, which is the highest recorded to date [37].

Studies of metabolic/immune response and rifampicin pharmacokinetic were assessed in TB-DM patients recruited from Infectology and Endocrinology Services in the Hospital Central Dr. Ignacio Morones Prieto in San Luis Potosi, Mexico. This study demonstrated that the levels of CD8+ T lymphocytes and NK cells were diminished in both, TB and TB-DM patients, who exhibited high concentrations of TNF-α but low levels of IL-17. Poor glycemic control without dyslipidemia accompanied by malnutrition was detected in most of the TB-DM patients, together with poor and slow rifampicin absorption [38].

In another study, the microbiological evolution and final outcome of MDR/extensively drug-resistant TB (XDRTB) patients with and without DM were compared. From 90 patients tested during the period 2010–2015, 54.4% had DM and 86% undergoing insulin treatment [39].

An important study of DM2 and TB patients from 15 primary care units in five states of Mexico during the period of July 2012–March 2014 were followed up. A bidirectional screening was performed in all patients, revealing that in some cases patients are unaware of their TB and DM2 comorbidity. Patients treated under joint management for both diseases experienced more success in the treatment than patients treated under routine DM and TB programs, demonstrating the feasibility of implementing this model in Mexico [40].

These observational studies allow to conclude that in Mexico, DM2 is closely related to TB infection, and this situation is commonly under-estimated and is more severe in border regions.

Future directions

From the studies selected here, it is clear that the most studied populations for prevalence and incidence estimation of TB/DM2 in Mexico are those living in the border region (Chihuahua, Tamaulipas, Nuevo Leon) and in Veracruz, which also limits with Tamaulipas, because there is a great and continuous movement of persons, including border workers and migrant farm workers. All the studies reported a prevalence over 20% for the comorbidity, associating DM2 as one of the principal risk factor for the development of pulmonary TB and related with TB drug-resistance. Moreover, taking into account the mutual interest of Mexico and USA, bilateral agreements have been made in terms of cooperation in health. Both countries belong to the PAHO and work together to increase the quality of control and surveillance programs. This could explain that some research groups constituted by experts in the field of TB and DM2 are located at both sides of the border. For this reason, in 2002 a consortium for TB control (Nuevo Santander Tuberculosis Trackers, NSTT) was formed by Restrepo and colleagues. [20].

The situation described in this paper for Mexico, is currently present in other parts of the world, where TB is also endemic. In a recent study conducted by Viswanathan (2012), the authors showed that prevalence rates of DM and pre-diabetes among TB patients in South India were 25.3 and 24.5%, respectively. This study also showed that those with TBDM were more likely to have the infective form of TB [41]. Effects of DM on TB severity were also investigated by a longitudinal cohort study in South India, assessing TB severity at presentation, treatment response, and recurrence in patients with new smear-positive results with or without DM. Of 209 screened patients with TB, 75.1% had DM or pre-DM according to WHO criteria [42]. In a cross-sectional survey of TB patients registered from June to July 2011 in the state of Kerala, India, to determine the prevalence of DM, it was shown that among 552 TB patients screened, 243 (44%) had DM2 [43].

Previously, it has been reported that an analysis of 2 years data on TB subjects from Saudi Arabia in 1998 revealed that 27% had DM [44]. Another study from Taiwan reported 16.9% of DM among TB patients [45]. These data allow to conclude that a similar situation of TB-DM comorbidity is taking place in other developing countries, such Asian countries, where life conditions could lead to the establishment of both diseases.

Conduction of epidemiological studies in such regions are quite difficult due to, in part, the mobility of patients, which make

impossible in some cases the attendance to medical dates, mainly in longitudinal studies. These difficulties are reflected in the principal limitations of the studies conducted, with a direct consequence in the under or over-estimation of infection and comorbidity rates. By other way, as principal limitations for most of the studies, are inconsistencies in the data contained in databases in health institutions, differences in the diagnostic criteria, self-reported data without verification, small amount of patients enrolled in the studies, genetic differences between different populations, among others. Despite this, a clear trend to the increase in both diseases is observed. In this moment, it is considered that prevalence of DM2-TB in the range of 10–30%, with the higher values (higher than national average) in the border regions.

As this situation is predicted to be worsened by 2030, there is an urgent need to efficiently diagnose both the diseases. According to the Official Mexican Standard for the control of TB (NOM-006-SSA2–2013) and DM (NOM-015-SSA2–2010), it is stipulated for a bidirectional screening in those patients in order to prevent complication associated to this comorbidity.

Several research groups are working on the development of diagnostic tools to anticipate the development of TB or latency reactivation among diabetic patients. Taking into account that both diseases have a metabolic component and biochemical basis of susceptibility has been enunciated, it is interesting to determine the class of compounds that could be altered in both diseases. Commonly, the stratification of individuals under risk for developing DM2 is based on well-established parameters, for example, age, body mass index, fasting glucose levels, among others. However, these individuals cannot be identified until symptoms of the diseases are present and the risk for contracting TB is more elevated, without any doubt, a big challenge.

Acknowledgements Authors acknowledge the National Council of Science and Technology (CONACyT), Mexico, Grant Infra 269795.

Compliance with ethical standards

Conflict of interest Lopez Hernandez Y declares that she has no conflict of interest.

Mendoza Almanza G declares that she has no conflict of interest.

Rivas-Santiago C declares that he has no conflict of interest.

Salgado Bustamante M declares that she has no conflict of interest.

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):153–157
DOI 10.1007/s13410-017-0563-4

ORIGINAL ARTICLE

Fibrocalculous pancreatic diabetes in Bangladeshi children and adolescents—a not so rare form of secondary diabetes

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Received: 19 September 2016 / Accepted: 16 May 2017 / Published online: 6 July 2017

Abstract Fibrocalculous pancreatic diabetes (FCPD) is a unique form of diabetes with classic triad of abdominal pain, pancreatic calculi, and diabetes. This study was undertaken to identify the clinical characteristics in newly diagnosed children and adolescents with FCPD and compare them with children with Type 1 diabetes (T1D). The study was carried out at the Changing Diabetes in Children (CDiC) Diabetes Clinic of tertiary care hospital, BIRDEM in Bangladesh. All patients underwent abdominal X-rays and careful analysis of history of abdominal pain to differentiate the two diagnoses. Pancreatic auto antibodies were not available. Four hundred twenty-nine patients were aged under 18 years at diabetes presentation: 106 (25%) fulfilled the criteria for FCPD and 323 (75%) for T1D. When comparing FCPD with T1D at diagnosis, it was found that they were older (median age 14.0 [IQR 12.0–15.0] years vs 12.0 [IQR 10.0–14.0] years ($p < .0001$)). Few had a positive family history of diabetes (31% vs 48% ($p = .002$)). DKA was less common (4 vs 12% ($p = .020$)). HbA1c was higher (13.6 ± 4.2% (125 ± 22) vs 12.1 ± 3.4% (109 ± 14) ($p = .001$)). More FCPD patients had atypical symptoms compared to T1D (25 vs 14%, ($p = .010$)). The median duration of symptoms was significantly longer (3.0[1.0–6.0] vs 1.0[1.0–2.0] months ($p = .0001$)). Cataracts were more common in FCPD (9 vs 3% ($p = .010$)). The median dose of insulin was higher in FCPD patients than T1D (1.41[0.91–2.03] vs 1.02[0.78–1.48] ($p = .0001$)). In addition to pancreatic calcification, FCPD showed atypical symptoms with longer prodrome and higher HbA1c than T1D.

Keywords Type 1 diabetes · FCPD · Abdominal pain · Pancreatic calcification

Abbreviations

BIRDEM	Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders
CDiC	Changing diabetes in children
DKA	Diabetic ketoacidosis
ERCP	Endoscopic retrogarde cholangio-pancreatography
FCPD	Fibrocalculous pancreatic diabetes
T1 D	Type 1 diabetes

Introduction

Type 1 diabetes mellitus (T1DM) is the most common type of diabetes seen in children and adolescents. Fibrocalculous pancreatic diabetes (FCPD) is a unique form of diabetes secondary to non-alcoholic, chronic calcific pancreatitis occurring in tropical countries [1]. FCPD was initially classified as a form of malnutrition-related diabetes mellitus (MRDM) which was recognized by the 1985 WHO study group on diabetes mellitus, with the other form being protein deficient pancreatic

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diabetes [2]. Later, the entity MRDM was deleted and FCPD has been reclassified as a disease of the exocrine pancreas [3].

Reports from tropical parts of the world [4, 5] have confirmed the widespread occurrence of this syndrome in several developing countries of the world. The classical triads consist of abdominal pain, pancreatic calculi, and diabetes [1]. Environmental factors such as protein-calorie malnutrition [6–14] or the consumption of cassava, a source of cyanogenic glycosides [12, 15], are believed to be important in its pathogenesis. Ingestion of a tuber, cassava (tapioca), protein energy malnutrition, and oxidative stress had been earlier implicated in the etiology of FCPD [1], but most of these have now been disproved. More recently, there has been overwhelming evidence for a genetic susceptibility of pancreatic calculi linked to the SPINK1 N34S gene mutation in a study done with population from India and Bangladesh [16–18]. Histopathology of the pancreas in FCPD showed a spectrum of changes ranging from moderate to severe atrophy, fibrosis of the parenchyma, and degeneration of the ducts [19].

FCPD is predominantly a disease of youth and the usual age at onset is before 30 years [20]. Onset in childhood is less common but has been reported by Geevarghese [15]. Mohan described 11% patients with onset below 20 years of age had FCPD [21].This study was undertaken to identify the clinical characteristics in newly diagnosed children and adolescents with type 1 diabetes and FCPD.

Methodology

The study was carried out at Changing Diabetes in Children (CDiC) Diabetes Clinic of tertiary care Hospital, BIRDEM (Bangladesh Institute of Research and Rehabilitation of Diabetes, Endocrine and Metabolic disorder) in Bangladesh. Records of all patients registered at this program were in diabetes medical record system and data were collected retrospectively, so the formal consent was not required.

A total of 925 children and adolescents under 18 years of age were diagnosed as FCPD or T1D between 2006 and 2011. Among them, 429 patients were randomly selected to be included in this study. Classification of diabetes was based on WHO, ISPAD, and Mohan's criteria [1, 22, 23] and all patients underwent abdominal X-ray. Pancreatic autoantibodies were not available in our clinic so patients were classified clinically as T1D with abrupt onset of typical symptoms of diabetes, usually those who were non obese, absence of signs of insulin resistance, severe diabetes with markedly elevated HbA1c, presenting with diabetic ketoacidosis (DKA), and requiring insulin from time of onset. Patients were classified as FCPD if they had pancreatic calcification on X-ray or ultrasonography reported by radiologist and absence of alcohol intake, hypercalcemia, or biliary duct stones.

All patients were evaluated at the time of diagnosis and demographics including age, sex, height, and weight were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity and overweight were defined by the age and sex-specified cutoffs by Cole et al. [24]. Family history was taken regarding the presence of diabetes. HbA1c was assessed by Clover A1C using Photo Electric Method. All patients were followed up prospectively and nutritional status and glycemic control were reassessed.

Statistical analysis

Descriptive statistics were presented as mean ± SD score for normally distributed data and median (interquartile range IQR) for skewed data. Continuous data was compared using parametric test ANOVA, skewed data using nonparametric Kruskal-Wallis test and categorical variables by the chi-square test or Fisher's exact test. A two-tailed p value <0.05 was considered to be significant.

Results

Pancreatic calcification was found in 106 (25%) of 429 patients and thus 25% were classified as FCPD. The median age at presentation of diabetes was 14.0 [IQR 12.0–15.0] years; in comparison, patients with type 1 diabetes were younger: 12.0 [IQR 10.0–14.0] years, ($p < .0001$) (Table 1). More than 94% of FCPD presented after 10 years of age with the youngest presentation being 9 years of age. Classical symptoms of diabetes, namely polyuria, polydipsia, and weight loss, were present in 86% of T1D and 75% of FCPD atypical presentations like abdominal pain and blurring of vision were more common in FCPD than T1D (25 vs 14%) ($p = .010$). A past history of abdominal pain was present in 15% of FCPD which was significantly higher than in T1D (4%) ($p = .0001$). The pain in FCPD was usually described as severe, epigastric in location and characterized by periods of remission and exacerbation. It radiated to the back on either side, was relieved by bending forward or lying in a prone position or administration of analgesic or antispasmotic drugs. On follow-up, FCPD patients developed severe episodic pain with remission and relapse. In two patients, ERCP was done and multiple pancreatic stones were removed.

Blurring of vision was found in 25% of FCPD patients and 14% in T1D ($p = .010$). Compared to T1D, FCPD had longer duration of symptoms with 5% of FCPD patients having had symptoms for more than 12 months. Cataract was more common in FCPD (9%) in comparison with T1D (3%) ($p = .010$).

Table 1 Characteristics of Type 1 diabetes and FCPD at presentation

Characteristics	Type 1 diabetes (323)	FCPD (106)	*p* value
Age at diagnosis (years)	12.0 [IQR 10.0–14.0]	14.0 [IQR 12.0–15.0]	.0001
Gender (male)	158 (82)	34 (18)	.003
Atypical symptoms	44 (14)	25 (25)	.010
Duration of symptoms (months)	1.0 [1.0–2.0]	3.0 [1.0–6.0]	.0001
Family history of DM	154 (48)	32 (31)	.002
DKA at presentation	37 (12)	4 (4)	.020
Cataract at presentation	9 (3)	9 (9)	.010
BMI SDS	−1.6 [−3.3–0.00]	−0.71 (−2.9–0.13)	.0001
Insulin U/kg	1.02 [0.78–1.48]	1.41 [0.91–2.03]	.0001
HbA1c (%)	12.1 ± 3.4	13.6 ± 4.2	.001
HbA1c (mmol/mol)	109 ± 14	125 ± 22	.001

Around 4% patients with FCPD presented with DKA whereas 12% of T1D presented with DKA (p = .020). Nutritional status was analyzed. Around 23% FCPD and 30% Type 1 were underweight whereas 3% were obese in FCPD and 6% in type 1 (p = .4). Mean HbA1c was significantly higher in FCPD patients 13.6 ± 4.2% (125 ± 22) vs 12.1 ± 3.4% (109 ± 14) (p = .001) at the time of presentation. At follow-up, mean HbA1c was markedly reduced in FCPD patients 8.3 [7.2–11.3] than T1D 9.1 [7.1–10.8] (p = .995) (Table 2).

Discussion

In this cohort, we found 25% were FCPD which was 29.6% in our previous report [25]. FCPD has been described in several other countries including India, Brazil, Indonesia, Jamaica, Madagascar, Nigeria, Srilanka, Uganda, Zair, and Zambia while South India has the highest prevalence of FCPD in the world [1, 7]. FCPD as termed earlier is a highly heterogeneous and multifactorial condition with respect to clinical, biochemical, and histopathological features [1].

The median age at onset in FCPD patients was significantly higher than T1D in this study and in other report [20]. Comparing the clinical characteristics during presentation in children and adolescents with diabetes, atypical symptoms were more common in FCPD than in T1D patients which are consistent with our previous report [25].

Duration of symptoms was also significantly longer in FCPD patients. In five (5%) patients with FCPD, the duration was more than 1 year. In our previous study, we also reported significant difference in duration of symptoms between T1D and other types.

In our study, population median BMISDS was normal in 69% of FCPD patients that reflects most of them were having normal weight which differ from earlier reports where it was suggested that FCPD patients were emaciated and suffered from nutritional deficiencies [7, 9, 10, 15, 21, 26, 27]. However, recent reports suggest a change in the clinical presentation that may be attributed to improve nutritional status [28, 29]. Around 3% of FCPD patients were obese which was consistent with one study [29].

A striking feature in our population was four FCPD patients presented with diabetic ketoacidosis, although ketosis is rare in FCPD patients found in different studies [1, 23, 30, 31]. In one of our previous reports, only one patient presented with DKA [25].

In addition, cataract was significantly found during presentation with 9% of FCPD and 3% T1D. Cataract has been found to be at presentation in 16 (19%) newly diagnosed

Table 2 Features of Type 1 diabetes and FCPD patients at follow-up

Characteristics	Type 1 diabetes	FCPD	*p* value
Current age (years)	16.0 [IQR 14.0–18.0]	19.0 [IQR 16.0–20.0]	.0001
Duration of diabetes (years)	5.2 [4.0–6.7]	5.0 [4.2–6.1]	.0001
DKA	3%	0%	.08
Cataract	2%	7%	.013
BMI SDS	−0.56 [−1.57–0.50]	−0.71 [−1.43–−0.08]	.236
Insulin U/kg	1.06 [0.77–1.42]	1.00 [0.75–1.46]	.695
HbA1c (%)	9.1 [7.1–10.8]	8.3 [7.2–11.3]	.995
HbA1c (mmol/mol)	76 [54–95]	67 [55–100]	.995

FCPD patients in our previous study [32].The pathogenesis of diabetic cataract is not well understood. Prolonged duration of symptoms before treatment is thought to play a role and hyperglycaemia alone may not be the only factor as not all patients developed cataract [33, 34]. Higher HbA1c was significantly found in FCPD patients during presentation, which was consistent with our previous study where glycemic control was worst in FCPD patients than other types of diabetes.

Positive family history was found in FCPD patients (31%) in our study, although we found only 2.7% patients had positive family history in our previous study. But 27.2% were found to have positive family history in a recent study conducted in BIRDEM [20]. Familial aggregation of FCPD patients has been reported from India and Bangladesh suggesting genetic susceptibility [35, 36].

Limitations in the study should be noted. The beta cell antibody sampling and genetic testing are not routinely done in the clinical setting in Bangladesh, so we had to classify the T1 D clinically.

Conclusion

In addition to pancreatic calcification, FCPD presented with atypical symptoms with longer prodrome and higher HbA1c than T1D. The higher rate of cataracts as well as worse glycaemic control also suggests that the disease process may have a more insidious course. Although rare in the western world, FCPD is not very common in children and especially adolescents in Bangladesh. Plain X-ray or ultrasonogram of abdomen should be routinely done to detect FCPD especially in young diabetic patients of this region. The cause of the pancreatic calcification remains unknown but requires further investigation.

Compliance with ethical standards

Funding Funding is supported by CDiC programme of Diabetic Association of Bangladesh.

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 research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):158–164
DOI 10.1007/s13410-017-0565-2

ORIGINAL ARTICLE

Prevalence and risk factors of cognitive dysfunction in patients with type 2 diabetes mellitus receiving care in a reference hospital in Cameroon: a cross-sectional study

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Received: 24 February 2017 / Accepted: 29 May 2017 / Published online: 21 June 2017

Abstract Diabetes mellitus has been associated with cognitive impairment but this has not been well investigated in sub-Saharan Africa. The aim of this study was to determine the prevalence and risk factors of cognitive dysfunction in patients with type 2 diabetes and to assess its influence on medication adherence. We carried out a cross-sectional study over a period of 4 months at the outpatient diabetes and endocrinology clinic of the Douala General Hospital. Consecutive patients with type 2 diabetes attending the clinic underwent cognitive assessment using the Mini Mental State Exam (MMSE). The patient Health Questionnaire-9 (PHQ-9) was used to rule out the confounding effect of depression, and the Morisky score was used to assess medication adherence. Data were analyzed using SPSS version 20 for Windows. Of the 223 participants (54.3% females) with a mean age of 56 ± 9.5 years, 33 (14.8%) had cognitive dysfunction (12.6, 1.8, and 0.4% having mild, moderate, and severe cognitive dysfunction, respectively). Level of education ≤7 years (OR = 5.314, 95% CI 2.443–11.561, $p < 0.001$) was the only factor significantly associated with cognitive dysfunction. No significant association was found between cognitive dysfunction and treatment adherence. In conclusion, one out of seven of our patients with type 2 diabetes have cognitive dysfunction, which is strongly associated with low level of education and does not affect treatment adherence and glycemic control. Our results suggest that screening for cognitive dysfunction in our patients should focus on patients with a low level of education.

Keywords Cognitive dysfunction · Cognitive dysfunction · Diabetes mellitus, type 2 · Prevalence · Risk factors · Medication adherence

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Abbreviations

BMI	Body mass index
CI	Confidence interval
DGH	Douala General Hospital
FCG	Fasting capillary glucose
IDE	Insulin degrading enzymes
MMSE	Mini Mental State Examination
PHQ	Patient health questionnaire
T2DM	Type 2 diabetes mellitus
WHO	World Health Organization

Introduction

Diabetes mellitus is a global epidemic that afflicted 422 million people worldwide in the year 2014. The World Health Organization (WHO) African region had 25 million people living with diabetes in 2014, which is the second WHO Region with the fastest risen prevalence [1]. According to the International Diabetes Federation, Cameroon had 567,300 cases of diabetes in 2015 accounting for 6.5% of the adult population [2]. Diabetes mellitus is a common chronic condition in the elderly population and thus they are at an increased risk for developing micro- and macrovascular complications [3]. Recent studies suggest that type 2 diabetes mellitus (T2DM) is associated with higher risk of cognitive dysfunction, dementia, and depression in the elderly [4].

Hyperglycemia and hypoglycemia, insulin resistance and insulin insufficiency, have been shown to lead to cognitive dysfunction [5] though the exact pathophysiology is unclear. Cognitive dysfunction is also an age-related condition with an increase in its incidence and prevalence with age [6]. In 2010, an estimated 35.6 million people were living with dementia which is projected to rise to 65.7 million by 2030 [7]. In sub-Saharan Africa, the prevalence of mild cognitive impairment in the general population in 2013 was estimated between 6.3 and 25%, and the prevalence of dementia was between 2.3 and 10.1% [8]. A hospital-based study carried out in Cameroon in 2013 reported a prevalence of dementia of 2.85% [9].

Cognitive dysfunction is a major hindrance in receiving treatment adequately; this would thus impede in the management of patients with diabetes mellitus who are required to be on long-term therapy. Despite these, there is no available data in Cameroon on the prevalence and risk factors of cognitive impairment among patients with type 2 diabetes, and its impact on adherence to treatment or attainment of treatment targets.

We therefore designed this study to assess the prevalence of cognitive dysfunction among patients with type 2 diabetes, identify the risk factors of cognitive dysfunction in these patients, and determine the influence of cognitive dysfunction on medication adherence and glycemic control.

Material and methods

Study design and participants

This cross-sectional study was carried out at the outpatient diabetes and endocrinology clinic of the Douala General Hospital (DGH). Patients were enrolled from 18 November 2015 to 04 March 2016. The DGH is one of the major reference hospitals in Cameroon; it receives patients referred from all over the Regions of Cameroon. Patients living with diabetes and receiving care in the unit undergo an annual evaluation, which includes: a clinical evaluation, an assessment of diabetes control (HbA_1c level), chronic complications (fundoscopy, dipstick proteinuria, and serum creatinine), and cardiovascular risk factors assessment (lipid profile). The neurology unit of the hospital employs four neurologists who interact with other specialists including endocrinologist, for the diagnosis and management of neurological complications of diabetes.

All patients with T2DM attending the diabetic unit of DGH during the study period, and who consented to participate in the study were included. Patients with severe major depression, patients on neuroleptic drugs, or those who could not read nor write were excluded. We also excluded patients with epilepsy, HIV/AIDS, stroke, and alcohol abuse.

Study procedure and data collection

To eliminate the confounding effect of depression that is frequent in people living with diabetes, patients were screened for severe major depression using the MacArthur initiative on depression Patient Health Questionnaire-9 (PHQ-9) [10]. All

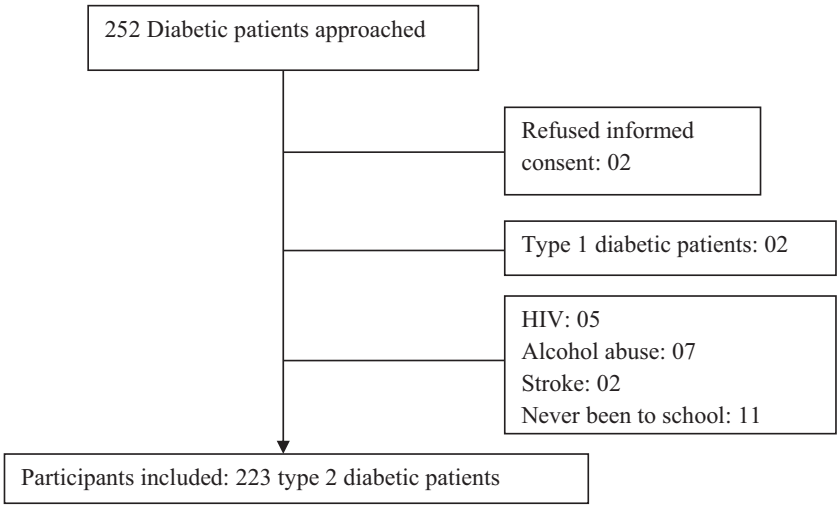

Fig. 1 Flow chart of the inclusion process of patients with type 2 diabetes included from November 2015 to March 2016 in the Douala General Hospital. Of the 252 patients approached, 223 were finally included in the study. Reasons for excluding participants are shown

Table 1 General characteristics of patients with type 2 diabetes included from November 2015 to March 2016 in the Douala General Hospital

Variables	*n* (%)
Categorical variables	
Female	121 (54.3)
Level of education	
Primary school	53 (23.8)
Secondary school	68 (30.5)
High school	52 (23.3)
University	50 (22.4)
Smoking	
Current smokers	4 (1.8)
Past smoker	37 (16.6)
Physical inactivity	97 (43.5)
Hypertension	126 (56.5)
Dyslipidemia	72 (32.3)
Medications	
OAD	160 (71.7)
Insulin	24 (10.8)
OAD+ insulin	35 (15.7)
Lifestyle modification only	4 (1.8)
Other drugs	154 (69.1)
Complications	
Diabetic retinopathy	13 (5.8)
Diabetic neuropathy	76 (34.1)
Diabetic nephropathy	5 (2.2)
Diabetic foot	6 (2.7)
Erectile dysfunction (n = 102)	28 (27.5)
Continuous variables	Mean (SD) or median (IQR)
Age, years	55.9 (5.6)
Diabetes duration, years	5 (1–30)
BMI, kg/m^2	29.5 (5.5)
FCG, mg/dl	150.6 (75.5)
Hb, g/dl	12.24 (1.54)
Creatinine, mg/dl	1.02 (0.49)
HbA1c, %	8.7 (2.7)
Total cholesterol, mg/dl	180.0 (54.1)
Triglycerides, mg/dl	104.5 (61.2)
HDLc, mg/dl	48.8 (21.1)
LDLc, mg/dl	111.8 (47.4)
Serum uric acid, mg/dl	5.78 (2.08)

BMI body mass index, *FCG* fasting capillary glucose, *Hb* hemoglobin, *HbA1c* glycated hemoglobin, *HDLc* high density lipoprotein cholesterol, *LDLc* low density lipoprotein cholesterol, *OAD* oral antidiabetic drug

patients with severe depression (PHQ-9 score > 19) were not included. Cognitive function was assessed by the Mini-Mental State Examination (MMSE) [11], and cognitive dysfunction was defined by a score of <25. Adherence to medication was assessed using the 8-item Morisky Medication Adherence Scale (8MMAS) [12] and a score ≥ 1 defined medication non-adherence.

We also collected data on medical and family history, and behavioral factors (smoking, physical exercise, and alcohol consumption). The weight, height, blood pressure, and waist circumference were measured using standard methods. The body mass index (BMI) was calculated as weight (in kilograms)/height x height (in meters).

The fasting capillary glucose (FCG) was measured using a glucometer (One Touch Ultra2®). The Jaffe method was used to measure serum creatinine levels using a Roche–Hitachi Cobas C311®analyzer. Standard laboratory procedures were used in the measurement of HbA_1c (immuno-turbidimetric method) and parameters of the lipid profile (enzymatic method). Proteinuria were determined by dipstick.

Definition of variables

Hypertension. Patient known to have hypertension or patient on blood pressure lowering drugs.
Educational level. ≤ 7 years (primary education) and >7 years (secondary or high school or university).
Smoking**.** Never smoked (non smoker), ever smoked (current and past smoker)
Physical activity. Regular exercise of 30 or more minutes at least 3 times/week.
Overweight. BMI between 25 and 29.9 kg/m^2.
Obesity. BMI ≥ 30kg/m^2.
History of dyslipidemia. Patient diagnosed to have dyslipidemia or on lipid-lowering drugs.
Hyperuricemia. Serum uric acid level > 7.0 mg/dl in males and >6.0 mg/dl in females [13].
Treatment targets. Fasting capillary glucose: 80–120 mg/dl; HbA1c: ≤ 7%; blood pressure: ≤ 130/80 mmHg (for patients with hypertension); low density lipoprotein: ≤ 100 mg/dl; high density lipoprotein: ≥ 50 mg/dl; triglyceride: ≤ 150 mg/dl.

Statistical analysis

SPSS version 20 for Windows was used for all analyses. Categorical variables are presented as proportions and continuous variables as median (interquartile range) or mean (standard deviation) as appropriate (non-normal or normal distribution, respectively). The chi-square test was used to analyze the associations between cognitive dysfunction and predictor variables which were age, gender, level of education, smoking, hypertension, physical activity, obesity, hypertension, dyslipidemia, chronic complication, or outcomes such as poor adherence to drug therapy or treatment targets. A p value <0.05 was used to characterize statistically significant results.

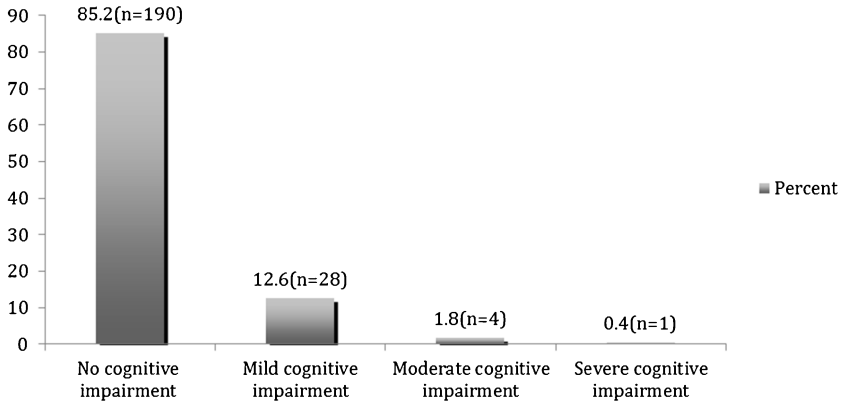

Fig. 2 Distribution of MMSE-diagnosed cognitive dysfunctions in patients with type 2 diabetes included from November 2015 to March 2016 in the Douala General Hospital. MMSE: mini mental state examination

Results

General characteristics of participants

Of the total of the 223 participants included in this study (Fig. 1), 121 (54.3%) were female. Participants' age ranged from 25 to 82 years, with a mean of 56 ± 9.5 years. The median duration of diabetes was 5 (1–30) years. Medications other than antidiabetic drugs consisted mostly of antihypertensive drugs and statins. Other characteristics are presented in Table 1.

Prevalence, distribution, and risk factors of cognitive dysfunction

The MMSE score of participants ranged from 12 to 30, with a mean of 26.92 ± 2.8. Out of the 223 participants, 33 had a score of less than 25 that defined cognitive dysfunction giving a prevalence of 14.8% (95% CI: 10.6–19.9). The prevalence and classification of participants' cognitive function are shown in Fig. 2. Among all factors explored, only the level of education ≤7 years was significantly associated with cognitive dysfunction (Table 2).

Cognitive dysfunction and outcome

Out of the 219 participants on treatment, 83 (37.9%) had strong adherence while 78 (35.6%) and 58 (26.5%) had moderate and low adherence to medication, respectively. There was no significant association between cognitive dysfunction and treatment adherence (OR 0.80, 95% CI 0.38–1.70; $p = 0.56$).Also, cognitive dysfunction was not significantly associated with poor glycemic control (OR 1.44, 95% CI 0.65–3.19; $p = 0.37$).

Discussion

In this population of 223 patients with type 2 diabetes with median diabetes duration of 5 years, the prevalence of cognitive dysfunction was 14.8%. We also found that the level of education ≤7 years was the only significantly associated factor with cognitive dysfunction. There was no significant association between cognitive dysfunction and treatment adherence or poor glycemic control.

Many studies in other countries have addressed the same topic and have reported variable prevalence. Higher prevalence of 44% was reported in Nigeria in a study where they included patients who have never attended school, and they did not screen participants for severe depression [14]. In United States of America, Saudi Arabia, and India, the prevalence of cognitive dysfunction was 12, 17.1, and 10%, respectively [15–17].

Multiple pathways have been suggested to explain the link between diabetes and cognitive dysfunction. Diabetes is associated with changes in the blood brain barrier and transport functions of the cerebral microvessels [18] that may result in thickening of the basement membrane which increase the accumulation of focal amyloid β peptides [19]. Chronically, higher blood glucose level exerts a negative influence and causes structural changes in the hippocampus. Chronic hyperglycemia leads to the enhanced formation of advanced glycation end products [20], which have potentially toxic effects on neurons. Inflammation might also link T2DM and dementia through activation of glia by inflammatory cytokines hence damaging neurons [21]. Insulin resistance also plays a role in cognitive dysfunction through principally two mechanisms. Firstly, reduced amounts of insulin-degrading enzymes following desensitization of insulin receptors may result in greater amyloid deposition [22]. Another pathway is the tau protein phosphorylation, which leads to formation of neurofibrillary tangles [23]. Dysfunction of cerebral autoregulation with increasing age along with structural and functional alterations in cerebral blood vessels due to diabetes mellitus impairs the functioning of neurovascular units. These phenomena may induce functional deficits in neurons and increase neuronal degeneration and the susceptibility to hypoxia and ischemia [24].

Cognitive dysfunction among type 2 diabetic patients was significantly associated with the level of education ≤7 years.

Table 2 Risk factors of MMSE-diagnosed Cognitive Dysfunction in patients with type 2 diabetes included from November 2015 to March 2016 in the Douala General Hospital

Variable	Normal cognitive function	Cognitive dysfunction	Odds ratio	95% CI	*p* value
Age					
≤ 56	96 (86.5)	15 (13.5)	1.226	0.451–1.323	0.591
> 56	94(83.9)	18 (6.1)			
Gender					
Male	87(85.3)	15(14.7)	1.014	0.483–2.129	0.972
Female	103(85.1)	18 (14.9)			
Level of education					
≤ 7 years	35 (66.0)	18 (34.0)	5.314	2.443–11.561	<0.001
> 7 years	155(91.2)	15(8.8)			
Duration of diabetes					
≤ 5 years	100(87.7)	14(12.3)	1.508	0.715–3.182	0.279
> 5 years	90 (82.6)	19(17.4)			
Smoking					
Never smoked	154(87.6)	28 (15.4)	0.763	0.276–2.115	0.603
Smoked	36(87.8)	5(12.2)			
Physical activity					
No	79(81.4)	18(18.6)	1.686	0.802–3.546	0.165
Yes	111(88.1)	15(11.9)			
Obesity (kg/m^2)					
< 30	105(82.0)	23(18.0)	0.537	0.242–1.190	0.122
≥ 30	85(89.5)	10(10.5)			
Hyperuricemia					
No	172(30)	30(14.9)	0.956	0.265–3.445	0.945
Yes	18(85.7)	3(14.3)			
Dyslipidemia					
No	128(84.8)	23(15.2)	0.898	0.403–2.002	0.792
Yes	62(86.1)	10(13.9)			
Hypertension					
No	87(89.7)	10(10.3)	1.942	0.877–4.304	0.097
Yes	103(81.7)	23(18.3)			
Chronic complications					
No	102(83.6)	20(16.4)	0.753	0.354–1.602	0.461
Yes	88(87.1)	13(12.9)			
OAD					
No	52(82.5)	11(17.5)	0.754	0.342–1.662	0.482
Yes	138(86.3)	22(13.8)			
Insulin					
No	171(85.9)	28(14.1)	1.607	0.555–4.653	0.378
Yes	19(79.2)	5(20.8)			
OAD + insulin					
No	161(85.6)	27(14.4)	1.234	0.468–3.251	0.671
Yes	29(82.9)	6(17.1)			
Lifestyle modification					
No	186(84.9)	33(15.1)	–	–	–
Yes	4(100)	0(0.0)			
Other drugs					
No	59(85.5)	10(14.5)	1.036	0.464–2.313	0.931
Yes	131(85.1)	23(14.9)			
HDLc (mg/dl)					
> 50	97(87.4)	14(12.6)	1.511	0.653–3.498	0.275
< 50	55(82.1)	12 (17.9)			
LDLc (mg/dl)					
> 150	72(88.9)	9(11.1)	1.700	0.713–4.051	0.288
< 150	80(82.5)	17(17.5)			

MMSE mini mental state examination, *OAD* oral antidiabetic drug

Other studies have suggested low level of school attained to be an independent risk factor of cognitive dysfunction [14]. This is probably due to the low development of interconnections between neurons resulting in reduced cognitive reserve.

In a context of an overall poor level of adherence (37.9%) in our population (mainly results of poor access to drugs), there was no significant association between cognitive dysfunction and medication adherence, probably because in the African context, family members are often available for support in taking drugs.

We also did not find any significant association between cognitive dysfunction and parameters used to assess glucose

control (FCG and HbA1c), though cognitive dysfunction was more frequent in people with poorer glycemic control. A study carried out by Lee et al. in 2014 showed similar results [25]. In addition, no association was observed between diabetes duration and cognitive dysfunction, as also reported in Nigeria [14]. This probably pertains to similar treatment adherence in the two groups that yielded similar results in long-term glucose control.

Contrary to Gorska-Ciebiada et al., no association was found between obesity and cognitive dysfunction [26]. A study in Saudi Arabia instead found an inverse (protective) association between obesity and cognitive dysfunction [16]. As reported earlier in Nigeria [14], we did not observe any association between hypertension and cognitive dysfunction. Lee et al. and Gorska-Ciebiada et al. [25, 26] found a significant association between hypertension and cognitive dysfunction with prevalence of hypertension being 75.8 and 77.1%, respectively. This difference may be due to the fact that their study population was made up of elderly type 2 diabetic patients with mean ages of 70.9 ± 0.5 and 73.6 ± 4.8 years, respectively.

Among potential limitations of our study is the use of the MMSE to assess cognitive dysfunction; this tool may lack sensitivity in early detection of mild cognitive dysfunction and hence can give false-negative result but nevertheless it remains the most useful and reliable tool easily used in clinical practice. It is universally known and provides rapid information of mental state of patients that can be communicated to and easily interpreted by professionals. Also, though we excluded all patients with obvious confounding illnesses or on potentially interfering drugs, the fact that we could not assess all potential metabolic confounders is another shortcoming.

Conclusion

Cognitive dysfunction affects 1 out of 7 of our patients with type 2 diabetes aged 25 to 82 years, most of whom have mild cognitive dysfunction. Low level of education is associated with a fivefold increase in risk. In this population, cognitive dysfunction does not affect medication adherence or disease outcome.

Acknowledgements We appreciate the immense gratitude of all patients who accepted to participate in this study and to the nurse staff of the outpatient Diabetes and Endocrine unit of the Douala General Hospital. The 2HD Research Network is supported by a Cruddas Link Fellowship (SPC), Harris Manchester College, University of Oxford, UK.

Author's contribution ZA, YMD, YNM, CN, and SPC helped in the study design and data collection. ZA, YMD, and SPC contributed in the data analysis and drafting of the manuscript. ZA, YNM, CN, HNL, and SPC helped in the data interpretation, editing, and reviewing the manuscript. All authors read and approved the final manuscript. SPC takes responsibility of the integrity of the work and is the guarantor.

Compliance with ethical standards The study was approved by the Institutional Ethics Committee for Research in Human Health of the University of Douala, Cameroon. Administrative clearance was obtained from authorities of the DGH. Patients who presented at the unit were approached, they received a description of the study, and they were informed about the purpose, risks, and benefits. Written informed consent was obtained from each participant before inclusion.

Conflict of interest Zainab I Abba, YannickMboue-Djieka, Yacouba N Mapoure, CyrilleNkouonlack, Henry N Luma, and Simeon-Pierre Choukem declare that they have no conflict of interest relevant to this article.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):165–172
DOI 10.1007/s13410-017-0570-5

ORIGINAL ARTICLE

Preventing and managing diabetic foot ulcers: application of Orem's self-care model

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Received: 25 December 2016 / Accepted: 19 June 2017 / Published online: 6 July 2017

Abstract One of the most common complications of diabetes mellitus is a diabetic foot ulcer. Thus, managing and preventing this complication is a main priority for health professionals, especially for nurses. This study was designed to investigate the application of Orem's self-care deficit theory on prevention and management of diabetic foot ulcer. This quasi-experimental study was conducted in Urmia, Iran. Purposive sampling was used to select 60 patients and they were allocated into two groups. Two patients in the intervention group were excluded due to amputation and four patients from each group left the study because of unwillingness to complete the study. The intervention group received two self-care training sessions and home visits for 12 weeks, but the control group received the routine care. Data were collected using a questionnaire which consisted of four parts (demographic data, self- care status, need assessments based on Orem model, and Saint Elian Wound Score System). Data were analyzed by SPSS software (ver. 16). A significant difference was found between two groups regarding self-care mean scores, number of affected zones, ischemia, infection, and wound healing phase ($p < 0.05$). Application of Orem's self-care model could be helpful for the management of diabetic foot ulcers and could change patients' lives by lowering the risk of amputation and medical costs.

Keywords Diabetes complications · Foot ulcer · Nursing care · Orem self-care model · Self-care

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Introduction

Diabetes has afflicted 150 million people all over the world and it is estimated to grow two times more by 2025 [1]. Diabetes is a major health problem in Iran. More than 1.5 million Iranian people have been diagnosed with diabetes in 2014, and 7.5% of the patients had type 2 diabetes [2]. Diabetes and its complications impose a significant economic burden and health problems on the societies and health care systems [3]. Diabetic foot ulcers (DFUs) are the most significant and debilitating complications of this disease [4]. Poor circulation caused by peripheral vascular disease could lead to DFUs [5]. However, incidence rates vary by gender, age, race, and geographic area. The annual incidence of foot ulcer was reported 6.0% for males and 5.9% for females in 2008 [6]. DFUs are the most leading cause of hospitalization, which is hard to manage and often leads to amputation [7]. Foot ulcer and lower-limb amputation are responsible for significant morbidity, mortality, and health care costs in patients with diabetes [8]. In addition to thoroughly affecting the patients' quality of life, this accounts for a significant economic burden on health care systems [9, 10].

A significant aspect in diabetes care is self-care management [11]. However, patients are often faced with challenges in making the behavioral changes necessary to achieve optimum blood glucose control in order to minimize the risk of diabetic complications [12]. These patients may lack adequate skills and support needed to improve their self-care

management. A systematic review showed that training for self-care management of diabetic patients enhanced their knowledge, the regularity and accuracy of blood glucose self-monitoring, and dietary habits [13]. The evidence has revealed that self-care behaviors are important for prevention of DFU [14]. These behaviors are influenced by patient's attitudes toward foot care, which also affect foot ulcer outcomes such as wound healing and recurrence [10].

Numerous theories have been created to clarify the concept of self-care. Among these, the Orem's self-care deficit nursing theory (SCDNT) [15] is widely accepted and used by nurses internationally [16]. Therefore, the conceptual framework of Orem's self-care model was used to guide this study. This study aimed to investigate the effect of Orem's self-care model on prevention and management of DFU.

Materials and methods

Design and sample

This is a quasi-experimental (pretest-posttest with control group) study. It was conducted on 60 patients with DFU who were admitted to the endocrinology wards of two educational centers affiliated to Urmia University of Medical Sciences in Iran. The diagnosis was made based on the American Diabetes Association (ADA) criteria for diagnosing diabetes mellitus [17]. The study was conducted from July to December 2014. The results of a previous study conducted by Adib Hajbaghery et al. were used to calculate sample size. In their study μ1, μ2, sd1, and sd2 were respectively equal to 1.05, 0.35, 0.94, and 0.74 [18]. Therefore, by considering a type I error of 0.05 and a power of 0.80, the sample size was estimated to be 22.89 patients for each group. However, a total of 60 patients (30 patients for each group) was recruited because of the attrition rate. Patients' allocation was carried out by utilizing a random number table. (Fig. 1).

After obtaining permission from the ethics committee of Urmia University of Medical Sciences (code number: umsu. rec. 1392.2016), the researchers referred to two educational centers and carried out required coordination with the related authorities to be able to collect data. The inclusion criteria were as follows: patients ranging in age from 20 to 60 years, being literate, being able to perform self-care, willingness to participate in the study, not having significant communication impairments (i.e., visual or auditory loss), being able to make phone calls, and having no severe physical or psychological co-morbidities. Taking immunosuppressive drugs during the study period and patient's decision to leave the study were considered as exclusion criteria.

Measures

A questionnaire used as a data collection tool comprised of four parts:

1. Demographic data: this consists of the personal information, marital status, educational levels, and clinical status and laboratory tests including the blood sugar, hemoglobin A1c, etc.
2. Self-care status: this part consisted of 22 questions about self care activities related to diabetic foot. The questions were developed in the Likert scale (from never = 1 to always = 5).
3. Need assessments based on Orem model: it consisted of 40 questions about patients need in three domains according to the Orem's Self-Care Theory (universal self care requisites, 13 questions; developmental self-care requisites, 7 questions; and health deviation self-care, 20 questions). The questions were developed in the Likert scale (from never = 1 to always = 5). The universal self-care was divided into the following sub-categories, as Orem presents: (a) maintaining sufficient intake of food, air, and

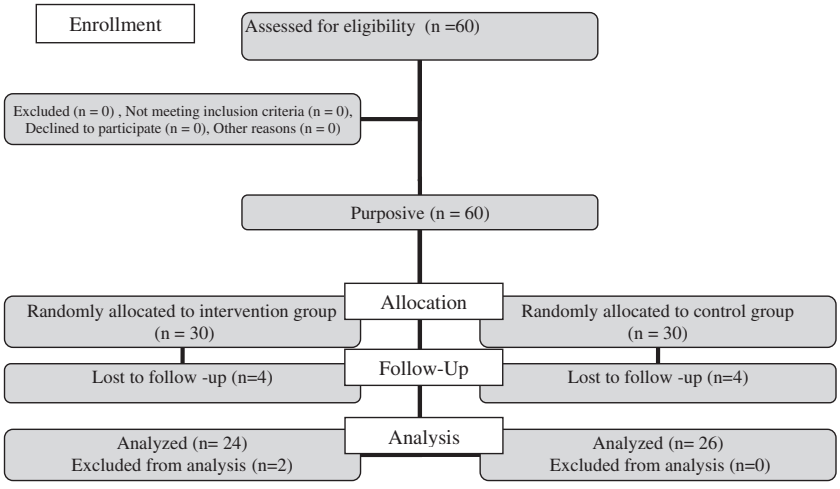

Fig. 1 The sampling framework of the study

water, (b) providing care about process of elimination, (c) balance between rest and activity, between social interaction and solitude, (d) preventing hazards to well being of human life, and (e) promoting of human functioning. The items about the developmental self-care requisites aimed to find what actions the individual performs to promote self-care during developmental processes/or associated with an event. Health deviation self-care investigates the conditions related to the therapy, knowledge about specific foot ulcer care, control of complications, and access to health services. According to the results, patients' needs were categorized in three basic variations in nursing systems: wholly compensatory, partly compensatory, and supportive-educative.

4. Saint Elian Wound Score System (SEWSS): this is used for scoring of the severity of diabetic foot from mild to severe (1 to 3) in the following categories: primary location, area, depth, topographic aspects, edema, ischemia, infection, neuropathy, number of affected zones, and wound healing phase. The score sum was considered as I (score was ≤10, better prognosis for healing wound), II (score was 11–20, partially, foot threatening), and III (score was 21–30, limb & life threatening) [19]. In this study, Rydel-Seiffer (128 Hz) semi-quantitative tuning fork was struck to the dorsal head of the first metatarsal joint of the patients and their sensation was assessed. When the subjects said they were unable to feel the vibration, the scale was read at the apex of the single triangle formed from the initial two triangles. Having no sensation was recorded as 0. The score was graded as 1 when subjects could feel the fork vibration. If subjects could feel the fork vibration when the scale of fork showed five or less, the score was considered as 0.5 [20].

We used content validity for ensuring the validity of data collection tools. The developed tool was assessed by 10 faculty members of Urmia University of Medical Sciences and we modified questionnaires based on their comments. The reliability of the questionnaires was assessed by the test-retest method on 10 patients with an interval of 1 week. The correlation coefficient for self- care status, need assessments based on Orem's model, and SEWSS was 90, 80, and 94%, respectively.

Intervention

The presuppositions of the Orem's theory were used for collecting data, planning, and implementing effective care. After each patient consented to take part in the study, he/she was asked to carefully fill out the questionnaire. Before starting of Orem's self-care program, the intervention group was divided into three small subgroups of 10 patients each. Then, each subgroup was invited to participate in two 60-min training sessions of self-care. The content of these sessions consisted of self-care activities related to diabetic foot care. Then, each patient in the intervention group received home visits once a week for 3 weeks in July 2014. On an average, the visits lasted 1 h. The meetings were scheduled by phone, according to the patient's convenience. During the first visit, we requested the patients to sign the consent form and used the therapeutic requirements form for collecting data. In the other meetings, we referred to the recorded data, emphasized the needed care and evaluated the self-care capacity of the patient. Some steps were adopted during the visits, including:

First visit; we investigated the patient's health situation through filling out the data collection form. After this visit, we obtained the nursing diagnoses, based on the Taxonomy II provided by the North American Nursing Diagnosis Association International (NANDA International) [21]. Then, we detailed the potential interventions which are appropriate with the first phase of Orem's nursing process. If nursing care was actually necessary it was determined in this phase. For each diagnosis, we established goals that served as a guide to assess the interventions delivered.

Second visit; we discussed the intervention priorities with the patient regarding his/her health and adopted a care plan, which was adequate to meet the perceived needs. The goals were compatible with the diagnosis and aimed to enable the patient to become a self-care agent.

Third visit; we evaluated the effectiveness of the interventions. Then, we investigated nursing diagnoses and the need for further interventions. This pointed to the third phase of Orem's theory. The patient was prepared for the independence phase, in which he/she could perform the self-care activities. The patients in the intervention group received twice a week follow-ups at home to reinforce self-care activities for 4 months. Then, all patients filled out the questionnaires again. The patients in the control group received only their usual care during the study. The result indicates no harm or disadvantage toward patients in this study.

Statistical methods

Two patients in the intervention group were excluded due to amputation and four patients from each groups (intervention and control) left the study because of unwillingness to complete the study. Therefore, data were analyzed with 24 patients in the intervention group and 26 patients in the control group. Data were analyzed using SPSS software Version 16.0 (IBM, USA). Descriptive and inferential statistics were used in this study. The frequency of characteristics was presented as number (%), and quantitative results as mean (±standard deviation). Fisher's exact test was performed to compare qualitative

demographic data and student *t* test was used for quantitative data between two groups. All *p* values were two tailed and significance level was considered as $p < 0.05$.

Results

The socio-demographic attributes of both the groups are presented in Table 1. No significant differences were found between the two groups in terms of age, gender, marital status, type of diabetes, smoking, education level, previous amputation, employment status, duration of the disease, family history of diabetes, BMI, FBS, BS, and HbA1c level ($p > 0.05$).

The results of this study showed that the majority of patients in the intervention group allocated to the partly compensatory system, regarding universal self-care, developmental self-care requisites, and health deviation self-care (75, 58.3, 88.3%, respectively) before starting the program (Table 2).

Results showed no significant difference between the control group (63.76 ± 9.77) and the intervention group (70.0 ± 16.65) regarding baseline self-care mean scores ($p = 0.11$). However, a significant improvement was observed in the intervention group (94.25 ± 9.45) compared to the control group (67.26 ± 9.62) regarding self-care means scores at the end of the study ($p = 0.001$) (Table 3).

The Fisher's exact test showed no significant difference between the two groups regarding location, topographic aspects, number of affected zones, infection, ischemia, edema, neuropathy, depth, area, and wound healing phase before the intervention. However, a significant difference was observed between the two groups in terms of ischemia, infection, edema, neuropathy, topographic aspects, depth, area, and wound healing phase at the end of the study (Table 4).

Discussion

The results indicate that application of Orem's self-care program has a positive impact on improving patients' self-care behaviors. Orem believes that patients' self-

Table 1 Comparison of demographic characteristics of the control and the intervention groups

Variable		Group		Fisher's exact test
		Control, *N* (%)	Intervention, *N* (%)	
Gender	Woman	14(53.8)	13(54.2)	$p = 0.603$
	Man	12(46.2)	11(45.8)	
Marital status	Single	7(26.9)	3(12.5)	$p = 0.072$
	Married	17(65.4)	20(83.3)	
	Widow	2(7.7)	1(4.2)	
Type of diabetes	Type 1 diabetes	19(73.1)	16(66.7)	$p = 0.426$
	Type 2 diabetes	7 (26.9)	8(33.3)	
Smoking	Yes	7(26.9)	2(8.3)	$p = 0.089$
	No	19(73.1)	22(91.7)	
Education level	Primary and guidance	18(69.2)	14(58.3)	$p = 0.175$
	High school	8(30.8)	7(29.2)	
	University	0(0)	3(12.5)	
Previous amputation history	No	18(69.2)	21(87.5)	$p = 0.912$
	Yes	8(30.8)	3(12.5)	
Employment status	Unemployed	5(19.2)	5(20.8)	$p = 0.974$
	Housewife	14(53.8)	12(50.0)	
	Employee	7(26.9)	7(29.2)	
Disease duration	Less than 5 years	2(7.7)	8(33.33)	$p = 0.59$
	5–10 years	6(23.1)	6(25.0)	
	More than 10 years	18(69.2)	10(41.7)	
Family history of diabetes	Yes	11(42.3)	11(45.8)	$p = 0.513$
	No	15(57.7)	13(54.2)	
Age (Mean ± SD, years)		54.57 ± 6.04	53.70 ± 9.25	$p = 0.69$*
BMI (kg/m^2)		27.45 ± 5.35	28.46 ± 4.65	$p = 0.48$*
FBS (mg/dl)		207.0 ± 83.28	204.0 ± 70.59	$p = 0.92$*
BS (mg/dl)		290.0 ± 118.64	293.0 ± 84.90	$p = 0.28$*
HbA1c (%)		9.24 ± 1.83	9.13 ± 1.93	$p = 0.83$*

*Independent *t* test result

Table 2 Determining nursing system according to requirements in the intervention group before the self-care program performance

Requisites	Educative development *N* (%)	Partly compensatory *N* (%)	Wholly compensatory *N* (%)
Universal self-care	2(8.3)	18(75)	4(16.7)
Developmental self-care requisites	10(41.7)	14(58.3)	0(0)
Health deviation self-care	3(12.5)	20(88.3)	1(4.2)

care capabilities could be improved and regulated by providing the nursing care to meet their self-care needs [22]. A study by Rubin et al. indicated significant differences in HbA1C levels before and 6 months after self-care education in patients with type 2 diabetes [23]. It has been found that self-care behaviors are effective in preventing DFUs, but there are limited studies to show the impact of self-care behaviors in preventing the progression of DFU. The results of Chin et al.'s study showed that self-care behaviors significantly related to lower risk of DFUs [14]. In another study, Ghafourifard and Ebrahimi showed the positive effect of Orem's self-care model on self-care agency of patients with diabetes [11]. Naji et al. showed Orem's self-care model is effective on recovery of patients with heart failure and their self-care abilities [24]. Our result showed significant differences in terms of ischemia after application of Orem's self-care program between the two groups which indicated the positive impact of the program on improving DFU perfusion in the patients. Diminished perfusion is considered a limiting factor for healing of DFU [25]. However, this finding is not supported by other studies. Parisi et al. revealed that there is no connection between peripheral vascular disease and healing DFUs [26]. However, Gürlek et al. showed that peripheral vascular disease is the most significant reason for lower-limb amputation [27]. Loss of sensation and peripheral artery disease associates with poor outcomes in healing DFU. Prompers et al. reported that one of the baseline predictors for not healing DFUs was peripheral vascular disease [28].

The results showed a significant decrease of diabetic foot infection in the intervention group compared to the control group. Islam et al. reported that educational organizations and institutions can prevent secondary infections of DFUs by training patients about the risk of barefoot walking, the importance of proper shoes, checking feet daily, and importance of immediate visit to the doctor rather than self-treatment [29]. Our results showed that education and following-up of self-care behaviors can significantly prevent the progression of diabetic foot infection. Chiovetti recommended nurses to design and implement educational programs for clients based on the individual educational needs [30]. Moreover, Ren et al. showed that the intensive nursing education could prevent diabetic foot ulceration in patients with high-risk diabetic foot [31]. This study supports the results of our study. Furthermore, results of Horswell et al.'s study indicated that a managed diabetic foot care program could significantly lead to decreasing emergency visits, the number of hospitalizations in health care facilities, and complications such as osteomyelitis and amputation [32]. In line with our study, Chiang et al. showed that applying Orem's theory could improve the knowledge of diabetes and foot care of patients with diabetes [33]. Bakker et al. in their study entitled" Practical guidelines on the management and prevention of the diabetic foot 2011" proposed a structured and organized education as effective strategy for prevention of DFU [34].

This study had some limitations to be considered. The study period was short and it was done in a particular geographical area; several elements such as patients' culture may affect the outcomes. Thus, this study's findings must be analyzed with caution. Therefore, we suggest similar studies to be conducted in other regions with different educational and cultural systems so that the impact of the application of Orem's self-care model can be investigated broadly and used effectively for DFUs if further study corroborates our results. The result of this study can be used for nursing management, diabetes associations, patients with diabetes, patients' families, and further research projects.

Table 3 Comparison of the self-care means scores between the two groups before and after intervention

Self-care status	Intervention Mean ± SD	Control Mean ± SD	Independent *t* test result
Before intervention	70.0 ± 16.65	63.76 ± 9.77	$p = 0.11$
After intervention	94.25 ± 9.45	67.26 ± 9.62	$p = 0.001$
Mean differences between before and after	24.25 ± 10.92	3.50 ± 1.92	$p = 0.001$

Table 4 Comparison of the ulcer-related data between the two groups before and after intervention

Variable		Category	Intervention group *N* (%)	Control group *N* (%)	Fisher's exact test
Ischemia	Before the intervention	No	2(8.33)	5(19.25)	$p = 0.921$
		Mild	2(8.33)	1(3.85)	
		Moderate	10(41.67)	9(34.65)	
		Severe	10(41.67)	11(42.25)	
	After the intervention	No	7(29.2)	2(7.7)	$p = 0.001$
		Mild	10(41.67)	5(19.25)	
		Moderate	7(29.2)	13(50.0)	
		Severe	0(0.0)	9(34.6)	
Infection	Before the intervention	No	3(12.51)	8(30.5)	$XP = 0.296$
		Mild	1(4.15)	3(11.5)	
		Moderate	10(41.67)	5(19.25)	
		Severe	10(41.67)	10(38.5)	
	After the intervention	No	7(29.2)	5(19.24)	$p = 0.009$
		Mild	10(41.67)	2(7.7)	
		Moderate	6(24.98)	9(34.65)	
		Severe	1(4.15)	10(38.5)	
Edema	Before the intervention	No	2(8.33)	2(8.0)	$p = 0.694$
		Periwound	6(24.98)	8(30.5)	
		Affected leg only	6(24.98)	5(19.25)	
		Bilateral secondary to systemic disease	10(41.67)	11(42.25)	
	After the intervention	No	12(50)	1(3.85)	$p = 0.004$
		Periwound	5(20.83)	7(27.0)	
		Affected leg only	5(20.83)	12(53.15)	
		Bilateral secondary to systemic disease	2(8.33)	4(16.0)	
Neuropathy	Before the intervention	No	3(12.48)	5(19.25)	$p = 0.744$
		Protective sensation diminished	5(20.83)	5(19.25)	
		Loss of protective sensation	7(29.2)	7(27.0)	
		Diabetic neuro-steo artropathy	9(37.49)	9(34.5)	
	After the intervention	No	6(24.98)	1(3.85)	$p = 0.012$
		Protective sensation diminished	5(20.83)	8(30.5)	
		Loss of protective sensation	6(24.98)	7(27.0)	
		Diabetic neuro-steo artropathy	7(29.2)	10(38.65)	
Location	Before the intervention	Phalanges/digits	5(20.83)	8(30.5)	$p = 0.942$
		Metatarsal	10(41.67)	11(42.25)	
		Tarsal	9(37.49)	7(27.25)	
	After the intervention	Phalanges/digits	6(24.98)	7(27.0)	$p = 0.403$
		Metatarsal	10(41.67)	12(46)	
		Tarsal	8(33.35)	7(27.0)	
Topographic aspects	Before the intervention	Dorsal or planter	3(12.48)	7(27.0)	$p = 0.826$
		Lateral or medial	12(50)	11(42.5)	
		Two or more	9(37.49)	8(30.5)	
	After the intervention	Dorsal or planter	7(29.2)	3(11.5)	$p = 0.012$
		Lateral or medial	10(41.67)	15(58.0)	
		Two or more	7(29.2)	8(30.5)	
Number of affected zones	Before the intervention	One	5(20.83)	7(27.0)	$p = 0.424$
		Two	7(29.2)	11(42.5)	
		Multiple wounds	12(50)	8(30.5)	
	After the intervention	One	5(20.83)	8(30.5)	$p = 0.107$
		Two	9(37.49)	10(39.0)	
		Multiple wounds	10(41.67)	8(30.5)	
Depth	Before the intervention	Superficial	10(41.67)	7(27.0)	$p = 0.918$

Table 4 (continued)

Variable		Category	Intervention group *N* (%)	Control group *N* (%)	Fisher's exact test
		Deep ulcer	7(29.2)	7(27.0)	
		All layers	7(29.2)	12(46.0)	
	After the intervention	Superficial	13(54.17)	8(30.5)	$p = 0.007$
		Deep ulcer	9(37.49)	10(39.0)	
		All layers	2(8.33)	8(30.5)	
Area (cm^2)	Before the intervention	Small <10	10(41.67)	8(31.0)	$p = 0.714$
		Medium (10–40)	9(37.49)	9(34. 5)	
		Big >40	5(20.83)	9(34. 5)	
	After the intervention	Small <10	13(54.17)	7(27.0)	$p = 0.033$
		Medium (10–40)	9(37.49)	12(46)	
		Big >40	2(8.33)	7(27.0)	
Wound healing phase	Before the intervention	Epithelialization	7(29.2)	8(31.0)	$p = 0.169$
		Granulating	8(33.35)	9(34. 5)	
		Inflammatory	9(37.49)	9(34. 5)	
	After the intervention	Epithelialization	13(54.17)	10(38.5)	$p = 0.014$
		Granulating	10(41.67)	11(42.5)	
		Inflammatory	1(4.15)	5(19.0)	

Conclusion

Diabetes is one of the most common and disabling chronic diseases in the globe. We need to have standardized nursing language to improve communication among nurses. This will increase clients' capability to control their disease and its complications, subsequently. Effective disease control requires the patient's readiness to take care of themselves. This study showed that utilizing nursing theory as a standardized nursing language has an effective role in promoting self-care in patients with type 2 diabetes. Thus, dedicated self-care behaviors to prevent diabetes related morbidity and mortality are vitally needed. In summary, application of Orem's self-care model has been shown helpful for patients with DFUs and could change patients' lives by lowering the risk of amputation and medical costs.

Acknowledgements This article was part of a thesis in Masters of Science in Nursing with the registration number of 9201331279. Hereby, the authors appreciate and are thankful of the honorable Deputy of Research of Urmia University of Medical Sciences and all patients who participated in this research. We wish to also thank Mariam Angelica Parizad for her review of the manuscript.

Compliance with ethical standards

Funding This study was financially supported by the Deputy of Research Council of Urmia Medical Science University.

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

Ethical approval This study is approved by the ethics committee of Urmia University of Medical Sciences (Approval code: umsu. rec. 1392.2016).

Informed consent Informed consent was taken from all the subjects to use their personal data for research purposes.

Ethical issues None to be declared.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):173–178
DOI 10.1007/s13410-017-0572-3

ORIGINAL ARTICLE

Dicer expression is impaired in diabetic cutaneous wound healing

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Received: 19 February 2017 / Accepted: 26 June 2017 / Published online: 14 July 2017

Abstract Diabetes, as a fast growing non-communicable disease, is one of the major health problems of the twenty-first century. Several complications such as cardiovascular disease and renal failure are accompanying diabetes. The chronic cutaneous wound is another diabetes complication, results from the reduced body healing potential. At genome level, diabetic wound environment displays disorganized gene functions emphasizing the critical role of gene regulatory networks in the control of chronic wound repair. MicroRNAs, major regulators of gene activity, have been shown to be impaired in several pathological conditions such as chronic wounds. A reason behind that can be sought in the impaired activity of enzymes involved in the development and production of miRNAs. In current study, streptozocin-induced diabetic rats and non-diabetic controls were used to study the effect of diabetes on *Dicer* presence in wound environment. Unwounded skin of diabetic animals showed significantly lower level of *Dicer* expression compared with non-diabetic animal-derived skin. However, at day 7 post-wounding, diabetic animal-derived wounds contained a higher level of *Dicer* expression compared with non-diabetic ones. Parallel to these findings, the granulation tissue formation and wound closure are impaired in diabetic wounds. This study highlights the dysregulated presence of *Dicer* at different stages of the diabetic cutaneous wound.

Keywords Diabetes · miRNA · Dicer · Wound repair

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Introduction

MicroRNAs (miRNAs) are non-protein coding ribonucleic acid sequences which unstable mRNA and prevent mRNA translation [1]. For the first time, these 18–25 nucleotides sequences have been introduced in *Caenorhabditis elegans* as developmental regulators [2, 3]. These small sequences are involved in several body processes such as cell proliferation and differentiation. MicroRNA degradation at developing mouse embryo results in premature loss of embryo [4]. More than thousands of miRNAs have been found in human genome. These miRNAs are able to control multiple aspects of gene functions simultaneously; therefore, they are known as master regulators [5].

Cutaneous wound repair phases consist of a complex network of cell reactions and various gene activity modulations. Under tight control of gene regulators, these complicated cellular functions result in wound repair. Any aberration of this necessary arrangement will result in repair complications such as diabetic chronic wounds. Some gene regulators are able to control the functions of more than one gene at the same time, such as most transcriptional factors and miRNAs. Several miRNAs are involved in the healing process such as miRNA-126 which promotes angiogenesis through VEGF production [6]. miRNA-203 induces keratinocytes differentiation and several other miRNAs are involved in the healing process through modulation of fibroblasts functions [7–9].

In wounds with poor repair potential such as diabetic wounds, the organized healing process is disturbed, which results in prolonged inflammation and lack of angiogenesis in the case of diabetes [7]. It has been shown that miRNAs have different expression patterns in diabetic wounds compared with non-diabetic [10]. In diabetes, the miRNA processor enzyme, Dicer has also shown to be expressed differently in diverse tissues such as bone marrow progenitor cells and

retinal cells that show lower levels of *Dicer* expression [11]. This enzyme has critical roles in most cells and tissue functions such as sperm maturation [12], thyroid gland [13], liver [14], pancreas [15], and kidney [16]function. Down or upregulation of *Dicer* activity has been reported in different types of cancer [17], highlighting the significant role of this enzyme in maintaining the normal state of the body. This study signifies novel insights emphasizing the dysregulated presence of the Dicer enzyme in different phases of diabetes cutaneous wound repair. This might be a major reason for delayed healing in diabetes.

Methods

Animals

Animals used in this study were housed at the Mashhad University of Medical Sciences animal care facility. All procedures were approved by the university ethical review committee. Male Wistar rats (body weight 200–250 g) were purchased from the Mashhad University of Medical Sciences animal care facility. Diabetes was induced in animals using intraperitoneal single dose injection of 65 mg/Kg streptozocin (STZ) which was freshly dissolved in cold 10 mM citrate buffer, pH 4.5. The control group has received the buffer. Rats fasted for 12-h before STZ injection. The blood glucose level was measured using glucometer (EasyGluco) before the induction of diabetes and 10 days after injection to confirm diabetes. Animals with blood glucose level >300 mg/dl were considered diabetic. Diabetic animals were housed with standard husbandry conditions for 4 weeks before wounding experiments. Blood glucose levels were estimated at the time of creation of the wounds to rule out the possibility of reversal of diabetes.

Wounding model

Four weeks after diabetic induction, diabetic and nondiabetic controls were anesthetized by diethyl ether and the dorsum shaved and sterilized with antiseptic wipes. A 1-cm-diameter full-thickness wound was excised including the *panniculus carnosus* layer. Animals were housed in separate cages until the tissue was harvested at the described time points by removing the entire wound area, including a 2-mm perimeter. Wounds were harvested at day 2, 4, and 7 post-wounding. Six animals were used in each group.

Real-time RT-PCR analysis

RNA was extracted using RNA extraction kit (Pars Tous), following the manufacturer's instructions. Reverse transcription was performed using AccuPower® CycleScript RT PreMix (dN6) following the manufacturer's instructions (K-2046, Bioneer). Quantitative analyses of *Dicer* gene expression were performed using Step One machine (Applied Biosystem) and TaqMan gene expression assay (Applied Biosystems): *Dicer1*, Rn01518055_m1; and *B2M*, Rn00560865_m1 was used as reference gene.

Wound closure

Photographs were taken at pre-selected time points. Wound area was measured using ImageJ software.

Histochemistry

Wound tissues at different time points were harvested and fixed in 10% formalin, embedded in paraffin and sectioned into 5 μM thickness. Hematoxylin and eosin staining was performed on sections using standard techniques.

Statistical analysis

All values are reported as mean ± S.E.M. A Student *t* test or one-way ANOVA and post hoc *t* tests were used to assess differences, with probability values of $p \leq 0.05$ denoting statistical significance. Statistical analyses were performed using GraphPad Prism5.

Results

Dicer expression in wound repair phases: a comparison between diabetic and non-diabetic wounds

Animals were wounded 1 month after diabetic induction. Wound tissues were harvested at day 2, 4, and 7 post-wounding representative of inflammatory, granulation tissue, and angiogenesis phases of wound repair, respectively. *Dicer* gene expression at different time points was analyzed using TaqMan gene expression assay. Comparison of diabetic and non-diabetic wounds has been demonstrated in Fig. 1. Diabetic and non-diabetic wounds are statistically significantly different in *Dicer* gene expression at day 0 (unwounded skin) and day 7.

Dicer expression in inflammatory phase

We considered day 2 post-wounding as a time point with a high level of inflammation. *Dicer* gene expression was checked in RNA isolated from whole wound tissue. Figure 1b compares *Dicer* expression level between wounds of diabetic and non-diabetic animals.

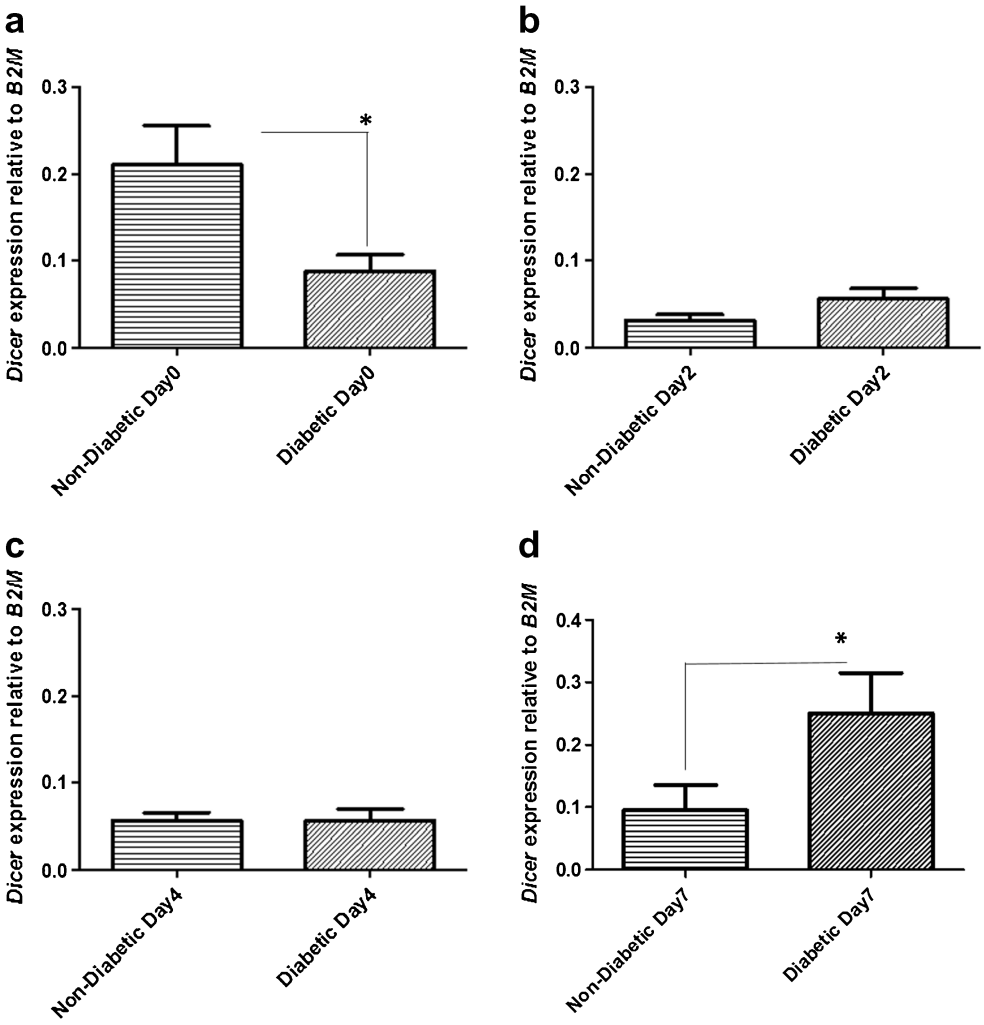

Fig. 1 Comparison of *Dicer* expression between diabetic and non-diabetic wound repair phases. **a** *Dicer* expression at unwounded skin (day 0) ($p = 0.035$). **b** Comparison of *Dicer* expression at day 2 post-wounding ($p = 0.054$). **c** Expression of *Dicer* 4 days post-wounding ($p = 0.49$). **d** Comparison of *Dicer* expression at day 7 post-wounding ($p = 0.043$)

Dicer expression in granulation tissue formation phase

Day 4 has been considered as a transition point in which the inflammation gradually settle down and environment will be prepared for neoangiogenesis. Figure 1c shows the *Dicer* expression level at day 4 post-wounding.

Dicer expression during neoangiogenesis phase

Day 7 post-wounding represented as a hallmark time point for neoangiogenesis phase studies. Figure 1d represents *Dicer* expression at day 7 in diabetic and non-diabetic-derived wound tissues.

Diabetic and non-diabetic animals have different patterns of *Dicer* gene expression during wound repair

A one-way between groups analysis of variance was conducted to explore the differences of *Dicer* expression in various phases of wound healing (Fig. 2). In non-diabetic control group, wounding is followed by a sharp decrease in the level of *Dicer* expression at day 2 post-wounding. While in diabetic wound, the *Dicer* expression remained unchanged until day 7 in which there is a boost in the level of *Dicer* expression.

Wound closure and H&E staining results confirm the impaired healing in diabetic wounds

Photographs were taken on days 2, 4, and 7 post-wounding to assess the closure rate. As Fig. 3 represents the rate of wound closure at day 4 and 7 is significantly less ($p \leq 0.01$) in diabetic compared with non-diabetic control. H&E staining demonstrates a denser and thicker granulation tissue at different time points post-wounding in the control group compared with the diabetic ones.

Discussion

miRNAs are very key gene regulators with disturbed functions in many pathological conditions such as diabetes.

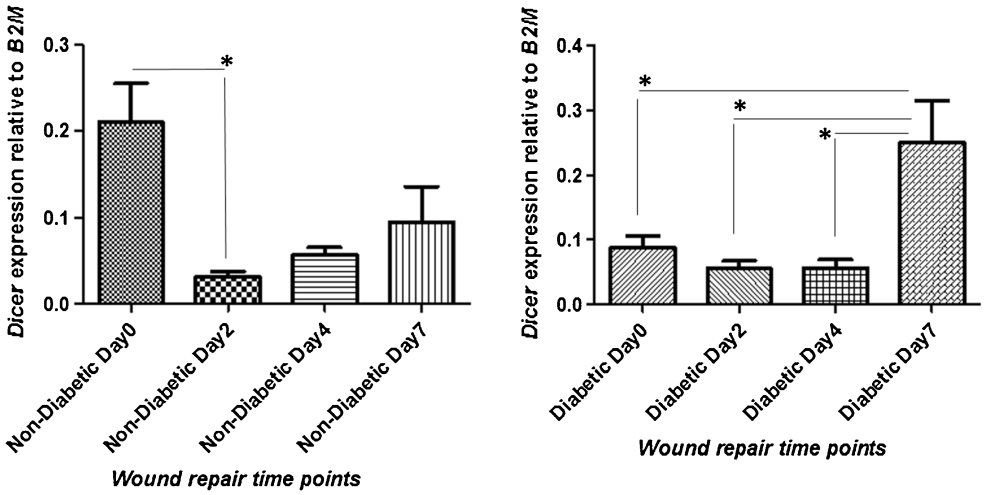

Fig. 2 *Dicer* expression at different phases of wound repair in diabetic and non-diabetic animals. ($p \leq 0.05$)

Diabetic chronic wounds are composed of a dysregulated environment in which various proteins and effectors are present. In this between miRNAs as major gene regulators are also impaired and different miRNAs show altered expression levels in diabetic wound healing phases compared with non-diabetic wounds [10]. In this study, we have evaluated the expression level of *Dicer*, coding for an enzyme responsible for miRNA processing, at different phases of wound repair in diabetic and non-diabetic animals. Results are presenting a different pattern of *Dicer* expression in diabetic wound compared with non-diabetic. As Fig. 2a shows during non-diabetic wound repair phases there is a subtractive *Dicer* gene expression trend which is opposite for diabetic wounds in which the highest expression level of *Dicer* occurs at day 7 post-wounding. We also compared the *Dicer* expression at different phases of repair between diabetic and non-diabetic-derived wounds. There is a substantial level of *Dicer* expression in unwounded skins which is significantly higher in non-diabetic compared with diabetic animals. However, in non-diabetic animals, this amount significantly decreased as skin

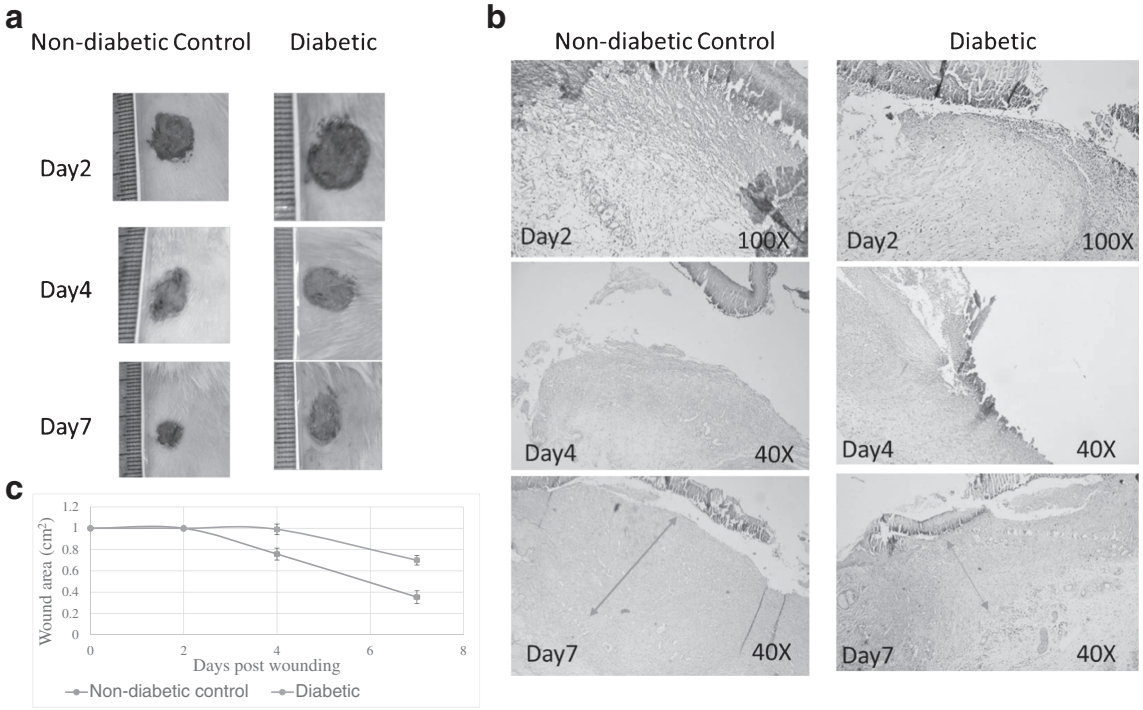

Fig. 3 **a** Representative photographs of wound closure at different time points in non-diabetic control and diabetic animals. **b** H&E staining of wound tissues at different time points. **c** Comparison of wound area at different time points in non-diabetic control and diabetic animals ($p \leq 0.01$)

injured (day 2). In diabetic animals, no significant changes can be seen at day 2 post-wounding. Diabetic and non-diabetic-derived wounds are also different at the level of *Dicer* gene expression at day 7 post-wounding which is the phase of angiogenesis. Although it is already known that diabetic animals have impaired wound healing, we analyzed the wound closure rate in our model to confirm our model. In the same direction with dysregulated *Dicer* expression in diabetic wounds, the wound closure is much slower in diabetic animals. Moreover, there is less granulation tissue in diabetic wounds.

The dysregulated expression of *Dicer* has also been reported in various pathological conditions by several studies. *Dicer* expression has prognostic value in some cancers such as cervical and non-small-cell lung cancer [18, 19]. In Akita mouse model of diabetes (insulin 2 mutant) upregulation of Dicer and spread downregulation of miRNAs has been reported in diabetic cardiomyopathy [20]. The *Dicer* overexpression in this model of diabetic cardiomyopathy has been also reported by another study in which Dicer has been recognized as a regulator of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-4 (TIMP-4) [21]. In fact Dicer depletion in heart results in dilated cardiomyopathy and heart failure [22]. Deletion of Dicer1 enzyme early in pancreas development results in defects in all pancreatic lineages as well as insulin producing β cells [23].

Moreover, Dicer is a key regulator of epidermis formation and maintenance of hair follicle stem cells. Dicer knockout mice cannot survive after birth as a result of dehydration [24]. In this study, we have presented that during cutaneous wound repair in diabetic animals the expression of *Dicer* is impaired which may be responsible for the dysregulated miRNA networks reported in both diabetic cutaneous and corneal wound repair. A high level of several miRNAs has been reported in diabetic wounds [10, 25]. In diabetes, the plasma level of miRNAs changes in such a way that plasma level of some miRNAs such as miR-126 presents a diagnostic value [26]. It has been displayed that in diabetes the diurnal pattern of Dicer shows a phase shift which results in a decrease in the level of Dicer in diabetic progenitor cells in both human and mouse [11]. Dysregulated levels of Dicer and Drosha have been reported in pregnant women with gestational diabetes [27]. Loss of Dicer in platelets of diabetic mice and patients has been shown to be responsible for platelet dysfunctions in diabetes [28]. In this study, we found that in non-diabetic animals the *Dicer* expression level decreases immediately after skin injury and as the wound repair proceeds there is a gradual increase in the Dicer level. This is in direct of a study showed that after injury the miRNA gene silencing function is reduced and coming back to normal levels following the wound closure [29]. We also showed that in diabetic animals *Dicer* expression level remains unchanged after wounding until day 7 in which there is a high level of *Dicer* expression. Considering the evidence which showed that Dicer silencing enhances cell proliferation and invasion capacity [30] and inactivation of *Dicer* results in an impaired postnatal angiogenic response [31], our findings may indicate a novel clue describing the reason of enhanced inflammatory cells recruitment and lack of angiogenesis during diabetic wound healing.

In conclusion, results from this study demonstrate a dysregulated *Dicer* expression in diabetic wound may have a major role in delayed wound repair. This finding is well supported by previous studies demonstrating a dysregulated presence of miRNAs in diabetic wounds. In this study, we have used STZ-induced diabetic model which may not fully represent the diabetic state in human. However, these preliminary data have opened a new insight in the understanding of the mechanism of diabetic wound repair and has offered a novel therapeutic possibility to prevent chronic wounds.

Acknowledgements This work was financially supported by the Research Council of the Mashhad University of Medical Sciences, Mashhad, Iran.

Compliance with ethical standards

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

Statement of human and animal rights All procedures followed in animal studies were in accordance with the ethical standards of the Mashhad University of Medical sciences and were approved by the university ethical review committee.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):179–184
DOI 10.1007/s13410-017-0580-3

ORIGINAL ARTICLE

Diabetic foot ulcers—comparison of performance of ankle-brachial index and transcutaneous partial oxygen pressure in predicting outcome

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Received: 27 February 2017 / Accepted: 28 August 2017 / Published online: 25 September 2017

Abstract Diabetic foot ulcer (DFU) is the commonest condition for hospital admission and usually the starting point of most diabetic related lower limb amputations. Considering the significant role played by vascularity in the outcome of ulcer healing, we undertook this study to find out the comparative utility of commonly used vascular assessment methods. This study was a single center prospective non-randomized observational study, conducted for a period of 6 months, in diabetic patients presenting with foot ulcers of Wagner Grade II and III. The aim of our study was to compare the performances of ankle-brachial index (ABI) and transcutaneous partial pressure of oxygen ($tcPO_2$) measurement in predicting wound healing in diabetic ulcers and to define the optimal cut-off value for Indian patients. Five hundred sixty-four patients were included in this study, with the mean age of 58 years. Eighty-seven patients (15%) had peripheral arterial occlusive disease. Four hundred seventy ulcers (83%) healed with the mean healing days of 42.6 days. Age, duration of diabetes, serum creatinine level, and presence of infection were the factors with negative impact in wound healing. In our study, ABI value of 0.6 was found to have 100% sensitivity and 70% specificity, and $tcPO_2$ value of 22.5 was found to have 75% sensitivity and 100% specificity in predicting wound healing. Both ABI and $tcPO_2$ are complementary, but $tcPO_2$ is a better predictor for amputation while ABI is a better predictor for ulcer healing. While assessing the ischemic status of foot ulcer, the cut-off values should be higher in diabetics than non-diabetics.

Keywords Diabetic foot ulcer · Peripheral arterial occlusive disease · Ankle-brachial index · Transcutaneous partial pressure of oxygen · Amputation · Cut-off values

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Introduction

Diabetic foot ulcers (DFU) affect 15% of diabetic patients during their life time [1], and it is one of the commonest cause for hospital admissions. Unless an early holistic approach of the patient is not taken, this devastating complication ends up in loss of limb and possibly life. A total of 85% of diabetes-related amputations are due to DFU, causing a leg amputation in every 30 s somewhere in the world [2]. With a general rise in elderly population and wider availability of better medical facilities, in India, more and more patients are being diagnosed with diabetes mellitus (DM), and we see more complications related to diabetes in a major way. DFU itself is worrisome, causing not only morbidity, but also lots of financial burden to the patient. Long-term morbidity can be avoided by determining the contributory factors for ulcer healing. Predicting the wound healing in a diabetic patient is difficult, considering the

complex and contributory role of various factors in play, but not impossible. One of the important and established factors, determining the outcome of any ulcer in the extremities, is peripheral arterial occlusive disease (PAOD). Assessment of vascularity plays a significant role in the management of DFU.

The primary goal of any physician treating DFU is to obtain epithelial cover as early as possible. Predicting the ulcer healing is an essential step in the management of DFU, especially when there are variety of factors like age, sex, body mass index, smoking, associated infection, elevated blood sugar, elevated serum creatinine, and local blood supply, affecting the healing [3]. To evaluate the degree of role of PAOD in the outcome of DFU, a range of various non-invasive techniques have been proposed—ankle-brachial index (ABI), transcutaneous tissue oxygen tension (tcPO2), and toe pressure and toe-brachial index. ABI is the conventional test, but not useful in calcified pedal vessels, which is a very common finding in diabetic patients, showing normal or high values [4–6]. TcPO2 measures subclinical micro-vascular involvement, which shows the local tissue oxygen supply available for wound healing, rather than blood supply, but this method is not useful in major vessel involvement [7–10].

There is a physician's dilemma in the management of DFU, in assessing the course of disease and to predict the outcome, so as to take a well-informed decision. When there is a clear cut lesion causing critical ischemia, the role of revascularization is clear to the treating physician to define the course of management. His dilemma starts when he finds diffuse arterial disease, associated with neuropathy and/or severe infection, which is a very common presenting picture in DFU. Whether the patient will, or not, benefit with revascularization, cannot be decided purely on the basis of ABI alone. The role of tcPO2 in such difficult cases is to be defined, to help the physician to take an informed decision. To assess the extent of PAOD, thereby assessing and predicting the wound outcome, the utility of ABI and tcPO2 measurement should be validated. Unclear about which one will accurately predict the wound outcome, this study was done to find out the comparative utility of ABI and tcPO2 in predicting wound outcomes in DFU and to define the cut-off values for both measurement in Indian diabetic patients.

Methodology

This was a single-center prospective non-randomized observational study, conducted for a period of 6 months from September 2015 to February 2016, at MV Diabetic Research Center, Chennai. All diabetic patients who attended the outpatient department with Wagner Grade II and III foot ulcers were included in this study. On admission, basic demographic, medical details and clinical features of all patients were recorded. On evaluation, biochemistry investigation reports, microbiology lab results, ABI, and tcPO2 were recorded for all patients. Comprehensive wound care was offered by a multidisciplinary team as per institute protocol, which includes strict control of hyperglycemia and co-morbid factors, wound debridement by mechanical, chemical, and surgical methods, offloading, and management of complications. All patients were followed up to complete wound healing or up to 3 months. Complete epithelialization of the ulcer or healed amputation stump was considered as primary wound outcome.

The outcome was categorized as healed and amputation. Amputations were defined as minor which includes mid-foot and distal amputations, while major amputation was defined as amputations above the ankle. Patients, who had clear treatable lesions, underwent revascularization procedures and who had non-healing ulcers at the end of the study period, due to lost to follow-up or extensive infection were excluded from the study. All data were entered in a Microsoft excel sheet and statistical analysis was done using SPSS. Binary demographic factors, baseline risk factors, and outcome variables were compared between the outcome groups using the χ^2 test, the Fisher exact test, and the odds ratio with 95% confidence interval, wherever appropriate. Continuous demographic factors and outcome variables were summarized using a mean with 95% standard deviation and compared between outcome groups by using t test. The performances and optimal cut-off values for the studies factors were calculated and presented using an ROC curve.

Results

A total of 582 patients attended the hospital with DFU of grade II and III, during the study period. Out of that, 5 patients who underwent revascularization procedures and 13 patients with a non-healing ulcer at the end of the study period were excluded. The remaining 564 patients were included in this study, including 380 males and 184 females [Table 1]. The mean age of the study group was 58.23 ± 10.11 years. The age distribution in both groups is shown in Fig. 1. Neither sex of the patient ($p = 0.039$) nor body mass index (BMI) ($p = 0.07$) had any effect in the wound outcome. Various studies have proven the effect of BMI in the occurrence of diabetic complications. In our study as the mean BMI was 23, it may not have any effect in the wound healing. The mean BMI for the healed group was 26.8 ± 4.8, while that of the amputation group was 25.7 ± 4.3. Only 7 patients in our study group were current smokers, which is an insignificant number to come to any conclusion ($p = 0.865$).

Duration of DM in our study group showed a wide variation, ranging from recently diagnosed within 1 month to disease of 43 years duration, with an average of 23 years. There

Table 1 Basic demographic factors in the study group

	Healed	Amputation	*p* value
Total	470	94	
M:F	308:162	72:22	0.039
Age (mean in years)	51.3	63.7	0.014
BMI (mean ± SD)	26.8 ± 4.8	25.7 ± 4.3	0.070
Smoking	1	6	0.865
Duration of DM (mean in years ± SD)	11.96 ± 7.3	14.22 ± 7.1	0.007
Abnormal HbA1c	418	77	0.058
Abnormal Sr creatinine	116	38	0.002
Presence of infection	247	62	0.017
ABI < 0.9	48	39	< 0.001
$TcPO_2$ < 40	75	41	< 0.001

were 54 patients (9.8%) who had the disease for more than 20 years and 218 patients with the duration of 10–20 years. The mean duration of the disease in the amputation group was 14.22 ± 7.1 years and that of the healed group was 11.96 ± 7.3 years. Duration of DM has got a significant negative role in wound healing as well as higher degree of amputation ($p = 0.007$). HbA1c levels were measured on admission and reassessed periodically to treat and bring it under control. On admission, 495 patients (88%) had their HbA1c level more than 7, indicating poor control (mean HbA1c on admission = 9.28). But on statistical analysis, p value was 0.058, excluding its role in wound outcome. These may be due to the in-house control of blood sugar level with insulin, oral anti diabetic drugs, and proper diet, which controls the hyperglycemia and prevents its adverse effects. All patients with uncontrolled hyperglycemia were given priority treatment with a strict regime on diet and drugs. And, in the end of study period, all of them had their blood sugar under control and their HbA1c level less than 7. This strict control may be the reason in their negative role in the poor DFU outcome.

With so many patients in our study group having DM for prolonged duration (> 10 years in 272 patients), incidence of chronic kidney disease and elevated serum creatinine is not an unexpected finding. In our study group, 154 patients (27.3%) had elevated serum creatinine levels > 1.2 g/dl. With the outcome of amputation, the p value was 0.002, establishing level of serum creatinine as a significant factor in the wound outcome. Infection in the patients was diagnosed by clinical signs of cellulitis, abscess, local lymphangitis, presence of associated systemic signs, such as fever, hyperleucocytosis, and bacteriological samples were taken, in cases of clinically established infection. A total of 309 patients were admitted with local infection in the DFU, of whom 62/309 (20%) patients had amputation, while 87.5% (223/255) of patients without infection had their ulcer healed. p value = 0.017 was significant, but not on a greater scale.

Out of 470 healed ulcers, 65% (304 patients) healed within 41 to 45 days. Only 81/470 patients (17%) had delayed healing of > 45 days. The average healing days were 42.6 ± 2.7 days. A total of 94 patients underwent amputation, 32 patients with minor and 62 patients with major amputation. The most difficult situation in the management of DFU is to take decision at the appropriate time and appropriate level of amputation. The decision for minor amputation is comparatively easier, considering the less morbidity and tissue loss associated. The decision for major amputation is taken considering the grade of DFU (higher the grade more proximal the level), associated infection (whether controlled or severe

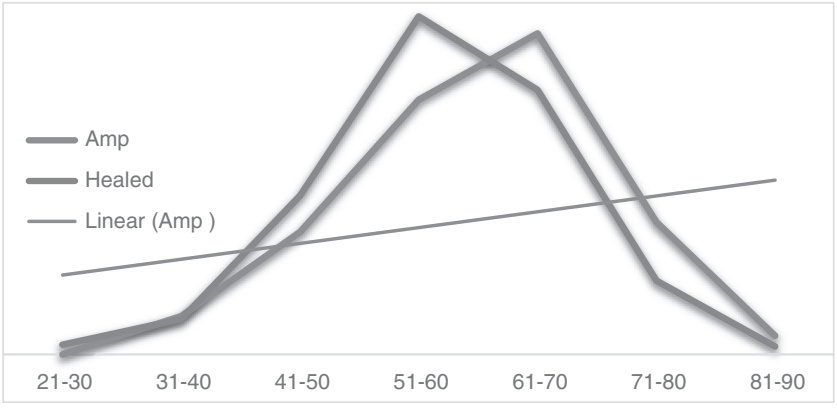

Fig. 1 Effects of age in wound outcome

uncontrollable infection to regular antibiotics), and the general functional capacity of the patient. Out of 62 patients who underwent major amputation, 46 DFU were graded up to Wegener grade IV/V, in spite of adequate antibiotic control and 16 patients had severe ischemic foot, with non-reconstructable vessels. Most major amputations are considered as a life-saving procedure rather than trying for limb salvage.

The prevalence of PAOD is 18% (87 patients) in our study. Out of 470 patients with healed ulcers, 422 (90%) had recorded ABI > 0.9 and 395 (84%) patients recorded tcPO2 > 40. In the category of amputation, the values were 55/94 (59%) patients and 53/94 (56%) patients, respectively. A total of 39/94 patients (42%) in the amputation category and 48/470 patients (10%) in the healed category showed abnormal ABI measurement of < 0.9. In the measurement of tcPO2, 41/94 patients (44%) in the amputation group and 75/470 patients (16%) in the healed group showed low values of < 40. The diagnostic ODDS ratio for wound healing for ABI was 23 and for tcPO2 was 4.23, with a positive predictive value of 75 and 35%, respectively [Table 2]. By plotting the ROC curve, for the state of healed, a tcPO2 value of 22.5 must have 100% sensitivity and 75% specificity. The corresponding value for ABI was 0.6, with sensitivity of 99% and specificity of 68%. By decreasing the value of ABI to 0.4, it had the sensitivity of 100% and specificity of 80% [Fig. 2a]. By plotting the ROC curve, for the positive state of amputation, an ABI value of < 0.6 was found to have 68% sensitivity and 99% specificity. The corresponding value for tcPO2 was 22.5, with sensitivity of 75% and specificity of 100% [Fig. 2b]. Multivariate logistic regression analysis showed an ODD ratio of 3.5 (95% CI 2.2–5.7) for the effect of wound healing with ABI and an ODDS ratio of 3.0 (95% CI 2.1–4.3) for the effect of abnormal tcPO2 in amputation [Table 3].

Table 2 Sensitivity, specificity, and diagnostic odds ratio (DOR) of ABI and $tcPO_2$

	ABI < 0.9	$TcPO_2$ < 40
Sensitivity	41.5%	43.6%
Specificity	97%	84%
DOR	23	4.23
Positive predictive value	75%	35.3%
Negative predictive value	88.5%	88.2%

Discussion

In 2014, the prevalence of DM in India had increased to 9.1% and is expected to continue rising [11]. With the rise in diabetic patients, complications related to DM are also in rise. In this study, we undertook to analyze the utility of ABI and tcPO2 in predicting wound outcomes in DFU, one of the commonest morbidity in DM. Over a period of 6 months, 564 patients with DFU are evaluated. Our average wound healing days of 42.6 ± 2.7 is much less than those reported in other studies. Average ulcer duration of 133 days had been reported in the literature [12]. The better result of our study might be due to the strict wound care protocol, followed at the institute, comprising a multidisciplinary team, involving physician, surgeon including plastic and vascular surgeon, podiatrist, special wound care nursing team, and specific consultancy for diet and life-style modification, including smoking cessation. A recent study by Wang et al. showed an average age of patients with DFU as 66.96 years [13]. Our study showed a relatively younger population of mean age of 58 years, which is similar to other Indian statistics. Age, as a contributory factor, is proven to be statistically significant in our study and another interesting factor revealed is that the age of amputation (mean = 63.7 years) is one decade later than the healed group (mean = 51.6 years). Older patients are more prone for amputation than younger patients. In a study by Marston [14], it was found to have female gender as a positive effect in healing, but our study did not find any significant difference based on sex of the patient ($p = 0.039$), even though females formed one third of the study group (33%). Another noted factor, which was proven negative in our study, is smoking [15]. This may be due to very small number of patients ($N = 7$), who were active smokers in our study. As suggested by previous studies [13], a significant correlation to the duration of disease is noted. In our study, the range was so wide, from a recently diagnosed patient with less than a month to a 71-year-old patient with 43 years of disease. Another proven factor is that of serum creatinine level, which had a negative impact on healing [16, 17]. Several risk factors proven to affect healing were not found to be significant in this study. BMI is a known factor, proven by several studies [18]. The non-significance of BMI in our study may be due to lower near normal values (mean of 25). Improved glucose control is a proven factor that promotes wound healing [15]. This may be the reason why there is no significant role in wound healing for abnormal HbA1c ($p = 0.058$) in our study, because strict glycemic control is one of the basic management in our protocol and we have only taken the glycemic level at admission, which may not affect the course of the disease. This proves the effective role of strict glycemic control in preventing poor wound outcome and possibly, amputation. A recent report by Lee et al. also stated higher HbA1c levels in the successful wound treatment group [16].

Previous studies have suggested a negative role of infection in wound healing [19, 20]. Our study established a weak role for infection ($p = 0.017$). This may be due to the present availability of higher antibiotics, appropriate use of antibiotics after determining extent and severity of the infection, and thorough surgical debridement to remove the infected tissue

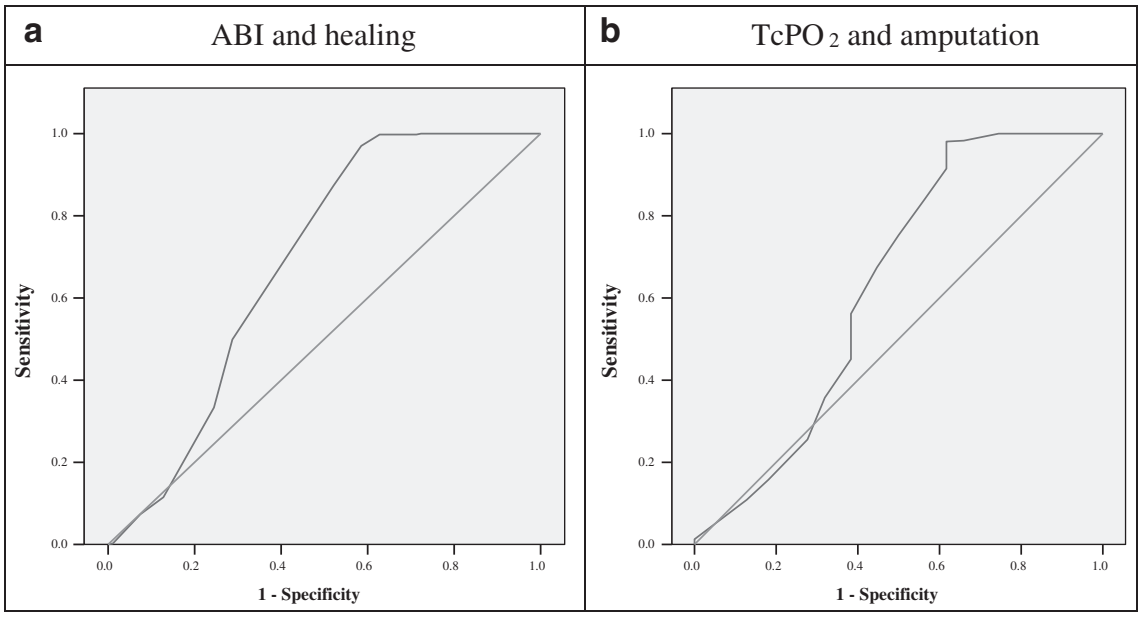

Fig. 2 ROC curves

and earliest epithelial cover, as per our protocol. The prevalence of PAOD among diabetics has been found to be low in Indian population, but is on the rise, and may be due to better diagnostic facilities, increasing duration of diabetes, and increasing older population. Previous Indian studies published in the later years of the twentieth century reported 3–6% [21, 22] prevalence, while recent studies from India, reported 12.6–31.6 and 19.7% [23–25]. In the west, the prevalence is said to be high, ranging 25–45% [26–28]. To assess the ischemic nature of the wound, if ABI is used, we have 87 patients (15%), but if tcPO2 is used, we have an increase by 29 patients, i.e., 116 patients (21%). As tcPO2 measures the microangiopathic involvement by DM, it scores as a better marker to diagnose the vascularity of a distal wound. By assessment of diagnostic odds ratio, ABI with a score of 23, proves itself as a better indicator for predicting wound healing. Even though the negative predictive value of both parameters is the same, the positive predictive value of ABI is much higher than tcPO2, signifying its position as a better indicator for wound healing. Even the results of multivariate regression analysis confirm the significance of ABI with an odds ratio of 3.5 for wound healing. But, for the predicament of amputation as outcome, tcPO2 scores better than ABI with an odds ratio of 3.

Though ABI value of < 0.9 is commonly used to predict the PAOD, and value of < 0.3 is used for indication of critical ischemia, in our study, ABI of 0.6 is found to have optimum sensitivity and specificity in predicting wound outcome. An ABI of < 0.6 predicted poor outcome in 32% of DFU, with a sensitivity of 99%. It may be suggested that in diabetic patients, the consideration of critical wound ischemia should be at a lower level than that of non-diabetics, and in patients with DFU and an ABI < 0.6, immediate and adequate treatment measures should be taken to improve vascularity and to save the limb. Same way, the optimal cut-off value for tcPO2 to be considered might be 22.5 mm of Hg, rather than the universally accepted value of 40. A tcPO2 value of < 42.50 predicts 56% of amputation, while it was 70 and 92%, respectively at tcPO2 values of < 22.50 and < 12.50. With the consideration of amputation, thus, tcPO2 values of 22.5 may be considered with more sensitivity and specificity. These results are similar to the results by Lalithambika et al. [29], which has near about the same values of ABI of 0.77 and tcPO2 value of 22.5 mm of Hg, in their study in Indian population. Thus, in patients with DFU and tcPO2 value < 20 mm of Hg, the patient may be alerted to the high probability of amputation.

Conclusion

Indian patients were younger, with a long history of disease duration, moderate BMI, and with high HbA1c. A dedicated

Table 3 Results of the multivariate logistic regression analysis

Variable	Odds ratio for healing (95% confidence interval)	Odds ratio for amputation (95% Confidence Interval)
ABI < 0.9	3.539 (2.208, 5.673)	0.514 (0.115, 0.216)
$TcPO_2$ < 40	0.733 (0.633, 0.842)	2.988 (2.099, 4.252)

multidisciplinary team approach, with strict control of hyperglycemia, infection, and life-style modification such as avoidance of smoking, will result in better wound healing and reduce drastically the average healing days. Due to biological variability of individuals, no parameter can be 100% effective under all conditions. Nevertheless, it has been demonstrated that, within the two parameters assessed, ABI is a better predictor for wound healing and tcPO2 is for predicting amputation. A value of 0.6 for ABI and 22.5 for tcPO2 may be considered for Indian diabetic patients while assessing DFU outcome. However, in the absence of a prospective randomized study with more patients, opinions will continue to be divided.

Compliance with ethical standards

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest None.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):185–190
DOI 10.1007/s13410-017-0567-0

ORIGINAL ARTICLE

How good is procalcitonin as a marker in case of sepsis in diabetes mellitus?

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Received: 16 February 2017 / Accepted: 11 June 2017 / Published online: 25 July 2017

Abstract Prompt diagnosis and treatment with appropriate antimicrobial chemotherapy are of paramount importance to reduce morbidity and mortality associated with sepsis. The limitations of blood cultures have fostered interest in the development of sensitive and rapid laboratory biomarkers of sepsis. Procalcitonin has been proposed as a marker of disease severity in septicemia. The aim of the study was to assess the accuracy and clinical value of procalcitonin for diagnosis of sepsis in diabetes mellitus. A descriptive, cross-sectional study was conducted on 30 cases of known diabetes mellitus admitted in the Endocrinology I.C.U. with suspected bacterial infection and sepsis. The bacterial isolates were identified by standard protocol. A double-antibody sandwich enzyme-linked immunosorbent one-step process assay was used to assay the levels of procalcitonin. There was positive correlation between procalcitonin levels and the number of days for total leukocyte count to subside though duration of hospital stay or time taken for fever to subside was not significantly correlating. Positivity in blood culture was significantly correlating with median procalcitonin levels though C-reactive protein levels were unable to demonstrate the same. Other markers of severity of sepsis were not significantly correlating with procalcitonin levels. No glycemic marker showed any significant correlation with procalcitonin levels. With good clinical judgment and judicious use of antimicrobial agents, procalcitonin could serve as a valuable adjunct in the diagnosis and management of sepsis with diabetes mellitus. Search for more validated biomarkers should be emphasized upon.

Keywords Procalcitonin · Sepsis · Diabetes mellitus

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Introduction

Sepsis is a potentially dangerous or life-threatening medical condition, found in association with a known or suspected infection whose signs and symptoms fulfill at least two of the following criteria of a systemic inflammatory response syndrome (SIRS): elevated heart rate (tachycardia) >90 beats per minute at rest, body temperature either high (>or 38.3 °C) or low (<36 °C), increased respiratory rate of >20 breaths per minute or a reduced $PaCO_2$ (partial pressure of carbon dioxide in arterial blood level), and abnormal white blood cell count (>12,000 cells/μL or <4000 cells/μL or >10% bands, an immature type of white blood cell) [1].

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in secretion and action of insulin or both. Because of its high prevalence and potential to alter critical elements of sepsis pathophysiology, diabetes is likely an important comorbid condition [2]. Hyperglycemia impacts different components of the host response including function of immune cells and regulation of cytokines. Increased endothelial cell activation and procoagulant changes are found in diabetic subjects. Patients

with diabetes mellitus have an increased risk of developing infections and sepsis [3, 4] and constitute 20.1–22.7% of all sepsis patients [5]. In a series of cases in the pre-insulin era, diabetic coma was usually precipitated by infection, and infection remains an important cause of death in diabetics that may be associated with better prognosis [6–8]. Mortality of sepsis is more in diabetics when compared with nondiabetics. Severity of disease increases mortality and tight control of blood glucose. It is an accepted opinion that diabetes worsens prognosis of infection, particularly sepsis, although there is not much data published on this subject [9].

Blood cultures are the current "gold standard" for diagnosis of septicemic conditions. However, on some occasions, blood cultures have intrinsic limitations in terms of sensitivity and rapidity, and it is not expected that these drawbacks will be overcome by significant improvements in the near future. The limitations of blood cultures have also fostered interest in the development of sensitive and rapid laboratory tests aimed at detecting nonspecific biomarkers of sepsis [10]. Procalcitonin (PCT) is the precursor peptide, or prohormone, of the mature hormone calcitonin. Procalcitonin is a polypeptide present in the plasma of healthy subjects in minimal levels (<0.5 mg/ml). It consists of 116 amino acids with a molecular weight of 13 kD [11, 12]. Under normal circumstances, it is produced in the C-cells of the thyroid gland, though during severe infections with systemic manifestations, procalcitonin levels may rise to over 100 ng/ml produced mostly by extrathyroid tissues.

Since early identification of infections and sepsis is crucial for patient management, an effective marker specific for bacterial infection is very useful in the critical care settings. There are several markers of sepsis, like C-reactive protein (CRP), serum procalcitonin, interleukin-6, interleukin-8, and lactate, of which PCT has been found to be the most effective. PCT has been proposed as an indicator of the presence of infection and as a useful marker of the severity of sepsis [13]. The present study was initiated to see the diagnostic utility of procalcitonin in diabetic sepsis.

Materials and methods

A hospital-based descriptive, cross-sectional study was conducted in the Department of Microbiology and Endocrinology Division of Department of Medicine at UCMS and GTB Hospital, Delhi, from December 2013 through April 2015. Thirty consecutive patients irrespective of age and sex with known diabetes mellitus admitted in the Endocrinology I.C.U. with suspected bacterial infection and sepsis, severe sepsis, and septic shock were included. Patients on antibiotics for last 48 h or with coexisting systemic complications like chronic kidney disease, congestive heart failure, and chronic respiratory infections or immune disorders were excluded. After approval from the Institutional Ethical Committee of UCMS, blood was collected for routine hemogram, blood culture, C-reactive protein, procalcitonin levels, and other relevant investigations along with glycemic markers like blood glucose levels, fasting, and postprandial and glycosylated hemoglobin. Samples for PCT were centrifuged to separate the serum which was kept at −70 °C for further analysis.

Bacteriological processing

Blood samples were collected for blood culture in the BACTEC blood culture bottles. Each vial contained 40 ml enriched soybean-casein digest broth, 0.02% SPS resin, CO_2O_2, and sensor for the detection of fluorescence. The vials were placed in BACTEC 9120 system. A positive result was indicated by an audible alarm and yellow illumination of the positive indicator lamp at the site of positive vial. The bottles were incubated for 5 days before being reported as negative. Positive vials showing presumptive presence of viable organisms were subjected to subculture on blood agar, MacConkey agar, and chocolate agar followed by Gram staining.

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was performed to determine the susceptibilities to routinely used antibiotics and results were interpreted as per CLSI (Clinical Laboratory Standard Institution) guidelines [14].

Estimation of CRP

Estimation of CRP was done by latex slide agglutination test (RHELAX CRP) with a cutoff value of 0.6 mg/dl.

Estimation of procalcitonin

Human Procalcitonin ELISA Kit The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the level of procalcitonin in samples. Optical density (OD) was measured at 450 nm. According to concentration and the corresponding OD values, standard curve linear regression equation was calculated and then applied the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. Assay range was 9.3–300 ng/ml.

Outcome measures

Severity of sepsis was assessed by urine output, mental status of patient, breathing difficulty, blood pressure, platelet count, time taken for fever to subside, duration of hospital/I.C.U. stay, total leukocyte count (TLC) blood glucose level, and mortality.

Data management and statistical analysis

The data was analyzed by statistical software SPSS version 20.0. Spearman correlation was calculated between PCT levels and severity of sepsis parameters, glycemic markers, and outcome measures of sepsis. Mann-Whitney *U* test was applied to compare PCT levels between CRP (<0.6 vs >0.6 mg/dl) and blood culture (positive vs negative). The *p* value less than 0.05 was considered as significant.

Results

Enrolled subject in the study either had diabetes mellitus type 1 or 2 with clinical sign and symptom of sepsis. Out of the thirty subjects enrolled in this study, 27 (90%) were of type 2 diabetes and 3 (10%) were of type 1 diabetes. The median age in years was 45.7 (16–67). Thirteen (43.3%) were males whereas 17 (56.7%) were females. Fasting blood sugar and postprandial blood sugar were found to be elevated in 96.7% of patients; glycosylated hemoglobin was more than 7 in 93.3% of patients, whereas 3.3% had range in between 6.5 and 7%.

In this study, minimum PCT level came out to be 2.1 ng/ml and maximum was 120.6 ng/ml. About 83.3% were in range of 1–40 ng/ml, 6.7% were in 41–80 ng/ml, 6.7% were in 81–120 ng/ml, and 3.3% had levels up to 120.6%. In blood culture-positive subjects, PCT levels were from 9.11 to 120.60 ng/ml, higher than the normal value in healthy individuals.

Blood culture revealed some etiological agents in 9/30 (30%) subjects. PCT was found to be elevated in all the 30 subjects. Out of 30 patients, 2 patients died whose PCT levels were 120.6 and 58.8 ng/ml.

Age and sex distribution of subjects on the basis of type of diabetes and levels of procalcitonin is shown in Table 1.

The correlation of various parameters of diagnosis or severity of sepsis in diabetes with levels of procalcitonin is depicted in Tables 2 and 3. There was positive correlation between the number of days for TLC to subside and PCT levels though duration of hospital stay or time taken for fever to subside was not significantly correlating. Positivity in blood culture was significantly correlating with median PCT levels though CRP levels were unable to demonstrate the same. There was inverse correlation, though not statistically significant, between the platelet count and PCT levels. Other markers of severity were not significantly correlating with PCT levels. No glycemic marker showed any significant correlation with PCT levels.

Table 1 Age and sex distribution with type of diabetes and procalcitonin levels among the study subjects

Age (in years)	Sex (M—male) (F—female)	Type of diabetes mellitus (1 or 2)	Procalcitonin (ng/ml)
17	M	1	6.16
50	F	2	5.10
40	F	2	10.95
16	M	1	11.56
64	F	2	17.46
56	M	2	83.15
50	M	2	16.51
21	F	1	22.06
48	M	2	17.05
56	M	2	58.8
27	F	1	10.13
39	M	2	6.2
60	F	2	10.23
35	F	2	120.6
60	F	2	9.11
49	F	2	56.1
48	M	2	10.12
60	F	2	25
67	F	2	10.55
55	F	2	20.65
65	F	2	15.63
45	M	2	5.68
42	M	2	2.15
65	F	2	2.96
18	F	1	5.01
38	M	2	115.6
39	F	2	6.22
54	F	2	20.5
48	M	2	11.55
40	M	2	7.06

Mean procalcitonin level is 18.63 ± ng/ml and range is 2.1 to 120.6 ng/ml

It was nearly a coincidence that all blood culture-positive subjects had diabetes mellitus type 2, which was not statistically significant ($p = 0.2860$).

Discussion

Microbiological diagnosis of patients suffering from diabetes mellitus having sepsis/infection at the time of admission takes

Table 2 Correlation of procalcitonin levels with diagnostic parameters of sepsis in diabetes mellitus

Parameters		No. of subjects	Coefficient of correlation[a]	*P* value	Significance
Severity of sepsis	Respiratory rate	29	−0.041	0.834	NS
	Mean arterial pressure	30	−0.099	0.603	NS
	Platelet counts	30	−0.382	0.130	S
	Total leucocyte count	30	0.165	0.785	NS
Glycemic markers	Random blood sugar	30	−0.019	0.922	NS
	Fasting blood sugar	30	−0.064	0.742	NS
	Postprandial blood sugar	30	0.142	0.462	NS
	Glycosylated hemoglobin	30	−0.109	0.567	NS
Outcome measures of sepsis	No. of days for TLC to subside	28	0.518	0.005	S
	Time taken by fever to subside	30	0.338	0.079	NS
	Duration of hospital stay	30	0.004	0.984	NS

NS nonsignificant, *S* significant

[a] Spearman correlation

48–72 h to reach the causative agents with antimicrobial susceptibility testing. Biomarker like procalcitonin helps in overcoming this problem of time by indicating sepsis/infection earlier than any microbiological procedures. The main reason for which diabetes predisposes to infection and sepsis to infection appears to be abnormalities of the host response, particularly in neutrophil chemotaxis, adhesion, and intracellular killing, defects that have been attributed to the effects of hyperglycemia. There is also evidence for defects in humoral immunity, and this may play a larger role than previously recognized [15]. Few studies have shown the association between diabetes mellitus and poor chronic glycemic control and blood stream infection in critically ill patients [16–18].

The critical point in patients with sepsis is the absence of documented infection at admission to the endocrinology ward. Also, the use of cultures to identify the presence of bacteria requires time, which makes it inaccessible for clinical use in the endocrinology ward, and negative cultures do not exclude the sepsis. Bacteriological evidence of infection, although considered a gold standard, may have some drawbacks. Furthermore, positive or negative cultures may not develop concurrently with clinical signs of sepsis. Because these common clinical and laboratory parameters lack sensitivity and specificity, others are needed to provide an early marker of the infectious etiology of a generalized inflammatory response and thus allow early diagnosis and the application of more specific therapeutic interventions.

In a previous study, it was reported that in one-third of sepsis patients, the causative pathogens could not be identified for which various reasons like history of prior antibiotic therapy, unavailability of previous reports suggestive of infections, or other limitations of blood culture technique could be cited [19]. The present study revealed *Staphylococcus aureus* (77.7%) and *Klebsiella pneumoniae* (22.3%) as the causative agents of sepsis in 30% of the subjects which had shown blood culture positivity. In regard to antimicrobial susceptibility testing, all *S. aureus* isolates were methicillin sensitive while *K. pneumoniae* strains showed variable sensitivity to antimicrobials tested. In contrast as per earlier studies, Gram negative bacilli were predominant in such cases whereas in the last two decades, Gram positive cocci have emerged as major microbial agents causing septicemia in diabetes mellitus patients [20].

Appropriate therapy is often delayed and empirical antimicrobial therapy has to be started as soon as sepsis is presumed, before results from blood culture become available, and this diagnostic uncertainty is compensated for by the liberal use of broad-spectrum antibiotics, contributing to the increasing

Table 3 Correlation of C-reactive protein levels and blood culture with procalcitonin levels in diabetes with sepsis

Variable	Category	Median (IQR)	Significance[a]
C-reactive protein (mg/dl)	≤6	16.78 (9.21–21.00)	0.448 (NS)
	>6	10.34 (6.20–47.20)	
Blood culture	P	56.10 (10.34–99.37)	0.022 (S)
	N	10.23 (5.92–17.26)	

IQR interquartile range (25th to 75th percentile), *P* positive, *N* negative, *NS* not significant, *S* significant

[a] Mann-Whitney *U* test

resistance of antimicrobial drugs a growing public health problem. Researchers and clinicians have been investigating and implementing various methods of early diagnosis for sepsis before documentation of infection.

According to a study, elevation of inflammatory marker was shown to be in correlation with HbA1c and AGE concentrations in diabetic patients without actual infection [21]. In this study, no statistically significant correlation was found between random blood sugar, fasting blood sugar, and postprandial blood sugar.

PCT level is increased in bacterial and fungal infections, but not in viral infections [22]. In our study, 100% subjects came out with elevated PCT levels, though only 30% were blood culture positive. Four of six meta-analyses performed on the diagnostic accuracy of PCT to detect infection in different patient populations identified PCT as helpful for the diagnosis of clinically or microbiologically documented infection [23–26] whereas one meta-analysis identified only a moderate benefit in the detection of bacteremia [25], and another one found the benefit moving toward a null effect in larger studies [26]. Procalcitonin levels increase with increasing severity of the inflammatory response to infection. A study compared procalcitonin values in patients with bacterial pneumonia and septic shock. Procalcitonin values were moderately increased in patients with bacterial pneumonia (mean 2.4 ng/ml) but were markedly increased in patients with septic shock (means 72–135 ng/ml). PCT showed a more favorable kinetic profile than CRP and other cytokines: its levels increased within 4 to 12 h upon stimulation, and circulating PCT levels halved daily when the infection is controlled by the host immune system or antibiotic therapy [12].

Compared to any other currently available sepsis marker, PCT seems to have some potential to discriminate between infectious and noninfectious systemic inflammation. Recent guidelines issued by the Infectious Diseases Society of America and the American College of Critical Care Medicine recommended the use of PCT as an adjunctive diagnostic marker to differentiate sepsis from systemic inflammatory response syndromes of a noninfectious origin [27]. Although it is established that hyperglycemia has a definite role to play in infection/sepsis in case of diabetes mellitus, but in the present study, no correlation was established among PCT levels and glycemic markers.

While blood cultures are still considered the "gold standard" for the diagnosis of bacteremia and sepsis, PCT provides important information in early stages of sepsis as well as during antimicrobial treatment and can be useful for antimicrobial stewardship. However, PCT is also less than a universal and perfect biomarker, as it can also be increased in noninfectious disease conditions. Laboratories and clinicians must appreciate the complexity of diagnostic algorithms for sepsis and understand the particular information that biomarkers, such as PCT, can offer [28]. The advantage of PCT as a biological marker of sepsis is that it appears to be specific for infection or infection-induced organ dysfunction, while other proteins that have been studied such as CRP, neopterin, cytokine, and chemokine are not [29]. In our study too, the significance of increased procalcitonin levels could be demonstrated in positive blood cultures. A recent study done on 2952 hospitalized patients including 440 blood culture-positive cases raised median PCT values and further concluded that PCT could be used in clinical algorithm to diagnose positive infections and sepsis where different values could even be related to different kinds of microbemia, different infection sites, and different severity of infections [30].

In conclusion, PCT serum levels might be a useful diagnostic tool in management of sepsis in diabetes mellitus before documentation of bacteria. The use of PCT measurement to decide the need of antibiotic therapy should be a practical approach in patients of diabetes mellitus with sepsis. For wider application and acceptance of such finding, large number of subject size will be required to state the findings with definite conviction.

Compliance with ethical standards

Funding There was no source of funding for the study.

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

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):191–194
DOI 10.1007/s13410-017-0575-0

ORIGINAL ARTICLE

Feasibility of Ramadan fasting in adults with type 1 diabetes: an observational study

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Received: 7 February 2017 / Accepted: 1 August 2017 / Published online: 11 August 2017

Abstract Ramadan fasting is usually a challenge for both Muslim patients with diabetes and their health care providers. The challenge is even greater in patients with type 1 diabetes. Existing clinical guidelines categorize patients with type 1 diabetes as very-high-risk patients, and therefore, they are advised not to fast. However, many patients with type 1 diabetes choose to fast despite medical advice. In this study, we examine the feasibility of Ramadan fasting in adult patients with type 1 diabetes and define the frequency of acute complications in these patients during Ramadan. In this retrospective study, patients aged 18 years or older with type 1 diabetes who choose to fast during the month of Ramadan in the year 2015 were interviewed during their first visit after Ramadan, and their records were reviewed to determine the number of days in which patients broke the fast, frequency of hypoglycemia, hyperglycemia and diabetic ketoacidosis, and frequency of hospitalization during Ramadan. The 73 patients included in the study were able to fast an average of 26.8 days which corresponds to 92% of total fasting days. Hypoglycemia and hyperglycemia caused the patients to break the fast in 2.36 and 2.78% of the total fasting days, respectively.

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Keywords Type 1 diabetes · Ramadan · Fasting

Introduction

Ramadan is the ninth month of Islamic lunar calendar during which Muslims are required to observe an absolute fast by abstaining from eating and drinking from the dawn to the sunset. The length of the month varies between 29 and 30 days, and the length of the fasting hours usually varies between 12 and 18 h depending on the season (being longer in summer and shorter in winter) and the geographical latitude.

Ramadan fasting is usually a challenge for both Muslim subjects with diabetes and their health care providers. The challenge is even greater in subjects with type 1 diabetes than that in those with type 2 diabetes. Individuals with type 1 diabetes are at higher risk for developing hypoglycemia and diabetic ketoacidosis (DKA), and this risk is predicted to be greater during Ramadan fasting [1].

Few guidelines on managing patients with diabetes during Ramadan exist [2–4]. These guidelines categorize subjects with type 1 diabetes as very-high-risk subjects, and therefore, they are advised not to fast. However, since Ramadan fasting is considered one of the five pillars of Islam, most individuals with diabetes, including those with type 1 diabetes, insist on fasting.

Although available guidelines are mostly based on weak evidence, most of them are based on data from studies of type 2 diabetes. There are limited data on feasibility and safety of Ramadan fasting in individuals with type 1 diabetes.

In this study, we examine the feasibility of Ramadan fasting in adults with type 1 diabetes and define the frequency of acute complications in these subjects during Ramadan.

Method

In this retrospective study, patients with type 1 diabetes who choose to fast during the month of Ramadan in the year 2015 were interviewed during their first visit after Ramadan, and their records were reviewed. The subjects were on follow-up to diabetes clinic at the Benghazi Medical Center and Ibn Sina Clinic, Benghazi, Libya. Only adult subjects aged 18 years or more were included as this is the age range at which patients with type 1 diabetes are transferred from pediatric clinics to adult care. Data collection included demographic data, insulin doses, types and regimens before and after Ramadan, use of self-monitoring of blood glucose (SMBG), the level of HbA1c within 3 months of the start of Ramadan, number of days in which patient broke the fast, frequency of hypoglycemia, hyperglycemia and DKA, and frequency of hospitalization during Ramadan.

Data were analyzed using the SPSS Statistics (SPSS Inc., Chicago, USA) version 17.0. Discrete variables were expressed as numbers and percentages, and continuous variables as mean and standard deviation (SD).

Differences between categorical values were analyzed using the chi-squared test while the Student's *t* test was used for continuous values. A *p* value less than 0.050 was considered statistically significant.

Results

Clinical characteristics

The study included 73 subjects with type 1 diabetes who decided to fast during Ramadan. They were 31 (42.5%) males and 42 (57.5%) females. The subjects' ages ranged from 18 to 50 years with mean age of 31 years. The mean duration of diabetes in the study subjects was 11.6 years (range 1–28 years).

HbA1c measurements within 3 months before the start of Ramadan were available for 67 (91.8%) of the 73 subjects. The mean HbA1c in these subjects was 8.8% (range 5.8–14%). Only 9 (13.2%) subjects had HbA1c of ≤ 7.0% and 21 (30.9%) subjects had HbA1c of ≤ 8.0%.

Sixty-one (83.6%) subjects were on multiple daily injections (MDI) of basal-bolus regimen and 12 (16.4%) subjects were on twice-daily injections of a pre-mixed insulin. Of the 61 individuals who were on MDI, 38 subjects were on insulin analogs (aspart plus either detemir or glargine) and 23 individuals were on human insulin (regular insulin plus NPH). Thirty-nine (53.4%) subjects were using self-monitoring of blood glucose at home during the fasting hours while 34 (46.6%) subjects did not monitor their blood glucose during the fasting.

Insulin adjustment during Ramadan

Insulin dose was decreased in 52 (71.2%) subjects, increased in 6 (8.2%), and remained unchanged in 15 (20.6%) subjects.

Overall, the total insulin dose was decreased by an average of 16.6% (SD ± 17).

The basal insulin dose was decreased by an average of 13% (SD ± 18.7) and the bolus insulin dose was reduced by 18% (SD ± 29.7%).

Fast feasibility

On average, our subjects fasted 26.8 days. Of the 73 subjects included in the study, 43 (58.9%) subjects were able to complete the 29 days of fasting without complications.

Of the total 2117 fasting days (73 subjects × 29 days), our subjects fasted 1959 days (92.5%).

There was no statistically significant difference in the number of fasted days between males and females, those who were on insulin analogs and those who were on human insulins, those on basal-bolus regimens and those on pre-mixed insulin regimens, those with HbA1c ≤ 8.0% and those with HbA1c > 8.0%, and those who used SMBG and those who did not use SMBG (Table 1).

Causes of breaking the fast

Of the total 2117 fasting days (73 subjects × 29 days), our subjects broke the fast in 158 days (7.46%) because of different reasons including hypoglycemia, hyperglycemia, and ketoacidosis.

Overall, the subjects broke the fast in an average of 2.16 days (SD ± 4.17) during the month of Ramadan.

There was no statistically significant difference in the number of subjects developing acute complications (hypoglycemia, hyperglycemia, and ketoacidosis) necessitating breaking the fast according to sex, insulin type, insulin regimen, insulin dose change, HbA1c, and use of SMBG (Table 1).

Hospital admission was required in four subjects, two because of DKA and two because of other reasons (pilonidal sinus and renal problem).

Hypoglycemia

Hypoglycemia necessitating breaking the fast for 1 or more days occurred in 22 (30.1%) subjects. Out of the total 2117 fasting days, there was 50 days (2.36%) without fasting because of hypoglycemia.

Hyperglycemia

Hyperglycemia necessitating breaking the fast for 1 or more days occurred in ten (13.7%) subjects. Out of the total 2117

Table 1 Number of fasted days and frequency of acute complications in study subjects according to sex and clinical parameters

	Number of fasted days		Number of patients developing acute complications (%)	
Sex				
Males (n = 31)	27.5	p = 0.212	11 (35.5)	p = 0.402
Females (n = 42)	26.3		19 (45.2)	
Insulin type				
Analogs (n = 38)	26.3	p = 0.277	19 (47.5)	p = 0.221
Human (n = 35)	27.4		11 (33.3)	
Insulin regimen				
MDI (n = 61)	26.7	p = 0.601	26 (42.6)	p = 0.550
Twice daily (n = 12)	27.4		4 (33.3)	
Insulin dose				
Reduced (n = 52)	27.1	p = 0.692	23 (44.2)	p = 0.392
Not reduced (n = 21)	26.7		7 (33.3)	
HbA1c				
≤ 8.0% (n = 21)	26.8	p = 0.787	9 (42.9)	p = 0.723
> 8.0% (n = 52)	27.1		18 (38.3)	
Use of SMBG				
Yes (n = 39)	26.6	p = 0.721	19 (48.7)	p = 0.156
No (n = 34)	27		11 (32.4)	

HbA1c glycated hemoglobin, *MDI* multiple daily injections, *SMBG* self-monitoring of blood glucose

fasting days, there was 59 days (2.78%) without fasting because of hyperglycemia.

Diabetic ketoacidosis

Two (2.7%) subjects developed DKA during the month of Ramadan.

In these two subjects, there were identifiable precipitating factors for DKA (missing the basal dose in one patient and respiratory tract infection in the other).

Other reasons

Seven (9.6%) subjects had to break their fast for 1 or more days due to reasons other than hypoglycemia and hyperglycemia (most commonly gastrointestinal illnesses). Out of the total 2117 fasting days, there was 18 days (0.85%) without fasting because of these reasons.

Discussion

Current guidelines on management of subjects with diabetes during Ramadan fasting consider subjects with type 1 diabetes at high risk to fast in the holy month and therefore recommend them not to fast [2–4].

However, different studies, both in adult and pediatric populations, showed that significant number of subjects with type 1 diabetes can fast during Ramadan safely [5–7].

In our study, adult subjects with type 1 diabetes could fast most days of Ramadan without significant complications with almost 60% of them able to fast the whole month. Even in those who had to break their fasting, most of them did so for 5 days or less (85%) with only 11 (15%) subjects had to break their fast for more than 5 days.

On average, our subjects fasted for almost 27 days. This was greater than the average length of fasting in the EPIDAR study (23 days), a large population-based study of diabetes during Ramadan which was conducted in 13 countries [5]. Of note, all our subjects were managed by endocrinologists while the diabetes population in the EPIDAR study was a mixture of subjects treated by endocrinologists, general practitioners, and general internists [5].

Recommendations on managing diabetic subjects during Ramadan recommend that basal insulin dose should be decreased by about 20% during Ramadan in individuals with type 2 diabetes [1]. There is no similar consensus on the optimal dose change in those with type 1 diabetes. In a small study on the change of insulin requirement during Ramadan, the optimal recommendation is that insulin dose should be decreased by 15% during Ramadan [8].

This is comparable to the change in the insulin dose in our subjects which was reduced by an average of 16.6%. Dose reduction during Ramadan should be based on the level of glycemic control before the fasting month with poorly controlled subjects needing less dose reduction than those with good control.

Hypoglycemia was the most frequent reason for breaking the fast in our subjects with almost one-third of them had to break the fast for 1 or more days because of an episode of hypoglycemia. In the EPIDAR study, the overall incidence of hypoglycemia during Ramadan was very low mainly due to

restricting the definition of hypoglycemia to severe hypoglycemia requiring hospitalization which was increased by 4.7-fold [5].

In our study, we did not observe any episode of severe hypoglycemia requiring hospitalization. Similarly in a study including 23 adolescents with type 1 diabetes, no single episode of severe hypoglycemia was reported [7].

Severe hyperglycemia without DKA required ten of our subjects to break the fast for 1 or more days during the month of Ramadan. None of these subjects required hospitalization because of hyperglycemia.

Only two subjects developed DKA in our study, and in both of them, a precipitating factor was clearly identified. The American Diabetes Associations recommendations for management of diabetes during Ramadan consider subjects with type 1 diabetes at high risk for developing DKA during fasting [2, 3]. However, these recommendations were not based on outcome studies. In EPIDAR study, there was no significant increase in incidence of severe hyperglycemia and DKA during Ramadan compared with that before Ramadan [5]. In a study on incidence of DKA during Ramadan, there was no increase in the incidence or mortality from DKA during Ramadan compared with that in other months in the same lunar year [9].

Furthermore, in a study of 56 subjects with type 1 diabetes who are subjected to prolonged fasting (> 25 h), most of them could complete the fast successfully and no serious side effects were reported [10].

The feasibility of fasting in our subjects and the frequency of acute complications (hypoglycemia, hyperglycemia, and DKA) showed no statistical difference between subjects with HbA1c ≤ 8.0% and those with HbA1c > 8.0% and those who used SMBG and those who did not use SMBG. The small number of subjects may have led to the lack of statistical significance which is an issue of all similar studies in patients with type 1 diabetes owing to the relatively small number of this population compared to type 2 diabetes [6–8]. Therefore, despite this lack of difference, it is strongly recommended that subjects with type 1 diabetes should have good control before deciding to fast during Ramadan as well as be advised to use SMBG more frequently during this month, a recommendation that is endorsed by existing international management guidelines [2–4].

In conclusion, subjects with type 1 diabetes wishing to fast during the month of Ramadan can do so provided that their insulin dose is adjusted based on their pre-Ramadan glycemic control.

They should be educated about dose adjustment, SMBG, and possible complications before starting the fast and preferably be managed by an experienced endocrinologist.

Funding No funding was received.

Compliance with ethical standards

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

Ethical approval For this type of study, formal consent is not required; thus, verbal consent was obtained from all the subjects during the interview. The study did not include any active involvement of human subjects, and since there was no dedicated ethical review committee in the institution at the time of our study, we obtained ethical and administrative clearance from department of medicine at the institution which oversees the study clinics.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):195–201
DOI 10.1007/s13410-017-0576-z

ORIGINAL ARTICLE

The relationship between GRACE risk score and glucose fluctuation in patients with acute coronary syndrome and abnormal glucose metabolism

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Received: 16 May 2017 / Accepted: 11 August 2017 / Published online: 7 September 2017

Abstract The aim of this study was to determine the relationship between glucose fluctuation and global registry of acute coronary events (GRACE) risk score in patients with acute coronary syndrome (ACS) and abnormal glucose metabolism using continuous glucose monitoring system (CGMS). A total of 76 patients with ACS admitted into coronary care unit (CCU) were divided into two groups based on their blood glucose level and were further divided into subgroups based on GRACE risk score. A CGMS was used to real-time monitor blood glucose for 72 h in all patients after admitted into CCU. Twenty-four-hour mean blood glucose (24-h MBG) and 24-h mean amplitude of glycemic excursion (24-h MAGE) were calculated. Among the 76 patients, 52 patients had abnormal glucose metabolism. Among the 52 patients, there were 8 patients in low-risk group, 19 patients in moderate-risk group, and 25 in high-risk group. Among the 24 patients with normal glucose metabolism, there were 6 patients in the low-risk group, 6 patients in the moderate-risk group, and 12 in the high-risk group based on GRACE score. For patients with abnormal glucose metabolism, the 24-h MBG and 24-h MAGE of moderate- and high-risk groups were significantly higher than that of the low-risk group, which were not found in patients with ACS and normal glucose metabolism. High prevalence of abnormal glucose metabolism was found in ACS patients admitted into CCU and larger blood glucose fluctuation is associated with moderate and high GRACE risk scores in patients with ACS and abnormal glucose metabolism.

Keywords Acute coronary syndrome · Continuous glucose monitoring system · Glucose fluctuation · GRACE risk score

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Glucose metabolism disorders are closely associated with poor prognosis of patients with cardiovascular disease [1–3]. Acute coronary syndrome (ACS) is the most common risk status in patients with cardiovascular diseases, which have higher morbidity of abnormal glucose metabolism than the general population (25.2%) [4]. Kuhl et al. found that the incidence of abnormal glucose metabolism was 72% in Swedish patients with ACS [5]. EARLY ACS study, a multiple-center study targeting 8795 patients with ACS by Giraldez et al., found that the incidence of abnormal glucose metabolism could reach up to 55.5% [6]. China Heart Research Group investigated the prevalence of newly diagnosed abnormal glucose tolerance in 1328 patients with acute coronary syndrome and without known diabetes mellitus (DM) from 52 hospitals of 7 cities in China in 2005. It was found that abnormal glucose metabolism was seen in 887 (67%) of the 1328 patients [7]. These studies suggest that patients with ACS have high prevalence of abnormal glucose metabolism.

It is known that fasting blood glucose, blood glucose level at admission, and HbA1c are closely associated with

cardiovascular disease prognosis [2, 3, 8, 9]. More recently, studies have shown that there is a close relationship between intermittent high blood sugar and blood sugar fluctuations and oxidative stress, endothelial dysfunction, and atherosclerosis [10–14]. Currently, there are few studies that reported the relationship between blood glucose fluctuation and the prognosis in patients with ACS: it is unknown what the relationship between blood glucose fluctuation and GRACE risk score is and how blood glucose fluctuation in patients with normal and abnormal glucose metabolism affect GRACE score. To answer these questions, we employed continuous Glucose Monitoring System (CGMS) to determine glucose fluctuation in patients with ACS and abnormal glucose metabolism.

Subjects and methods

The study was approved by the ethics committee of Nanjing Hospital. Informed consents were signed by all patients.

Subjects

A total of 76 patients with ACS, admitted into coronary care unit (CCU) between July 2015 and January 2016 in Nanjing First Hospital, Nanjing Medical University, China, were recruited. Among them, 8 patients had unstable angina pectoris, 53 patients had ST segment elevation myocardial infarction (MI), and 15 patients had non-ST segment elevation MI. The exclusion criteria are the following: (1) Patient had history of mental illness and was not suitable for using the CGMS and (2) blood glucose > 22.2 mmol/L at admission.

Methods

Patient baseline characteristics and clinical data were collected by the same person throughout the study. Age, sex, histories of diabetes and hypertension, blood pressure, blood creatinine, HbA1C, fasting blood glucose, and fructosamine were measured and recorded.

A CGMS (Model REF-MMT 7102W, Medtronic MiniMed, USA) was used to real-time monitor blood glucose for 72 h after patient was admitted into CCU. The first 24-h data collected by CGMS were analyzed since ACS affects blood glucose significantly in the first 24 h during acute stage [15].

The CGMS data was collected and analyzed by the same nurse to record the incidence of hypoglycemia and to observe any regional adverse reaction and alarm from CGMS. The following parameters were calculated based on CGMS data:

1) Twenty-four-hour mean blood glucose (24-h MBG) and 24-h standard deviation of blood glucose (24-h SDBG).
2) Mean amplitude of glycemic excursion every 24 h (24-h MAGE).
3) Percentage of time hypoglycemia and hyperglycemia: the cut-off point for hypoglycemia is 3.9 mmol/L and for hyperglycemia is 10.0 mmol/L. The periods of hypoglycemia and hyperglycemia will be calculated and divided by 24 h. The area under curves (AUC) of blood glucose > 10.0 mmol/L and < 3.9 mmol/L were calculated [16].

All patients had standard diet and were treated according to standard ACS examination and treatment protocol. ACS patients with diabetes would continuously receive anti-diabetic treatment.

Seventy-six patients were further divided into two groups based on their glucose metabolism according to the 2010 American Diabetes Association (ADA) diagnostic criteria [17]: patients with abnormal glucose metabolism (n = 52, 68.4%) and normal glucose metabolism (n = 24, 31.6%). Patients with abnormal glucose metabolism include 1; patients who had history of diabetes, 2; newly diagnosed patients with HbA1c ≥ 6.5%, 3; and patients who had glucose intolerance with HbA1c 5.7–6.4%, 4. Acute stress hyperglycemia: though patients had no history of diabetes and glucose intolerance, they had FBG ≥ 7.0 mmol/L and/or random blood glucose ≥ 11.1 mmol/L. The normal glucose metabolism is defined as no history of diabetes and normal glucose tolerance with HbA1c < 5.7%.

The GRACE risk score is the sum of eight quantified parameters, including age, heart rate, systolic blood pressure, creatinine level, heart failure (Killip class), elevated cardiac enzyme levels, ST segment deviation, and cardiac arrest at admission (Table 1) [18–20]. By giving a score based on each of the parameters, we can make a risk score, which is useful for making predictions on in-hospital mortality and risk of death within the 6 months after discharge from the hospital, the long-term prognosis, and effect of interventional therapies [21].The higher the GRACE risk score, the higher the risk for patients to have adverse cardiovascular events. The patients were divided into three groups based on GRACE risk score [18, 19]: low risk, GRACE score ≤ 108; moderate risk, 109 ≤ GRACE score ≤ 140; and high risk, GRACE ≥ 141 (Table 1). Based on this, patients were also divided as low- (n = 14), moderate- (n = 25), and high- (n = 37) risk groups.

Statistical analysis

CGMS parameters were analyzed using CGMS Software 3.0 (Medtronic MiniMed). All statistical analyses were performed using the SPSS statistical program, version 20 (SPSS, Chicago, IL, USA). Data are presented as mean ± SD for continuous variables. χ^2 test was used to compare the categorical variables. Intergroup differences were tested with one-

Table 1 GRACE risk score system

Age (year)	Points	Heart rate (beats/min)	Points	SBP (mmHg)	Points	Creatinine (mg/dl)	Points	Killip class	Points	Other risk factors	Points
≤ 30	0	≤ 50	0	≤ 80	58	0–0.39	1	I	0	Elevated cardiac enzyme levels	14
30–39	8	50–69	3	80–99	53	0.4–0.79	4	II	20	ST segment deviation	28
40–49	25	70–89	9	100–119	43	0.8–1.19	7	III	39	Cardiac arrest at admission	39
50–59	41	90–109	15	120–139	34	1.2–1.59	10	IV	59		
60–69	58	110–149	24	140–159	24	1.6–1.99	13				
70–79	75	150–199	38	160–199	10	2–3.99	21				
80–89	91	≥ 200	46	≥ 200	0	> 4.0	28				
≥ 90	100										

SBP systolic blood pressure

way analysis of variance (ANOVA). Results identified as significant via ANOVA were re-analyzed with the LSD correction. $p < 0.05$ was considered significant.

Results

Patients baseline characteristics

The clinical characteristics and demographics of all the subjects are shown in Table 2. Among the 76 patients, 8 patients had unstable angina (10.5%), 53 patients had ST segment elevation myocardial infarction (69.7%), and the remaining 15 patients had non-ST segment elevation myocardial infarction.

According to 2010 American Diabetes Association (ADA) diagnostic criteria [17], 24 patients had history of diabetes (31.6%), 8 patients were newly diagnosed (10.5%), 12 patients had glucose intolerance (15.8%), and 8 patients had acute stress hyperglycemia (10.3%). The remaining 24 patients had normal glucose metabolism.

According to GRACE score, the low-risk group had 14 patients, the moderate-risk group had 25 patients, and the high-risk group had 37 patients (Table 3).

The association between glucose fluctuation and GRACE risk score

It was found that 24-h MBG, 24-h MAGE, and AUC of blood glucose >10 mmol/L of moderate- and high-risk groups were significantly higher than those of the low-risk group ($p < 0.05$). There was no significant difference found in the incidence of hypoglycemia episode. The percentages of patients that experienced hypoglycemia episodes were: low-risk group = 35.7% (5/14), moderate-risk group = 24% (6/25), and high-risk group = 8.1% (3/37), $p > 0.05$. Similarly, no significant differences were found in AUC of BG < 3.9 mmol/L and percentage of BG < 3.9 mmol/L among groups (Table 3).

Though 24-h MBG, 24-h MAGE, and AUC of the high-risk group were higher than those of the moderate group, no significant difference was found between them (Table 3). To determine the relationship between MAGE and GRACE risk score, we performed χ^2 test and found that higher MAGE (≥ 3.9 mmol/L) is associated with higher GRACE risk score (Table 4), indicating patients with high MAGE have a trend to have high GRACE risk score.

Table 2 Characteristics of patients

Cases	76
Male/female (*n*)	57/19
Age (years)	63.25 ± 12.57
Systolic blood pressure (mmHg)	142.53 ± 20.50
LVEF (%)	52.19 ± 10.75
Urea nitrogen (mmol/L)	7.23 ± 3.81
Creatinine (μmol/L)	79.32 ± 74.44
Admission blood glucose (mmol/L)	10.56 ± 5.59
Fructosamine (μmol/L)	248.71 ± 69.64
HbA1c (%)	6.77 ± 2.00
Hemoglobin (g/L)	140.92 ± 19.46
CK (U/L)	661 ± 703.50
CK-MB (U/L)	79.32 ± 74.44
Uric acid (μmol/L)	369.53 ± 115.59
Fibrinogen (g/L)	3.01 ± 1.08
Cholesterol (mmol/L)	4.46 ± 1.16
Triglycerides (mmol/L)	1.80 ± 1.12
HDL cholesterol (mmol/L)	1.06 ± 0.34
LDL cholesterol (mmol/L)	2.87 ± 1.01

LVEF left ventricular ejection fraction, *HbA1c* glycosylated hemoglobin, *CK* creatine kinase, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

Table 3 The relationship between GRACE risk score and blood glucose in patients with ACS

Risk groups (cases)	Low-risk group (n = 14)	Moderate-risk group (n = 25)	High-risk group (n = 37)
Blood glucose (mmol/L) at admission	7.89 ± 4.36	10.66 ± 4.48	11.51 ± 6.41*
Fructosamine (μmol/L)	230.62 ± 64.73	246.68 ± 65.10	257.10 ± 75.62
HbA1c (%)	6.29 ± 1.69	7.04 ± 2.05	6.78 ± 2.09
24-h MBG (mmol/L)	6.59 ± 1.62	8.20 ± 2.60*	8.50 ± 2.87*
24-h MAGE (mmol/L)	2.60 ± 1.34	4.18 ± 2.52*	4.51 ± 3.98*
24-h SDBG (mmol/L)	1.19 ± 0.52	1.63 ± 0.91	1.75 ± 1.37
AUC of BG < 3.9 mmol/L	0.04 ± 0.08	0.02 ± 0.07	0.01 ± 0.04
AUC of BG > 10 mmol/L	0.14 ± 0.45	0.68 ± 1.04*	0.91 ± 1.60*
BG < 3.9 mmol/L (%)	0.05 ± 0.07	0.03 ± 0.07	0.01 ± 0.04
3.9 ≤ BG ≤ 10 mmol/L (%)	0.87 ± 0.23	0.71 ± 0.30	0.73 ± 0.31
BG > 10 mmol/L (%)	0.08 ± 0.23	0.27 ± 0.31*	0.26 ± 0.32*

*vs low risk group: $p < 0.05$

24-h MBG 24-h mean blood glucose, *24-h MAGE* mean amplitude of glycemic excursion every 24 h, *24-h SDBG* 24-h standard deviation of blood glucose, *BG* blood glucose; *AUC* area under curve

The association between glucose metabolism and GRACE risk score

We analyzed the CGMS data of patient groups: previously known diabetes, newly diagnosed diabetes, abnormal glucose tolerance, stress hypoglycemia, and normal glucose metabolism (Table 5). Twenty-four-hour MBG, MAGE, AUC of BG > 10 mmol/L, AUC of BG between 3.9 and 10 mmol/L, and AUC of BG < 3.9 mmol/L were significantly bigger or higher in patient groups of previously known diabetes and newly diagnosed diabetes as compared with those in patient groups with abnormal glucose tolerance, stress hyperglycemia, and normal glucose metabolism.

Among 52 patients with abnormal glucose metabolism, 8, 19, and 25 patients were in low-, moderate-, and high-risk groups, respectively (Table 6). Twenty-four-hour MBG and 24-h MAGE increased with increasing GRACE risk scores. Both moderate- and high-risk groups had significantly higher 24-h MBG and 24-h MAGE than those of the low-risk group. No difference was found between moderate- and high-risk groups.

Among 24 patients with normal glucose metabolism, 6, 6, and 12 patients were in low-, moderate-, and high-risk groups, respectively (Table 7). No difference was found between any two groups.

Table 4 Cases of patients with MAGE < 3.9 or ≥ 3.9 in three risk groups

Risk groups (case)	MAGE (mmol/L)		p
	< 3.9	≥ 3.9	
Low-risk group	12	2	
Moderate-risk group	11	14	0.038
High-risk group	20	17	

MAGE mean amplitude of glycemic excursion

Discussion

The findings of the current study suggested bigger blood glucose fluctuation is associated with moderate and high GRACE risk scores in patients with ACS and abnormal glucose metabolism. However, glucose fluctuation is in normal range (24-h MBG is < 6.5 mmol/L and 24-h MAGE is < 3.9 mmol/L) [22, 23] even in patients with moderate and high GRACE risk scores.

It was found that the prevalence of abnormal glucose metabolism was as high as 68.4% which is significantly higher than that of the patients in ordinary wards of the Department of Cardiology of the Hospital (37.5%) [24]. This is consistent with studies reported by Kuhl et al. [5], and Yan et al. [7]. They found that the prevalence of abnormal glucose metabolism reached up to 72 and 67% in Sweden and Chinese ACS patients, respectively. These indicate that ACS patients in CCU (coronary care unit) are more vulnerable to abnormal glucose metabolism which might be associated with stress-induced hyperglycemia. The mechanisms of stress-induced hyperglycemia were caused by an inflammatory and adrenergic response to ischemic stress [25, 26].

It is known that glucose metabolism disorders are closely correlated with the prognosis of the patients [1–3]. A study by Kosiborod et al. [2] investigated 141,680 patients with myocardial infarction (MI) in hospital and found that there is a close correlation between elevation of blood glucose and the mortality of elderly patients with MI, especially the patients with no diabetic history. Cakmak et al. [3] performed a follow-up study on 100 patients with MI and suggested that blood

Table 5 CGMS data of patient groups based on their glucose metabolic conditions

Group	Previously known diabetes	Newly diagnosed diabetes	Abnormal glucose tolerance	Stress hyperglycemia	Normal glucose metabolism
Cases	24	8	12	8	24
24-h MBG (mmol/L)	10.54 ± 2.65	9.31 ± 2.47	7.09 ± 1.33*#	6.65 ± 1.59*	6.08 ± 0.61*
MAGE (mmol/L)	6.66 ± 3.66	5.47 ± 2.93	2.90 ± 1.86*#	2.65 ± 1.86*	2.01 ± 1.36*
AUC of BG > 10 mmol/L	1.75 ± 1.75	1.05 ± 1.16	0.12 ± 0.22*#	0.05 ± 0.11*	0.00 ± 0.02*
AUC of BG (3.9–10) mmol/L	1.17 ± 1.16	1.74 ± 1.50	2.99 ± 1.18*#	3.38 ± 1.47*	3.89 ± 0.60*
AUC of BG < 3.9 mmol/L	0.03 ± 0.07	0.00 ± 0.00	0.03 ± 0.07	0.03 ± 0.07	0.02 ± 0.05

*vs previously known diabetes and newly diagnosed diabetes groups, $p < 0.05$

vs normal glucose metabolism group, $p < 0.05$

glucose and HbA1c at the time of admitting were related to the mortality of patients with MI.

The hyperglycemia, especially the blood glucose fluctuation plays an important role in the development of atherosclerosis and acted as independent hazards for cardiovascular diseases and correlates with MI patients' prognosis, myocardial remodeling, and rupture of blood vessel plaques [27–29]. To be more comprehensive and accurate in monitoring glucose concentration, the CGMS is employed in this study to real-time monitor blood glucose levels. The CGMS can continuously record patients' blood glucose concentration every 5 minutes. The data derived from CGMS include 24-h MAGE, 24-h SDBG, and MODD (mean of daily differences) etc., and MAGE is generally accepted as a golden criterion for determining blood glucose fluctuations [22].

A follow-up study by Wang et al. on MACE (major adverse cardiac events) incidences of patients with MI suggested that those who had MACE had higher 24-h MAGE (gibber glucose fluctuation) than those who did not have MACE [30]. *Bigger glucose* fluctuation increases mortality of patients [31]. Thus, decreased blood glucose fluctuation is also considered as a sign of better blood glucose control [31–33].

In the present study, we applied CGMS to 76 ACS patients for 72 h and divided them into 3 groups based on GRACE score. We found that 24-h MAGE of 3 groups were 2.60 ± 1.34 mmol/L, 4.18 ± 2.52 mmol/L, and 4.51 ± 3.98 mmol/L, indicating that patients with moderate and high GRACE scores had larger glucose fluctuation as compared with patients with low GRACE score. This is partially in agreement with other studies [27, 34]. Su et al. studied 222 patients with acute MI and found that patients with 24-h MAGE ≥ 3.9 mmol/L had higher GRACE risk score than patients with 24-h MAGE < 3.9 mmol/L [27]. Another study investigated 186 elderly Chinese patients with MI and found that patients with high 24-h MAGE had high GRACE risk score and found correlation between 24-h MAGE level and GRACE risk score [34]. These findings suggest that patients with higher GRACE are associated with bigger glucose fluctuation and need an intensive anti-diabetic therapy, which will reduce mortality and improve the prognosis in patients with acute MI. Zhang et al. studied 237 patients with MI and found that higher 24-h MAGE led to significant increase in the incidence of combined MACE and non-infarct-related vascular remodeling in patients with ACS and diabetes, which was not found in patients with ACS only [15]. Krinsley et al. performed a study including 4084 ICU patients and found that improved blood glucose fluctuation was positively correlated with the patients' mortality in non-diabetic patients after doing a multiple regression analysis. However, this was found in patients with diabetes [35]. *Thus*, *bigger glucose* fluctuation is associated with high mortality and poor prognosis in patients with ACS and diabetes.

Table 6 The relationship between blood fluctuation and GRACE risk score in the patients with ACS and abnormal glucose metabolism

Risk groups	Case	24-h MBG (mmol/L)	24-h MAGE (mmol/L)	24-h SDBG (mmol/L)
Low-risk group	8	6.88 ± 2.09	3.08 ± 0.78	1.33 ± 0.58
Moderate-risk group	19	8.99 ± 2.50*	4.94 ± 2.31*	1.94 ± 0.80
High-risk group	25	9.60 ± 2.88*	5.64 ± 4.30*	2.18 ± 1.43

*vs low risk group $p < 0.05$

24-h MBG 24-h mean blood glucose, *24-h MAGE* mean amplitude of glycemic excursion every 24 h, *24-h SDBG* 24-h standard deviation of blood glucose

Table 7 The relationship between blood glucose fluctuation and GRACE risk score in patients with ACS and normal glucose metabolism

Subgroup	Cases	24-h MBG (mmol/L)	24-h MAGE (mmol/L)	24-h SDBG (mmol/L)
Low-risk group	6	6.20 ± 0.63	1.96 ± 0.78	1.0 ± 0.39
Moderate-risk group	6	5.72 ± 0.56	1.75 ± 1.45	0.63 ± 0.27
High-risk group	12	6.21 ± 0.60	2.16 ± 1.60	0.84 ± 0.57

24-h MBG 24-h mean blood glucose, *24-h MAGE* mean amplitude of glycemic excursion every 24 h, *24-h SDBG* 24-h standard deviation of blood glucose

In the present study, ACS patients were also divided into 2 groups based on blood glucose: the abnormal glucose metabolism group and the normal glucose metabolism group. In the abnormal glucose metabolism group, the 24-h MAGE values of the low-, moderate-, and high-risk groups were 3.08 ± 0.78 mmol/L, 4.94 ± 2.31 mmol/L, and 5.64 ± 4.30 mmol/L, respectively. The 24-h MAGE value of the low-risk group was in normal range, while those of the moderate- and high-risk groups were significantly higher than that of the low-risk group. However, the 24-h MAGE values of low-, moderate-, and high-risk groups were 1.96 ± 0.78 mmol/L, 1.75 ± 1.45 mmol/L, and 2.16 ± 1.60 mmol/L, respectively, in patients with the normal glucose metabolism, indicating that glucose fluctuation is not a risk factor in patients with ACS.

There are several limitations inherent in this study. First, the sample size is relatively small. Second, we did not determine the effect of micro-vascular complication on glucose fluctuation. Third, we did not register major adverse cardiac event (MACE) in this patient cohort.

In conclusion, the findings of the current study suggested that high prevalence of abnormal glucose metabolism was found in ACS patients admitted into CCU. Bigger blood glucose fluctuation is associated with moderate and high GRACE risk scores in patients with ACS and abnormal glucose metabolism. However, glucose fluctuation is in normal range even in patients with moderate and high GRACE risk scores.

Acknowledgements The study was supported by the fund of Scientific and Technological Development Program of Jiangsu Province of China (BL2014010).

Compliance with ethical standards

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):202–208
DOI 10.1007/s13410-017-0579-9

ORIGINAL ARTICLE

Changes in vascular structure in diabetic patients after 8 weeks aerobic physical exercise: a randomized controlled trial

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Received: 22 January 2017 / Accepted: 14 August 2017 / Published online: 7 September 2017

Abstract Diabetes is a highly prevalent metabolic disease with macrovascular and microvascular complications. Insulin resistance plays a major role in the pathogenesis of atherosclerosis in type 2 diabetes patients. The risk of atherosclerosis progress in diabetes is of 2–4 orders of magnitude**.** Early atherosclerosis can be detected by carotid intima-media thickness (cIMT) ultrasonography. There is an inverse relationship between intima-media thickness and physical activity. The aim of this study was to investigate the effects of an 8-week aerobic exercise program on the vascular structure in different segments of the carotid artery. This study was a randomized control trial. Thirty individuals were selected out of 642 volunteers who fulfilled the inclusion criteria. They were randomly divided into aerobic exercise and control groups by a block randomization method. This study was carried out during May–October 2016 in Iran. The aerobic protocol comprised of 24 sessions of aerobic exercise on a treadmill for 30 min per session, 3 days a week. The intensity of the training protocol was 50–70% of the patient's max heart rate. Measurements of vascular parameters were evaluated by the same person, before and after the 24-session intervention. There were no significant differences between anthropometric, sex, age, diabetic history, and cardiac ejection fraction, compared to baseline characteristics. Intima-media thickness (0.52 ± 0.12), intima-media/lumen (0.07 ± 0.02) in bulb carotid, common carotid, and internal carotid, as well as bulb wall, were reduced significantly in the training group compared to the control group after 8 weeks ($p < 0.05$). Twenty-four sessions of aerobic exercise improved vascular parameters in type 2 diabetics.

Keywords Exercise · Diabetes mellitus · Carotid intima-media thickness

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Introduction

Diabetes is a highly prevalent metabolic disease, growing in epidemic proportions. It was estimated in 2013 that diabetes affected at least 347 million individuals (9.2–9.8% of the total world population) [1]. This disorder is caused by insulin resistance, hyperglycemia, and vascular dysfunction, which is an independent risk factor for vascular disease. Reductions in nitric oxide, as well as increased amount of reactive oxygen species, result in serum lipids and glucose increasing in type 2 diabetes, which leads to impaired endothelial-dependent vasodilation. This issue, in turn, causes an increased vascular smooth muscle contractile response and ultimately leads to atherosclerosis [2].

Atherosclerosis is the main cause of death in cardiovascular disease around the world [3]. Insulin resistance is an important cause in the pathogenesis of atherosclerosis in patients with type 2 diabetes [4]. The progression of atherosclerosis is 2–4 times higher for those who are suffering from diabetes [5]. Unlike microvascular complications, macrovascular disease is associated with hyperglycemia and the duration of the patient's

diabetes. Therefore, diagnosing macrovascular complications is difficult before the development of glycemic symptoms. In many studies, early atherosclerosis is detected by carotid intima-media thickness ultrasonography [6]; due to the fact that the severity of atherosclerosis is correlated with the thickness of carotid intima-media [7], it is a useful clinical marker [8]. Preclinical atherosclerosis is associated with an increase in the amount of common carotid, internal carotid, and carotid bulb artery thickness [9]. Moreover, the rate of increased carotid intima-media thickness in type 2 diabetics is two times higher than in non-diabetic individuals [10]. Carotid intima-media thickness has a higher ability to predict strokes than other factors, such as carotid plaque [11]. A 0.1-mm increase in carotid intima-media thickness is associated with a 10–15% increase in risk of myocardial infarction and a 13–18% increase in the risk of developing a stroke [3].

Intima-media thickness is decreased by taking drugs such as amlodipine and pravastin, as well as by quitting smoking, consuming fiber, reducing cholesterol intake by 100 mg/day, and regular exercise [12]. Due to the adverse side effects of drugs and the various advantages of exercise, physical activity has been recommended in multiple guidelines [13]. It has been variously reported that vascular structure is modified by aerobic exercise over different periods of practice, e.g., 6 months, 1 year, 2 years, and 4 years (Kim et al. [14]; Anderssen et al. [15]; Okada et al. [16], and Wildman et al. [17], respectively). More than half of cardiovascular risk factors are reduced by the positive effects of exercise [18]. Functional arterial adaptations are one of the primary results of exercise, and if it continues, structural adaptations will occur [7]. Interestingly, doing exercise increases endothelial functional improvement [18] by 2.23% and also changes peripheral and carotid arterial thickness [7]. There is an inverse relationship between carotid wall thickness and physical activity in type 2 diabetes patients [19], and also in hypertension patients [9, 20]. It has been shown that exercise is more efficient than diet in improving vascular performance [21]. Despite numerous documents confirming the effectiveness of exercise in reducing intima-media thickness in diabetics, patients often ignore it as a treatment therapy. One of the possible reasons for this might be due to the prolonged duration of exercise sessions. Although this study is not the first one to explore the area, it takes into account the short-term effects of exercise on vascular structure. In aiming to examine the effects of short-duration exercise, the effects of 24 sessions of aerobic exercise on vascular parameters were measured.

Materials and methods

Subjects

This study was conducted in parallel as a randomized control trial (RCT). Volunteer diabetic patients were recruited from all diabetic centers and specialized and general governmental hospitals, through advertisements. The study was performed from May–October 2016 in the Sheikhorreis Rehabilitation Center, Hamedan University of Medical Sciences, Iran. It was approved by the Human Ethics Committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RETECH.REC.1395.577). The study was registered as a clinical trial study on the Iranian Registry of Clinical Trials (registration ID in IRCT: IRCT2016121831443N1, www.irct.ir). Six hundred and twenty-four individuals volunteered to participate in this study. Inclusion criteria included 2–10 years duration of diabetes, 40–60 years old, male and female, HbA1c value of 6–10%, BMI (body mass index) 20–30 kg/m^2, inactive lifestyle (less than 30 min exercise per week), non-smoking, non-alcoholic, non-insulin user, and no history of the following: cardiovascular, musculoskeletal, neurological, and metabolic disorders. Exclusion criteria consisted of absence for two successive sessions, respiratory problems during exercise, and reluctance in following the protocol for the aerobic group. After being screened by telephone and having an interview with laboratory and endocrinology specialists, the participants underwent cardiac examinations (echocardiography and exercise test) carried out by a cardiologist to exclude patients with cardiac problems. The sample size allowed the study to detect the effects of exercise with a 95% confidence interval. In each group were 12 individuals, calculated according to criteria laid out by Meyer et al. [21]. Sample size requirements were as follows: M = 1.3, SD = 1.8, effect size (delta) = 1.088. Out of 624 individuals, 30 volunteers that passed inclusion criteria were entered into this study. A block randomization method was designed to randomize subjects into the aerobic group and control group, with an allocation ratio of 1:1. Participants were asked not to change their consumption of prescription drugs, and lifestyle factors. The control group completed 8 weeks without intervention. Figure 1 shows the stages of this study in consort diagram.

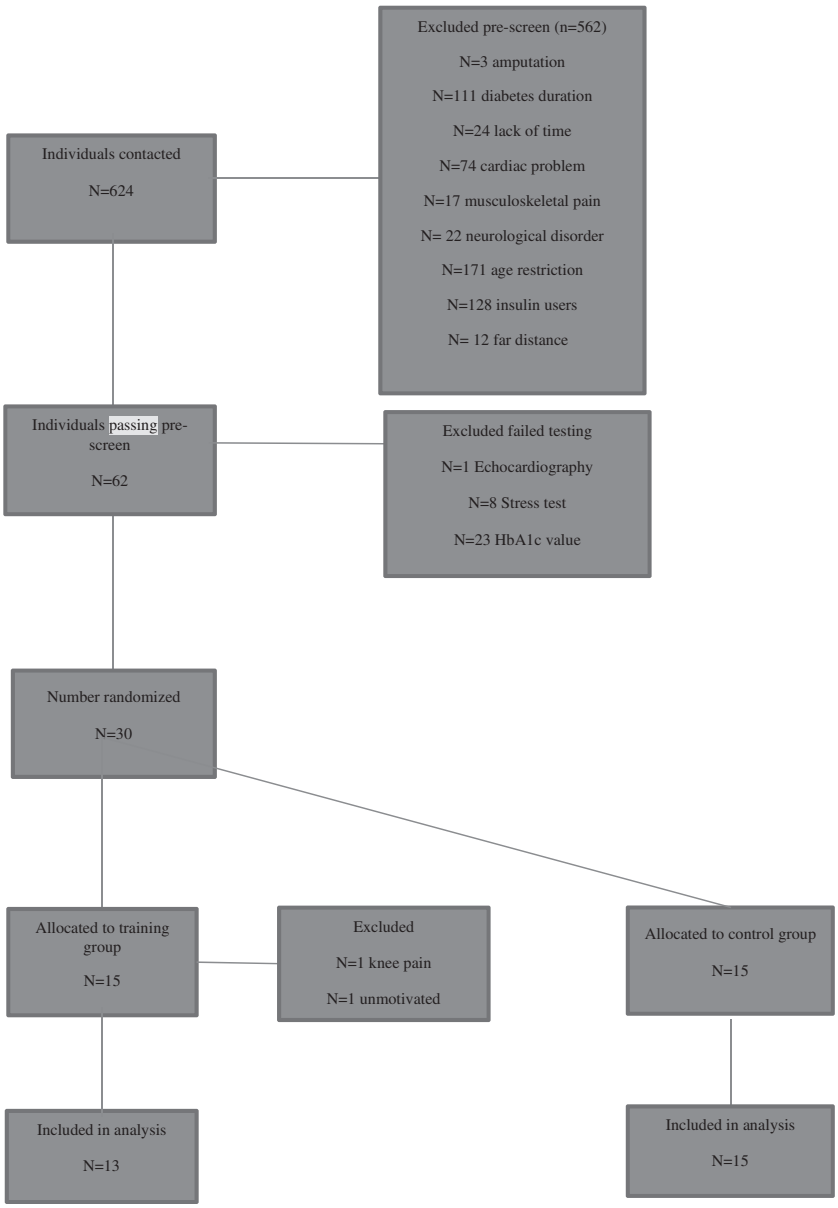
Intervention

The aerobic protocol included 24 sessions (three sessions a week for a total of 8 weeks) of aerobic exercise on a treadmill (Motorized Treadmill®, USA) with zero incline, with each session lasting 30 min. The intensity of the training protocol was set at 50–70% of maximum heart rate, estimated from the Bruce protocol test. The target heart rate was calculated from the Karvonen formula.

$$HR_{target} = HRrest + 50{-}70\%(HR\max{-}HRrest)$$

Exercise intensity was gradually increased during the 8 weeks of the study. Diastolic and systolic blood pressure and blood glucose were measured during the exercise

Fig. 1 CONSORT diagram



period (Omron digital pressure® RS2 and Accu-Check glocometer® performa, respectively). If the patient's blood pressure was more than 160/90 mmHG, the patient was instructed to sit and rest for 10 min. However, if there was no change in blood pressure, training was not performed. Also, 15 g carbohydrate was consumed if the glucose level was below 100 mg/dl. Blood glucose was measured again after 20–30 min, and exercise was only resumed if the blood sugar was higher than 100 mg/dl. It should be noted that if the patient's blood sugar was higher than 250 mg/dl, exercise was not commenced. Heart rate was monitored throughout the training program using a digital heart rate meter, placing the belt around the chest and wearing a wrist-strapped heart rate monitor (Beurer® PM60, Germany).

Measurements

Measurements of vascular parameters (intima-media thickness, wall thickness (intima, media, and adventitia), lumen thickness, and intima-media/lumen ratio in the right carotid bulb, internal carotid, and common carotid) were carried out in a supine position with a 45° rotation of the head to the opposite direction, by non-invasive B-mode sonography (Mindray® DC7, China) before and after the 24 sessions [3]. Its validity and reliability have been proven in various labs [22].

Statistical analysis

All data were analyzed using SPSS software package, version 16. Data normality was checked by the Kolmogorov-Smirnov

Table 1 Baseline characteristics of the aerobic and control groups (mean ± SD)

Variable	Training group (n = 13)	Control group (n = 15)	p value
Age	48.31 (5.02)	48.60 (4.80)	0.876
Height (cm)	165.92 (7.84)	166.66 (6.95)	0.792
Weight (kg)	75.80 (13.64)	75.03 (9.91)	0.862
BMI(kg/m2)	27.40 (3.65)	26.93 (2.42)	0.692
EF max (%)	56.15 (2.23)	56.00 (2.07)	0.850
EF min (%)	53.08 (3.25)	53.67 (2.29)	0.580
Disease duration (year)	4.61 (2.14)	5.33 (1.99)	0.366

EF ejection fraction, *BMI* body mass index

test. Independent t test was used to compare data in two groups. Paired t test was used to determine the differences between variables before and after 24 intervention sessions in each group. The alpha level was set at .05.

Results

Thirty patients with type 2 diabetes were part of this study. Two patients were excluded due to their incapability of completing the treatment after 12 sessions. Finally, 28 patients (control = 15 and aerobic group = 13) completed the study. Table 1 shows the basic characteristics of the control group and the aerobic group. The results showed that there were no significant differences at baseline in anthropometrics, age, duration of diabetes, and cardiac ejection fraction variables ($p > 0.05$).

Vascular values before and after 8 weeks are shown in Table 2. The intima-media/lumen thickness measurements in three areas are presented (Figs. 2, 3, and 4).

After 8 weeks, intima-media thickness, intima-media/lumen in the bulb carotid, common carotid, and internal carotid, as well as bulb wall, were reduced significantly in the training group and between the two groups.

Discussion

This study evaluated the intima-media thickness, wall, lumen and intima-media/lumen ratio (wall thickness normalized [23]) of the carotid bulb, internal carotid, and common carotid in type 2 diabetic patients. Reduced intima-media thickness and IMT/lumen in the carotid bulb, internal carotid, common carotid, and bulb wall were reported in the aerobic group compared to the control group after 24 sessions of aerobic training.

Some authors have confirmed the effect of diabetes on intima-media thickness. Increasing intima-media thickness resulted in vascular structural changes and, consequently, changes in atherosclerosis in type 2 diabetes [24]. It has been revealed that physical activity reduced carotid intima-media thickness and atherosclerosis in diabetic patients [25]. The mechanism of decreasing intima-media carotid thickness by

Table 2 Ultrasonography parameters in aerobic and control groups before and after intervention (mean ± SD)

Variable carotid(mm)	Training group		p value	Control group		p value	p value between two group
	Pre	Post		Pre	Post		
Bulb intima-media	(0.16) 0.60	(0.12) 0.52	0.001	(0.17) 0.66	(0.25) 0.75	0.118	0.05
Bulb wall	(0.24) 1.09	(0.21) 1.01	0.002	(0.17) 1.17	(0.35) 1.25	0.305	0.227
Bulb lumen	(0.50) 7.24	(0.54) 7.20	0.356	(0.97) 7.35	(1.04) 7.51	0.156	0.034
Bulb IMT/lumen	(0.02) 0.08	(0.02) 0.07	0.000	(0.03) 0.09	(0.04) 0.10	0.146	0.002
Common intima-media	(0.12) 0.49	(0.10) 0.45	0.008	(0.24) 0.59	(0.25) 0.61	0.271	0.359
Common wall	(0.16) 0.97	(0.15) 0.90	0.002	(0.36) 1.09	(0.35) 1.10	0.433	0.299
Common lumen	(0.59) 5.68	(0.66) 5.68	1.000	(0.72) 6.03	(0.54) 5.93	0.145	0.987
Common IMT/lumen	(0.02) 0.09	(0.02) 0.08	0.15	(0.03) 0.1	(0.04) 0.1	0.160	0.358
Internal intima-media	(0.11) 0.45	(0.09) 0.39	0.001	(0.09) 0.48	(0.07) 0.49	0.433	0.740
Internal wall	(0.21) 0.88	(0.17) 0.81	0.044	(0.03) 0.09	(0.04) 0.10	0.670	0.318
Internal lumen	(0.80) 4.57	(0.65) 4.38	0.193	(0.69) 4.53	(0.63) 4.45	0.262	0.814
Internal IMT/lumen	(0.02) 0.1	(0.01) 0.08	0.003	(0.02) 0.1	(0.02) 0.1	0.568	0.134

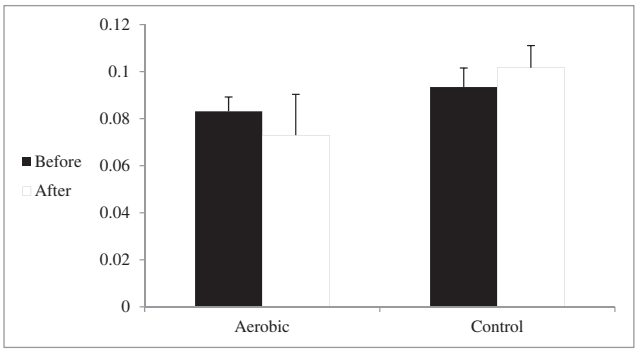


Fig. 2 Intima-media thickness/lumen ratio changes of carotid bulb artery in aerobic and control groups before and after intervention (mean ± SE)

exercise is through increased nitric oxide bioavailability due to reduced oxidative stress and increased improvement in the antioxidant defense system [2], weight loss, reduced insulin resistance, lipid profile control, improved endothelial function, and increased aerobic capacity [10], balance between vasoconstriction and vasodilatation [26], inflammation reduction, resting sympathetic activity reduction, reduced vascular tone, increased artery wall stretching due to increased blood pressure during exercise, and increased shear stress [7].

However, there are inconsistent results regarding the effect of exercise on carotid intima-media thickness changes. There are studies in which the effect of exercise and diet on carotid intima-media thickness was examined [14–17, 27]. The results of these studies proved the positive influence of dieting and exercising at different intensities: 60–70% max HR [27], 12–14 RPE [14], and increased consumption of up to 1400–1500 kcal/week [17]. There are different follow-up programs (e.g., 1 year [15], 6 months [14], 2 years [16], and 4 years [17]) which have been applied to monitor any reduction in atherosclerosis symptoms. It has rarely been reported that common carotid and bulb carotid parameters did not change in patients with hypertension after exercise [15]. Some other studies also employed type 2 diabetic patients, similar to this study [14]. Carotid structural change is slower and requires more time for older people [28]; therefore, vascular changes were observed after six months, due to the fact that participants were older on an average, compared to this study [14]. In the current study, the mean age of participants was lower; therefore, aerobic exercise resulted in a change in the vascular structure just after 24 sessions.

Different studies have documented contradictory results regarding the effects of exercise on the cardiovascular parameters of healthy people after training. According to Tanaka et al. [29], one of the first studies about exercise on cIMT (Thijssen et al. [7]), 3 months of aerobic exercise with an intensity of 60–75% max HR did not change IMT and lumen/intima-media ratio in healthy individuals [29]. In a study by Thijssen [23], cycling at 65–85% HRR changed femoral artery wall thickness in healthy men, but did not cause changes in the carotid and brachial. A reduction of intima-media carotid bifurcation thickness in healthy people, who did not use statins after three years, with low to moderate intensity aerobic exercise was also reported (Rauramaa et al. [28]). Age is one of the reasons for the long-term effects of exercise on cIMT. In one study, a positive effect of training on decreasing cIMT in older individuals was demonstrated. The subjects were all postmenopausal healthy women, divided according to receiving estrogen and exercise. Reduced carotid thickness was observed in the training group over the age of 65, who received hormones [30]. It should be noted that no intervention was carried out in that study, and the level of exercise intensity was not clearly determined. It might be possible that if the exercise intensity was determined and carried out under supervision, the influences of training could also be observed in younger individuals. Another research exhibited that 6 months of aerobic exercise intervention reduced intima-media thickness in 67 children (aged from 11 to 16 years) [21]. Moreover, there was a reduction in intima-media thickness after 6 months of exercise intervention with a 55–65% VO_2 max intensity in obese children (6–11 years) [31]. On the other hand, there were no reported changes in the common carotid artery thickness with plaques and reduced thickness in patients without plaques, after 12 months of a combination of aerobic and resistance exercise, with an intensity of RPE ≥ 15, in type

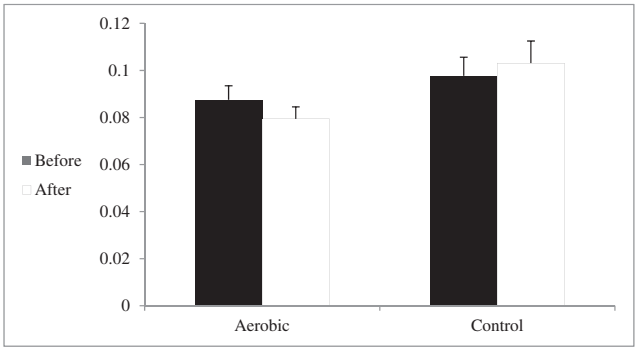


Fig. 3 Intima-media thickness/lumen ratio changes of common carotid artery in aerobic and control groups before and after intervention (mean ± SE)

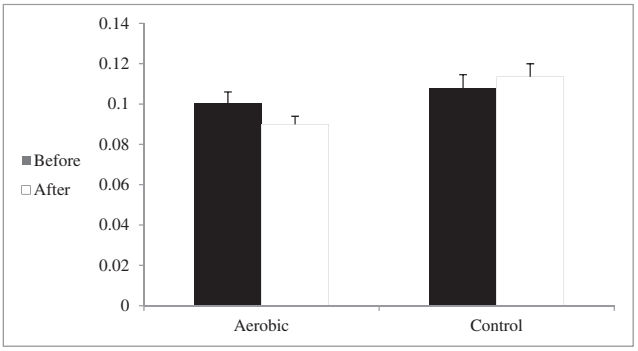


Fig. 4 Intima-media thickness/lumen ratio changes of internal carotid artery in aerobic and control groups before and after intervention (mean ± SE)

2 diabetic people and CAD [25]. Similarly, our results are in line with Byrkjeland's et al. results with regard to patients without plaques [25]. Perhaps the ineffectiveness of exercise on cIMT could be attributed to the use of statins as a medication. It has been proven that statins block the effects of exercise on cIMT. Additionally, some medications such as aspirin, metformin, and calcium channel blockers, if taken with Glybanglamyd, will independently reduce cIMT [25].

The positive results of 24 aerobic exercise sessions on some vascular parameters in this study are important clinical findings. It was proven that the thickening of the artery walls reduces arterial compliance and increases peripheral and systemic vascular resistance, which consequently increases heart disease incidence [30]. The carotid artery is an elastic artery with fewer smooth muscle cells in the middle wall layer and less plasticity compared to muscular arteries such as the femoral. Physical activity resulted in sympathetic-adrenergic activity modulation by influencing the smooth muscles in the artery walls [30]. Thus, more intense and longer training is needed to change the different segments of the carotid artery [7]. However, according to Thijssen et al., artery wall thickness is a vascular dynamic system and could change the elastic and muscular vasculature acutely, even in a short-term period [32]. There is a study which confirmed these short-term vascular changes. Duijnhoven et al. reported that 8 weeks of bed rest increased carotid wall thickness by as much as 17% (0.0013 mm), which is 75 times higher than the effect of age on this parameter. Notably, the annual increase of intima-media thickness has a value of 0.0087 mm [33]. Carotid intima-media thickness change could be observed if smooth muscle relaxing agents are used (10% reduction). Other interventions such as statin medications, lipid-decreasing agents, and vitamin C all cause reduction in intima-media thickness within 1–4 years [32]. Therefore, it is important to take these into account when measuring intima-media. Such factors can prevent exercise from being effective on intima-media thickness. Individual and methodological variations could be the causes of those differences in similar studies. Many studies employed healthy or diabetic individuals suffering from cardiac problems, while the target group of this study was diabetic patients only. Positive intima-media changes are questionable if cardiovascular disease risk factors were in the inclusion criteria.

One of the main limitations of this study is the exhaustion imposed by long periods of assessment and intervention. It was also recommended that high-intensity exercise be used with precise monitoring of participants' diets.

Conclusion

The result of this study indicated that 24 sessions of aerobic exercise resulted in vascular changes in diabetic patients. Therefore, the exercise training program which was used in this study is advised in order to attain healthy cardiovascular function with reduced complications.

Acknowledgments We gratefully acknowledge the support of Shahid Beheshti Medical University, Tehran, Iran.

Funding This study was not financially supported by any governmental institute. All costs have been paid by the first author.

Compliance with ethical standards

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

Ethical approval All procedures performed involving human participants were in accordance with the ethical standards of Shahid Beheshti Medical University 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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Int J Diabetes Dev Ctries (April–June 2018) 38(2):209–215
DOI 10.1007/s13410-017-0578-x

ORIGINAL ARTICLE

Regression-based prediction of seeking diabetes-related emergency medical assistance by regular clinic patients

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Received: 11 January 2017 / Accepted: 14 August 2017 / Published online: 14 September 2017

Abstract This study aims to examine diabetes-related emergency medical assistance (DREMA)-seeking factors among type-2 diabetes patients and predict likelihood of patients seeking DREMA before their next scheduled clinic visit. This case-control-designed study comprised a systematic random sampling of 228 patients who completed a structured interview (mean age = 62.6 years). DREMA prediction model was developed based on parameter estimates of a logistic regression model on DREMA (≥ 1 admission vs. 0 admissions), with variable selection from a forward stepwise selection process, considering all 24 potential independent variables. For the final DREMA prediction model, four variables were retained via forward stepwise logistic regression analysis: (1) age, (2) type of rice consumed, (3) physical activity outside of a regular job, and (4) leisure time exercise frequency. Likelihood of seeking DREMA increased with aging, regular or frequent consumption of white rice rather than brown or parboiled rice, and being physically inactive outside of occupation. Odds of seeking DREMA were directly associated with frequency of exercise during leisure time. With further validation and model updating based on local population characteristics, clinicians will be able to predict the DREMA-event likelihood for each clinic patient diagnosed with type-2 diabetes. Modifiable DREMA-seeking variables suggest possible interventions for preventing undesired DREMA events.

Keywords Type-2 diabetes · Clinic patients · Logistic regression · Prediction · Diabetes-related emergency medical assistance

Introduction

Diabetes is a costly worldwide pandemic. Many patients diagnosed with type-2 diabetes mellitus have associated complications, including cardiovascular diseases, nephropathy, neuropathy, and foot ulcers, all of which can generate emergency conditions [1–4]. For diabetes alone, occurrence of hyperglycemia and hypoglycemia primarily leads to medical emergencies [5–8]. As paramedic emergency services are limited to trauma care [9], only three types of diabetes-related emergency medical assistance (DREMA) are available for diabetes patients in many low-income countries like Sri Lanka, visit the following: a family practitioner; the outpatient department of a public or private hospital; or the OPD of a hospital with subsequent transfer to emergency room, inpatient department (non-elective hospitalization), or a larger hospital. All three options will be hereafter referred to as "DREMA," without specifying the form of assistance.

Both meaningful and accurate validated mathematical clinical prediction models have added advantages over human clinical prediction approaches [10]. Mathematical models can integrate more factors than a clinician is capable of considering during decision making [11]. Although human clinical decision making is inconsistent and biased, especially when time and/or clinician's experience are limited, mathematical models consistently produce the same output with identical data inputs [11, 12]. Furthermore, empirical research

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findings demonstrate that several prediction models are more accurate than human clinical prediction [13]. Models based on multivariate logistic regression provide output in the form of odds ratios (odds of an event occurring vs. the odds that it will not), are user-friendly and interpretable with a less arbitrary nature of assigning weights compared with univariate models [14, 15].

This study aimed to retrospectively examine factors that contributed to worsening of symptoms among type-2 diabetes patients who attended a suburban medical clinic in Sri Lanka and, utilizing a user-friendly logistic regression model, to predict the likelihood of a patient seeking DREMA before his/her next visit to a clinic within 3 months. Patients with either cardiovascular complications (e.g., hypertension, a history of myocardial infarction) or other comorbidities (e.g., asthma, epilepsy) and those with type-1 diabetes were excluded in order to reduce the number of testable hypotheses as well as to simplify the final prediction model [16].

Methods

Conceptual model Based on empirical research evidence, an informal model was developed using a set of logical hypotheses. Likelihood of preventing DREMA was viewed as a complex outcome of general health status, pre-existing condition of diabetes, pre-disposing factors (e.g., age, education), reinforcing factors (e.g., social norms), and enabling factors (e.g., diabetes education) [17–19]. Variables that were thought to influence DREMA seeking were categorized under six domains: (1) anthropometry and blood sugar (measured at many diabetes clinics), (2) demography and occupational posture, (3) diabetes history and management, (4) food types and intake frequency, (5) physical activity and exercise, and (6) smoking and alcohol consumption.

Study setting The study was conducted in the medical clinic of Homagama Base Hospital, Sri Lanka located in a suburb of the capital, Colombo. The medical clinic had been held twice every week, whereas each clinic patient was expected to have follow-up visits at least once every 3 months for consultation and treatment—a free service. The Institutional Review Board of Indiana University, USA, approved the recruitment of human subjects in this study.

Study design Case-control study design was utilized in a medical clinic [20]. The study compared type-2 diabetes patients who had DREMA between two subsequent clinic visits with type-2 diabetes patients who had no DREMA during a 6-month period between February and July. The study design involved the retrospective collection of behavioral data (corresponding to the time period prior to previous clinic visit) and retrospective review of clinic records for fasting blood sugar level (during previous clinic visit), along with on-site measurement of weight, height, waist, and hips.

Study population Approximately a one third of medical clinic patients had type-2 diabetes. The study population consisted of clinic patients who had diagnosed type-2 diabetes mellitus but had no other chronic illness (e.g., asthma, epilepsy). Patients were recruited when they came to the pathology laboratory for the fasting blood glucose (FBG) test prior to visiting the clinic.

Sampling method Based on these established exclusion criteria, slightly less than half of type-2 diabetes patients were eligible. As clinic procedures required all diabetes patients to complete a FBG prior to follow-up management during their visit, FBG testing was the mechanism used to systematically draw a random sample. Hence, every fifth diabetes patient who arrived for FBG testing was invited to participate in the study. The first interviewee of each clinic day was selected by using random numbers.

Instrument Data were collected through use of a 31-item structured interview administered by a qualified physician. A study information sheet was administered to participants prior to the interview and special consent was obtained to examine clinic records in addition to the informed consent. The survey was originally developed for this study and had no prior validation. Survey questions were formulated by four experts representing four different disciplines (medicine, health behavior, nutrition, and biostatistics) and were based on evidence from literature and work experience in similar clinics. Interview questions revealed information about the variables of interest in the conceptual model. All questions were appropriate to the culture and the health-related behaviors of urban Sri Lanka.

Data collection Data collection occurred during regular morning sessions of the twice weekly clinic. During the informed consent process, 228 of the 251 total invited clinic patients responded to the structured interview (Table 1), yielding a high response rate of 90.8% during the 3-month survey period. The interviewer—a physician working in the pathology laboratory of the same hospital and not a diabetes clinic service provider—clarified the patients' answers and recorded them appropriately. In order to minimize patients' possible response biases, interviews were carried outside the diabetes clinic but within the hospital. Any contradicting or confusing information provided by a patient was resolved by consulting his/her clinic records in order to minimize interviewer bias and response bias. Total time taken for interview, anthropometry, and clinic records review was approximately 30 min.

Data analysis Data were input into Excel and then analyzed with IBM SPSS statistical software, version 24.0; 18 of the 24 independent variables were treated as categorical data (see Table 1 for details). The dependent variable, DREMA was coded as 0 admissions vs. 1 or more admissions. Pearson Chi-square tests (χ^2) were

Table 1 Description of Variables and Associations between DREMA and 24 Independent Variables (18 Categorical and 6 Continuous)

Variables			EMA (Emergency Medical Assistance)				
			0 Admission		>= 1 Admission		
Categorical variables		Overall frequency	n	%	n	%	*p* value
Gender	Female	113	93	82.3%	20	17.7%	0.281
	Male	115	88	76.5%	27	23.5%	
Posture at work	Sitting and standing	121	93	76.9%	28	23.1%	<.001
	Walking	97	79	81.4%	18	18.6%	
	Heavy physical work	10	9	90.0%	1	10.0%	
Having regular clinic visits?	Yes	168	134	79.8%	34	20.2%	0.814
	No	60	47	78.3%	13	21.7%	
Diabetes education	Yes	120	96	80.0%	24	20.0%	0.809
	No	108	85	78.7%	23	21.3%	
Barriers in changing life style	Economic	39	30	76.9%	9	23.1%	0.895
	Social	27	22	81.5%	5	18.5%	
	No barriers	162	129	79.6%	33	20.4%	
Add sugar to tea/coffee?	Yes	82	64	78.0%	18	22.0%	0.708
	No	146	117	80.1%	29	19.9%	
Usual lunch	Rice	158	124	78.5%	34	21.5%	0.004
	Bread	12	12	100.0%	0	0.0%	
	Other	21	21	100.0%	0	0.0%	
	Don't take lunch	37	24	64.9%	13	35.1%	
Usual dinner	Rice	101	84	83.2%	17	16.8%	0.466
	Bread	13	10	76.9%	3	23.1%	
	Other	38	31	81.6%	7	18.4%	
	Don't take dinner	76	56	73.7%	20	26.3%	
Frequency of eating bread	More than once a day	62	45	72.6%	17	27.4%	0.122
	Once a day	45	33	73.3%	12	26.7%	
	More than once a week	31	25	80.6%	6	19.4%	
	Once a week	68	61	89.7%	7	10.3%	
	Less than once a week	22	17	77.3%	5	22.7%	
Frequency of eating rice	Never	25	24	96.0%	1	4.0%	0.044
	Once a day	35	31	88.6%	4	11.4%	
	Twice a day	57	42	73.7%	15	26.3%	
	Three times a day	111	84	75.7%	27	24.3%	
Rice type	Red	112	100	89.3%	12	10.7%	<.001
	White	116	81	69.8%	35	30.2%	
Physical activities during past month	Yes	209	174	83.3%	35	16.7%	<.001
	No	19	7	36.8%	12	63.2%	
Frequency of eating fruit	At least twice a day	41	36	87.8%	5	12.2%	0.007
	At least once a day	82	72	87.8%	10	12.2%	
	More than once a week	54	36	66.7%	18	33.3%	
	Less than or equal to once a week	51	37	72.5%	14	27.5%	
Exercise time	None	135	96	71.1%	39	28.9%	0.040
	Less than 30 minutes	21	20	95.2%	1	4.8%	
	Greater than or equal to 30 minutes	23	19	82.6%	4	17.4%	
Exercise in leisure time	Not at all	135	96	71.1%	39	28.9%	0.002
	Less than once a month	12	10	83.3%	2	16.7%	
	Less than once a week	38	35	92.1%	3	7.9%	

Table 1 (continued)

Variables			EMA (Emergency Medical Assistance)				
			0 Admission		>= 1 Admission		
		43	40	93.0%	3	7.0%	
Smoke	Never	204	163	79.9%	41	20.1%	0.263
	Given up temporarily or permanently	10	9	90.0%	1	10.0%	
	Less than or equal to daily	14	9	64.3%	5	35.7%	
Alcohol	Never	202	161	79.7%	41	20.3%	0.339
	Given up temporarily or permanently	15	13	86.7%	2	13.3%	
	Less than or equal to daily	11	7	63.6%	4	36.4%	

Continuous variables	0 Admission		>= 1 Admission		*p* value
	Mean	SD	Mean	SD	
BMI	30.99	7.53	34.07	6.39	0.011
Waist Hip Ratio	0.78	0.17	0.82	0.13	0.051
Fasting Blood Glucose Level (mmol)	6.30	2.23	7.45	2.56	0.002
Age	62.12	9.30	64.30	10.95	0.169
Time duration since diagnosis of diabetes	9.92	4.07	9.68	3.08	0.711
How many meals do you usually eat?	3.04	0.32	3.00	0.47	0.508

performed to test associations between DREMA and the 18 categorical variables. *t* test was performed on the six continuous variables; (1) BMI, (2) waist-to-hip ratio (WHR), (3) FBG, (4) age, (5) time duration since diagnosis, and (6) number of meals per day. Finally, the DREMA prediction model was developed based on parameter estimates of a logistic regression model on DREMA (≥ 1 admission vs. 0 admissions), with variable selection from a forward stepwise selection process, considering all 24 potential independent variables. In the forward selection procedure, each variable was entered into the model one at a time, and the most significant variable was selected to enter into the model first. The remaining variables were then tested again one at a time, with inclusion of the next most significant variable, and so-on until all significant variables had been added ($p < 0.05$). Table 2 provides the logistic regression for the retained variables. Receiver operating characteristic (ROC) curve was calculated for each model. Area under the curve (AUC) used to compare the ability of the model to accurately predict DREMA; AUC = 1 indicates a perfect predictor and ≤ 0.5 a worthless predictor [21]. Youden's Index, *J* (*J* = sensitivity + specificity – 100), identified optimum cutoff for the probability of DREMA seeking, which best classified patients as DREMA seeking and not seeking. Youden's Index is commonly used as a summary measure of the ROC curve, because it can identify the maximum potential effectiveness of a biomarker [22].

Results

Of the 228 respondents (mean age = 62.6 years), 113 (49.6%) were female and 115 (50.4%) were male. Majority of the participants did not have high school diplomas (74.1%) and were either retired (53.1%) or unemployed (24.1%). Pearson Chi-square tests (χ^2) for the 18 categorical variables showed that seven distinct variables were statistically significant ($p < 0.05$) in predicting DREMA: (1) type of lunch ($\chi^2(3) = 13.41$, $p = 0.004$), (2) frequency of eating rice ($\chi^2(3) = 8.09$, $p = 0.044$), (3) rice type ($\chi^2(3) = 13.18$, $p < 0.001$), (4) physical activities ($\chi^2(3) = 22.93$, $p < 0.001$), (5) frequency of fruit intake ($\chi^2(3) = 12.12$, $p = 0.007$), (6) duration of exercise time ($\chi^2(3) = 6.44$, $p = 0.040$), and (7) frequency of exercise on leisure time ($\chi^2(3) = 14.41$, $p = 0.002$). Those who did not eat lunch were most likely to seek DREMA (35.1%) followed by those who ate rice (21.5%), compared with those eating bread or "Other" (0%). Subjects who ate rice two or three times a day required DREMA more often (26.3 and 24.3%, respectively) than those who never ate rice (4%) or ate it once a day (11.4%). Those eating white rice were more likely to seek DREMA (30.2%) than those eating red rice (10.7%). Respondents who reported not exercising regularly were more likely to seek DREMA (28.9%) than those who exercised.

No significant association existed between DREMA and the remaining 11 variables; gender, posture at work, having regular clinic visits, diabetes education, barriers in changing lifestyle, adding sugar to tea/coffee, type of dinner, frequency of eating bread, educational level, smoking, and alcohol use.

DREMA demonstrated a significant positive association with FBG ($t(226) = 3.06$, $p = 0.002$) and BMI ($t(226) = 2.58$, $p = 0.011$), where higher FBG (M = 7.45, SD = 2.56) and higher BMI (M = 34.1, SD = 6.39) were found

Table 2 Logistic Regression on DREMA (>=1 admission vs 0 admissions) (N=228)

	Beta	S.E.	Wald	df	*p*-value	Odds Ratio
Age	0.07	0.02	9.07	1	0.003	1.07
Rice Type (White vs Red)	1.22	0.43	8.20	1	0.004	3.39
Any Physical Activity outside regular job (No vs Yes)	3.91	0.85	21.15	1	<.0010	49.90
Leisure Exercise: Less than once a month (Level 2) vs Not at all (Level 1)	-0.29	0.86	0.11	1	0.736	0.75
Leisure Exercise: Less than once a week (Level 3) vs Not at all (Level 1)	-1.63	0.69	5.61	1	0.018	0.20
Leisure Exercise: Less than or equal to everyday (Level 4) vs Not at all (Level 1)	-3.55	1.12	10.00	1	0.002	0.03

among respondents with at least one admission compared with those with no admissions (FBG: M = 6.30, SD = 2.23; BMI: M = 30.99, SD = 7.53).

However, WHR, time duration since diagnosis, and the number of meals per day were not significantly important in predicting DREMA.

For the final DREMA prediction model, four variables were retained through a forward stepwise logistic regression analysis: (1) age, (2) the type of rice, (3) physical activity outside of a regular job (yes/no), and (4) exercise in leisure time (not at all, once a month or less, once a week or less, every day). The likelihood of seeking DREMA increased with aging, where those who were 10 years older were twice as likely to seek DREMA (OR = e^ (0.07 × 10) = 2.01) than someone 10 years younger. Regarding the type of rice, the odds of seeking DREMA increased with regular or frequent consumption of white rice rather than brown or parboiled rice. Thus, a patient who regularly eat white rice tended to have 3.39 times the odds of seeking DREMA compared with those who regularly eat brown or parboiled rice. With respect to patients' physical activity levels, being physically inactive outside of occupation aggravated the unfavorable outcomes associated with increasing age and increased the likelihood of seeking DREMA. Specifically, patients who did not engage in any physical activities outside of occupational posture during the past month had 50 times higher odds of seeking DREMA compared with those who regularly participated in physical activities during the same time duration. Also, the odds of seeking DREMA were directly associated with the frequency of exercise during leisure time. That is, a patient with higher frequency of leisure time exercise had lesser odds of seeking DREMA. For example, compared with those who exercised almost every day during their leisure time for the past 1 month (leisure exercise level 4), patients who did not exercise at all during their leisure time (leisure exercise level 1) had a 33.33 times higher odds of seeking DREMA. The final DREMA prediction model selected can be presented as a Y-prediction equation in the form of ($y = a + b^{*}\times 1 + c^{*}\times 2 + d^{*}\times 3 + e^{*}\times 4$), where y is on the logit scale to model the probability of DREMA, a through e are the parameter estimates from the logistic regression model, and ×1 through ×4 are each respondent's data values for the four independent variables in the model:

$$Y = \log\left(\frac{p}{1-p}\right) = -2.53 + 0.07 \times (\text{age}) + 3.91 \times (\textit{physically inactive}) + 1.22 \times (\textit{white rice consumption}) - 0.291 \times (\textit{leisure activity level } 2) - 1.63 \times (\textit{leisure activity level } 3) - 3.55 \times (\textit{leisure activity level } 4)$$

To calculate the probability of DREMA, $p = \exp(Y)/(1 + \exp(Y))$.

The probability of a given patient seeking DREMA, i.e., P(DREMA), can be calculated using the equation $p = \exp(Y)/(1 + \exp(Y))$.

Finally, the AUC value for this DREMA-seeking prediction model was 0.812 (Fig. 1), indicating a good classification accuracy. Using Youden's Index, the optimal cutoff point for the probability of DREMA seeking based on the prediction model is 0.285. Thus, a predicted probability of 0.285 or above was classified as seeking DREMA, with moderate sensitivity and high specificity values of 0.66 and 0.81, respectively [23].

Discussion

Overall, the final DREMA prediction model was both meaningful and simple (user-friendly), and with integration of a tool (e.g., computer program) to help with calculations, may prove useful to both clinicians and non-clinicians. With further validation and updating of this model (and its underlying concept) based on characteristics of local populations,

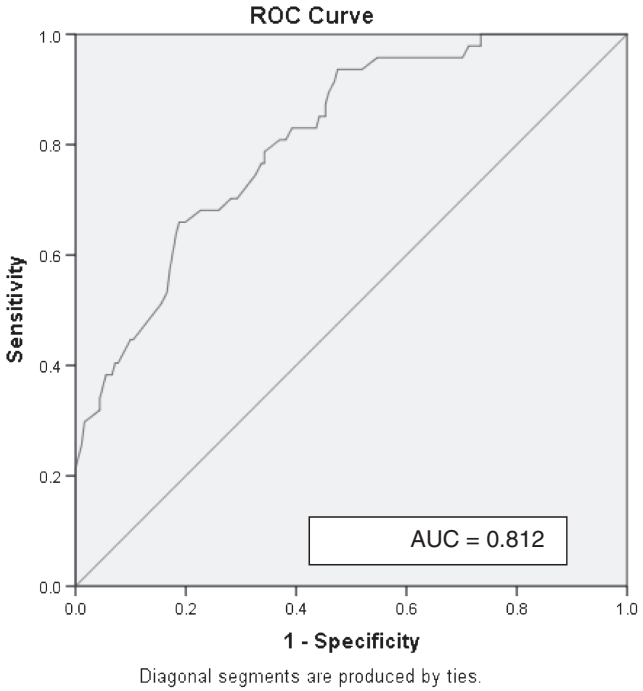

Fig. 1 AUC value for DREMA-seeking prediction model

healthcare providers will be able to predict the likelihood of seeking DREMA for each clinic patient diagnosed with diabetes.

Several difficulties were encountered during this study. Firstly, absence of a control group (e.g., non-daibetic patients) made it impossible to investigate DREMA seeking by persons who were not diagnosed with diabetes as well as those who were diagnosed with diabetes and its common cardiovascular complications. Secondly, data were missing for six variables (removed) either because a patient refused to report them or because the interviewer failed to collect the information from a patient with adequate clarity. Student *t* test was performed to compare means for patients with complete data profiles and those with missing data, which excluded the existence of any systematic difference [24, 25]. Thirdly, many variables that were not significant in bivariate analysis may have multidimensional constructs that are not easy to quantify. Put differently, the real-world scenario would be far more complicated than the developed conceptual model [24]. Fourthly, the study failed to collect data on patients who had not been discharged from the hospital, were terminally ill, or had died following a DREMA event during the study period because only those who visited the clinic were investigated. In order to validate the proposed model, further iterations of this study may use a prospective approach. Moreover, the model may not be sensitive to patients with either cardiovascular complications or other comorbidities (e.g., asthma, epilepsy) and those with type-1 diabetes since patients with such comorbidities were excluded from the study. Therefore, the safer way of using the prediction model would be recognizing low-risk patients rather than leaving the model to identify high-risk patients. Thus, future research should attempt to validate the model in a real-world scenario involving patients with cardiovascular complications and other diabetes-related comorbidities.

Finally, model selection was based on statistical significance and meaningfulness of the combination of variables. Generalizing to a different community may require model updating based on unique characteristics of the local population [25].

Alternative methods, such as artificial neural networks and decision trees, may also be useful to clinicians for predicting DREMA events. Artificial neural networks are computational models, which resemble neurons that generate an action potential based on an input from other neurons of the network [26]. Software of the artificial neural network interprets multiple inputs fed into it and produces a dichotomous output. Both input data and outcomes help the neural network to implicitly identify complex linear or non-linear relations and possible interactions between predictor and outcome variables in order to calculate the probability of a specific outcome [27–29]. However, artificial neural networks have several limitations, such as restricted ability to explicitly identify causal relationships, not being user-friendly, greater need for computational resources and training, and tendency to effectively memorize irrelevant data (overfitting) that reduces predictive accuracy [27]. A mathematical algorithm, which is set to maximize the predictive accuracy, produces variables, cutoff values, and suitable sequence of splitting, resulting in a "decision tree" that can be easily translated into routine clinical practice [14]. Clinicians can predict the outcome for patients by simply following the answers to questions in each of the boxes [30]. However, this method can be less accurate than other prediction models because of the limited number of variables available on the trees [31].

Several new questions arise from this study. The attitude of clinicians toward using the model is critical and must be verified. Finally, model selection was based on statistical significance and meaningfulness of the combination of variables. Research should investigate model applicability in different types of communities [25], and, if not applicable, determine required modifications based on unique characteristics of the local population, e.g., anthropometric indicators, educational attainment, diabetes education, food habits, and physical activity. An impact analysis is required to measure the usefulness of the prediction model in the clinical practice with regard to patient satisfaction, cost-benefit, and time/resource constraints [10].

Findings of this study may influence clinical practice; factors associated with increasing the likelihood of DREMA events in individual patients were identified, suggesting interventions that might prevent those undesired events. In

particular, increasing the level of physical activity at home, implementing a weight-loss plan to reduce central obesity (WHR), executing a dietary plan to prevent hyper- and hypoglycemia, consuming brown or parboiled rice as a family habit, and performing physical exercises five times per week, either singly or in combination, are useful behavioral interventions that may reduce the probability of DREMA events for individual patients.

Acknowledgements We would like to thank Dr. Ahmed YoussefAgha and Jessie He for their contributions to the first draft of the manuscript and data analysis, respectively.

Funding disclosure The authors have no relevant financial or non-financial relationships to disclose i.e., there was no funding or support.

Compliance with ethical standards

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

Ethical approval Ethical approval for the recruitment of human subjects and review of medical records were obtained from the Institutional Review Board, Indiana University Bloomington, USA.

Informed consent The authors obtained informed consent from all individual participants included in the study.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):216–220
DOI 10.1007/s13410-017-0583-0

ORIGINAL ARTICLE

Severe hypoglycemia in diabetics requiring hospitalization and short-term mortality

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Received: 8 June 2017 / Accepted: 6 September 2017 / Published online: 25 September 2017

Abstract We planned a prospective study to observe short-term mortality in diabetic patients with severe hypoglycemia requiring hospitalization. Total 50 patients were enrolled in the study. Diabetic patients presenting to hospital with plasma glucose <70 mg/dl requiring hospitalization for correction were enrolled in study. Patients with underlying malignancy, acute cerebro-vascular event, symptomatic congestive heart failure, and creatinine clearance <10 ml/min/1.73 m^2 were excluded. All subjects were followed for 3 months from date of admission. Majority of patients were type 2 diabetics (41, 82%) and remaining were type 1 diabetics. Mean age of type 1 diabetic was 31.77 ± 8.9 years and for type 2 diabetic was 65.97 ± 10.5 years. Mean plasma glucose at presentation was 36.62 ± 11.57 mg/dl. Fourteen (28%) patients had previous admission with hypoglycemia. Thirty patients did not have knowledge about hypoglycemia management at home. Seventeen (34%) were on single drug (insulin/SU), 19 (38%) were on two drugs, and 14 (28%) were on three or more drugs. Mean creatinine clearance was 55.83 ± 26.5 ml/min/1.73 m^2. Mean HbA1C was 8.58 ± 1.94%. Total in-hospital mortality was two (4%), and two more deaths were observed during 3-month follow-up. All four deaths occurred in type 2 diabetics who were illiterate and had no previous history of severe hypoglycemia. Total in-hospital mortality was 2/50 (4%) and 90-day all-cause mortality was 4/50 (8%). All patients who died were type 2 diabetic; hence, all-cause mortality was 4/41 (9.7%) in type 2 diabetes. Our study suggests that severe hypoglycemia, particularly in type 2 diabetes, is associated with very significant all-cause mortality in short term.

Keywords Diabetes · Hypoglycemia · Mortality

Introduction

Diabetes is a chronic disease requiring continuous medical care with multifactorial risk-reduction strategies along with good glycemic control. Patient self-management education and support are critical in preventing acute complications and reducing the risk of long-term complications [1].

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals that are currently diagnosed with the disease [2, 3]. Many studies have shown that modern management with good glycemic control can limit, delay, or prevent the chronic complications of diabetes. However, aggressive diabetes treatment could be associated with an increased risk of hypoglycemia [4], particularly in patients with type 1 diabetes mellitus and patients with insulin-treated type 2 diabetes mellitus [5–9]. Hypoglycemia is not uncommon in diabetic population and approximately 90% of all patients who receive insulin have experienced hypoglycemic episodes [10].

Methods

We planned this study, as there are very limited Indian data available. This was a prospective study of 50 diabetic patients admitted with severe hypoglycemia at Dr. S.N. Medical College and attached Mahatma Gandhi Hospital. The clinical profile, risk factors, and short-term outcomes in these patients were assessed.

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The inclusion criteria were as follows: any diabetic patient presenting to the hospital with severe hypoglycemia: plasma glucose ≤70 mg/dl, inability to take orally after 30-min management in emergency department, and assessed by the physician that patient requires hospitalization for correction of hypoglycemia. We excluded patients with associated malignancy, acute cerebro-vascular event, symptomatic congestive heart failure, and creatinine clearance <10 ml/min/1.73 m^2. A full history was taken by the attending physician concentrating on risk factors including age, educational status, duration of known diabetes, previous history of admission to hospital due to hypoglycemia, and drug history. History of diabetes was determined by previous history of use of any anti-diabetic medications. Two venous samples were drawn at the time of inserting the intravenous line before initiating any medical intervention and sent for routine blood examinations.

The statistical analysis was performed using chi-square test for comparison of dichotomous variables, and p value <0.05 was considered statistically significant. All subjects were followed for 3 months from date of admission to the hospital. All the information obtained were recorded on a standardized proforma.

Results

A total of 50 patients were enrolled. Forty-one (82%) patients were type 2 diabetics and remaining were type 1 diabetics. The mean plasma glucose at presentation was 36.62 ± 11.57 mg/dl (distribution of glucose level, Table 1). Out of 50 patients, 44 (88%) patients presented to the emergency department with Glasgow Coma Scale (GCS) ≤8; remaining six patients were having GCS >8 but were having altered behavior and inability to take orally after 30-min management in emergency department. Majority of patients with type 1 diabetes were between 20 and 40 years of age (67%, mean age 31.77 ± 8.9 years), and majority of patients with type 2 diabetes were of age >60 years (73%, mean age 65.97 ± 10.5 years). Fourteen (28%) patients had previous admission with hypoglycemia. Thirty patients (60%) did not have knowledge about hypoglycemia management at home. Baseline characteristics and other details are in Table 2.

Thirty-two (64%) patients had a creatinine clearance value <60 ml/min/1.73 m^2. The mean creatinine clearance was 55.83 ± 26.5 ml/min/1.73 m^2. However, mean creatinine clearance in non-survivors was 30.49 ± 20.51 ml/min/1.73 m^2. Distribution of creatinine clearance is in Table 3. Relation between mean creatinine clearance and drugs/combinations used is shown in Fig. 1.

Overall, out of 50 subjects (type 1 and type 2 both), 17 (34%) were on single drug (insulin/SU), 19 (38%) were on two drugs, and 14 (28%) were on three or more drugs (Table 4).

In type 2 diabetes subjects (n = 41), sulfonylurea (SU) was most commonly prescribed drug alone or in combination with other drugs. SU + metformin was most commonly used combination in type 2 diabetes subjects (15 (36%). Details are in Fig. 2.

Out of the 35 patients in whom HbA1c was measured, 42.9% had HbA1c levels in the <8 range, 34.3% had HbA1c levels in 8–10 range, and the remaining 22.9% had HbA1c >10. The mean HbA1C in current study is 8.58 ± 1.94. Distribution of HbA1c is shown in Table 5.

Two patients (4%) died during hospitalization and another two subjects (4%) died during 3-month follow-up. All patients who died were type 2 diabetic, all were illiterate, were above 65 years of age, all had glucose level less than 36 mg/dl at presentation, and their mean creatinine clearance was 30.49 ± 20.51 ml/min/1.73 m^2. None of the non-survivors had previous history of hospitalization for hypoglycemia.

Table 2 Baseline characteristics and results

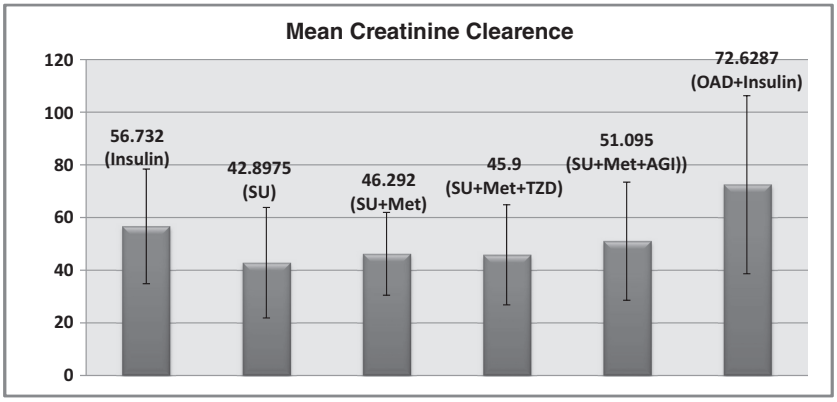
Parameters	Patients (mean ± SD)
No. of patients	50
Type 1/type 2	9/41
Male/female	25/25
Age (type 1/type 2)	31.77 ± 8.9 years/65.97 ± 10.5 years
Literate/illiterate	24/26
Duration of known diabetes	7.83 ± 3.1 years
Previous history of admission	14 (28%)
Knowledge about hypoglycemia	20 (40%)
Plasma glucose	36.62 ± 11.57 mg/dl
Creatinine clearance	55.83 ± 26.5 ml/min/1.73 m^2
HbA1c	8.58 ± 1.94%
All-cause mortality	4 (8%)

Table 1 Distribution of glucose level

Glucose level (mg/dl)	Number (%)
<30	16 (32%)
30–50	29 (58%)
>51–70	5 (10%)
Total	50 (100%)

Table 3 Distribution of creatinine clearance

Creatinine clearance (ml/min/1.73 m^2)	Number (%)
<30	8 (16)
30–60	24 (48)
>60	18 (36)
Total	50 (100)



(SU= Sulfonylurea, Met= Metformin, TZD= Thiazolinediadiones, AGI= Alpha glucosidase inhibitors, OAD= Oral anti-diabetic drugs)

Fig. 1 Creatinine clearance in patients on different drug combinations. *SU* sulfonylurea, *Met* metformin, *TZDs* thiazolinediadiones, *AGIs* alpha glucosidase inhibitors, *OADs* oral anti-diabetic drugs

Seventy-five percent (three out of four) non-survivors were on sulfonylurea.

Discussion

Were it not for the barrier of hypoglycemia, people with diabetes could have normal HbA1c levels over a lifetime of diabetes [10]. It is now well established that glycemic control makes a difference in diabetes. Reduction of mean glycemia over time prevents or delays microvascular complications—retinopathy, nephropathy, and neuropathy—in both type 1 and type 2 diabetes. It may also reduce macrovascular events [11, 12]. However, iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes [10].

Previous studies [13, 14] have shown that most hypoglycemia episodes are associated with SU and insulin combination. In a study conducted by Bruderer et al. [13] on incidence of and risk factors for severe hypoglycemia in treated type 2 diabetes mellitus patients, it was observed that current use of sulfonylureas and current insulin use were both associated with an increased risk of severe hypoglycemia. In our study, SU + metformin combination was associated with 78% episodes of hypoglycemia in type 2 diabetics (Table 4). Most likely explanation for this may be that in our sub-set of patients, oral anti-diabetic drugs (OADs) were used more frequently.

Table 4 Frequency of oral anti-diabetic drugs used in type 2 diabetes subjects ($n = 41$)

Drugs	Number of patients (%)
Sulfonylurea	32 (78)
Metformin	32 (78)
Insulin	12 (30)
Thiazolidenediones	8 (20)
α-Glucosidase inhibitors	6 (14)

Compromised renal function may be another important factor in our patients. It is a well-established fact that risk of hypoglycemia increases with renal failure. More than 60% subjects in current study had a creatinine clearance value <60 ml/min/1.73 m^2 and mean creatinine clearance was 55.83 ± 26.5 ml/min/1.73 m^2. However, mean creatinine clearance in non-survivors was 30.49 ± 20.51 ml/min/1.73 m^2. This suggests that risk of death in patients with hypoglycemia is much higher when renal functions are more severely compromised.

The mean HbA1C in current study is 8.58 ± 1.94. This suggests that glycemic variability was the major reason for hypoglycemia rather than tight glycemic control.

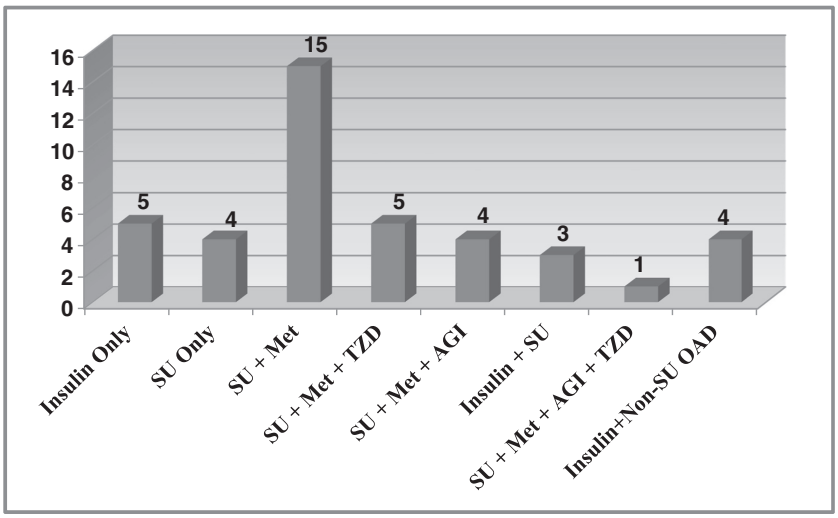
In the current study, only 18% were type 1 diabetics. Most of other studies also reported smaller type 1 diabetes population. In a study conducted by Liatis et al., 8.1% subjects were with type 1 diabetes [15]. The main reason for this is smaller type 1 diabetic population.

No death was observed in type 1 diabetics as compared to 9.7% mortality in type 2 diabetics and overall mortality was 8%. This may be because of more advanced age of type 2 diabetics and more cardiovascular risk. In contrast, type 1 diabetics had more knowledge about home management of hypoglycemia.

In comparison, a study done by Rajendran et al. [16] in patients with diabetes requiring emergency department care for hypoglycemia showed that the 30-day, 90-day, and 1-year all-cause mortality were 10.6, 16.7, and 28%, respectively, indicating significant short-term as well as long-term mortality.

All deaths occurred in illiterate patients and these patients had no history of previous severe hypoglycemia. This highlights importance of literacy in general and patient education in diabetic population in particular. All diabetics should be made aware about signs and symptoms of hypoglycemia and management of hypoglycemia at home.

Small sample size was the limitation of our study.



(SU= Sulfonylurea, Met= Metformin, TZD= Thiazolinediadiones, AGI= Alpha glucosidase inhibitors, Non-SU OAD= Oral anti-diabetic drugs other then Sulfonylurea)

Fig. 2 History of drugs/ combination used in 41 type 2 diabetics. *SU* sulfonylurea, *Met* metformin, *TZDs* thiazolinediadiones, *AGIs* alpha glucosidase inhibitors, *non-SU OAD* oral anti-diabetic drugs other than sulfonylurea

Table 5 Distribution of HbA1C

HbA1C (%)	Frequency	Percent
<8	15	42.9
8–10	12	34.3
>10	8	22.9
Total	35	100.0

Conclusion

Most episodes of hypoglycemia were observed in patients with type 2 diabetes. Most commonly prescribed drug, associated with severe hypoglycemia, was sulfonylurea. In 57% of patients, HbA1c was 8% or more (and mean HbA1C was 8.58 ± 1.94), suggesting that apart from tight glycemic control, severe hypoglycemia also occurs in patients with significant glycemic variability. Sixty-four-percent patients had a creatinine clearance value <60 ml/min/1.73 m^2 (mean creatinine clearance was 55.83 ± 26.5), suggesting that decline in renal function increases the risk of severe hypoglycemia.

Total in-hospital mortality was 2/50 (4%) and 90-day all-cause mortality was 4/50 (8%). All patients who died were type 2 diabetic; hence, all-cause mortality was 9.7% in type 2 diabetes. Our study suggests that severe hypoglycemia, particularly in type 2 diabetes, is associated with very significant all-cause mortality in short term.

Compliance with ethical standards

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

Ethical approval Study was approved by institutional ethical committee.

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):221–227
DOI 10.1007/s13410-017-0584-z

ORIGINAL ARTICLE

Effect of vitamin D supplementation on reduction of cardiometabolic risk in patients with type 2 diabetes mellitus and dyslipidemia

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Received: 2 July 2017 / Accepted: 6 September 2017 / Published online: 16 October 2017

Abstract Endothelial progenitor cells (EPCs) participate in endothelial regeneration. Previous studies link vitamin D deficiency, inflammatory cytokines, and cardiovascular disease (CVD) risk. This study evaluates the impact of vitamin D supplementation on EPCs, inflammatory markers, and glycemia in type 2 diabetes. This is prospective open-label randomized controlled study. Sixty-five patients with type 2 diabetes, dyslipidemia, HbA_{1c} below 9%, and vitamin D deficiency (below 30 ng/ml) attending the outpatient clinic between April and December 2015 were randomized to active vitamin D (60,000 IU of vitamin D orally once a week for 8 weeks, followed by once a month for 4 months) or control for 6 months. Data was analyzed with STATA 14. Demographics include median age 54 (range 48.5–60) years, median duration of diabetes 7 (4–12.5) years, mean BMI 26.86 ± 3.8 kg/m^2, mean HbA_{1c} 7.22±0.8%, and median vitamin D 13.42 (range 10.24–17.23) ng/ml; 50% were men. Vitamin D supplementation increased vitamin D levels in the active group compared to control ($p < 0.01$). EPCs decreased in both groups from baseline. There was no difference in change in EPCs, hsCRP, IL-6, IL-10, TNF-α, and HbA_{1c} or insulin resistance (HOMA-IR) between the active- and control-groups at the end of the study. Vitamin D supplementation did not alter EPCs or inflammatory markers, or improve glycemic control at the dose and duration investigated. Further studies are needed to study the long-term effects on markers of endothelial repair.

Keywords Vitamin D · EPC · Endothelial progenitor cell · Endothelial dysfunction · Type 2 diabetes · Cardiovascular disease · Inflammatory cytokines

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Introduction

Type 2 diabetes is an independent risk factor for cardiovascular disease (CVD), and CVD is the prime cause of mortality in patients with diabetes [1]. Despite evidence that multifactorial risk management offers cardiovascular benefits, patients with diabetes continue to have residual excess cardiovascular risk. Approaches beyond conventional risk management are needed. Recent studies support inflammation [2–7] and endothelial dysfunction participates in hyperglycemia and CVD risk. Endothelial progenitor cells (EPCs) are bone marrow-derived cells in the peripheral blood, which migrate to areas of ischemia, promote angiogenesis and endothelial repair. An inverse relationship exists between cardiovascular risk factors, and the number and migratory capacity of EPCs [8]. Quantification of CD34 and CD133 positive EPCs by flow cytometry is a novel approach to identify patients with defects in endothelial repair [8–10].

Several studies report conflicting evidence on the role of vitamin D in inflammation, cardiovascular risk reduction and glycemic control [11–19]. The effect of vitamin D on inflammatory markers such as hsCRP, IL-6, Il-10 and TNF-α, and

EPCs in patients with low levels of vitamin D and type 2 diabetes is uncertain. Increase in inflammation and a decrease in the number of EPCs are associated with increased cardiovascular risk. A novel approach achieved by treating vitamin D deficiency, a highly prevalent, unrecognized and untreated conditions may be a cost effective alternate to the measurement of EPCs and can have paramount implications in cardiovascular residual risk reduction.

The aim of this study was to evaluate the impact of vitamin D repletion on the levels of inflammatory markers, EPC numbers, and glycemic control in patients with type 2 diabetes and dyslipidemia.

Methods

Design and drug administration

This study evaluated the effects of vitamin D supplementation on EPC, inflammatory cytokines, glycemic control, and insulin resistance in patients with type 2 diabetes. This randomized, parallel group, open-label 24-week study enrolled patients attending the outpatient at Diacon Hospital, a tertiary, university recognized hospital for diabetes care, research, and postgraduate studies, in Bangalore, India, between April 2015 and January 2016.

Patients who met the eligibility criteria at screening were randomized in a 1:1 allocation ratio alternatively by the trial site personnel to the intervention arm which received vitamin D supplementation and a control arm which did not receive vitamin D. Patients, trial site personnel, and investigators were aware of the randomization group.

Participants randomized to active intervention received 60,000 IU of vitamin D orally once a week for 8 weeks, followed by once a month for 4 months. Patients in both arms received rosuvastatin 10 mg once a day (or 20 mg once a day for patients with known cardiovascular disease) during the course of the study. Rosuvastatin was initiated in four patients (three in the active arm and one in the control arm) at the time of randomization. All other patients had been on the stable doses of statin for at least 3 months prior to enrollment. There was only one patient in the control arm with cardiovascular disease receiving 20 mg of rosuvastatin. Vitamin D and rosuvastatin were dispensed by the hospital at each scheduled study visit. Patients were to maintain their background medication for diabetes throughout the trial. Patients on sulfonylureas were allowed to reduce the dose if hypoglycemic episodes occurred. A treat to target approach was followed to allow patients on insulin to titrate their insulin dose as per standards of care.

Patients with baseline hypertension were allowed to continue all anti-hypertensive medications throughout the study. These medications included thiazide diuretics, angiotensin receptor antagonists, calcium channel blockers, and beta blockers. No changes were made in the dose of anti-hypertensive medications during the study. Low-dose aspirin therapy (75 to 150 mg per day) was continued for patients receiving aspirin prior to enrollment; no subsequent changes were made during the course of the study.

Trial population

Inclusion criteria

Eligible trial patients were men and women, aged 25–65 years (inclusive), previously diagnosed with type 2 diabetes and dyslipidemia, had A1c below 9%, low vitamin D levels (< 30 ng/ml) and were on stable doses of oral/injectable anti-diabetic medications and rosuvastatin for at least 90 days prior to screening. The following background anti-diabetic medications were allowed as monotherapy or in combination: metformin, sulfonylureas, DPP4 inhibitors, pioglitazone, alpha glucosidase inhibitors, and insulin (regular insulin, NPH, rapid acting analogues, premixed insulin, and basal insulin based on the prescribed insulin regimen).

Exclusion criteria

Exclusion criteria included prior use of vitamin D supplementation, acute infections, sepsis, any malignancy, hyperparathyroidism, chronic renal insufficiency or failure (eGFR < 60 ml/min/1.73 m^2 MDRD formula), nephrocalcinosis, statin intolerance, women in reproductive age group planning pregnancy, pregnancy and lactation, patients with type 1 diabetes or chronic fibrocalculous pancreatic diabetes, malabsorption, chronic inflammatory autoimmune disorders e.g., rheumatoid arthritis, patients on steroids, patients on immunosuppressive drugs, or medications used in the treatment of autoimmune and rheumatological disorders (e.g., hydroqxychloroquine, methotrexate), patients on drugs which may impair hydroxylation of vitamin D ,such as isoniazid, and patients on drugs which induce cytochrome P450 and cause accelerated loss of vitamin D, such as rifampicin.

Study end points and assessments

The primary efficacy end points were the changes from baseline in levels of EPCs 24 weeks after treatment. Secondary efficacy end points included changes in inflammatory marks: hsCRP, IL-6, IL-10, and TNF-α from changes in HbA1c levels and insulin resistance 24 weeks after treatment.

Vitamin D, hsCRP, IL-6, IL-10, TNF-α, fasting insulin levels, HbA_{1c}, and lipid profile were measured at baseline, 12 and 24 weeks. Patients were categorized based on insulin resistance (IR) into normal IR (< 3), moderate IR

(3–5) and severe IR (> 5) using homeostatic model assessment (HOMA) [20].

Insulin resistance—fasting insulin in mU/l × fasting glucose in mg/dl / 405

Endothelial progenitor cells were isolated by flow cytometry using PE-CD34, PE-CD45, and FITC-CD133 antibodies at baseline and 24 weeks [using mouse anti-human CD34 PE (555822; BD Biosciences, CA, USA), mouse anti-human CD 45 PE (555484; BD Biosciences, CA, USA), and mouse anti-human CD 133 VioBright FITC (130-105-225/226; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany)]. Quantification of serum levels of cytokines was by ELISA (BD Biosciences, USA).

Patients were evaluated for micro and macrovascular complications of diabetes as per the American Diabetes Association Standards of Care clinical practice recommendations [1, 21]. Safety assessments included adverse events (AEs), physical examinations, vital signs (pulse, blood pressure) electrocardiogram, and laboratory assessments at the study site, (hemoglobin, RBC count, WBC count, differential count, urea, creatinine, and urinalysis). A plasma glucose < 70 mg/dl was used as cut-off to define hypoglycemia.

Compliance with ethics guidelines

The study protocol was approved by the institutional ethics committee and the Research Society for the Study of Diabetes in India (RSSDI). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients for being included in the study.

Statistical analysis

This pilot study planned to recruit at least 50 patients (25 in each arm) and a sample size of 65 was considered to include loss to follow-up rates. As there was a lack of preliminary data on the effect of vitamin D supplementation on EPC, the results of this pilot will help provide estimates to aid in sample size calculations for similar studies in the future.

Descriptive statistics summarized the data using mean, standard deviation, median, and interquartile range (25th to 75th percentiles). Baseline differences between groups were evaluated using the *t* test, Wilcoxon rank-sum test or the χ^2 test based on the distribution and type of variable appropriately. The primary and secondary end points were assessed using analysis of covariance (ANCOVA) model for continuous

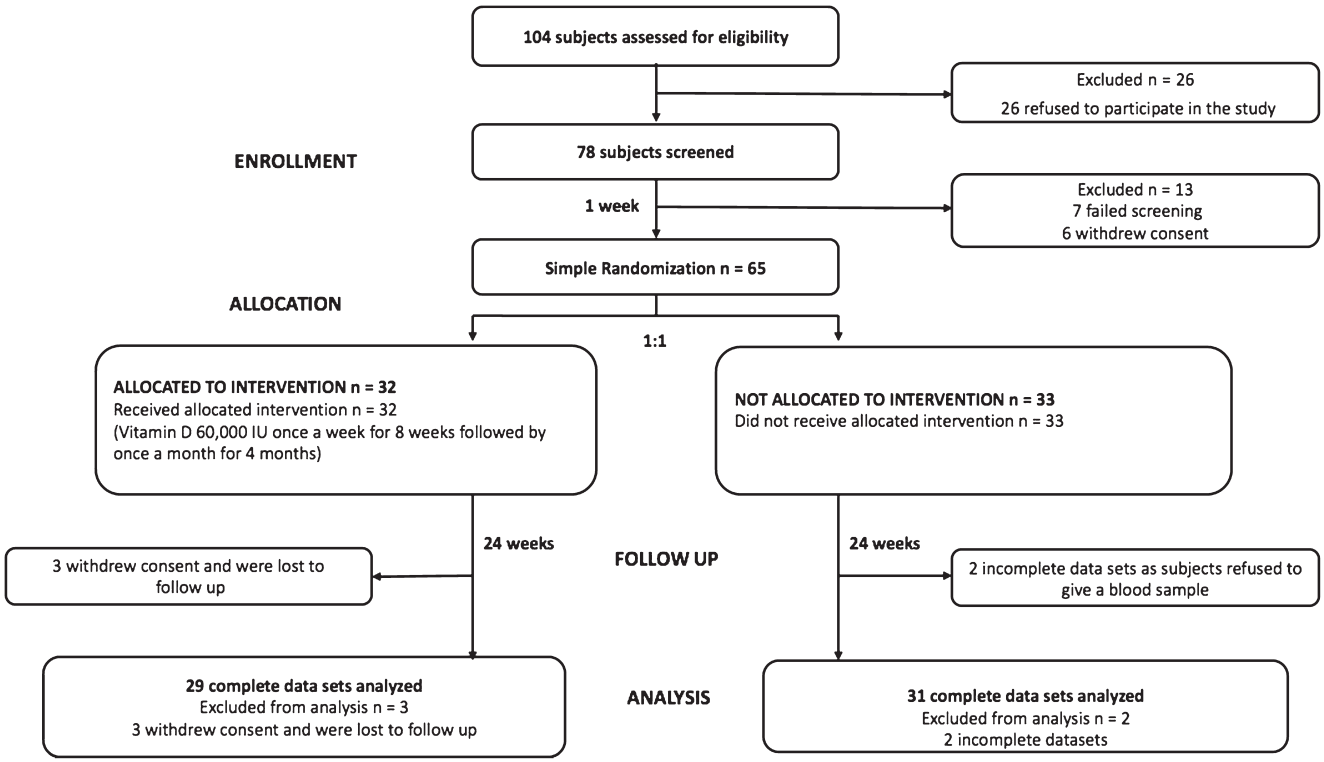

Fig. 1 Flow diagram of efficacy of vitamin D supplementation on reduction of cardio-metabolic risk in patients with type 2 diabetes mellitus and dyslipidemia: enrollment, randomization, follow-up, and data analysis

outcomes with treatment group, age, and sex as fixed effects and baseline value as covariate; and the χ^2 test for categorical outcomes. A two-sided p value at 5% alpha was considered statistically significant. The primary analysis was a complete case analysis; patients with missing data sets and loss to follow-up were excluded from the analysis. Data was analyzed using Stata/IC version 14.2 (StataCorp LP, college Station, TX).

Results

Eligible participants were recruited from April 2015 to August 2015. Study participants attended clinic visits at the time of randomization (baseline), 12 and 24 weeks. Figure 1 shows the flow of participants throughout the study. Characteristics of the 60 subjects who completed the study are as follows: median age 54 (48.5–60) years, 50% male, mean BMI 26.86 ± 3.8 kg/m^2, mean HbA_{1c} 7.22 ± 0.8%, duration of diabetes 7 (4–12.5) years, and median vitamin D levels 13.42 (10.24–17.23) ng/ml. At baseline, eight patients in the active and nine patients in the control arm were receiving aspirin (most patients were on 75 mg aspirin per day, only one patient in the control arm with cardiovascular disease was on 150 mg aspirin per day). None of the patients received anti-inflammatory doses of salicylates (3 to 6 g per day).

The primary analysis was a complete case analysis. Baseline subject demographics are summarized in Table 1. Subjects in the active group had higher levels of total serum cholesterol and LDL at baseline; and a higher percentage were women. All patients with baseline hypertension were well controlled with blood pressure < 140/90 mmHg throughout the study. All other measures, clinical and laboratory were distributed equally across both groups.

Evidence of adherence to vitamin D supplementation was observed by the increase in levels of vitamin D in the intervention compared to the control arm [20.48 (95% CI 16.35 24.61) versus − 3.49 (95% CI − 5.42 − 1.56); $p < 0.01$].

Effect on endothelial progenitor cells and inflammatory markers

There were no differences in EPCs or the inflammatory cytokines between groups at 24 weeks when adjusted for baseline covariate, age, and sex. There were no interactions between sex and the dependent variable (Table 2). A reduction in EPCs, hsCRP, and TNF-α and an increase in IL-6 were observed in both groups at 24 weeks compared to baseline. IL-10 levels remained below detectable limits in both groups.

Table 1 Comparison of baseline demographic data between active and control group

	Intervention, n = 29	Control, n = 31
Age years	53 (48–60)	55 (50–61)
Duration of diabetes years	7.98 ± 6.14	8.89 ± 5.72
Male	10 (34.48)	20 (64.52)*
Female	19 (65.52)	11 (35.48)
Hypertension	17 (58.62)	18 (58.06)
Neuropathy	3 (10.34)	4 (12.90)
Retinopathy	5 (17.24)	5 (16.13)
Ischemic heart disease	0	1 (3.23)
Peripheral arterial disease	0	0
Cerebrovascular accident	0	0
Body mass index (kg/m^2)	25.9 (24–30)	26.5 (23.52–28.78)
HbA1c %	7.21 ± 0.88	7.24 ± 0.73
Total cholesterol (mg/dl)	149 (124–170)	131 (114–148)*
Triglycerides (md/dl)	125 (100–193)	127 (95–162)
HDL (mg/dl)	39 (35–47)	35 (33–41)
LDL (mg/dl)	83.02 ± 33.81	67.03 ± 18.86*
VLDL (mg/dl)	25 (20–38.6)	25 (19–30.4)
Vitamin D (ng/ml)	13.09 (9.07–19.84)	13.51 (10.73–16.22)
hsCRP (mg/l)	2 (0.82–3.24)	1.25 (0.54–1.82)
IL-6 (pg/ml)	0.01 (0–1.96)	0 (0–0.83)
IL-10 (pg/ml)	0 (0–0.009)	0 (0–0)
TNF-α (pg/ml)	9.16 (7.41–9.63)	8.53 (7.69–9.36)
Insulin resistance		
Normal < 3	18 (62.07)	15 (48.39)
Mild–moderate 3–5	4 (13.79)	15 (48.39)
Severe > 5	7 (24.14)	8 (25.81)
EPC cells/μl	5 (2–8)	4 (3–6)

Continuous variables are shown as mean ± SD or as median (interquartile range). Categorical variables are presented as n (percentages). T test for normally distributed continuous variables, Wilcoxon rank-sum test for non-normally distributed continuous variables, and χ^2 test for categorical variables

EPC endothelial progenitor cells

*p values < 0.05.

Effect on glycemic control and insulin resistance

There was no significant difference in HbA_{1c} between groups at the 24 weeks when adjusted for baseline covariate, age, and sex. There were no interactions between sex and the dependent variable No differences were observed in the proportion of patients with no insulin resistance, mild to moderate insulin resistance, and severe insulin resistance from baseline within groups or between groups. However, a higher proportion i.e., 41.94% of patients in the control arm had severe insulin resistance compared to 20.69% of patients in the intervention arm at 24 weeks (Table 2).

Table 2 Summary of results: Differences in outcomes in intervention and control arm at 24 weeks

	Intervention, $n = 29$	Control, $n = 31$	p value
Vitamin D (ng/ml)	20.48 (16.35 24.61)	− 3.49 (− 5.42 − 1.56)	< 0.01*
hsCRP (mg/l)	− 1.2 (− 2.66 0.25)	− 0.42 (− 0.85 0.01)	0.4
IL-6 (pg/ml)	1.73 (− 0.35 3.82)	2.48 (0.28 4.69)	0.93
IL-10 (pg/ml)	0.04 (− 0.36 0.46)	− 0.09 (− 0.2 0.01)	0.63
TNF-α (pg/ml)	− 3.68 (− 4.37 − 2.99)	− 4.1 (− 4.56 − 3.64)	0.63
EPC cells/μl	− 1.65 (− 3.09 − 0.21)	− 0.06 (− 2.17 − 2.04)	0.74
HbA1c %	− 0.003 (− 2.34 0.22)	− 0.22 (− 0.5 0.04)	0.15
Insulin resistance			
Normal < 3	12 (41.38)	12 (38.71)	0.13
Mild–moderate 3–5	11 (37.93)	6 (19.35)	
Severe > 5	6 (20.69)	13 (41.94)	
Body mass index (kg/m^2)	− 0.12 (− 0.45 0.21)	− 0.22 (− 0.5 0.45)	0.42
Total cholesterol (mg/dl)	− 20.51 (− 33.72 − 7.3)	− 7.48 (− 21.04 6.08)	0.62
Triglycerides (mg/dl)	− 6.72 (− 27.84 14.39)	− 18.74 (− 49.03 11.55)	0.46
HDL (mg/dl)	6.27 (4.23 8.31)	8.75 (6.60 10.90)	0.07
LDL (mg/dl)	− 25.42 (− 36.94 − 13.89)	− 12.89 (− 23.7 − 2.07)	0.61
VLDL (mg/dl)	− 1.33 (− 5.56 2.88)	− 3.19 (− 9.3 2.91)	0.43

Continuous variables are shown as change in means from baseline (95% CI). Categorical variables are presented as n (percentages). For continuous outcomes: ANCOVA adjusted for baseline covariate, age, and sex

EPC endothelial progenitor cells

*significant p value

Safety

The study showed no differences in BMI, total cholesterol, serum triglycerides, HDL, LDL, and VLDL between groups at 24 weeks when adjusted for baseline covariate, age, and sex (Table 2). No adverse symptoms related to vitamin D toxicity necessitating investigation for hypercalciuria or hypercalemia, or hypoglycemia (defined as plasma glucose < 70 mg/dl) occurred during the study.

Discussion

In the past decade, inflammatory markers have come to light as independent risk factors for CVD[2, 4, 22–25]; and EPCs [7–10] have emerged as regenerative cells, offering a novel target of untapped therapeutic potential. To our knowledge, this is the first randomized clinical trial investigating the effect of vitamin D supplementation on both inflammatory cytokines and EPCs in patients with type 2 diabetes.

Vitamin D: Inflammatory cytokines and EPCs

Inflammation plays a complex, intricate, and yet incompletely understood role in diabetes and CVD. The inflammatory response is initiated when IL-1 and TNF-α are released from the site of inflammation, resulting in a cascade of changes including release of IL-6 and acute-phase reactants like fibrinogen and hsCRP among others [22]. IL-6, TNF-α, and hsCRP have been implicated in the progression of atherosclerosis and plaque rupture [23–28]. IL-10 on the other hand is considered to be an anti-inflammatory cytokine that reduces the production of other inflammatory cytokines, and is associated with better acute coronary syndrome outcomes and inversely with stroke mortality [29]. In large prospective cohort studies like the Framingham Offspring Study, vitamin D has been associated with an increase in cardiovascular risk over and above traditional risk factors [30]. In a few smaller clinical trials, vitamin D supplementation has reduced the levels of inflammatory cytokines [16]. In this study, a reduction in hsCRP, TNF-α, and IL-10, and an increase in IL-6 were observed in both groups compared to baseline. The lack of an unidirectional change in inflammatory cytokines in the group which received vitamin D, compared to the control arm, demonstrates that vitamin D supplementation had no consistent or significant effect on levels of inflammatory cytokines.

Endothelial injury is now recognized as a pathophysiological process in patients with diabetes, hypertension, acute myocardial infarction, heart failure, stroke, and peripheral vascular disease [31–35]. Assessment of endothelial function includes flow-mediated dilatation (FMD) and quantification of circulating endothelial cells (CECs) and EPCs [8–10]. CECs have been identified as markers of endothelial damage and EPCs as biomarkers of vascular repair. EPCs are bone

marrow-derived immature cells that home in, differentiate, and maintain the endothelium by reendothelialization and neovascularisation at sites of trauma and ischemia [9, 10, 36, 37]. Increased levels of EPCs are inversely associated with cardiovascular outcomes [8]. The impact of vitamin D on EPCs has not been previously studied. In this study, a reduction in number of EPCs was observed in both groups compared to baseline. No quantitative differences were observed between the vitamin D and the control group at the end of the study.

Vitamin D: Glycemic control and insulin resistance

Conflicting reports on the role of vitamin D and glycemic control are available in medical literature. Initial studies showcased a promise of improvement in hyperglycemia and reduction in insulin resistance; more recent evidence has highlighted contrasting outcomes [17, 19]. In this study, vitamin D supplementation did not improve glycemic control or insulin resistance measured by HOMA-IR. Vitamin D supplementation did not result in changes in BMI and levels of total cholesterol, triglycerides, LDL, HDL, or VLDL.

The limitations of this study include small sample size and relatively short study duration. The study methods did not include measurement of migratory and functional ability of EPCs. In retrospect, all patients in the study had normal hsCRP at baseline. It is well known that hsCRP > 10 mg/l confers high cardiovascular risk to individuals. The inability to detect anti-inflammatory effects or changes in EPCs could perhaps be attributed to this low risk study population.

Patients in this study were on other potentially confounding background medications like aspirin and rosuvastatin, known to reduce inflammation and CV risk. However, only low-dose aspirin was used in this study and none of the patients received anti-inflammatory doses of aspirin. The use of both aspirin and statin was also similar across groups, thus highlighting the importance of randomization in balancing known and other unknown confounders.

Even though there appears to be a lack of benefit of vitamin D on EPCs in this study, based on previous evidence from studies of acute coronary syndrome and EPCs [9, 38–41], it is possible that a biologically meaningful benefit or a trend towards benefit could be seen if the baseline risk is high. A better approach for future studies would be to augment identification of high risk individuals based on hsCRP levels and to study newer targets of intervention in the high risk population. Individuals with high baseline risk perhaps would be a more suitable study population, in whom new markers and therapeutic targets for intervention would also provide additional benefit. This would also facilitate a better use of resources while offering the greatest benefits to the high risk individuals.

A greater understanding of inflammation and the role of EPCs are necessary to further explore the implications of additional markers and therapeutic targets. Larger studies of longer duration are required to validate our findings. In this study, vitamin D supplementation failed to demonstrate a reduction in inflammation and improvement in endothelial repair.

Compliance with ethical standards

Ethical approval The study protocol was approved by the institutional ethics committee and the Research Society for the Study of Diabetes in India (RSSDI). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013.

Statement of informed consent Informed consent was obtained from all patients for being included in the study.

Conflict of interest Authors declare they have no conflict of interest.

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):228–237
DOI 10.1007/s13410-017-0574-1

ORIGINAL ARTICLE

Influence of high glucose and AGE environment on the proliferation, apoptosis, paracrine effects, and cytokine expression of human adipose stem cells in vitro

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Received: 19 April 2017 / Accepted: 23 July 2017 / Published online: 6 November 2017

Abstract The incidence of delayed wound healing in patients with diabetes has increased in recent years. However, the reason of delayed diabetic wound healing and the changes of human adipose stem cells (hASCs) in the diabetic environment are still unclear. We simulated diabetic microenvironment with high glucose and glycation end products (AGEs) in vitro. CCK-8 and flow cytometry were used to study the proliferation and apoptosis of hASCs in the simulated diabetic microenvironment. The paracrine of hASCs was studied by transwell co-culture system. Protein chip was used to measure the expression of cytokines in hASCs. We found that high glucose and AGEs did not affect the proliferation of hASCs but arrested them in the S phase. More hASCs appeared early apoptosis in the simulated diabetic microenvironment. The promoting effect on the proliferation of fibroblasts and endothelial cells was weakened when hASCs were cultured in diabetic microenvironment for 6 days. The five cell factors, granulocyte colony-stimulating factor (G-CSF), transforming growth factor-α (TGF-α), hepatocyte growth factor (HGF), tissue inhibitor of metalloproteinases-1 (TIMP-1), and vascular endothelial growth factor (VEGF), were all downregulated in hASCs of AGEs and the high glucose group. In this study, we simulated diabetic microenvironment with high glucose and AGEs in vitro to evaluate the changes of proliferation, apoptosis, paracrine, and cytokine expression of hASCs in the diabetic environment and tried to find the possible reason of delayed diabetic wound healing.

Keywords hASCs · AGEs · Glucose · Apoptosis · Paracrine · Cytokine

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Introduction

The advanced glycation end products (AGEs) are latter products of Maillard reaction or glycation and adversely affect the functional properties of proteins, lipids, and DNA [1]. In many clinical researches, AGEs in the diabetic skin are higher than normal tissue [2]. Also, positive correlations are found between the concentration of skin AGEs and age or incidence of foot ulcer [3]. A group of studies indicated that the AGEs inhibit the proliferation of endothelial cell [4], fibroblast [5], and nerve cell [6] and induce apoptosis. The former experiments indicated AGEs inhibit the proliferation and lead to human adipose stem cell (hASCs) apoptosis, decreasing the proliferation-promoting effect of hASCs on fibroblasts and endothelial cells. The formation of AGEs is closely related to the high concentration of tissue glucose, and AGEs accompany by high glucose can simulate a diabetic environment better. hASCs sited in skin follicles and subcutaneous fat may be important seed cells in wound repair [7]. The aim of our research was to study the changes of hASCs in the high glucose and AGE-simulated diabetic microenvironment, and refer another theoretical basis for diabetic wound healing and stem cell therapy.

Material and methods

Material

Human adipose-derived stem cells were provided by the stem cell technology platform of Shanghai Biochemistry and Cell Biology Institute (commercial cell line, cell No.: SCSP-404). The hASCs are derived from adipose tissue of an adult female donor and are negative for CD14, CD31, CD34, and CD45 and positive for CD29, CD44, CD73, CD90, CD105, and CD166.

Human fibroblasts were provided by Dr. Ying-kai Liu of Shanghai Burn Institute of Rui Jin Hospital.

Human umbilical vein endothelial cells were provided by ATCC (strain, PCS-100-010).

Methods

Preparing and grouping of hASCs

The hASCs were cultured in a mesenchymal stem cell growth medium (serum and antibiotics free, ready-to-use, PromoCell, Heidelberg, Germany) and were detached by a low serum detach kit (0.04% trypsin, PromoCell, Heidelberg, Germany). The primary hASCs were passed to passage 7 (P7) before experiment. The primary, passage 7 and passage 10 (P10) hASCs was photographed under a microscope (200×).

Three groups were designed as follows: hASCs cultured in a medium contained 100 mg/L AGEs (AGE full length protein, Abcam, Cambridge, MA, USA) and 28 mmol/L high glucose as an experimental group (AG group), 100 mg/L BSA and 28 mmol/L mannitol as a control group (BM group) [8], and normal hASCs without any addition in the medium as a blank group (BK group).

Culturing the hASCs in simulated diabetic microenvironment

The hASCs were cultured in the mesenchymal stem cell growth medium with 100 mg/L AGEs and 28 mmol/L high glucose. The medium was changed every 48 h and remove the non-adherent cells. The hASCs cultured 2, 4, and 6 days were collected to be used in the following experiments. Three groups of hASCs were photographed after 6 days culture.

Proliferation and apoptosis of hASCs

The CCK-8 assay (Dojindo Laboratories, Tokyo, Japan) was applied to study the proliferation of hASCs cultured 2, 4, and 6 days. The CCK-8 test sample was measured three times, and the average OD value was taken as the final data. Ten repeat samples were collected from each group.

On day 2, day 4, and day 6, hASCs were stained by AnnexinV/PI (BD Pharmingen™, San Jose, CA, USA) to detect the percentage of early, late, and total apoptotic hASCs in each group by flow cytometry. Four repeat samples were collected from each group.

Cell cycle detection of hASCs

The hASCs cultured in simulated diabetic microenvironment for 6 days were fixed with 75% ethanol 30 min. The fixed hASCs were stained with PI/RNase staining buffer (BD Pharmingen™, San Jose, CA, USA) and detected by flow cytometry. Three repeat samples were collected in each group; the experiment was repeated three times.

Co-culture of hASCs with human fibroblasts and endothelial cells

Human fibroblasts (1×10^4) were seeded into the upper compartments of 24-well transwell inserts (Membrane pore size 0.4um, Corning, USA). 1×10^5, 0.5×10^5, and 0.25×10^5 hASCs suspended in a 500-μl stem cell growth medium were seeded into the lower compartments. Culture media was added into the lower compartment as the control group.

Endothelial cells (1×10^4) were seeded into the upper compartments. 1×10^5 hASCs in the 500-μl stem cell growth medium were seeded into the lower compartments. Culture media was added into the lower compartment as the control group.

Human fibroblasts (1×10^4) and endothelial cells (1×10^4) were seeded into the lower compartments. The upper compartments were seeded by 1×10^4 hASCs. The same volume of culture media was added into the lower compartment as the control group.

Human fibroblasts (1×10^4) and endothelial cells (1×10^4) were seeded into the upper compartments. 1×10^5 hASCs cultured in AGEs and high glucose for 6 days were seeded into the lower compartments (AG co-culture group). 1×10^5 hASCs cultured in BSA and mannitol for 6 days were seeded into the lower compartments as the control group (BM co-culture group). 1×10^5 hASCs were seeded into the lower compartments (BK co-culture group). Culture media were added into the lower compartment as the blank group (BK group).

All the co-culture systems (the upper and lower compartments) lasted for 4 days and we changed the medium on the second day. Then the cell proliferation of upper compartments was detected by CCK-8. Each co-culture system had 12 repeat samples.

Cytokine expression of hASCs in simulated diabetic microenvironment

Total cell proteins from 0, 2, 4, and 6 days intervened hASCs were extracted and the expressions were measured by using

QAH-ANG-1000-2 protein chip (RayBiotech, Norcross, GA, USA). Data of five interested cytokines were statistically analyzed. Each group on each time point had four repeat samples.

Data analysis

The cytokine data in the best confidence interval was chosen to be statistically analyzed. All data was identified by the normal distribution test (*W* test) and homogeneity test of variance (*F* test) with SPSS19.0. The data of each group were compared by *t* test using SPSS19.0. p value <0.05 indicates statistically significant difference.

Result

Cell culture and morphology of hASCs

Primary hASCs showed spindle, fusiform shape, 2–3 pseudopodia, large and oval nucleus and one to three nucleoli. The hASCs attached the culture dish firmly and distribution patterns were swirling or radial (Fig. 1a Primary). The shape and structure of P7 cells were not different from primary cells (Fig. 1a P7). P10 hASC volume increased, and the body became long and narrow with more pseudopodia. Some cells had intracellular clusters of vacuoles, transparent nuclei, and nucleolus pyknosis which were like "fried egg." Cell growth became slow and the number of floating cells increased (Fig. 1a P10).

The morphology of hASCs in simulated diabetic microenvironment

The AG group hASCs proliferated rapidly, reaching the peak in 2–4 days; cell count did not significantly increase in 4–6 days, and some cells became larger. The "fried egg"-like cells were more than the BM group and BK group (Fig. 1a).

The proliferation of hASCs cultured in simulated diabetic microenvironment

The OD values of three groups on days 2, 4, and 6 measured by CCK-8 are compared. The AG group is not different with the BM group on day 2 and day 6, but higher than the BM group on day 4. There were no significant differences between the BM group and BK group (Fig. 1b).

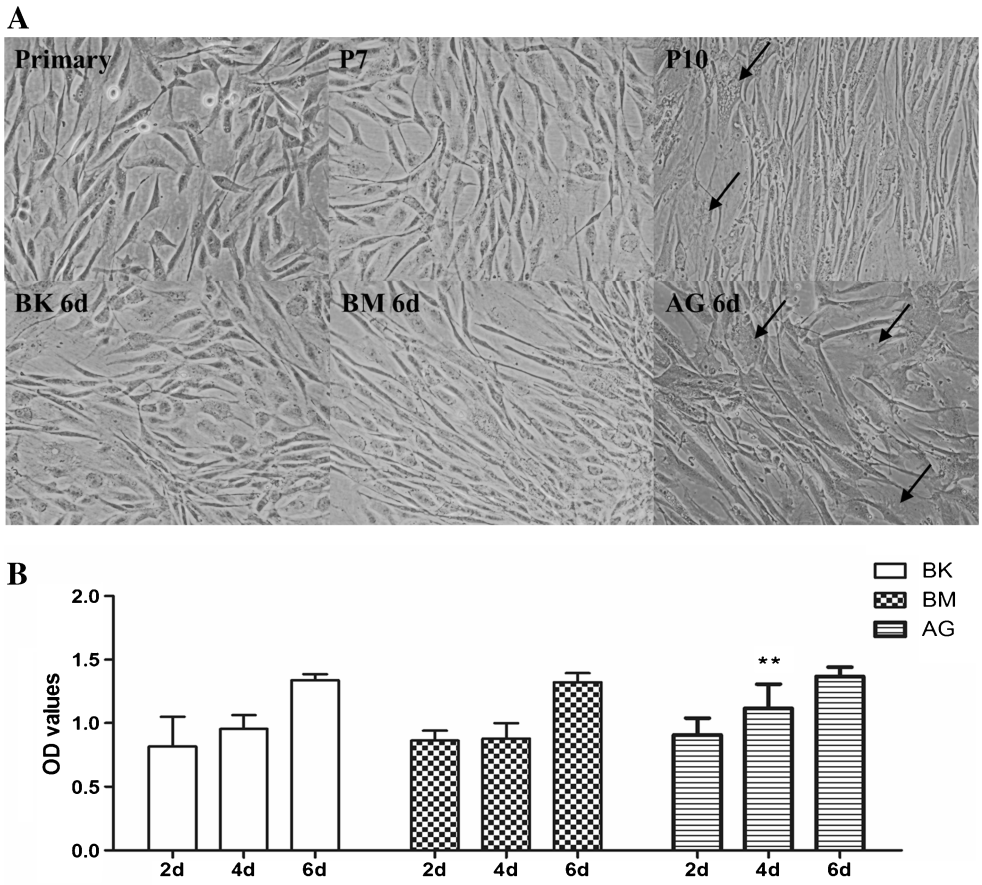
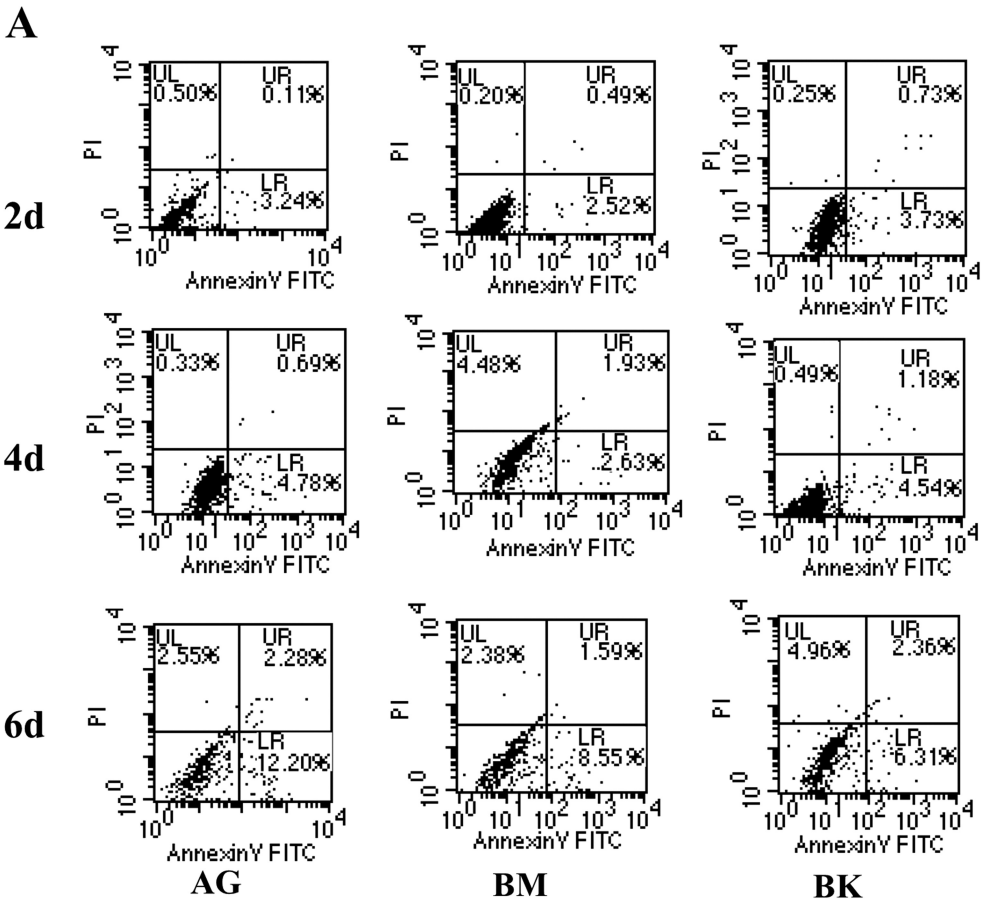


Fig. 1 The morphology and proliferation of hASCs in simulated diabetic microenvironment. The P10 hASCs contained more "fried egg" like cells than primary hASCs (*arrow*) (**a** *P10*) (200×). The hASCs in AG group could be found a few "fried egg" like cells on day 6 (*arrow*) (**a** *AG6d*) (200×).The differences of hASC proliferation between AG group and BM group (**b**) BM group and BK group (**c**). *$p < 0.05$, **$p < 0.01$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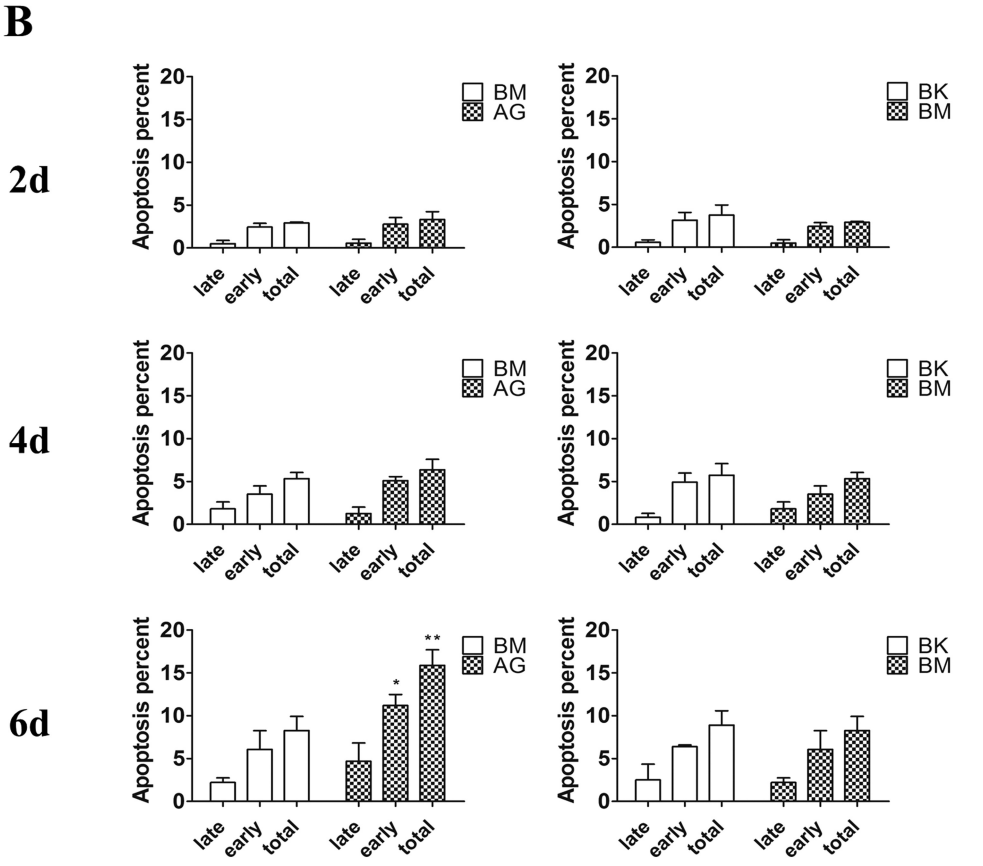
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◀ **Fig. 2** The apoptosis of hASCs cultured in simulated diabetic microenvironment. The flow cytometry results of the AG, BM, and BK groups on day 2, day 4, and day 6 (**a**) and the bar graph (**b**). *$p < 0.05$, **$p < 0.01$

The apoptosis of hASCs cultured in simulated diabetic microenvironment

The difference of apoptosis between the AG group and BM group was not significant on day 2 and day 4. The early apoptosis (AnnexinV+/PI-) and total apoptosis (early + late) of the AG group was higher than those of the BM group on day 6. The late apoptosis (AnnexinV+/PI+) of the AG and BM groups had no significant difference on day 6. The apoptosis of the BM and BK groups showed no significant difference (Fig. 2).

Cell cycle of hASCs in simulated diabetic microenvironment

The percentage of the G1 phase between the AG group and BM group showed no significant difference on day 6. The S phase percentage of the AG group was higher than that of the BM group, but the G2 phase percentage was lower than the BM group on day 6. The S/G2 value of the AG group was also higher than the BM group on day 6. The results of the BM and BK groups showed no significant difference (Fig. 3b).

The paracrine of hASCs in simulated diabetic microenvironment

The number of fibroblast in the 1×10^5 hASC co-culture group was significantly higher than that in the control group after 4 days (Fig. 4a). The number of fibroblast cultured with 0.5×10^5 or 0.25×10^5 hASCs was not significantly higher than that in the control group (Fig. 4b, c). The number of fibroblast in the 1×10^5 hASC group was significantly higher than that in the 0.5×10^5 hASC group and 0.25×10^5 hASC group. The number of endothelial cells co-cultured with 1×10^5 hASCs was significantly higher than that in the control group (Fig. 4d).

There were no significant differences between the number of hASCs in the upper compartments co-cultured with fibroblasts or endothelial cells and the number of hASCs in the control group (Fig. 4e, f).

The number of fibroblasts in the AG co-culture group was significantly lower than that in the BM co-culture group (Fig. 4g) but was still higher than that in the BK group (Fig. 4h).

The number of endothelial cells in the AG co-culture group was not different with that in the BM co-culture group (Fig. 4i). The endothelial cells in the AG co-culture group were no more than the BK group (Fig. 4j).

The number of endothelial cells and fibroblasts in BM co-culture group were not different with those in the BK co-culture group.

The protein expression of hASCs in simulated diabetic microenvironment

The expression of five interested cytokine, hepatocyte growth factor (HGF), granulocyte colony-stimulating factor (G-CSF), transforming growth factor-α (TGF-α), tissue inhibitor of metalloproteinases-1 (TIMP-1), and vascular endothelial growth factor (VEGF), were measured in the AG, BM, and BK groups on day 2, day 4, and day 6.

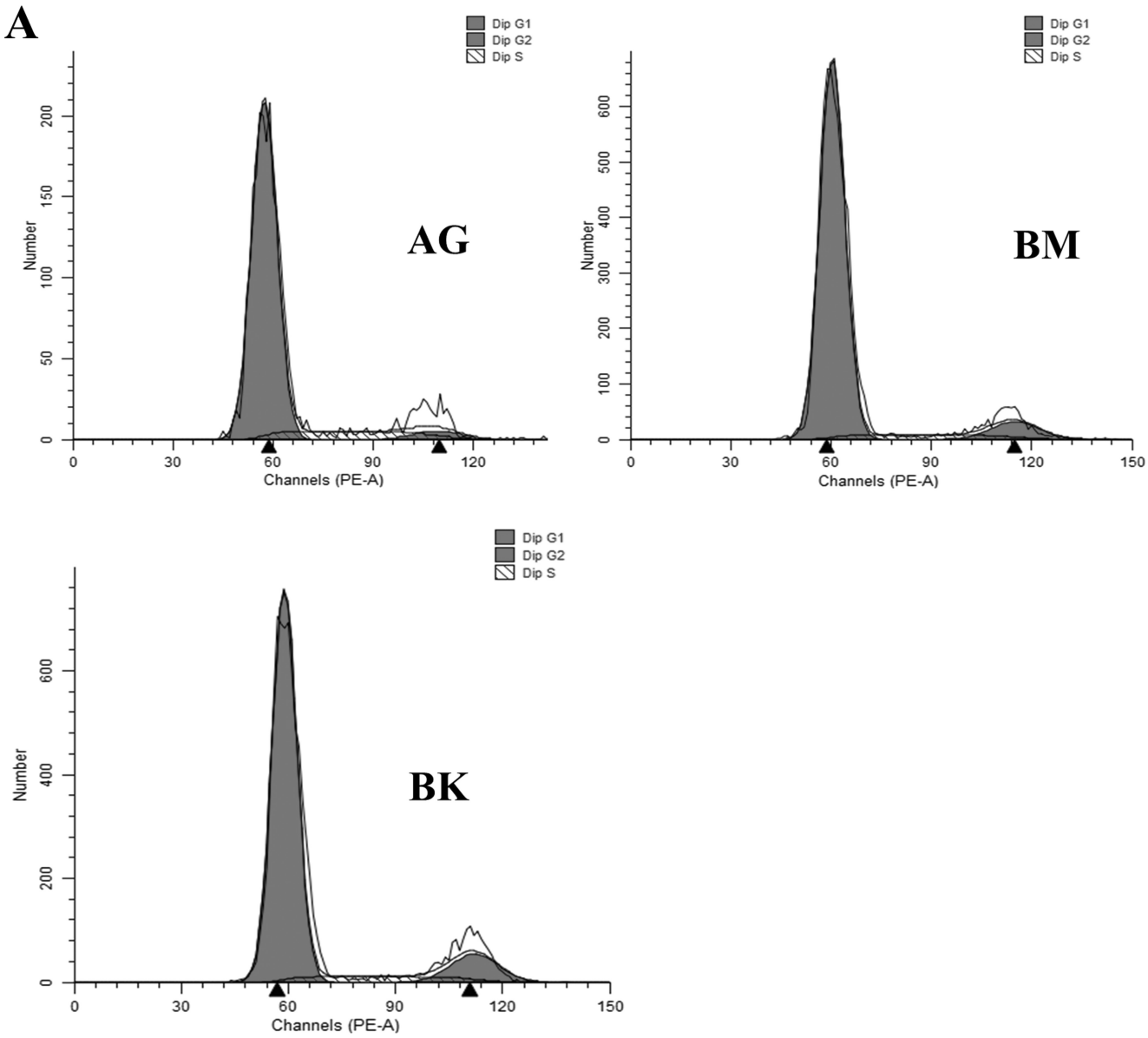
In simulated diabetic microenvironment, HGF was downregulated on day 2, day 4, and day 6 ($p < 0.01$). The expressions of G-CSF, TGF-α, and TIMP-1 in the AG group were not different from the BM group on day 2 and day 4. On day 6, the expressions of the three factors were downregulated ($p < 0.05$). VEGF expression was not different between the AG group and BM group on day 2, while the expression was downregulated on day 4 ($p < 0.05$) and day 6 ($p < 0.01$) (Fig. 5).

Discussion

The primary hASCs will grow old with time, morphology of P10 hASCs has changed, and the "fried egg"-like cells may no longer have stem cell properties [9]. P7 hASCs were chosen for experiments because they had enough number, morphology as primary cells, stable proliferation rate, and low apoptosis. The BK group was used to verify whether the BSA and mannitol environment has effect on hASCs.

The AGEs in our study were produced by the Maillard reaction of BSA and glucose which was similar to in vivo [10]. The combination of AGEs and high glucose in the culture medium can simulate the micro environment of diabetic tissues better than adding AGEs or high glucose only. The 28 mmol/L glucose can simulate the hyperglycemia of a diabetic patient [11]; while not reaching the blood glucose of ketoacidosis (33 mmol/L), 100 mg/L AGEs was chosen as former research [12].

The growth of hASCs in high glucose and AGEs was not inhibited; the simulated diabetic microenvironment might even promote the growth of hASCs on day 4. Many former researchers found that only high glucose would promoted the growth of hASCs [13, 14]. In our study, the promoting effect was not continuous for the number of hASCs in the AG group on day 6 and was no more than the BM group. In the cell cycle experiment, more hASCs in simulated diabetic environment were blocked in the S phase and unable to complete cell division. This may explain the low proliferation of hASCs on day 6.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◀ **Fig. 3** Cell cycle of hASCs in simulated diabetic microenvironment. The cell cycle results of the AG, BM, and BK groups on day 6. (**a**) and the bar graph contrast AG and BM group (**b**) and bar graph contrast BM and BK group (**c**) $*p < 0.05$, $**p < 0.01$

The percentage of early apoptotic hASCs increased in in vitro diabetic environment. Those early apoptotic hASCs could not complete differentiation and might not participate in wound healing. These results happened on hASCs just had been cultured in simulated diabetic environment for 6 days. The hASCs of diabetic patients may stay in the high glucose and AGE environment for several months or even years. It may lead to more severe cell cycle arrest and apoptosis [15, 16], may reduce the wound healing ability of hASCs, and may finally cause the delayed wound healing. AGEs and high glucose may also affect hASCs (local or transplanted) in the diabetic wound and weaken the efficacy of stem cell therapy.

The hASCs might promote the proliferation of fibroblasts and endothelial cells by paracrine [17]. The promoting effect of hASCs was positively correlated with cell number. In diabetic patients, apoptosis induced by AGEs in the diabetic microenvironment may decrease the number of hASCs and may weaken the paracrine promoting effect of hASCs on proliferation of fibroblasts and endothelial cells.

The HGF can upregulate the expression of VEGF and TIMP-1 by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor [18]. HGF plays a central role in angiogenesis and tissue regeneration. VEGF promotes the proliferation of vascular endothelial cells and angiogenesis [19]. The expression of HGF in hASCs of AG group was decreased, which may weaken the promoting effect of hASCs on the proliferation of endothelial cells. VEGF in hASCs was decreased on day 4 and day 6 after HGF which may further weaken the angiogenic promoting effect of hASCs and reduce the proliferative activity of endothelial cells.

The G-CSF can promote endothelialization of injured vascular. It may reduce expression of pro-inflammatory cytokines and enhance neurogenesis [20]. The low expression of G-CSF in the AG group may lead to decreased proliferation of endothelial cells and delayed repair of vascular injury.

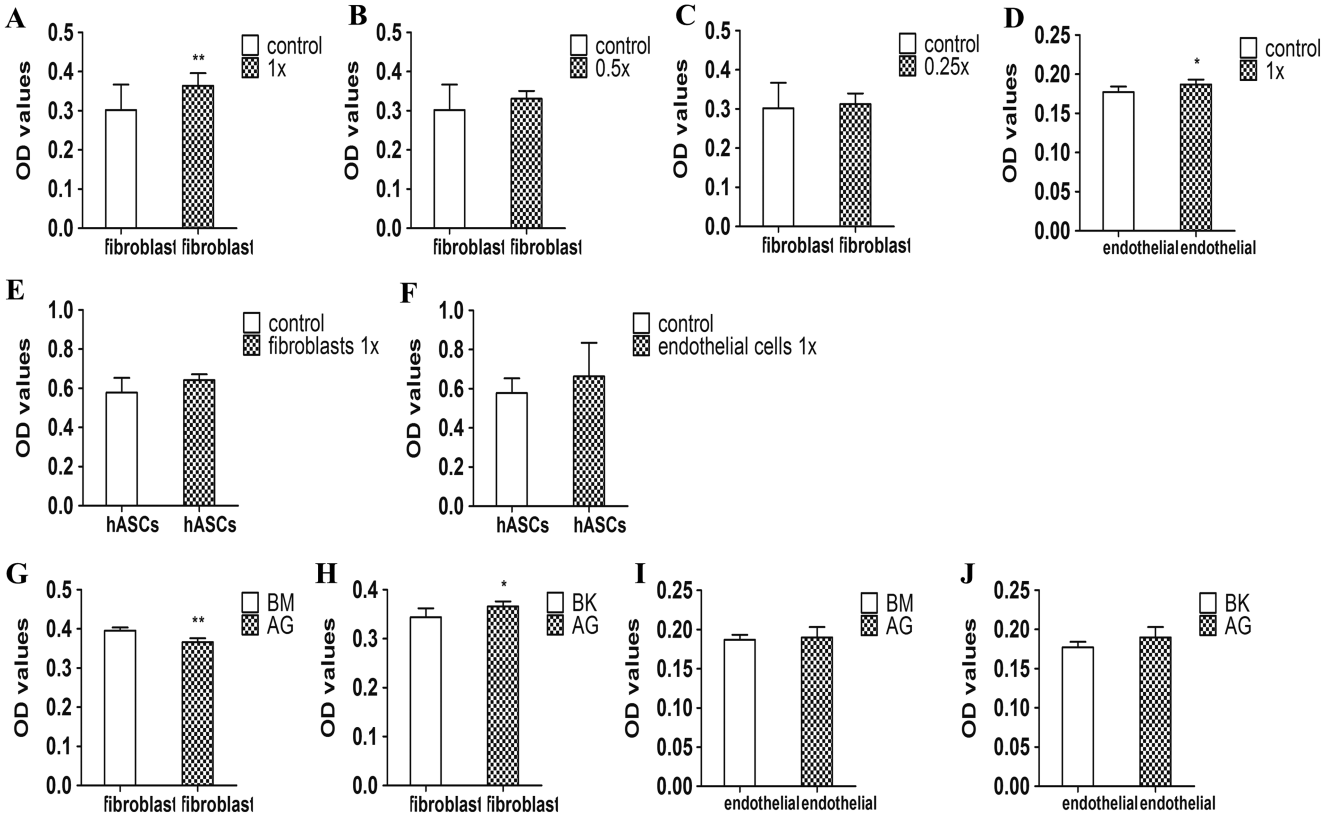

Fig. 4 The results of transwell co-culture system. The proliferation of fibroblasts co-culture with 1×10^5(**a**), 0.5×10^5(**b**), and 0.25×10^5(**c**) hASCs seeded in the lower compartments and with 1×10^5 hASCs of AG group in the lower compartments (**g**, **h**). The proliferation of endothelial cells co-culture with 1×10^5 hASCs seeded in the lower compartments (**d**) and with 1×10^5 hASCs of AG group in the lower compartments (**i**, **j**). The proliferation of hASCs co-culture with 1×10^5 \fibroblasts seeded in the lower compartments (**e**) and with 1×10^5 endothelial cells of the AG group in the lower compartments (**f**)

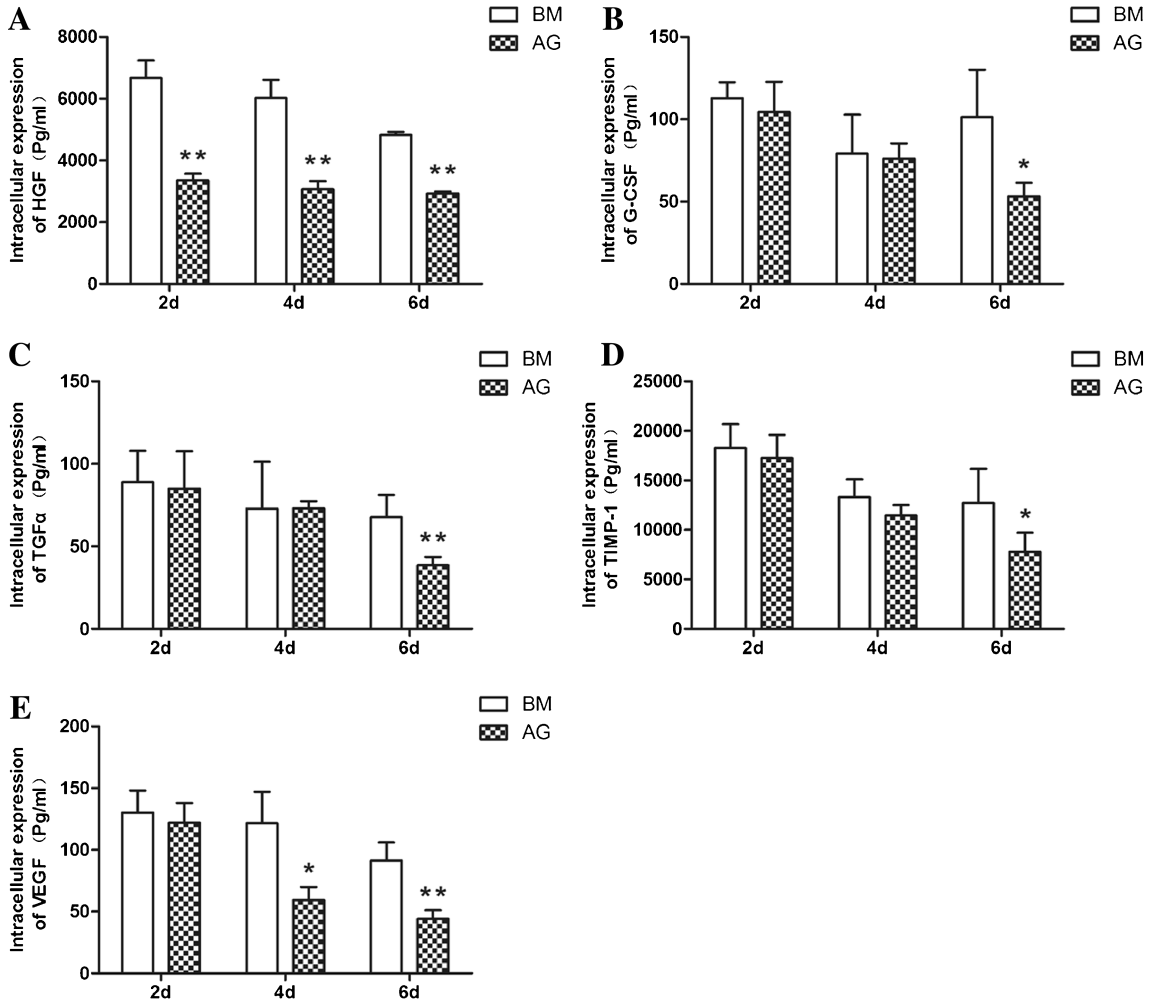

Fig. 5 Differences in protein expression of hASCs cultured in simulated diabetic microenvironment. The HGF (**a**), G-CSF (**b**), TGF-α (**c**), TIMP-1 (**d**) and VEGF (**e**) expression of hASCs cultured in the AG and BM groups. *$p < 0.05$; **$p < 0.01$

The TGF-α is a member of the epidermal growth factor (EGF) family, which activates cell proliferation and differentiation. TGF-α is also believed to promote angiogenesis and protect endothelial cell from apoptosis [21]. The TGF-α was downregulated in the AG group, which may decrease epithelial regeneration and may affect diabetic wound healing.

The TIMP-1 promotes cell migration and proliferation and is involved in degradation and remodeling of the wound matrix. Bullen et al. [22] found TIMP-1 levels were lower in chronic than in healing wounds. Imbalance of TIMPs and MMPs is a necessary factor for chronic wound formation [23]. TIMP-1 in AG group was decreased, which may accelerate the wound tissue degradation and aggravate the spread of inflammatory factors. The downregulation of growth factors such as G-CSF and TGF-α will reduce the proliferation of wound healing cells [24]. Fewer mesenchymal cells will lead to the decrease of HGF expression and further decrease the expression of VEGF and TIMP-1. A vicious cycle of wound healing factors was formed, and it may explain the causes of delayed diabetic wound healing.

Conclusions

High glucose and AGEs do not inhibit the proliferation but arrest the hASCs in the S phase. High glucose and AGEs lead to hASC apoptosis, decreasing the paracrine-promoting effect of hASCs on proliferation of fibroblasts and endothelial cells. The decrease of growth factors HGF, G-CSF, and TGF-α in hASCs may influence the proliferation of endothelial and epithelial cells. All the changes may lead to delayed diabetic wound repair and regeneration. The results of this study suggest that decreasing glucose and AGEs in the microenvironment of hASCs or increasing the expression of HGF and TGF

in hASCs may be a new therapeutic option in clinical to speed up diabetic wound healing. This study is not without limitations. We did not carry out these experiments on cells obtained from diabetic patients. More experiments are needed to study the changes of diabetic hASCs in the simulated diabetic microenvironment.

Acknowledgements The authors gratefully acknowledge Ying-kai Liu, Lei Yi, and Qing Pei for the technical assistance and Shu-wen Jin for the systematic guidance.

Authors' contributions Conceived and designed the study: JHG, SLL, JYD. Performed the experiments: JHG. Analyzed the data: JHG. Contributed reagents/materials/analysis tools: JHG, SLL, TX. Wrote the manuscript: JHG, SLL. Experiment skill advising: JYD, SLL. All authors read and approved the final manuscript.

Compliance with ethical standards

Funding This study was funded by the Chinese National Natural Science Foundation (81071566) and Key Project of Chinese National Programs for Fundamental Research and Development (973 program, 2012CB518105).

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

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):238–242
https://doi.org/10.1007/s13410-017-0588-8

ORIGINAL ARTICLE

The protective effect of the Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ (PPARγ) isoform 2 in progression to diabetes in a Pakistani cohort

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Received: 21 May 2017 / Accepted: 23 October 2017 / Published online: 13 November 2017

Abstract Diabetes is a serious health issue in Pakistan with 6.9 million cases of diabetes and 87,548 deaths due to diabetes in Pakistan in 2014. Peroxisome proliferators-activated receptors are transcription factors, regulating several physiological processes The aims of the current study were to determine the prevalence of Pro12Ala polymorphism (C>G) of PPAR-y isoform 2 gene and analyze its effect on selected anthropometric and biochemical parameters in a Pakistani cohort. We collected 926 samples, 500 healthy controls (fasting blood sugar < 99 mg/dL, random blood sugar < 126 mg/dL) and 426 cases with diabetes (fasting blood sugar > 99 mg/dL, random blood sugar > 126 mg/dL). The genotyping was done and serum biochemical parameters were determined. The results showed allelic frequency C = 0.716 and G = 0.284 in controls and C = 0.766 and G = 0.234 in diabetic cases; whereas, genotypic frequency was CC = 52.2%, CG = 38.8%, and GG = 9% in controls and CC = 57.7%, CG = 37.8%, GG = 4.5% in cases. The association of Pro12Ala polymorphism under additive, dominant, and recessive models was checked (additive OR 0.58, CI 0.44–0.77, *p* value 0.0002, dominant OR 0.60, CI 0.44–0.82, *p* value 0.001, recessive OR 0.36, CI 0.15–0.86, *p* value 0.016). The result showed that the polymorphism is significantly associated with the decreased risk of diabetes in the Pakistani study subjects under additive models. The variant showed an association with weight, BMI, blood glucose, TC, HDL-C, insulin, and HOMA-IR. In conclusion, the presence of minor allele lowers the risk of diabetes and the effect may involve modulating serum glucose and lipid levels.

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Keywords Diabetes · Pro12Ala · Peroxisome proliferators-activated receptor · Pakistan

Introduction

Diabetes is a metabolic disorder characterized by hyperglycemia, which may be due to altered metabolism of glucose. It is associated with many specific and non-specific microvascular complications. Diabetes can also be defined as the group of metabolic anomalies characterized by elevated blood glucose level, which can either result from improper functioning of the pancreatic β cells which decreases insulin production or due to the resistance of body cells to insulin wherein insulin production by β cells is normal but cells cannot respond to it [1].

Diabetes is a common cause of premature mortality and prolonged illness in Pakistan. It is highly prevalent in almost all regions of Pakistan, with an overall prevalence of 22.04% in urban and 17.15% in rural areas. The gender-wise distributions of diabetes in different provinces of Pakistan are the following: Punjab; males 16.6%, females 19.3%, Khyber PakhtoonKhuwa; 11.1% both sexes, Balochistan; 10.8% both sexes, and Sindh; males 16.2%, females 11.7% [2].

Peroxisome proliferators-activated receptors (PPARs) are transcription factors and members of the superfamily of steroid hormone receptors, activated by lipid soluble membrane permeable ligands and regulate the expression of several target genes. The physiological processes affected include insulin signaling, lipid and glucose metabolism, and adipogenesis [3].

PPAR has three iso-forms, PPARὰ, PPARβ, and PPARϒ [4]. The gene encoding for PPARϒ (PPARG) is located on the short arm of chromosome 3 (3p25). The gene is 100 Kb long containing nine exons [5]. The functions of this nuclear transcription factor include regulation of multiple genes involved in energy regulation, glucose, and lipid metabolism and plays

role in insulin sensitivity. It is a link between environmental factors and metabolic processes of the organism [6]. The PPARϒ contains three different promoters that on transcription yields further three iso-forms of PPARϒ, namely PPARϒ1, PPARϒ2, and PPARϒ3 [7]. PPARϒ1 is the isoform which have broad tissue range in body, while on the other hand, PPARϒ2 is only restricted to the adipose tissue of the body. PPARϒ3 is expressed abundantly in the macrophages, in large intestine, and in white adipose tissue [8].

There are many causes of diabetes, although the underlying mechanism of diabetes development remains to be established. Among genetic causes, mutations and variants in PPARϒ have been proposed to be a strong candidate. The most common gene polymorphism in the human PPAR?2 gene is cytosine-guanine exchange in exon B (codon12) which results proline to alanine (Pro12Ala) substitution in the protein. The Pro12Ala polymorphism (rs1801282) was first identified by Yen et al. in 1997 reporting a frequency of the rare Ala allele of 9.3% in subjects with normal glucose tolerance versus 2.2% in patients with type 2 diabetes was observed [9]. The alanine allele of the common Pro12Ala polymorphisms in the isoform PPARγ2 is associated with a 25% reduced risk for type 2 diabetes thus representing the first genetic variant with a broad impact on the risk of common type 2 diabetes. The effect of this polymorphism may involve increased insulin sensitivity, and suppression of free fatty acid release from fat tissue, where the isoform PPARγ2 is expressed exclusively. Modulation of expression and release of adipocytokines that influence insulin sensitivity are likely also to be involved, but this remains to be demonstrated in humans. The proposed underlying molecular mechanism of this polymorphism is that a moderate reduction of the ligand-independent activity of PPARγ2 results in the presence of Pro12Ala variant, but the findings for this variant differ depending on the superimposition of environmental factors. The understanding of how specific modulation of PPARγ influences metabolism in humans may accelerate the development of novel pharmacological agents useful for preventing or treating type 2 diabetes and related disorders.

Previous studies have investigated the role of this polymorphism in the development of obesity and diabetes, with equivocal results [10, 11], suggesting that environmental influences such as dietary intake may be involved. Moreover, the interaction with other independent modulators such as obesity, ethnicity, ratio of unsaturated to saturated fatty acids, and other common genetic polymorphisms emphasizes the need of further studies on this SNP. In addition, the contradictory findings observed in various studies highlight the importance to study the impact of *the Pro12Ala polymorphism* of the *PPARγ2* gene for diabetes [10, 12–14]. We, therefore, selected this polymorphism to find out its prevalence and see if it plays any role in diabetes in the Pakistani subjects.

Materials and methods

Subject recruitment

A total of 926 samples (426 diabetic cases and 500 normal controls) were recruited from the general population. Inclusion criteria for diabetic subjects were (i) diabetes diagnosed according to etiologic classification of diabetes by the International Diabetes Federation (IDF) and (ii) confirmation that all the grandparents of the subjects are of Pakistani origin. The exclusion criteria were the presence of any infectious disease, conditions where phlebotomy is contra-indicated, age below 10 years, body mass index (BMI) ≤ 18.5 kg/m^2, pregnancy, handicapped/mentally disturbed individuals, obesity, cancer, and ethnicity other than Pakistani. The recruitment was done between July 2014 and April 2015. The controls were healthy subjects from the general population with normal blood sugar levels (< 126 mg/dL random or < 99 mg/dL fasting). All the subjects were genetically unrelated and gave written informed consent. The procedures employed were according to the Helsinki declaration and an ethical approval was obtained from the institutional ethical board.

Blood sampling

Venous blood was collected from the subjects in the fasting state. Five milliliters of blood was collected from the median cubital vein using aseptic measures. Half was dispensed in an EDTA vial and used for DNA isolation, while rest half was poured in a gel clot activator containing vial and used for biochemical analysis.

Biochemical parameters' determination

Gel clot activator containing vial was centrifuged to separate plasma for determination of biochemical parameters. Serum was screened for HBV, HCV, and HIV. Serum fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high density and low density lipoprotein cholesterol (HDL-c, LDL-c) were determined using commercially available kits (Spectrum Diagnostics, Egypt); leptin was measured by LDN Nordhorn leptin ELISA kit, whereas insulin concentration was measured using an electrochemiluminescence method as described previously [15], and HOMA-IR was calculated.

Genotyping

For genetic analysis of Pro12Ala single nucleotide polymorphism tetra primers, ARMS PCR technique was used; for performing PCR reaction, two sets of primers both inner and outer primer pairs were selected from a reference article by Masud et al. [16]. Each PCR reaction was carried out with

25 μL of the volume with 10 ng of genomic DNA, 2.5 μM $MgCl_2$ and 200 μM each dNTP mix, 10 μmoles of each inner primer, and 1 μmole of each outer primer and 1 U of Taq Polymerase (Thermo Scientific, Cat#EP002) enzyme with no 3′ → 5′ and virtually negligible 5′ → 3′proofreading.

The reaction mixture was subjected to initial denaturation at 95 °C for 2 min followed by 40 cycles of reaction (each cycle with denaturation at 95 °C for 1 min, annealing at 58 °C for 45 s, and extension at 72 °C for 1 min) with a final extension at 72 °C for 10 min. After performing PCR, 2% agarose gel with 0.5× TBE buffer was used for electrophoresis at 90 V for 45 min. Ninety-five samples were randomly picked and regenotyped using same PCR conditions and 10 samples were sequenced to check for the accuracy of genotyping. The concordance rate was 100%.

Results

The diabetic cases studied were from the government hospitals, the diabetic centers, and laboratories in different areas of Lahore. The general characteristics of the subjects are given in Table 1 and show that, except height and gender (p value 0.314 and 0.108, respectively), all other parameters were found to be significantly different between cases and controls ($p < 0.05$). The mean age (years) of cases was 42.12 ± 9.53 and of controls was 49.84 ± 8.46. The proportion of diabetic patients suffering population from other disorders, including foot ulcer, cardiovascular disease, hypocholesteremia, hypertension, nephropathy, retinopathy, and obesity was higher in cases than controls (Table 2).

The distribution of observed genotypes is given in Table 3. The study was checked for the Hardy–Weinberg equilibrium and was found to be in equilibrium ($p = 0.304$ for controls and $p = 0.165$ for cases. The allelic frequency was C = 0.716 and G = 0.284 in controls and C = 0.766 and G = 0.234 in diabetic cases; whereas, genotypic frequency was CC = 52.2%, CG = 38.8%, and GG = 9% in controls and CC = 57.7%, CG = 37.8%, GG = 4.5% in the cases. The association of Pro12Ala polymorphism with diabetes was checked under additive, dominant, and recessive models. The result showed that the polymorphism is significantly associated with decreased risk of diabetes in the Pakistani study subjects under all models (additive OR 0.58, CI 0.44–0.77, p value 0.0002, dominant OR 0.60, CI 0.44–0.82, p value 0.001, recessive OR 0.36, CI 0.15–0.86, p value 0.016).

The association of this variant with different anthropometric and biochemical traits was checked and the results are given in Table 4. It showed a significant association with weight, BMI, height, blood glucose, TC, LDL-C, insulin, and HOMA-IR in cases, with weight, blood glucose, TC, and LDL-C and a marginal association with BMI and HOMA-IR in the controls. However, there was no association observed between this polymorphism and gender, TG, and HDL-C.

Table 2 Prevalence of comorbidities

Disorders	Frequency (%)	
	Cases (n = 426)	Controls (n = 500)
Cardiovascular disease	8.1%	2%
Nephropathy	7%	0
Retinopathy	3.4%	0
Foot ulcer	3.8%	0
Hypocholesteremia	7%	1%
Hypertension	8.9%	2.3%

Table 1 General characteristics of the study subjects

Parameters		Diabetic (n = 426)	Non-diabetic (n = 500)	p value
Gender	Male	228	272	0.108
	Female	198	228	0.108
Age (year)		42.12 ± 9.53	49.84 ± 8.46	0.0001*
Height (ft)		5.50 ± 0.3	5.49 ± 0.59	0.314
Weight (kg)		68.6 ± 13.83	65.38 ± 13.7	0.0004*
BMI (kg/m^2)		21.67 ± 5.3	22.7 ± 5.6	0.0043*
FPG (mg/dL)		103.71 ± 3.12	89.56 ± 5.09	< 0.001
Total cholesterol (TC) (mmol/L)		5.31 ± 0.54	4.21 ± 0.79	< 0.001
Triglycerides (TG) (mmol/L)		2.53 ± 0.83	2.17 ± 0.35	< 0.001
HDL-c (mmol/L)		1.32 ± 0.21	2.15 ± 0.23	< 0.001
LDL-c (mmol/L)		2.57 ± 0.56	2.02 ± 0.34	< 0.001
Insulin (μU/mL)		18.85 ± 4.13	11.43 ± 5.01	< 0.001
HOMA-IR		4.39 ± 3.86	1.95 ± 0.79	< 0.001

BMI body mass index, *n* total number

*Indicates significant differences

Table 3 Allelic and genotypic frequency in study subjects

Allele/genotype	Controls	Cases	OR (CI), *p* value
CC	261 (52.2%)	246 (57.7%)	Additive 0.58 (0.44–0.77), 0.0002
CG	194 (38.8%)	161 (37.8%)	Dominant 0.60 (0.44–0.82), 0.001
GG	45 (9%)	19 (4.5%)	Recessive 0.36 (0.15–0.86), 0.016
C	716 (0.716)	653 (0.766)	
G	284 (0.284)	199(0.234)	

CI confidence interval, *OR* odds ratio

Discussion

The current study tested a common SNP (Pro12Ala; rs1801282) from the coding region of PPARG gene for association with obesity in a Pakistani case control sample consisting of 926 subjects. The advances in the genetic era have led to the identification of multiple risk loci and sequence variants for monogenetic diseases, as well as common complex disease. The expression and effect of single nucleotide variants can be different across ethnicities. The Pro12Ala SNP is a well-studied genetic variant in Caucasians and many Asian countries including China, Japan, and India but has been investigated in Pakistan in the context of rheumatoid arthritis only [17] and its role has not been previously reported in Pakistan with respect to diabetes.

The present study determined the frequency of the proline to alanine substitution in the human *PPAR* γ2 gene in the Pakistani population and investigated its role on various anthropometric and biochemical parameters. The allelic frequency of *Pro12Ala*, a polymorphic variant of the *PPAR* γ2 gene, varies among different ethnicities. We found a high frequency of Ala allele in the control subjects and a significant association of the polymorphism with lowering the risk of diabetes in all tested genetic models an indication of a possible protective role of this SNP in the Pakistani subjects. This is in contrast to a recent study carried out in a Qatari population which could not find any significant association of this variant with diabetes [18]. The authors reported the frequency of *Pro12Ala* allele was greater (10.2%) in diabetic population than in non-diabetic group (9.4%), but the difference between the groups was not significant. Another study conducted in South Indian subjects reported lack of any protection by Pro12Ala variant from T2DM [19]. Some Caucasian studies have also observed high frequency of Ala allele in the diabetic subjects [13, 20], but most of the studies conducted in Caucasians and some Asian countries implicate a considerably high frequency of the Ala allele in the control subjects [10, 14, 21, 22] which suggests that the *Ala 12* allele of the

Table 4 Comparison of anthropometric and biochemical parameters among all three genotypes in diabetic and control subjects

Parameter	Diabetic cases (*n* = 426)			*p* value	Controls (*n* = 500)			*p* value
	GG	GC	CC		GG	GC	CC	
Age (years)	39.81 ± 3.08	41.23 ± 1.72	37.53 ± 1.43	0.51	46.13 ± 1.24	40.14 ± 2.49	47 ± 1.48	0.69
Weight (kg)	93.71 ± 1.84	90.24 ± 1.74	86.92 ± 3.75	0.04	65.29 ± 1.23	61.92 ± 1.41	58.06 ± 1.09	0.03
BMI(kg/m^2)	24.16 ± 1.91	23.67 ± 0.71	22.57 ± 1.76	0.021	23.76 ± 0.81	22.95 ± 0.71	22.04 ± 6.13	0.05
Height (cm)	160.33 ± 2.65	162.25 ± 1.25	163.14 ± 2.45	0.04	161.10 ± 1.65	161.30 ± 1.21	161.94 ± 1.04	0.09
FPG (mg/dL)	105.92 ± 6.54	103.01 ± 5.65	100.91 ± 4.52	0.003*	91.54 ± 6.81	90.22 ± 4.98	88.81 ± 5.41	0.011*
Total Cholesterol (TC) (mmol/L)	5.43 ± 0.23	5.23 ± 0.49	5.11 ± 0.52	< 0.001*	4.89 ± 0.79	4.59 ± 0.76	4.39 ± 0.59	< 0.001*
Triglycerides (TG) (mmol/L)	2.55 ± 0.43	2.51 ± 0.85	2.53 ± 0.82	0.131	2.12 ± 0.11	2.14 ± 0.19	2.05 ± 0.21	0.514
HDL-c (mmol/L)	1.13 ± 0.10	1.15 ± 0.15	1.09 ± 0.13	0.112	2.19 ± 0.44	2.21 ± 0.47	2.13 ± 0.52	0.135
LDL-c (mmol/L)	2.67 ± 0.45	2.49 ± 0.53	2.37 ± 0.56	0.021*	2.26 ± 0.62	2.14 ± 0.41	2.07 ± 0.34	0.04*
Insulin (μU/mL)	21.65 ± 4.19	20.02 ± 4.58	19.35 ± 4.91	0.03*	11.98 ± 4.31	11.45 ± 3.91	10.19 ± 3.21	0.07
HOMA-IR	5.37 ± 4.01	5.28 ± 3.23	5.16 ± 2.21	0.02*	2.37 ± 1.91	2.45 ± 1.11	2.55 ± 0.91	0.05

BMI body mass index, *FPG* fasting plasma glucose, *HDL-c* high density lipoprotein cholesterol, *LDL-c* low density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance

The table compares the anthropometric and biochemical characteristics of the study population with respect to genotypes. Values are indicated as mean ±SE

*Indicates any significant association

PPAR γ2 gene may protect subjects with this polymorphism from type 2 diabetes.

The correlation analysis between the *PPAR γ2* genotype and various anthropometric parameters of the case and control subjects revealed a significant association with BMI and only a marginal association with weight showing the important role of obesity in progression to diabetes, the variant appeared to significantly affect fasting plasma glucose, total cholesterol, LDL-C, insulin, and HOMA-IR. A previous study reported that *12 Ala* variant is associated with a lower level of insulin secretion in patients with diabetes [22]. These findings suggest that the variant may exert its effect by altering the metabolism of many serum parameters ultimately protecting from diabetes.

Conclusion

The study findings suggest that the Pro12Ala polymorphic variant is significantly associated with decreased risk of diabetes in the Pakistani subjects and may have a protective role against the disease. The results therefore support the role of this variant as the potential genetic marker for diabetes in the Pakistani population.

Acknowledgments Punjab Institute of cardiology is also acknowledged for providing the patients' blood samples.

Funding information The study was supported by the University of the Punjab research and project grant.

Compliance with ethical standards

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

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Int J Diabetes Dev Ctries (April–June 2018) 38(2):243–248
DOI 10.1007/s13410-017-0566-1

SHORT ARTICLE

Prevalence of diabetes and co-morbidities in five rural and semi-urban Kenyan counties, 2010–2015

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Received: 21 February 2017 / Accepted: 11 June 2017 / Published online: 30 June 2017

Abstract Globally, >80% of diabetes-related deaths occur in low- and middle-income countries. In 2014, the International Diabetes Federation ranked Kenya 31st in Africa in terms of diabetes with an estimated prevalence of 460 cases per 10,000 population. This study characterizes the prevalence and associated co-morbidities of diabetes in five rural and semi-urban counties in Kenya. We conducted a descriptive cross-sectional review of diabetes registry data in five selected rural and semi-urban hospitals between 2010 and 2015. Patients with clinical or laboratory diagnosis of diabetes were included in the study. Demographic and epidemiologic data were abstracted, entered into MS-Excel 2007, and descriptive and correlation statistics were calculated using Epi-Info 7. We identified 1548 cases (59% female) across the 5 sites, with a mean age of 58 ± 13.5 years. We calculated diabetes prevalence measures of 310, 30, 20, and 4 per 10,000 in Isiolo, Othaya, Mukurweini, Thika, and Meru, respectively. Type 2 diabetes comprised 98% of cases from Othaya and Mukurweini, 96% from both Isiolo and Meru counties, and 94% from Thika. The most common co-morbidity was hypertension, with 80% affected from Othaya and Mukurweini, 52% in Thika, and 34% in Isiolo County. The correlation between age, gender, and presence of a co-morbidity and diabetes varied across counties. Diabetes and its complications are prevalent in rural and semi-urban areas of Kenya and women seem to be more affected by the disease, indicating an increasing population who are at risk for type 2 diabetes and associated complications.

Keywords Diabetes · Prevalence · Rural · Semi-urban · Kenya

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Introduction

Diabetes is a significant global public health challenge [1]. More than 80% of diabetes deaths occur in low- and middle-income countries with developing countries contributing 75% of the global burden for diabetes [2].

In 2014, 387 million people worldwide had diabetes and by 2035, this number is expected to rise to 592 million. The greatest numbers of people with diabetes are between 40 and 59 years of age [3]. In 2014, diabetes caused 4.9 million deaths worldwide at a cost of at least USD 612 billion dollars; that is approximately 11% of total health and medical care spending on adult health care per year. There are approximately 500,000 children <15 years with type 1 diabetes in the world [4]; in 2013 alone, 79,000 more children developed type 1 diabetes [5]. In Africa, 76% of deaths due to diabetes are in people <60 years [6]. In 2014, Kenya was ranked 31st in Africa in terms of diabetes prevalence, reflecting an increasing burden of non-communicable diseases (NCDs) in addition to infectious diseases [7]. Diabetes cases between ages 20 and 76 were 749,000 with a comparative prevalence of 4.56%. Diabetes-related deaths were 20,350 with a

mortality of 97.3 (100,000 population) [6]. The World Health Organization estimates the prevalence of diabetes in Kenya is 3.6% and predicts a rise to 4.5% by 2025. Type 2 diabetes, being the most common type, has an earlier age onset in developing countries compared to developed countries; 45–55 and 64 years, respectively [8]. This means that Kenyans are at risk of developing diabetes-related complications at an earlier age, like blindness and amputations secondary to diabetic foot and thus a reduction in economic productivity [9, 10].

Preventing diabetes in Kenya will require diverse strategies that address the unique features of its diverse urban, semi-urban, and rural communities. This is especially relevant as rural residents continue to emigrate to newly urbanized towns and cities due to Kenya's economic growth [11]. We present data on the prevalence of diabetes among patients attending hospitals in the rural and semi-urban health facilities of Othaya, Mukurweini, Thika, Isiolo, and Meru county hospitals by socio-demography and to determine the common co-morbidities and complications among diabetes patients.

Fig. 1 Map of study area

Table 1 Demographic and clinical characteristics of patients in a study of diabetes in five hospitals in rural and semi-urban areas of Kenya, 2010–2015

Characteristic		Frequency (*n*)	Proportion (%)
Sex	Male	629	41
	Female	919	59
Age (years)	3–16	63	4
	17–30	90	6
	31–44	272	18
	45–58	463	30
	59–72	353	23
	73–86	245	16
	87–100	62	4
Type of diabetes	Type 1	60	4
	Type 2	1488	96
Co-morbidities	Hypertension	531	34
	Retinopathy	41	3
	Peripheral neuropathy	271	17
	Amputation	59	4

Methods

Study area

Kenya is made up of 47 counties with a total of 290 sub-county areas [12]. Our study focused on two counties and three sub-county hospitals (Fig. 1). Othaya and Mukurweini are sub-county level 4 hospitals that serve largely rural and low-socio-economic communities in central Kenya. Isiolo County Referral Hospital (ICRH) is a level 5 hospital that serves a very rural and low-SES population across north-central Kenya. Meru Teaching and Referral County Hospital (MTRH) is a level 5 hospital that serves a semi-urban county of mixed SES standing. Thika level 5 hospital (TL5H) is a semi-urban health facility that serves a community of mixed SES standing.

Othaya and Mukurweini Sub-County Hospitals (OSCH, MSCH) in Nyeri County have a catchment population of 134,333, ICRH has a catchment population of 10,580, MTRH has a catchment population of 1,277,500, and TL5H in Kiambu County has a catchment population of 153,000.

Level 4 hospitals in Kenya provide comprehensive clinical, laboratory, and surgical care to their communities and serve as referral points for smaller health care units. Level 5 hospitals serve as referral points for the level 4 hospitals when more complex and complicated care and clinical interventions are necessary [13].

Study design

We conducted a descriptive cross-sectional study at these five hospitals. These study sites were a convenience sample selected due to the comprehensive health services provided to their catchment populations. These facilities also are more likely to diagnose and treat emerging NCDs such as diabetes. Because multiple health facilities exist within each county and sub-county, we used each hospital's catchment area to determine the denominators for each facility to estimate the prevalence of diabetes [14].

Definitions of study criteria

A case was defined as any in- or outpatient with a clinical or laboratory diagnosis of type 1 or type 2 diabetes at any of the five facilities between January 2010 and December 2015. Co-morbidities were all underlying illnesses and conditions that were documented in the patient's clinical records before or after the diabetes diagnosis.

Statistical analyses

Demographic, clinical, and epidemiological data were collected from the patient records and entered into MS-Excel spread sheet. Descriptive statistics were calculated with Epi-Info 7 and summarized as means and standard deviation for continuous variables and proportions for categorical variables. We used Pearson's correlation to examine

Table 2 Prevalence of diabetes by facility in a study of diabetes in five hospitals in rural and semi-urban areas of Kenya, 2010–2015

Hospital	County	Geographic type	Type 1 (*n*)	Type 2 (*n*)	Total (*n*)	Catchment (*n*)	Prevalence (per 10,000)
Mukurweini Sub-County Hospital	Nyeri	Rural	3	177	180	72,374	25
Othaya Sub-County Hospital	Nyeri	Rural	4	219	223	61,959	36
Isiolo County Referral Hospital	Isiolo	Rural	13	315	328	10,580	310
Meru Teaching and Referral Hospital	Meru	Semi-urban	21	490	511	1,277,500	4
Thika Level 5 Hospital	Kiambu	Semi-urban	19	287	306	153,000	20

the associative relationship between key demographic (age, sex) and clinical (hypertension) variables and diagnosis of diabetes at each hospital location [15].

Results

The clinical and demographic profile of study participants are outlined in Table 1. A total of 1548 diabetes cases were reviewed from the 5 county hospitals, with a mean age of 58 (±13.52) years. MTRH had the most diabetic patients (511 [33%]), followed by ICRH (328 [21%]), TL5H (306 [20%]), OSCH (223 [14%]), and MSCH (180 [12%]). Type 2 diabetes was the most recorded at 96% in all five facilities. MTRH and ICRH both recorded 96%; TL5H had 94%, OSCH 97%, and MSCH 98%. Females comprised 59% in the five facilities. By facility, the female percentages were OSCH 74%, MTRH 70%, TL5H 60%, MSCH 58%, and ICRH 33%.

The diabetes prevalence measures were 310, 25, 36, 20, and 4 per 10,000 population in Isiolo, Othaya, Mukurweini, Thika, and Meru, respectively (Table 2). Hypertension was the most common co-morbidity in the whole population (Table 1). MTRH had 29% of the diabetes patients having peripheral neuropathy and 10% had amputations. ICRH had 15% of the diabetes patients having hypertension. TL5H had 52% of the diabetes patients having hypertension, 22% had peripheral neuropathy, 7% had retinopathy, and 2% had undergone amputation. OSCH had 80% of the diabetes patients having hypertension, 14% had peripheral neuropathy, 5% had retinopathy, and 1% had undergone amputation. MSCH had 80% of the diabetes patients having hypertension, 5% had retinopathy, and 14% had peripheral neuropathy.

Table 3 shows the correlation statistics results for age, sex, and presence of a co-morbidity on diabetes diagnosis. In summary, key demographic and clinical variables were positively correlated with a diagnosis of diabetes, but there were geographic variations in those correlations. Age was significantly correlated ($p < 0.001$) with diabetes in rural and semi-urban populations, except at the Mukurweini hospital site ($p = 0.057$). Gender was significantly correlated ($p < 0.001$) with diabetes in both populations, except at the Meru hospital site ($p = 0.039$). Presence of at least one co-morbidity was

Table 3 Means, standard deviations, and correlations for selected demographic and clinical variables in a study of diabetes in five hospitals in rural and semi-urban areas of Kenya, 2010–2015

Hospital	Variable	Unit	Mean	SD	Correlation[a]	*p* value
Mukurweini	Age	Years	60.3	13.1	0.142	0.057
	Gender	M = 1 F = 2	1.55	0.49	0.317	<0.001
	Co-morbidity[b]	Y = 1 N = 2	1.54	0.63	0.248	<0.001
Othaya	Age	Years	60.75	13.84	0.355	<0.001
	Gender	M = 1 F = 2	1.71	0.45	0.311	<0.001
	Co-morbidity	Y = 1 N = 2	1.18	0.56	0.306	<0.001
Meru	Age	Years	57.19	13.35	0.182	<0.001
	Gender	M = 1 F = 2	1.71	0.46	0.045	0.309
	Co-morbidity	Y = 1 N = 2	1.74	0.32	0.063	0.155
Thika	Age	Years	57.19	13.35	0.182	<0.001
	Gender	M = 1 F = 2	1.61	0.49	0.262	<0.001
	Co-morbidity	Y = 1 N = 2	1.58	0.56	0.237	<0.001
Isiolo	Age	Years	47.06	21.38	0.305	<0.001
	Gender	M = 1 F = 2	1.75	0.46	0.268	<0.001
	Co-morbidity	Y = 1 N = 2	1.29	0.56	0.293	<0.001

[a] Pearson's correlation coefficient

[b] Co-morbidity "yes" is documentation of ≥1 of the following diagnoses in the patient's medical file: hypertension, retinopathy, peripheral neuropathy, or amputation

significantly correlated ($p < 0.001$) in both populations, except at the Meru site ($p = 0.155$).

Discussion

The present study documented slight geographic variations in correlations of certain demographic and clinical variables in semi-urban and rural diabetes patients. However, rural populations had much higher prevalence proportions of diseases when compared to their semi-urban counterparts. These findings are similar to other studies on geographic variations in terms of impact of specific variables on diabetes. Studies by Assari et al. noted cross-country variations in socio-economic, behavioral, and co-morbidities on the health of diabetes patients from 15 diverse countries [16–18]. Results from this study indicate that those between 45 and 58 years old were most affected by diabetes. These results concur with that of Muthami [19] who did a study in Kiambu district and found similar results in terms of affected ages. Ayah et al. [20] also found increasing prevalence correlated with those between 45 and 54 years of age. A study by King et al. [21] found that people >46 years old were at a higher risk of developing diabetes.

In terms of gender, a study by Wild et al. [22] found that the prevalence of diabetes was greater in females than in males. This is similar to results of this study which found that there were more women than men who had diabetes in Othaya, Mukurweini, Thika, and Meru. However, this was not the case in Isiolo. In Isiolo, this finding could be because this study was done on inpatient admissions only thus is not a representation of all diabetes cases. Also, it could suggest more males were getting complications that warrant admission in Isiolo. This study also concurs with a study by the U.S. Centers for Disease Control and Prevention [23], which found that black females had a higher diabetes rate compared to black males and white males and females. Scavini et al. [24] also found similar results in that the prevalence of previously and newly diagnosed diabetes was higher among female Zuni Indians than in male Zuni Indians. Hypertension was the most common co-morbidity among diabetes admission cases in Othaya, Mukurweini, Thika, and Isiolo, similar to findings in throughout the Caribbean, Nigeria, and Ghana [5, 25–27].

The study was limited by poor documentation in patient records at each participating hospital. Patient files were missing important data such as patient history, physical examination, basic tests especially HBA1c for monitoring sugar control, and data related to patients' height and weight to estimate body mass indexes to determine whether the patient was overweight or obese. The records archive in most facilities was disorganized making it hard to retrieve old registers and files for the patients.

Conclusion

In conclusion, findings from our study suggest that older age (>44 years) and those with at least one co-morbidity seem to be more prone to have diabetes. The highest co-morbidity registered in the five facilities was hypertension. Females were more affected than males in Othaya, Mukurweini, Thika, and Meru. However, this was not the case in Isiolo. This study recommends comprehensive recording and storage of patient's information in the registers to improve data quality and further studies be done to find out why more females than males were having diabetes in Othaya, Mukurweini, Thika, and Meru.

Acknowledgements The authors thank faculty members of the Field Epidemiology and Laboratory Training Program and staff at the individual hospitals who helped us retrieve patient files and other registries.

Compliance with ethical standards

Funding This study was funded by the Field Epidemiology & Laboratory Training Program (FELTP), within the Ministry of Health, Nairobi, Kenya (G. Githinji, A. Hussein, T. Kimani, B. Mutuku, J. Githuku, Z. Roka, M. Obonyo), and by the Defense Threat Reduction Agency (J. Ransom, T. Galgalo).

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. Permission to collect and organize the secondary data was approved by the Medical Review Committee at each participating hospital.

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):249–250
https://doi.org/10.1007/s13410-017-0594-x

LETTER TO THE EDITOR

An online source of information for diabetes mellitus patients—a neglected opportunity for a developing region like Sub-Saharan Africa

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Received: 18 September 2017 / Accepted: 12 November 2017 / Published online: 23 November 2017

The world of current e-medicine not only in advanced countries focuses on the measurable benefits of using the Internet and mobile applications with the view of diabetes mellitus (DM), in particular on their impact on diabetes metabolic compensation (HbA1c) and hypoglycemia [1].

In general, simple educational portals as elementary source of information are underestimated. Nevertheless, they are increasingly important especially in places with insufficient education. When there was a significant lack of educational materials for type 1 DM (T1DM) patients and an insufficient network of educators in the Czech Republic, we created an online informational site with the help of medical experts and educators. Between 2009 and 2013, this was widely used by our patients, even without its active promotion (30,421 individual users spent at least 30 s on the site, i.e., more than a half of all T1DM patients in the Czech Republic) [2] and continues to be similarly used.

We have investigated implementation, in this respect, in Sub-Saharan African countries where there is a marked increase in DM [3] while healthcare enjoys little support; hence, the availability of medical care and professional information is very poor.

Google's keyword combinations of diabetes-Internet-education, diabetes-web-education, and diabetes-portal-education links together with "Africa" and individual country names were searched. The search was carried out in English, French, Portuguese, and Spanish. Except for South Africa, merely six informational sites were found (four in English, two in French). None of these, however, brings more than a little general information about diabetes; one even just promotes a nutritional supplement. There was not found any specific information for insulin-treated patients what-so-ever. The vast majority of national diabetes associations do not have their own websites.

Internet access is also an important issue. Although access is limited in Sub-Saharan Africa, there is a rapid growth of its users (from 2.4% of the population in 2005 to 25.1% in 2016), just as of cellphone users (from 12.4% in 2005 increased to 80.8% in 2016), thus increasing the potential for wider use of the Internet [4].

It may, therefore, be believed that the creation of a professional information resource, which could then be modified depending on the local needs of individual countries, would provide patients with necessary information and at a low cost partly bridge the current lack of trained educators and physicians.

Compliance with ethical standards

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

Ethics committee approval N/A

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):251–252
https://doi.org/10.1007/s13410-018-0628-z

LETTER TO THE EDITOR

Evaluating prescription adherence to evidence-based guidelines in diabetes management: a reply to Pingili et al. (2017)

Saurav Basu[1]

Received: 15 November 2017 / Accepted: 7 March 2018 / Published online: 16 March 2018

Dear Editor,

The study by Pingili et al. evaluated prescription adherence to AACE guidelines in a South Indian tertiary care hospital. The authors assessed the extent to which physician treatment strategies coincide with evidence-based guidelines for diabetes management [1]. Persistently poor glycemic control due to physician failure to intensify the antihyperglycemic treatment in diabetics not meeting their A1c goal increases their risk of adverse health outcomes. The following suggestions are with regard to the methodology employed in the study.

1. The study assessed glycemic control (HbA1c) in the patients from clinical records which were not older than 6 months. According to the AACE guidelines, failure to achieve A1c goal after 3 months of therapy requires progressively expanded regimens [2]. However, in this study, the validity of the prescription adherence in the subset of patients who had HbA1c investigation reports which were 4 to 6 months old at the time of enrolment should be lower compared to those who had more recent HbA1c reports. This is because medication adherence rates which determine glycemic control in a majority of diabetics are subject to variability with time. Moreover, non-adherence detrimental to glycemic control is frequently observed to coincide with religious festivities which may be of considerable duration extending for several days or even weeks which would render older HbA1c reports as non-representative of the current glycemic control [3, 4].
2. At least two consecutive A1c values at interval of 3–6 months apart with those of the former accepted as the entry A1c value are required for ascertaining appropriateness of therapy as per AACE guidelines. In this regard, the study methodology is deficient since only the latest HbA1c report was apparently used as a proxy for the entry A1c value. Since the inclusion criteria selected diabetics on treatment for at least 1-year duration, assessment of the A1c target achievement over the previous 12 months from the last known A1c value would have permitted a higher validity of the estimated prescription adherence.
3. When A1C targets are not met, for some physicians, initiating patients on insulin could be preferable to adding a third oral agent [5]. However, the study observations show that none of the patients with $A1c \leq 9\%$ were currently on insulin therapy whereas not all patients with $A1c > 9\%$ were on insulin either. Future studies can prospectively follow up such patients with suboptimal glycemic control ($A1C \geq 7$) at baseline and observe whether the physician-prescribed treatment for their management adheres to evidence-based practices.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):253
https://doi.org/10.1007/s13410-018-0629-y

REPLY

Pingili et al. Rejoinder to: Basu's "Letter to the Editor"

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Published online: 23 March 2018

Dear Sir,

As Dr. Saurav commented, a prospective or repeated cross-sectional study design would have been more desirable to assess glycemic control (HbA1c) in diabetic patients. We agree to that, but here we have conducted a cross-sectional study on risk factors and prescription adherence. Even though we have taken HbA1c values at the time of interview, all the patients are diabetic for the last few years. We have mentioned the same thing in the section of participant selection (i. e., patients of either sex diagnosed with T2DM of any duration (as per AACE guidelines) and willing to participate were included in the study) and we have excluded newly diagnosed diabetic patients and diabetic patients on anti-diabetic therapy for < 1 year. That indicates they are diabetic for the last several years and their HbA1c levels were checked by the physicians at stipulated time periods. So we requested the physicians to give the data not exceeding 6 months and we observed that most of the prescriptions (80–85%) are adhering to AACE guidelines. In this study, the observed non adherence was 15–20%.

As Dr. Saurav commented, glycemic controls in a majority of diabetics are subject to variability with time and religious festivities which may be of considerable duration extending for several days or even weeks which would render older HbA1c reports as non-representative of the current glycemic control. We also agree to that, if we conduct a repeated cross-sectional study or a prospective study or a case control study with sufficient number of participants, the non-adherence may reduce further. Our study focused mainly on risk factors and little on prescription adherence. Currently, we focused and working only on prescription adherence to several other standard guidelines in a prospective study along with quality of life and also working on diabetic complications. The study is not completed yet, and we are extending further. Soon we will complete the study. Other researchers also can conduct similar kind of study in other regions of India, and then we can improve the quality of life of our beloved people.

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International Journal of Diabetes in Developing Countries (April–June 2018) 38(2):254–255
https://doi.org/10.1007/s13410-018-0642-1

CORRECTION

Correction to: RSSDI clinical practice recommendations for the management of type 2 diabetes mellitus 2017

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Published online: 20 April 2018

Correction to: International Journal of Diabetes in Developing Countries https://doi.org/10.1007/s13410-018-0604-7

The aim of this erratum is to acknowledge that the original version of this article contained a mistake in the author group. The names of the numerous authors got missed, and instead

The online version of the original article can be found at https://doi.org/10.1007/s13410-018-0604-7

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were added as members of the steering committee. In the published version only one name is listed as the author, Sarita Bajaj. The correct and complete list of authors is:

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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-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 16 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

List of RSSDI Accredited Centres

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
17.	Lilavati Hospital & Research Centre	Bandra West, Mumbai

COURSE DETAILS

Name of the Course: Advanced Certificate 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: Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

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.

Registered with Registrar of News Papers, India vide no. RNI-43019/85

International Journal of Diabetes in Developing Countries

Volume 38 | Issue 2 | April–June 2018

Springer