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EDITORIAL

Melatonin: the sleeping hormone

Venkata Ranga Rao Kodali[1]

Published online: 24 December 2016

Pineal gland – "epiphysis cerebri" lies in the center of human brain. Phylogenetically, pineal gland became prominent in vertebrates—both aquatic and terrestrial. Pineal gland was thought to control circadian rhythms and play a role as "zeitgeber"—a German word for the natural phenomenon of cyclicity. The signals from the retina are relayed to the suprachiasmatic nucleus (SCN) which is the circadian clock. From the SCN, signals are relayed to the superior cervical ganglion and to the pineal gland. The possibility of other pathways is supported by finding that mice deficient in rods, cones, and melanopsin systems show no light suppression of the pineal melatonin synthesis pathway [1].

Pineal gland secretes melatonin and smaller quantities of many hormones including a putative β-carboline molecule pinoline. Melatonin ($C_{13}H_{16}N_2O_2$) levels are high at night and low at sunrise. Around 3 months of age melatonin secretion begins to establish circadian rhythm in life as the infant starts cyclical sleep. Entrainment happens every morning and evening to reset the clock. The retinal ganglion cells express melanopsin—a photopigment. The cues for pinealocytes come from melanopsin in the retina. Unlike the rods and cones, melanopsin is predominant in peripheral retina. The function of melanopsin appears independent of the rods and cones [2]. Or in other words, even in the absence of rods and cones the circadian entrainment exists. These physiological pathways give some credence to the concept of "vision beyond eyesight". Circadian rhythms run free in totally blind in contrast to the entrained. Trilateral retinoblastoma consists of retinoblastoma bilaterally along with pinealoblastoma. These findings lend support to the common progenitor cell origin. Interestingly, mutations in PAX genes cause the absence of pineal gland as well as aniridia [3], indicating connections to the ancient belief of third eye.

Melatonin is stored as serotonin precursor in pinealocytes until night time. Starting from dawn, the next steps of acetylation and methylation takes place. Melatonin is amphiphilic in nature. It is destroyed in the liver. All commercially available melatonin is synthesized and even the smallest available dose raises the levels to supraphysiological concentrations. Pineal gland calcification is seen in more commonly in Caucasians and this increases with aging. Pineal gland is shown to play a key role in the sense of direction. A study showed that subjects with pineal calcification lost their sense of direction. Similar observations were made in pigeons. Those with pineal calcifications lost their priming ability [4]. Melatonin is claimed to be beneficial in atopic dermatitis, neuroprotection, and ADHD. However, FDA approval is in animal husbandry for melatonin implants in minks to accelerate priming and molting [5]. There are inconclusive data on the effects of geomagnetic manipulation of the pineal gland to induce or suppress melatonin secretion or on sense of direction.

Melatonin effects beta cell insulin release through Melatonin 1 (MTNR1) and MTNR2 receptors. Mutations in MTNR 2 have recently been shown to increase the risk of diabetes [6]. Sleep deprivation can quickly reduce insulin sensitivity [7]. A meta-analysis showed an U-shaped association of diabetes with hours of sleep, i.e., those sleeping few hours and more hours having an increased risk [8]. Overall, the studies are indicating the association of poor sleep with detrimental effects on diabetes [8–10]. At a physiological level, melatonin decreases both insulin secretion and sensitivity. Melatonin also increases glucagon secretion [11].

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In this issue of the Journal, Rezvanfar and his team report results of an intervention study of melatonin in type 2 diabetics [12]. The researchers showed an improvement in glycemic control and HDL cholesterol. Large data on the effects of melatonin on lipids in vitro and experimental animals is available with very few well-conducted clinical trials. The indication from these studies is that the atherogenic lipid profiles benefit favorably. In the light of existing data [13], the current findings on lipids are not surprising.

The studies of melatonin on glycemic control are riddled with contrasting results in both experimental animals and humans. The field lacks robust human data. Some intervention studies showed benefits while others did not. A double blind cross-over trial in diabetics with insomnia used prolonged-release melatonin at 2 mg/d dose. A benefit was noted at 5 months but not as early as 3 weeks [14]. Melatonin administration has also been shown to worsen glycemia [15].

Genome-wide association studies (GWAS) have identified mutant alleles close to the MTNR1 gene for progression of normoglycemic status to prediabetes and prediabetes to diabetes [16]. MTNR2 mutations increase the risk for type 2 diabetes [6]. These receptor mutations and their link to glucose homeostasis were reviewed recently [17, 18].

Some of the caveats in the current study need to be highlighted. The study was blind designed to the patients. Melatonin was given after 12 weeks of placebo. This is not a cross-over trial. There is a huge variability in fasting glucose at baseline. The subjects were of average weight; the body mass indices were not shown. We assume that melatonin used was not a long acting preparation. Importantly, there is a significant reduction in HbA1c during the placebo period ($P < 0.01$) and a similar significant reduction in HDL cholesterol. It is possible that this effect could have been carried forward into the second phase of this study, representing a mere placebo effect.

Data are not available on the nocturnal profile of melatonin in the supplemented diabetics. As discussed above, melatonin is known to cause resistance to glucose induced insulin secretion, and this is more prominent in people with MTN receptor mutation. How is it possible to have an improved glycemic control with lessened insulin concentration? Some of the mechanisms: (1) with phase advancement of sleep rhythms, cortisol secretion may remain low early and possibly prolonged low phase. (2) Peripheral action and sensitivity and effects on other counter hormones: 3) we can also hypothesize that the benefits of melatonin exist outside alpha and beta cell.

This study was carried out in Arak, Iran, which is geographically located between the Caspian Sea and Persian Gulf. Fluoride levels in drinking water vary from suboptimal to toxic ranges in these areas. High environmental fluoride levels were reported from this area [19]. Fluoride accumulates in excessive concentration in the pineal gland. However, pineal calcification may not alter the melatonin or its photoperiodism. A baseline melatonin levels in the study population would have also helped to know whether the populations are melatonin depleted.

How to interpret and go forward from here? The study population is small and limited to a geographic area. Broader conclusions cannot be drawn from these data. We need a full profile of the melatonin, glucose, insulin, and counter hormone levels during such studies with the additional use of methods like Homeostatic Model Assessment and Insulin Resistance (HOMA-IR). The other simpler option could be using the continuous glucose monitoring with intravenous melatonin administration. The physiological relationship is such that the insulin levels go down while melatonin goes up during the night. Future studies addressing insulin, melatonin levels, and circadian rhythms in patients with pancreatectomy and pinealectomy/calcified pineal glands are all needed. We do not have any data on insulin sensitivity as a continuous variable in blind persons. Likewise, its role in pituitary and other tissues is underexplored. There is also paucity of data on what melatonin does in type 1 diabetes.

Melatonin remained a sleeping hormone so far. The field is rapidly evolving and soon its receptor agonists and antagonists will surface and hopefully we will be able to target these to the potential advantage to treat metabolic disorders.

While appears safe, a case control trial on melatonin has not been conducted so far despite its metaphorical popularity. At this point in time, the current findings are interesting but need long term physiological data to recommend melatonin use in diabetes practice.

Compliance with ethical standards

Conflicts of interest None.

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REVIEW ARTICLE

ODE models for the management of diabetes: A review

Saloni Rathee[1] · **Nilam**[1]

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Abstract Diabetes also known as diabetes mellitus is a chronic and complex metabolic disease due to the persistent raised blood glucose concentration for long duration. The mechanism behind the disturbed glucose-insulin dynamics is still not fully understood. The mathematical models which describe the glucose homeostasis, different aspects of diabetes and its consequences are growing rapidly, provide new insights into the biological mechanism involved and help in the management of diabetes. Here, contribution of diabetic's modelling using ordinary differential equations over the past five decades is discussed. Some parameter estimation techniques, softwares involved and some computational results are also presented.

Keywords Diabetes · Glucose-insulin · ODE models · Softwares · Parameter estimation techniques

Introduction

Diabetes is a disease of the glucose-insulin regulatory system. It is classified into three main categories: type 1 diabetes, type 2 diabetes and gestational diabetes. Type 1 diabetes is considered to be the result of an immunological destruction of the insulin-producing β cells [1]. Type 2 diabetes is the result of insulin resistance which increases resistance of the body to the effects of insulin on glucose uptake, metabolism or storage due to excessive hepatic glucose production and defective β cell function [2].

Diabetes is a condition in which high blood glucose concentration persists for long duration due to the disturbed insulin-glucose-glucagon dynamics in the body. Glucagon and insulin are two hormones secreted by α and β cells of the pancreas which take part to maintain the glucose level in normal range. In normal individual, blood glucose level is maintained in the physiological range (70-110 mg/dl) as the glucose-insulin regulatory system works properly. After glucose infusion (food intake, oral ingestion), raised blood glucose level triggers the pancreas to release insulin which helps the body cells (muscles and skeletal) to take up glucose. In case of low blood glucose concentration, glucagon helps the liver to break glycogen into glucose as shown in Fig. 1a. In diabetic individual, the glucose-insulin dynamics is disrupted resulting to persistent high blood glucose level as shown in Fig. 1b. Long-term persistence of diabetes affects the major organs of the body like the liver, kidney, eyes, nervous system and reproductive system and causes multiple organ failure [3].

A large number of research articles are published on diabetes, its types and related complications during the last decades [4–8]. Many mathematical models were developed and successfully captured the physiological changes occurring in the human body with or without diabetes [9–15]. Out of many, few mathematical models proved a milestone in the pathogenetic and physiological studies of diabetes [9, 13, 15]. Previously developed mathematical models are still used by researchers with suitable and significant modifications. The literature deals with different mathematical models and simulation of different aspects of diabetes is abundant. Several reviews based on the different mathematical models, tools and softwares are timely published and have proven to be useful for the academicians and researchers [16–18].

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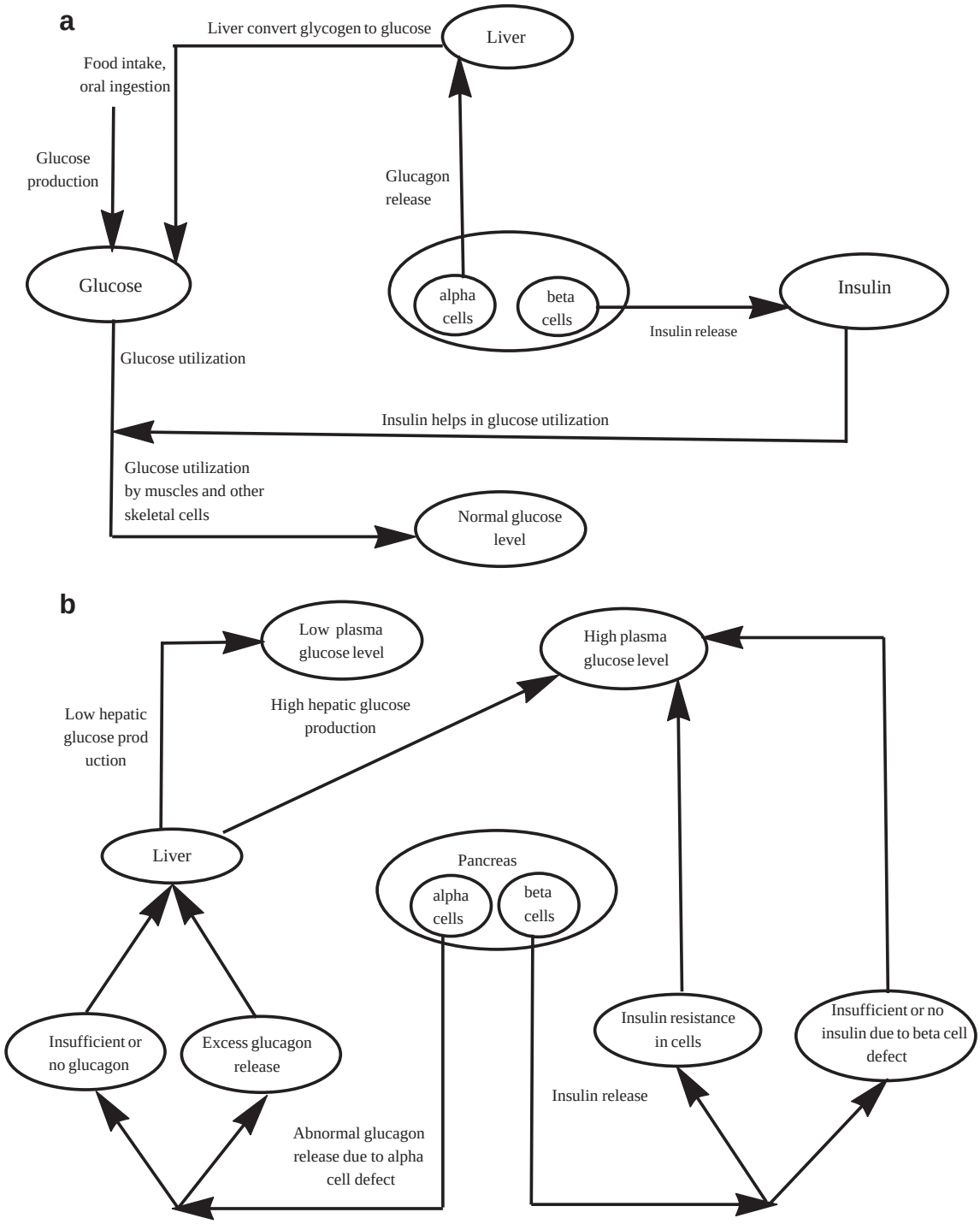

Fig. 1 Glucose-insulin dynamics under normal and diabetic condition. **a** In normal condition, β cells of the pancreas release insulin, which helps muscles and skeletal cells to take up glucose and maintain the normal glucose level. Also, the glucagon secreted by α cells of pancreas helps the liver to convert glycogen into glucose. **b** In diabetic condition, the glucose-insulin dynamics is disrupted and insufficient or no insulin is secreted from the β cells due to β cell defect which leads to high glucose level. Also, the glucagon secretion from α cells is disturbed, resulting in excess glucagon or insufficient glucagon release leading to high glucose level or low glucose level

The mathematical models may be simple/complex, deterministic/stochastic, continuous/discrete using ODE (ordinary differential equations), PDE (partial differential equations), DDE (delay differential equations), statistical differential equations (SDE), integral equations and many more. Mathematical models presented to the time can be classified into different categories based upon the physiology involved, complexity level of model and which type of data is used in the models. The models further can be classified according to the biological processes involved and also the motive for

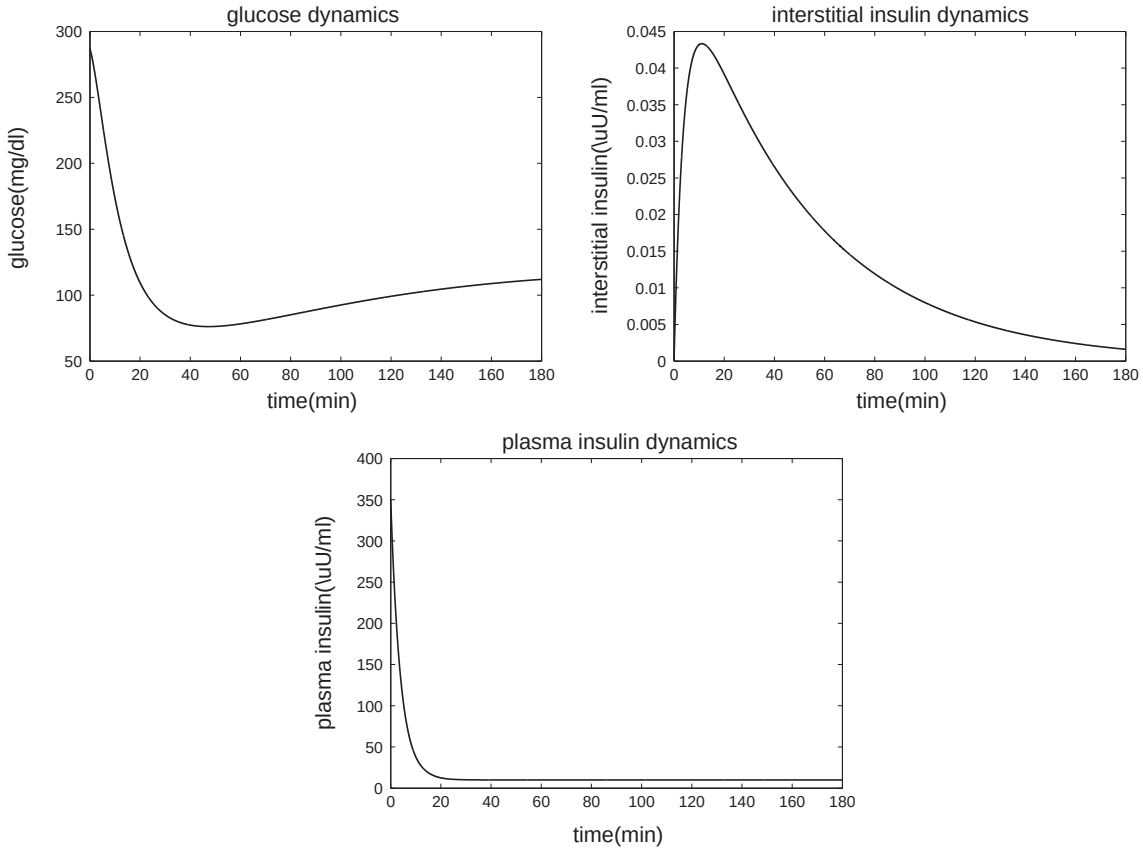

Fig. 2 Glucose, plasma insulin and interstitial insulin concentration levels are obtained using ODE45

which the models were proposed. Many attempts have been made to address the complexity behind the mechanism of the disease, but still an imbalance exists between the information obtained from the experimental theory and their mathematical representation.

Most of the ODE models were developed to evaluate the diagnostic tests such as intravenous glucose tolerance test (IVGTT), oral glucose tolerance test (OGTT) and meal glucose tolerance test (MGTT). The aim of these tests were to estimate the insulin sensitivity (S_I), glucose effectiveness (S_G), disposition index (DI), insulin secretion, insulin action and β cell function. To include all the mathematical models published so far in one review paper is difficult, but we tried our best to include the important mathematical models based on ordinary differential equations, which were used to manage the level and complexity of the disease.

To discuss the mathematical models of diabetes, it is necessary to get the knowledge of all the basic terms and definitions which are frequently used in the physiological and clinical study of diabetes. Some important basic terms and definitions which will be used in the paper are as follows:

- S_I (insulin sensitivity): effect of insulin to catalyse the glucose disappearance from the plasma [19]
- S_G (glucose effectiveness): ability of glucose to enhance its own disappearance independent of insulin presence [19]
- $\mathrm{AIR}_{\mathrm{glucose}}$: first-phase insulin response [19]
- DI (disposition index): ability of pancreatic β cells to compensate for insulin resistance [19]
- φ_1: first-phase pancreatic responsivity [6]
- φ_2: second-phase pancreatic responsivity [6]
- IVGTT (intravenous glucose tolerance test): a test in which glucose is injected intravenously and blood samples are collected following the glucose injection [5]
- OGTT (oral glucose tolerance test): a test in which glucose is given orally and blood samples are collected over 2 h following the glucose infusion [20]
- FSIGT (frequently sampled intravenous glucose tolerance test): a test to measure the blood glucose level in which nothing (drink and eat) is given for 8 to 12 h before the test

Ordinary differential equation models

To study the glucose-insulin regulatory system in the human body, many mathematical models were found in the literature. Out of those, mathematical models containing ordinary

differential equations are abundant, and approximately more than 500 papers can be found in the literature based on the ODE models [16]. Here, we tried to give the overview of the papers which consider ODE model to discuss different aspects of diabetes and its consequences.

Bolie [4] is considered as the pioneer in introducing the ODE mathematical model to capture the physiological changes in the glucose-insulin dynamics.

•In 1961, Bolie [4] developed a minimal model to evaluate the coefficients of normal blood glucose regulation.

The differential equations for glucose-insulin regulatory system are written as

$$\frac{dx(t)}{dt} = p - \alpha x + \beta y \quad (1)$$

$$\frac{dy(t)}{dt} = q - \gamma x - \delta y \quad (2)$$

Where x represents the deviation in insulin concentration from their mean physiological value, y represents the deviation in glucose concentration from their mean physiological value, p is the intravenous injection functions I divided by extracellular compartment value, q is the intravenous injection functions $\dot{G}$ divided by extracellular compartment value, α denotes the sensitivity of insulinase activity to elevate insulin concentration, β denotes the sensitivity of pancreatic insulin to elevate glucose concentration, γ represents the combined sensitivity of liver glycogen storage and tissue glucose utilization to elevate insulin concentration and δ represents the combined sensitivity of liver glycogen storage and tissue glucose utilization to elevate glucose concentration [4].

Results By the help of several assumptions like liver, pancreas and peripheral tissues were considered to communicate with each other in a single compartment, the values of four coefficients (α, β, γ, δ) and their biological variations were evaluated and used for managing the diabetes.

In 1964, Ackerman et al. [20] reviewed a model to predict the blood glucose level by simulating the behaviour of human regulating system. He compared the predictions made during OGTT to regulate the blood glucose and blood insulin concentration.

In 1970, Segre et al. [21] considered a two-compartment model and applied to the analysis of glucose and insulin control mechanism in 26 normal, 16 diabetic and 8 obese subjects. Glucose level for all the three groups were determined by infusing glucose (0.5 g/min for about 300 min).

Results A discriminant analysis for two groups gave a statistically significant separation between normal and diabetic subjects (with infused or impulsive glucose) and between normal and obese subjects (with infused glucose).

In 1978, Ruby et al. [22] presented a model which indicates the roles of both insulin and glucagon as regulators of blood glucose. The model simulations suggest that insulin plays the most important role in the control of hyperglycaemia, and glucagon is an important regulatory hormone under conditions of hypoglycaemia when the blood glucose value falls below 50 mg/dl.

In 1979, Bergman et al. [23] discussed the studies which led to definition and measurement of the characteristic parameters of metabolic regulation. They attempted to show that the parameter presents a novel and powerful way to conceive of metabolic regulation, which provides an improved means for investigating the environmental, dietary and activity-related factors which alter the regulation of metabolism in mammalian species.

In 1979, Bergman and Cobelli [5] estimated the insulin sensitivity after evaluating a mathematical model of glucose disappearance. Seven mathematical models of glucose uptake were compared to find the glucose disappearance. The parameter of the model was estimated from a single IVGTT to estimate the insulin sensitivity.

Limitations The study was for the animals, and experimental studies were required whether insulin sensitivity was estimated also for humans.

In 1980, Toffolo et al. [24] proposed the minimal model for the insulin kinetics in dog. The proposed minimal model was used for the physiological studies of insulin secretary function in dog by using IVGTT and proposed the idea to also apply the model for the pathophysiological studies in humans. Toffolo et al. compared six mathematical models to study the insulin kinetics and found that the model, given below, is superior in explaining insulin dynamics with respect to all aspects.

$$\frac{dI(t)}{dt} = -\gamma(G(t) - h)t, \text{if } G(t) \geq h \quad (3)$$

$$= 0, \text{if } G(t) \leq h \quad (4)$$

In 1981, Bergman et al. [6] introduced two separate mathematical models: one for glucose kinetics and another for insulin kinetics. Insulin model produce the parameters: φ_1, φ_2, responsivity of β cells to glucose, whereas glucose model produce the insulin sensitivity (S_I) parameter during IVGTT.

The minimal model for the glucose-insulin regulatory system is as follows:

$$\frac{dG(t)}{dt} = -X(t)G(t) - p_1(G(t) - G_b) \quad (5)$$

$$\frac{dX(t)}{dt} = p_2 X(t) + p_3 I(t) \quad (6)$$

$$\frac{dI(t)}{dt} = -nI(t) + \gamma(G(t) - G_c)^+ t \quad (7)$$

Where $G(t)$ (mg/dl) represents glucose concentration, $X(t)$ (min^{-1}) represents remote insulin concentration, $I(t)$ (μU/ml) represents the interstitial insulin, G_b (mg/dl) represents the basal glucose level, h (mg/dl) represents the threshold glucose level of glucose above which the endogenous insulin secretion will be stimulated, p_1 represents glucose effectiveness, p_2 is the fractional rate of insulin appearance in interstitial compartment, p_3 represents contribution of plasma insulin to the remote compartment from interstitial compartment, n represents the rate of plasma insulin clearance and γ is the degree by which glucose exceeds threshold or baseline glucose level.

Results The aim of the study was to determine the quantitative contributions of pancreatic responsiveness and insulin sensitivity to glucose tolerance by using "minimal model technique".

Limitations The results were limited to evaluation of IVGTT only. It remained to prove whether the model and the parameters are applicable on other dose such as OGTT and other stimulus pattern.

In 1984, Defronzo et al. [25] examined the tissue sensitivity to insulin in 36 control subjects and 19 insulin-dependent diabetics using insulin clamp technique. Following hyperinsulinaemia, suppression of hepatic glucose production was ~95 % in both diabetics and controls, suggesting that peripheral tissues are primarily responsible for observed impairment in insulin-mediated glucose uptake.

Results The result indicates that impaired insulin action is a common feature of insulin-dependent diabetics, despite daily insulin requirements that would not clinically characterize them as insulin resistant.

In 1985, Bergman et al. [7] examined the different approaches introduced by researchers for the evaluation of insulin sensitivity. He reviewed pancreatic suppression test ([26–28]), glucose clamp ([27, 28]) and minimal model approach ([6, 8]) to find the effect of closed loop feedback relation between insulin action and insulin secretion.

In 1986, Pacini and Bergman [9] proposed a mathematical model for measuring two main factors—insulin sensitivity and pancreatic responsivity—to control glucose tolerance. Bergman proposed MINMOD (minimal modelling approach)—a computer program to identify the model parameters S_G, S_I, φ_1 and φ_2 and analyse FSIGTT data.

The selected mathematical model is given as

1. For glucose disappearance

$$\frac{dG(t)}{dt} = -(p_1 + X(t))G(t) + p_1 G_b, G(0) = G_0 \tag{8}$$

$$\frac{dX(t)}{dt} = -p_2 X(t) + p_3(I(t) - I_b), X(0) = 0 \tag{9}$$

2. For insulin kinetics

$$\frac{dI(t)}{dt} = p_5(G(t) - h)^{+} t - n(I(t) - I_b), I(0) = I_0 \tag{10}$$

Results Program MINMOD successfully calculated S_G, S_I, φ_1 and φ_2 which represent an integrated metabolic portrait of any individual and help in managing diabetes.

Limitations

1. Insulin concentration and glucose concentration were treated as input data to derive the parameters of the equations.
2. Equilibrium does not exist and the solutions of the minimal model may not be bounded.
3. The variable $X(t)$ was introduced to consider the delay in action of insulin to stimulate glucose uptake.

In 1990, Welch et al. [10] determined the exogenous infusion of insulin in the minimal model FSIGTT analysis. He also extracted the information about insulin-mediated glucose uptake and noninsulin-mediated glucose uptake, insulin sensitivity and insulin secretion.

In 1991, Sturis et al. [13] developed a six-dimensional ODE model. Tolic et al. [29] simplified the model, and the model has been the basis of many DDE models [15, 30–33].

$$\frac{dG(t)}{dt} = G_{in} - f_2(G(t)) - f_3(G(t) f_4(I_i(t)) + f_5(x_3(t))$$

$$\frac{dI_p(t)}{dt} = f_1(G(t)) - E\left(\frac{I_p(t)}{V_p} - \frac{I_i(t)}{V_i}\right) - \frac{I_p(t)}{t_p}$$

$$\frac{dI_i(t)}{dt} = E\left(\frac{I_p(t)}{V_p} - \frac{I_i(t)}{V_i}\right) - \frac{I_p(t)}{t_i}$$

$$\frac{dx_1(t)}{dt} = \frac{3}{t_d}\left(I_p(t) - x_1(t)\right)$$

$$\frac{dx_2(t)}{dt} = \frac{3}{t_d}(x_1(t) - x_2(t))$$

$$\frac{dx_3(t)}{dt} = \frac{3}{t_d}(x_2(t) - x_3(t))$$

Where $G(t)$ is mass of glucose; $I_p(t)$ and $I_i(t)$ are the mass of insulin in the plasma and intercellular space; V_p is the plasma insulin distribution volume; V_i is the volume of intercellular space; E is the diffusion transfer rate; t_p and t_i are insulin degradation time constants in plasma and intercellular space; G_{in} is glucose supply rate to the plasma and $x_1(t)$, $x_2(t)$ and $x_3(t)$ are the three additional variables associated with certain delays of the insulin effect on HGP with total time delay t_d.

The function $f_1(G)$ represent the pancreatic insulin secretion; f_2, f_3, f_4 represent the glucose utilization in the body (brain (f_2), muscles and fat cells (f_3, f_4) and f_5 represents HGP) [29].

Results The occurrence of sustained insulin and glucose oscillations was found to be dependent on two essential features, a time delay of 30–45 min for the effect of insulin on glucose production and a sluggish effect of insulin on glucose utilization.

In 1991, Fisher [34] presented a mathematical model for glucose insulin interaction in the blood system. Mathematical optimization techniques are applied to mathematical model to derive insulin infusion program. A semi-closed algorithm is proposed for continuous insulin delivery to diabetic patients.

Results Insulin infusion program which incorporates an injection to coincide with the meal succeeds in achieving the most effective short-term control.

In 1995, Coates et al. [35] studied the minimal model (MINMOD) analysis of the frequently sampled intravenous glucose tolerance test (FSIVGTT) which depends on an adequate insulin response to the glucose load. Subjects with an insulin-dependent diabetes mellitus (NIDDM) was not included in MINMOD. Hence, in the paper, the technique has been modified by using intravenous bolus of insulin. They compared estimates of insulin sensitivity derived from minimal modelling of a 4-h insulin-modified FSIVGTT and the glucose clamp in subjects with NIDDM.

Results MINMOD analysis of the insulin-modified FSIVGTT provides a valid measure of insulin sensitivity in subjects with NIDDM.

In 1997, Vicini et al. [11] shows that 2CMM (two-compartment minimal model) provides indexes of glucose effectiveness (S_G), insulin sensitivity (S_I) and plasma clearance rate (PCR) and also overcomes the limitation of one-compartment minimal model [12] by providing the plausible profile of endogenous glucose production.

The 2CMM for the glucose-insulin regulatory system is as follows:

$$\dot{q}_1(t) = -\left[k_p + \frac{R_{d,0}}{Q_1(t)} + k_{21}\right] q_1(t) + k_{12}(t) q_2(t), q_1(0) = d \quad (11)$$

$$\dot{q}_2(t) = k_{21} q_1(t) - [k_{02} + x(t) + k_{12}] q_2(t), q_2(0) = 0 \quad (12)$$

$$\dot{x}(t) = -p_2 x(t) - s_k [I(t) - I_b], x(0) = 0 \quad (13)$$

$$g(t) = \frac{q_1(t)}{V_1}$$

Where q_1 and q_2 denote hot glucose masses in the first (accessible pool) and second (slowly equilibrating) compartments; $x(t)$ is insulin action; $I(t)$ and I_b are the plasma and basal insulin; $Q_1(t)$ is cold glucose mass in the accessible pool (mg/kg); $g(t)$ is plasma hot glucose concentration (mg/dl), d is the hot glucose dose (mg/kg); V_1 is the volume of the accessible pool (ml/kg); $R_{d,0}$ (mg kg^{-1} min^{-1}) is the constant component of glucose disposal; k_p (min^{-1}), k_{21} (min^{-1}), k_{12} (min^{-1}) and k_{02} (min^{-1}) are the parameters of glucose kinetics and p_2 and s_k (ml μU^{-1} min^{-1}) are the parameters describing insulin action.

Limitations

1. Effect of glucose on insulin-independent glucose uptake takes negative values.
2. Precision of 2CMM parameters estimation was not satisfactory every time.

In 2000, Topp et al. [14] developed a βIG model for β cell mass, insulin and glucose kinetics for diabetes.

The mathematical model for the glucose-insulin regulatory system is as follows:

$$\frac{dG(t)}{dt} = a - (b + cI)G \quad (14)$$

$$\frac{dI(t)}{dt} = \frac{d\beta G^2}{e + G^2} - fI \quad (15)$$

$$\frac{d\beta(t)}{dt} = \left(-g + hG - iG^2\right)\beta \quad (16)$$

Where a denotes hepatic glucose production, b is the rate of insulin-independent glucose utilization, c is the rate of insulin mediated glucose utilization, d denotes rate of insulin secretion by β cells, e determines inflection point of sigmoidal function, f denotes rate of insulin clearance, g is β cell natural death rate, h determines β cell glucose tolerance range, G is the blood glucose concentration (mg/dl), I is the blood insulin concentration (μU/ml) and β is the β cell mass (mg).

Results The model predicts three distinct pathways into diabetes: regulated hyperglycaemia, bifurcation and dynamical hyperglycaemia.

Limitations The model did not incorporate effects of hyperglycaemia on neogenesis, insulin sensitivity, insulin secretion rates and β cell heterogeneity. Also, the model does not incorporate the effects of insulin and incretin hormones on β cell mass dynamics.

In 2001, Ryan et al. [36] modified the mathematical model of β cell mass, insulin and glucose kinetics for diabetes developed by Topp et al. [14] by including the effects of insulin receptor dynamics which was important in the pathogenesis of diabetes and showed that insulin sensitivity can be increased by 36 % due to exercise, and required insulin level can also be decreased to maintain the glucose concentration. Also, the system of equations improves the quantitative prediction of β cell mass values and provides a theoretical justification for the fact.

Limitations The dimension of the mathematical model can be extended by incorporating other hormone secreting cells in the islets of Langerhans and incorporating the insulin sensitivity dynamics in the model.

In 2000, Gaetano and Arino [37] proposed another model known as "dynamical model" in order to overcome the limitations and drawbacks of the coupled minimal model.

The dynamical model for the glucose-insulin system is as follows:

$$\frac{dG(t)}{dt} = -b_1 G(t) - b_4 I(t) G(t) + b_7 \quad (17)$$

$$G(t) = G_b \forall t \in [-b_5, 0], G(0) = G_b + b_0$$

$$\frac{dI(t)}{dt} = -b_2 I(t) + \frac{b_6}{b_5} \int_{t-b_5}^{t} G(s)ds, I(0) = b_5 + b_3 b_0 \quad (18)$$

Where, b_0 (mg/dl) is the increase in plasma glucose concentration over basal glucose concentration at time zero after instantaneous administration of the i.v. glucose bolus; b_1 (min^{-1}) is the glucose disappearance rate constant, b_2 (min^{-1}) is the insulin disappearance rate constant, b_3 (pM $(mg/dl)^{-1}$) is the first-phase insulin concentration increase per (mg/dl) increase in the concentration of glucose at time zero due to the injected bolus; b_4 ($min^{-1}(pM)^{-1}$) is the constant amount of insulin-dependent glucose disappearance rate constant per pM of plasma insulin concentration; b_5 (min) is the length of the past period whose plasma glucose concentrations influence the current pancreatic insulin secretion; b_6 (min^{-1} pM $(mg/dl)^{-1}$) is the constant amount of second-phase insulin release rate per mg/dl of average plasma glucose concentration throughout the previous b_5 minutes; b_7 ((mg/dl) min^{-1}) is the constant increase in plasma glucose concentration due to constant baseline liver glucose release [37].

Results The original minimal model [9] was developed to cope for first 3 h after glucose infusion while the present dynamic model deals with many hours after the administration of glucose bolus and also prevailing a few minutes before glucose infusion. The model admits only one equilibrium point; the model is stable around that equilibrium point and the solutions are positive and bounded.

Limitations The assumptions taken in the paper might not be realistic, and the way in which the delay term was introduced was too restrictive based on the fact that pancreatic insulin secretion at time t was proportional to the average value of glucose concentration in b_5 min preceding time t.

Gaetano and Arino [37] reported that unstable steady state does not exist for the model while Li et al. [38] found that these models can posses unstable positive steady states producing oscillatory solutions.

In 2001, Li and Kuang [38] generalized the Arino's paper [37] to find an alternative way of incorporating time delay.

The mathematical model for the glucose-insulin regulatory system is as follows:

$$\frac{dG(t)}{dt} = -b_1 G(t) - \frac{b_4 I(t) G(t)}{\alpha G(t) + 1} + b_7 \quad (19)$$

$$\frac{dI(t)}{dt} = -b_2 I(t) + \frac{b_6}{b_5} \int_{-b_5}^{0} G(t+\theta)d\theta \quad (20)$$

With initial conditions, $G(0) = G_b + b_0$, $I(0) = I_b + b_3 b_0$, $G(t) \equiv G_b$ for $t \in [-b_5, 0]$, where $G_t(\theta) = G(t + \theta)$, $t > 0$, $\theta \in [-b_5, 0]$. The parameters b_0, b_3, b_5 and b_7 are the same as in model (17-18).

Results Sustainable oscillatory solutions (G and I) of mathematical model obtained for ($\tau > 60$ min) and no oscillations exist for ($\tau < 60$ min). The solution may converge to the steady state in an oscillatory way even the delay was small. Better ways of delivering insulin and timings of the intake of glucose were found. Also, they found that generalized dynamic model can produce oscillatory solutions without hepatic glucose production.

Limitations The obtained results were on the basis of only two subjects (1 male, 1 female). Insulin influenced on the hepatic glucose production were not taken and kept open for further studies.

In 2002, Cobelli et al. [39] proposed a new approach to estimate insulin sensitivity from an OGTT using an "integral equation". Three different model to determine R_a (rate of appearance of oral glucose in plasma) were presented in the paper: piecewise linear (P), spline (S) and dynamic (D). All the three models estimated the insulin sensitivity.

Limitations

1. The time course of (R_a) does not disclose explicitly in model.
2. It does not provide the recision with which S_I^I (I denoted integral) is estimated.
3. It assumed glucose and insulin concentration has returned to basal level at the end of the test to calculate the area under the curve correctly.

In 2002, Derouich and Boutayeb [40] introduced the effect of physical activities and exercise via parameters in a mathematical model given by Bergman et al. [6] and compared the behaviour of normal, NIDD and IDD people. The new added parameters demonstrated the effect of physical exercise on the diabetic body.

In 2002, Mari et al. [41] investigated β cell function and its relationship to insulin sensitivity by choosing 17 normal

volunteers. Insulin secretion and insulin sensitivity were measured by applying mathematical model on meal test (MT) and oral glucose tolerance test (OGTT) with the help of euglycaemic insulin clamp technique.

In 2003, Toffolo and Cobelli [42] introduced a new improved version of two-compartment minimal model (2CMM) [11]. The new improved version of 2CMM, proved a more reliable and precise parameter of glucose metabolism during an IVGTT.

In 2004, Dellaman et al. [43] used the reference method tracer two-step method and compared the results on database of 88 subjects. The method was compared with the homeostasis model assessment (HOMA) [44, 45], the quantitative insulin sensitivity (QUICK) [46] and MATSUDA-De Fronzo [47] to measure the insulin sensitivity during an OGTT. The results confirm that the OMM estimates the rate of appearance of glucose absorption and insulin sensitivity accurately.

In 2005, DallaMan et al. [48] presented a labelled oral minimal model (OMM*) in which a tracer was added to the oral dose and S_I (labelled insulin sensitivity) was determined. OMM* not only estimates the R_a (labelled rate of appearance of oral glucose in plasma) but also accurately measures S_I^*.

In 2005, Bergman [19] considered the minimal model and showed that insulin sensitivity or insulin sensitivity index (S_I) can be calculated from parameters of minimal model by

1. performing frequently sampled IVGTT
2. measuring glucose and insulin
3. fitting the data to the minimal model
4. calculating insulin sensitivity

Also, he showed that product of insulin sensitivity and insulin secretion would be approximately constant, i.e. insulin sensitivity × insulin secretion = disposition index($S_I \times \mathrm{AIR}_{\mathrm{glucose}} = \mathrm{DI}$).

In 2006, Boutayeb et al. [49] presented a mathematical model of the size of a population of diabetes mellitus. The nonlinear case was discussed and critical values of the population were analysed for stability.

The nonlinear ODE mathematical model is as follows:

$$\frac{dD}{dt} = I - (\lambda + \mu)D(t) + \gamma C(t)$$

$$\frac{dC}{dt} = \lambda \mathrm{D}(t) - (\gamma + \mu + \nu + \delta)C(t)$$

Since $N(t) = D(t) + C(t)$ gives rise to initial value problem (IVP).

$$\frac{dC}{dt} = -(\lambda + \theta)C(t) + \lambda N(t), t > 0, C(0) = C_0 \quad (21)$$

$$\frac{dN}{dt} = I - (\nu + \delta)C(t) - \mu \mathrm{N}(t), t > 0, N(0) = N_0 \quad (22)$$

Where $\theta = \gamma + \mu + \nu + \delta$ and C_0 and N_0 are the initial case of $C(t)$ and $N(t)$.

$C(t)$ is the number of diabetics with complications, $D(t)$ is the number of diabetics without complications and $N(t)$ denotes the size of population of diabetics at time t.

Results The result obtained estimates the size of the population of diabetics and the numbers with complications.

In 2006, Bergman et al. [50] performed dimensional analysis of MINMOD and found that with nondimensionalization, pathological DI is naturally defined in the model and it has the meaning of insulin sensitivity at unit first-phase pancreatic response. Using simulated data and human FSIVGTT data, they found the new approach which gives highly correlative parameter estimates to the original dimensional formulation.

In 2006, Wang et al. [51] formulated a mathematical model to deal with the question about heterogeneity between young- and adult-onset type 1 diabetes. It was found that if autoimmunity is initiated, then the turnover is slow, and a stable steady state can exist with the β cell turnover being rapid. Also, the model analysis pointed that pathway regulating β cell turnover can be a new target to interfere with the disease process of T1D.

In 2007, Silber et al. [52] developed an integrated model for healthy and type 2 diabetic patients to regulate the glucose and insulin concentration by using IVGTT data form 30 healthy and 42 diabetic individuals. Analysis of all the data by nonlinear mixed effect modelling was performed in NONEM.

Results The model could be used to analyse the effects of antidiabetic drugs on a physiological system and can be used to predict and stimulate data for different types of IVGTT in healthy and diabetic patients.

Limitations The design which were used to study glucose-insulin regulatory system seems very time consuming and expensive in sampling.

In 2007, Silber et al. [53] extended the previously developed integrated model [52] for glucose-insulin regulatory system by including the OGTT in healthy volunteers by simulation and bootstrap of the model. The base on which the new model developed was the incretin effect (i.e. oral glucose provocations results in stronger insulin response compared to intravenous provocations).

Results Glucose homeostasis parameters can be derived from the glucose provocations by the help of present model, which was most commonly used in the early stage of clinical drug development.

In 2007, Roy and Parker [54] extended the minimal model [6] and included the major effects of exercise on plasma glucose and insulin concentration level in the body.

In 2008, Gaetano et al. [55] made an attempt to discuss the progression of type 2 diabetes through a mathematical model. A model of the pancreatic islet compensation was formulated by the help of some physiological assumptions. The mathematical model was compared with the model developed by Topp et al. [14] and found to be more robust and useful for clinical purpose through assessment of the related parameters.

In 2008, Stahl and Johansson [56] made an attempt to show how system identification and control may be used to estimate predictive quantitative models to be used in design of optimal insulin regimens.

In 2008, Periwal et al. [57] examined a variety of mathematical models analogues to the minimal model of glucose disposal (MMG). To quantify the combined influence of insulin on lipolysis and glucose disposal during an insulin-modified frequently sampled intravenous glucose tolerance tests (FSIGT). The tested models contain compartments of plasma free fatty acids (FFA), glucose and insulin. Out of 23 models, they select the best fitted model by using Bayesian model comparison method which minimized model complexity. In the best model, insulin suppressed lipolysis via a Hill function through a remote compartment that acted both on FFA and glucose simultaneously, and glucose dynamics obeyed the classic MMG.

In 2010, Pacini et al. [58] compared the insulin sensitivity index (S_I) and glucose effectiveness (S_G) obtained in 16 normal subjects with two tests. The common protocol are regular (rFSIGT) and an insulin-modified test (mFSIGT), with an additional insulin administration at 20 min. Both FSIGTs with minimal model analysis provide the same S_I, which was a very robust index. S_G was different by 28 %, and the reason behind may be the relationship between S_G and the amount of circulatory insulin.

In 2011, Javier et al. [59] extended the model of Topp et al. [14] by proposing two models: one to show the adipose tissue effects on insulin sensitivity and another to show the effect of fat accumulation on the regulatory system. He discussed three different formulations for fat accumulation: a linear case and two nonlinear cases where the relationship between fat accumulation, insulin and glucose was discussed.

Other approaches

Other type of equations which are widely used in the mathematical models are the following: partial differential equations (PDE), stochastic differential equations (SDE), delay differential equations (DDE) and integral differential equations (IDE).

Parameter estimation techniques

- Bayesian parameter estimation technique to estimate the parameters of mathematical model [37, 60–63].
- Nonparametric stochastic deconvolution estimation technique [64–66].
- Pancreatic suppression test (PET) [67–69] and glucose clamp technique (GCT) used to evaluate the insulin resistance.
- Parameter estimation was performed on a digital computer (IBM 370/168, IBM corp.) using a nonlinear least square technique [70].
- Nonlinear mixed effects modelling using NONEM VI and the first-order conditional estimation method (FOCE) was used for data analysis [71, 72, 52, 53].

Software tools for numerical simulation

- Monte Carlo simulation are a broad class of computational algorithm to obtain numerical results [73].
- SAAM II software: Simulation, Analysis and Modelling software is widely used for tracer and pharmacokinetic studies [74, 75].
- Several physiology-based paradigm models are available for diagnosis like HOMA [44, 45], QUICKI [46] and MATSUDA [47].
- ODESOLVE is a MATLAB program for solving ordinary differential equations and described in the third edition of ordinary differential equations using MATLAB.
- MATCONT is a software for numerical bifurcation analysis of ordinary differential equations in MATLAB [76, 77].
- WinSAAM is a program used to model all types of biological systems [78].
- ODE23 and ODE45 are the tools used to solve ordinary differential equations in MATLAB and can be found in http://in.mathworks.com/help/matlab/ref/ode23.html and http://in.mathworks.com/help/matlab/ref/ode45.html.
- WINSTODEC is a stochastic deconvolution interactive program used for physiological and pharmacokinetic systems [66, 79].
- XPPAUT (XPP) is a tool for solving ordinary differential equations (ODE), difference equations (DE), delay differential equations (DDE), functional equations, boundary value problems and stochastic equations [80].

Computational results

Numerical simulation has been done for many mathematical models in the research papers. To include all the

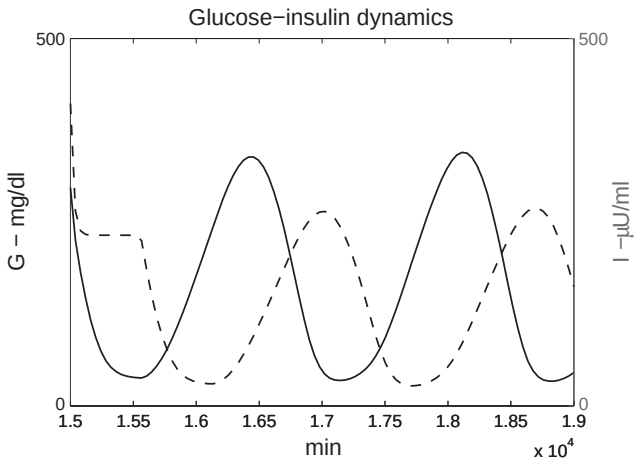

Fig. 3 DDE23 shows the sustained oscillations at $\tau = 550$ min

computational simulation in one paper is difficult or probably an impossible task. Here, we tried to give some computational results of few mathematical model.

Consider the Bergman model (8-10) [9], by using ODE45 tool in MATLAB 2012b, we plotted the glucose-insulin concentration level as shown in Fig. 2 and the steady point is taken as (G^*, X^*, I^*). Using ODE45 tool, it is easy to solve the mathematical model and helps to detect the glucose concentration, plasma insulin concentration and interstitial insulin concentration.

Consider a IVGTT model (19-20) [38], by using DDE23 tool in MATLAB 2012b, we can plot the glucose and insulin sustained oscillations for subject 6. The data has been taken from [37] having (G^*, I^*) as the equilibrium point. The tool DDE23 helps to detect the value of delay term τ at which sustained oscillations occur. The periodic and sustained oscillations at time delay $\tau = 550$ min is shown in the Fig. 3. No sustained and periodic oscillations obtained for $\tau < 550$ min.

Discussion

Mathematical models provide new insights for better understanding of the physiology involved in the disease for better management of the disease. They provide a justification of the theory; provide new information and software tools; help in estimating parameters and most importantly simulate the simple and complex mechanism involved in occurrence of any disease. Literature deals with many mathematical models (ODE, DDE, PDE, SE and IE models), and they are proven to be very informative for better understanding of the disease. In the present review paper, our motive was to give an overview of ODE models which deal with different aspects, diagnosis, care, cure and complications of diabetes. We indent to discuss the purpose behind every paper which deals with mathematical models. We discussed the theoretical, analytical and numerical results and also the limitations of every paper. The limitations of a previous paper motivate the occurrence of the next paper, and in this way, improved mathematical models were developed and presented which confirm the clinical and nonclinical results of the diabetes.

For example, Javier et al. [59] included the assumption of adipose tissue in the model of Topp et al. [14] to describe the effects of fat accumulation in diabetes. Similarly researchers may relax or include more assumptions in the model to describe more complex dynamics involved in the disease, which may throw light in the direction of controlling the disease.

In the three sections before the "Discussion section", a list of parameter estimation techniques, computer softwares and some computational results are presented in the paper.

Conclusion

During the last five decades, many research articles were published on different mathematical models and the computer algorithms. Besides the fact that many models were presented, still the exact mechanism involved in the physiology of the diabetes is not fully understood. The reason for the long persistence of hyperglycaemia acting differently in different individuals is not known. Here, we tried to present a panorama of all ODE models according to their year-wise publication so that it provides new insight to the researchers to think for further development in the diabetes research.

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Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

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

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ORIGINAL ARTICLE

Effects of a fixed-dose combination of sitagliptin and metformin versus respective monotherapies in newly diagnosed type 2 diabetic subjects

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Abstract Metformin is considered as a backbone therapy for type 2 diabetes (T2DM) management. Many patients need treatment with combination therapy. In India, multiple fixed-dose combinations with metformin are available. The study was done to compare the efficacy and safety of sitagliptin plus metformin combination versus metformin and sitagliptin monotherapy in newly diagnosed T2DM subjects. This was an open-label, randomized, parallel group, prospective and single centre study, in 60 subjects with T2DM. The subjects received either metformin 500 mg, sitagliptin 50 mg or fixed-dose combination of metformin 500 mg plus sitagliptin 50 mg. All study medicines were given twice daily for 12 weeks. Glycaemic control (HbA1c, fasting and postprandial blood glucose) and body mass index (BMI) were evaluated as efficacy parameters while safety was evaluated by reporting adverse events. Significant reduction in HbA1C level was seen in all three groups (P=0.0001). HbA1C reduction was significantly higher in the combination group compared to metformin monotherapy (P=0.0072). Fasting blood glucose (FBG) level reduced significantly in all three groups (P=0.0001). The reduction in fasting blood glucose was significantly higher with combination compared to sitagliptin monotherapy (P=0.0060). Postprandial blood glucose (PPG) also reduced significantly in all three groups (P=0.0001). The reduction with sitagliptin was statistically higher compared to metformin (P=0.0155). The combination treatment resulted in significantly higher reduction of PPG compared to sitagliptin monotherapy (P=0.0160). Body mass index reduced significantly in all three groups (P=0.0001). Reduction in BMI was significant with combination treatment compared to sitagliptin monotherapy (P<0.05). Overall, study medications were well tolerated. The incidence of adverse event was 11.7 %. No serious adverse event was reported in the study. In newly diagnosed, drug naïve, type 2 diabetes mellitus management, fixed-dose combination of sitagliptin plus metformin is effective and well tolerated. Due to its multiple benefits, it can be used as a suitable option for selected subjects requiring combination therapy in type 2 diabetes mellitus.

Keywords Efficacy · Metformin · Safety · Sitagliptin

Abbreviations

AACE	American Association of Clinical Endocrinologists
BMI	Body mass index
ICH/GCP	International conference on hormonization/good clinical practice
IERC	Institutional ethics research committee
FBG	Fasting blood glucose
FDC	Fixed-dose combination
HbA1c	Glycosylated haemoglobin
PPG	Postprandial blood glucose

Introduction

Type 2 diabetes mellitus is a progressive disease. Multiple antihyperglycaemic agents are usually required to achieve or

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maintain glycaemic control [1]. According to American Association of Clinical Endocrinologists (AACE), for patients with recent-onset type 2 diabetes or patients with HbA_1c <7.5 %, therapeutic life style changes with monotherapy is recommended. While selecting a glucose-lowering agent for the treatment of diabetes, goals of therapy for each patient should be considered. Other important factors to be considered include age of the patient, other specific conditions and tolerability and adverse event profile of the medicine [2]. Metformin is one of commonly used medicines in the management of type 2 diabetes mellitus. It has negligible risk of hypoglyceamia when used in monotherapy. The possibility of less clinically significant drug interactions adds to the good safety profile metformin. Metformin is a widely accepted first-line agent [3] and considered as the backbone therapy for type 2 diabetes mellitus [2].

Many new glucose-lowering drugs acting on novel pathways of diabetes have been introduced during the last decade. Dipeptidyl peptidase-4 (DPP-4) inhibitor is of the newer class of drugs [4]. Sitagliptin is one of the examples of DPP-4 inhibitor used in the management of type 2 diabetes mellitus [5].

Many patients with type 2 diabetes mellitus need treatment of combination therapy. It is important to consider the risks and benefits associated with combination therapy before using or modifying treatment with such therapy. Patients presenting with HbA_{1C} >7.5 % or those who do not achieve the target HbA_{1C} with metformin should be started on combination with metformin [2]. Many oral combination preparations containing metformin are available in India. The use of fixed-dose combination is popular because, as compared to two medicines given separately, fixed-dose combination (FDC) helps to reduce pill burden and improve adherence to therapy in chronic diseases like diabetes. In India, sitagliptin is available as single agent as well as in combination with metformin.

Objective

The study was done to compare the efficacy and safety of sitagliptin plus metformin FDC vesus metformin and sitagliptin monotherapy in newly diagnosed, drug-naive subjects with type 2 diabetes mellitus.

Material and methods

This was an open-label, randomized, parallel group, comparative, prospective and single centre study, involving a total of 60 newly diagnosed drug-naive subjects with type 2 diabetes mellitus. The subjects between 30 and 65 years of age with HbA_1c between >6.5 and <8.5 % at screening and body mass index (BMI) more than 27 kg/m^2 were enrolled in the study. Subjects with type 1 diabetes, those having BMI <27 kg/m^2, having clinically significant cardiovascular diseases, angina pectoris within 1 year and history of myocardial infarction within last 1 year, convulsive disorder, clinically significant gastrointestinal disease, renal disease, hepatic disease, haematological disease and those with known human immunodeficiency virus infection were excluded from the study. Similarly, pregnant or lactating females, smokers and alcoholic subjects were also not included in the study.

All the subjects were enrolled in the study after obtaining written informed consent. The selected subjects were randomized (1:1:1) by randomization blocks and assigned to receive one of the treatments from three groups. The subjects in group I received metformin 500 mg (Glycomet SR 500 mg, USV Ltd., India) twice daily while those in group II received sitagliptin 50 mg (Istavel 50 mg, Sun Pharmaceuticals Ltd.) twice daily. Group III subjects received a combination of metformin 500 mg plus sitagliptin 50 mg (Istamet, Sun Pharmaceuticals Ltd.) twice daily. The doses of study medications were selected based on the published clinical trials. Scott et al [6] in a randomized, double-blind, placebo and active controlled study evaluated efficacy and tolerability of sitagliptin with different (5, 12.5, 25 and 50 mg b.i.d.) dosages in type 2 diabetes patients inadequately controlled on diet and exercise. The results showed that out of all studied doses, sitagliptin 50 mg b.i.d. was the most effective dose [6]. Sitagliptin 50 mg/metformin 500 mg b.i.d and metformin 500 mg b.i.d. have also been studied in a randomized, double-blind, placebo-controlled study in type 2 diabetes either on or not on an OHA at the screening [7].

The patients were requested not to change the brand of medicines throughout study period. The total duration of therapy was 12 weeks in each group. Each group included 20 subjects. After the baseline examination, the subjects were followed at 4, 8 and 12 weeks. The efficacy was evaluated by comparing baseline parameters versus at the end of 12 weeks. The study was conducted in compliance with the protocol: the institutional ethics research committee (IERC), informed consent regulations and ICH/GCP guidelines. Before initiating the study, a written approval was obtained from IERC.

Evaluation parameters

The study drugs were compared on the following efficacy parameters: HbA_{1C}, fasting blood glucose (FBG), postprandial blood glucose (PPG) and body mass index (BMI). Fasting and postprandial sugar estimation was done using a semi auto analyser (by Transasia Biomedical Ltd.) by glucose oxidase/peroxidase [GOD/POD] method while HbA1c was measured by DS5 from Drew Scientific Ltd. The safety of the study drugs was assessed reporting adverse events during the study period.

Statistical analysis

Data describing categories or nominal data are expressed as numbers with percentages. Descriptive statistics is expressed as mean and standard deviation (SD). Paired *t* test, unpaired *t* test and ANOVA were used to measure the difference among the groups. *P* value <0.05 was considered as statistically significant. The statistical analysis was performed using SPSS 18.

Results

Table 1 shows distribution of subjects in three study groups. In all the three groups, the number of males was more compared to female subjects. All subjects completed the study.

Figure 1a shows comparative reduction in HbA_1c at week 12 compared to baseline in three groups. Statistically significant reduction in HbA_{1C} level was seen all three groups (paired *t* test; $P=0.0001$ for all three groups). The mean reduction in HbA_1c with combination of metformin plus sitagliptin was higher (−11.7 %) compared to respective monotherapies (metformin −6.5 %; sitagliptin −7.4 %). After applying ANOVA test, statistically significant difference was seen in the treatment groups in terms of reduction in HbA_{1C} level (ANOVA; $P=0.0180$). The reduction in HbA_{1C} was significantly higher in combination group compared to metformin monotherapy (unpaired *t* test; $P=0.0072$). However, there was no significant difference in metformin versus sitagliptin and sitagliptin versus combination therapy.

The reduction observed in FBG levels after 12 weeks of treatment compared to baseline was statistically significant in all the three groups (Fig. 1b; paired *t* test; $P=0.0001$). The percentage reduction in FBG after 12 weeks of treatment compared to baseline was 10.61, 10.12 and 11.69 % with metformin, sitagliptin and combination treatment, respectively. The reduction in FBG with combination treatment was significantly higher compared to sitagliptin monotherapy (unpaired *t* test $P=0.0060$).

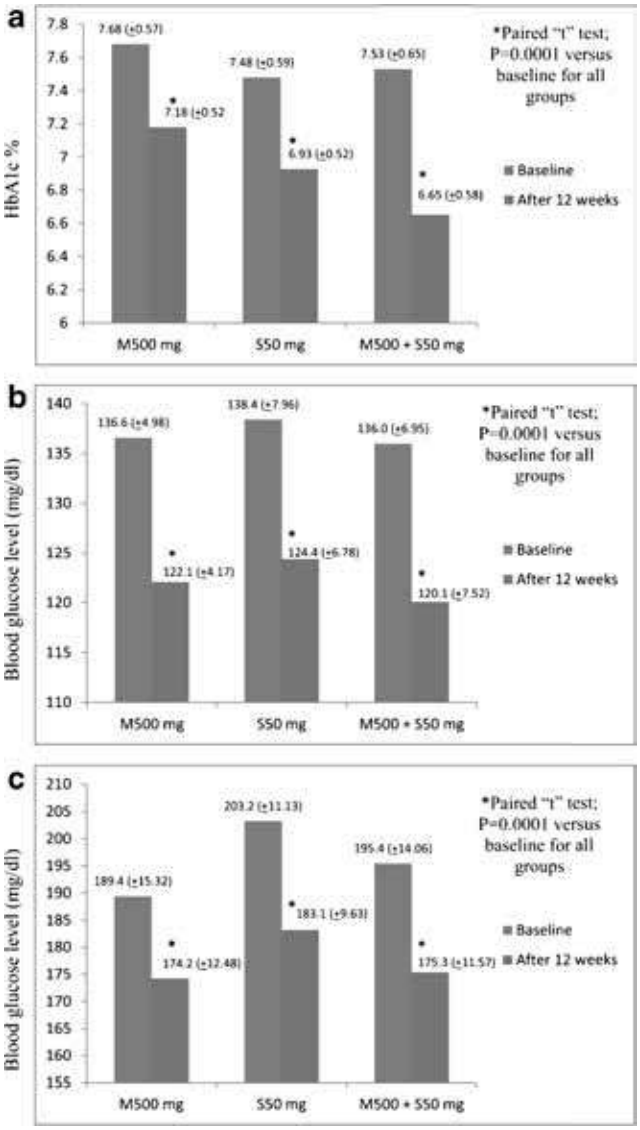

Fig. 1 **a** Effects of metformin (M500 mg), sitagliptin (S50 mg) and metformin+sitagliptin (M500+S50) given orally twice daily for 12 weeks on HbA1c, **b** on fasting blood glucose level and **c** on postprandial blood glucose level

Statistically significant reduction in PPG was seen in all three groups (Fig. 1c; paired *t* test; $P=0.0001$). The percentage reduction in PPG after 12 weeks of treatment compared to

Table 1 Demographic details

	Metformin (M500)	Sitagliptin (S50)	Metformin+sitagliptin (M500+S50)
Number of subjects	20	20	20
Age (years) mean (±SD)	47.90 (±7.16)	45.25 (±7.22)	45.70 (±7.26)
Body weight (kg) mean (±SD)	75.55 (±8.63)	75.40 (±7.08)	76.55 (±7.47)
Male, *n* (%)	12 (60 %)	14 (70 %)	13 (65 %)
Female, *n* (%)	8 (40 %)	6 (30 %)	7 (35 %)

Table 2 Effects of metformin (M500 mg), sitagliptin (S50 mg) and metformin+sitagliptin (M500+S50) given orally twice daily for 12 weeks on body mass index (kg/m^2)

Group		Mean (±SD)	Mean difference	*P* value
Metformin	Baseline	29.26 (±1.59)	0.59	<0.05
	End of study	28.66 (±1.53)		
Sitagliptin	Baseline	28.19 (±0.91)	0.64	<0.05
	End of study	27.55 (±0.98)		
Metformin plus sitagliptin	Baseline	28.53 (±1.30)	0.82	<0.05
	End of study	27.80 (±1.31)		

baseline was 8.03, 9.89 and 10.29 %, respectively. Statistically significant difference between groups was seen in the PPG after 12 weeks of treatment (ANOVA P=0.317). The reduction in PPG with sitagliptin was statistically significant compared to metformin therapy (unpaired t test; P=0.0155). Similarly, the combination treatment resulted in significantly higher reduction of PPG compared to sitagliptin monotherapy (unpaired t test; P=0.0160).

Statistically significant reduction in body mass index was observed in all three groups after 12 weeks of treatment compared to baseline (paired t test; P=0.0001 for all groups). However, the mean difference in BMI was more with combination therapy compared to metformin and sitagliptin monotherpy (Table 2). Applying ANOVA, statistically significant difference after 12 weeks of treatment was seen among three groups (P=0.0234). Reduction in BMI was significant with combination treatment compared to sitagliptin monotherapy (unpaired t test P= 0.0099). No significant difference was seen between remaining two groups.

Safety

Overall, study medications were well tolerated by the subjects. Adverse events such as hypoglycaemia and gastrointestinal symptoms were reported in 11.7 % of subjects. The incidence of adverse events in individual groups is given in Table 3. All adverse events were mild in nature. No serious adverse event was reported in this study.

Discussion

When lifestyle interventions are not successful or are not reasonable, pharmacological treatment is required in the management of type 2 diabetes mellitus [3]. Different drug classes acting on various pathophysiological mechanisms of type 2 diabetes are available for managing the disease. Metformin is old and still widely used medicine in type 2 diabetes.Safety of metformin is well documented in the literature. Metformin is known to produce durable anti-hyperglycaemic effect. In addition, it is associated with low risk of hypoglycaemia and beneficial effect on body weight and also has proven cardiovascular safety [2]. These advantages make metformin a first-line agent for the management of diabetes mellitus. The importance of early and optimal glycaemic control while managing type 2 diabetes is well known. Hence, for the optimal management, multiple drugs are combined, based on the different pathophysiologic mechanisms of diabetes [8]. Evidence suggests that aggressive glycaemic control in type 2 diabetes leads to improvement in the symptoms and short-term health and also helps to prevent long-term complications [8]. Sitagliptin, has been well studied in combination with metformin. The combination therapy is found to be effective and well tolerated when used in addition to metformin therapy [7, 9, 10]. In our study, more number of males compared to female in all three groups was just a finding, rather than an intentional inclusion in the study. Sitagliptin and metformin monotherapy as well as their combination were significantly effective in

Table 3 Incidence of adverse events (n=60)

Adverse event	Number of subjects		
	Metformin 500 mg	Sitagliptin 50 mg	Combination (metformin 500 mg+sitagliptin 50 mg)
	N (%)	*N* (%)	*N* (%)
Hypoglycaemia	–	1 (1.67 %)	–
Diarrhoea	2 (3.33 %)	–	–
Abdominal discomfort	2 (3.33 %)	–	
Nausea/vomiting	–	–	2 (3.33 %)

reducing both fasting and postprandial blood glucose and HbA_1c. The reduction in FBG and PPG with combination treatment was significantly higher compared to sitagliptin monotherapy. HbA_{1C} reduction at week 12 compared to baseline was significantly higher with combination therapy compared to metformin monotherapy but not with sitagliptin monotherapy. Thus, combination of metformin with sitagliptin is more effective than sitagliptin monotherapy for controlling blood glucose level.

Some antihyperglycaemic agents are known to be associated with an increase in bodyweight [11]. Weight gain is an undesired side effect in type 2 diabetes [5]. Sitagliptin is generally shown to be weight neutral [12, 13]. Similarly, metformin also has beneficial effect on body weight [2]. In a comparative study, Arechavaleta et al [5] showed that, in patients receiving combination of sitagliptin plus metformin, at 30 weeks, body weight decreased from baseline while there was an increase in the weight in patients receiving glimepiride plus metformin. The difference in weight change was significant between the groups [5]. In our study, there was significant reduction in BMI in all three groups, i.e. metformin monotherapy, sitagliptin monotherapy and combination of sitagliptin plus metformin.

There was no discontinuation because of adverse events. Similarly, serious adverse events were not reported. Thus, our study confirms the safety and efficacy of metformin alone and in combination with sitagliptin.

The limitations of our study include small sample size and open label study design. Larger studies are required to confirm the findings.

Conclusion

Fixed-dose combination of sitagliptin plus metformin is effective and well tolerated in the management of newly diagnosed drug-naïve type 2 diabetes mellitus. Apart from potential to improve compliance due to availability of fixed-dose combination, effective glycaemic control and beneficial effect on BMI make this combination a suitable option for selected patients requiring combination therapy for type 2 diabetes mellitus.

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ORIGINAL ARTICLE

A web-based interactive lifestyle modification program improves lipid profile and serum adiponectin concentrations in patients with metabolic syndrome: the "Red Ruby" study

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Abstract The effectiveness of internet-based programs in prevention and treatment of metabolic syndrome has not been fully explored. In the present study, we investigate the effect of a 6-month web-based interactive lifestyle modification program on anthropometric variables and biochemical risk factors of cardiovascular disease. The study had been carried out among 160 patients with metabolic syndrome (intervention, $n=80$; control, $n=80$). The primary outcomes were change in anthropometric variables, fasting serum glucose (FSG), lipid profile, insulin sensitivity, and serum adiponectin concentrations in intervention and control groups. Significant reductions in anthropometric variables and serum lipids were observed in both intervention and control groups; however, reduction in waist-to-hip ratio (WHR), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) was only significant in intervention group ($P<0.05$). Reduction in anthropometric variables and serum triglyceride, systolic and diastolic blood pressure, and liver enzymes were significant in intervention and control groups ($P<0.05$) but in women decrease in FSG, TC, and LDL-C were only significant in intervention group ($P<0.05$). The present study showed that a web-based intervention was effective in weight loss and improving cardio-metabolic factors in patients with metabolic syndrome after a 6-month intervention.

Keywords Web-based · Interactive · Internet · Lifestyle modification · Metabolic syndrome · Lipid profile · Adiponectin

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Introduction

Metabolic syndrome (MetS) including insulin resistance, abdominal fat distribution, dyslipidemia, and hypertension is associated with higher mortality and morbidity from coronary heart disease (CHD) and cardiovascular disease (CVD) [1, 2]. In fact, in persons with MetS but without diabetes, the increased risk of CVD and CHD mortality remain [3]. Several previous studies reported that metabolic syndrome is associated with 3- to 4.3-fold increase in mortality from CVD [4] and subjects with metabolic syndrome are 3.5 to 5 times more likely to develop type 2 diabetes mellitus [5]. The third national health and nutrition examination survey (NHANES III) reported an alarming roughly 30 % of metabolic syndrome in middle-aged men [6]. The prevalence of metabolic syndrome in Iran is increasing in parallel of increasing in coronary artery disease (CAD); over the last 20 years, age-adjusted mortality rate from CAD has increased from 20 to 45 %. The age-adjusted prevalence of metabolic syndrome in Tehran Lipid and Glucose Study (TLGS) was 33.7 %; while the prevalence in women was higher than men (42 versus 24 %) [7].

Therapeutic approaches in metabolic syndrome are multifactorial regimens of modifications in dietary habits, physical activity schedule, and drug therapy. Dietary interventions include mostly from reducing saturated fat intake and promoting weight loss [8]. Several reports propose the Mediterranean-

style diet [9] and dietary approaches to stop hypertension (DASH) eating plan [10] to reduce vascular inflammation and improve endothelial function in metabolic syndrome. These recommendations are mainly based on consuming more fruits, vegetables, whole grains, and lower amounts of saturated fats. These dietary plans also encourage participants to have higher physical activity and more exercise [9, 10]. Changing dietary habits and physical activity behaviors may lead to a healthier weight, improved serum lipids, and lower blood pressure and blood glucose [11].

Recently, innovation in technology and home-based care led to new approaches for chronic disease management; web-based interventional programs provide using the Internet to record, monitor, and deliver health care [12]. These web-based interventions have created new interactions between patients and health care provider; patients are capable to self-monitor the disease from home and providers, on the other hand, can easily communicate with patients and feedback on the management of their disease [12]. Additionally, unlike face-to-face interventions, a web-based intervention is available in all hours a day and therefore could be used by a broad population [13].

Several previous reports investigated the efficacy of web-based intervention programs in the control and management of obesity [14, 15], type 2 diabetes mellitus [13, 16], and several other chronic diseases [17–19]. It has been reported that interactive web-based programs for lifestyle modification produce significantly more weight loss and greater reduction in waist circumference compared with the control group [15]. Accordingly, we expect that a web-based interventional program might be beneficial in improving the metabolic parameters and anthropometric variables in metabolic syndrome, which has not been evaluated yet; therefore, the primary objective of the present study is to evaluate the effect of a 6-month web-based interactive intervention program on the metabolic and anthropometric features in patients with metabolic syndrome.

Subjects and methods

Design and participants

This study was a part of the "Red Ruby" study [20]. Red Ruby is an interactive web-based intervention program. The intervention consisted of an Internet web page (http://www.Heartresearch.com) designed to improve self-management nutritional and physical activity behaviors in patients with metabolic syndrome in order to improve the subject's awareness about prevention of cardiovascular disease. The advertisement procedure was performed through the Internet. All of the participants who registered in the web site and met the inclusion criteria enrolled in the study. Inclusion criteria consisted from the following: having metabolic syndrome according to the National Cholesterol Education Program's Adult Treatment Panel III report (NCEP-ATP III) criteria [21] (except for waist circumference which was defined as ≥90 cm for both genders for Iranian population [22, 23]), accessibility to the Internet at home or work, having simple skills to work with the Internet, aged 20 years old and above, and living in Tehran. Exclusion criteria included the following: a) having history of cardiovascular diseases, type 2 diabetes mellitus, cancer, and renal diseases; b) being pregnant; c) taking medications for hypertension; d) taking medications for dislipidemia; and e) having incomplete registration form. Participants were randomly assigned into intervention and control groups. The allocation sequence was performed in sequentially numbered, opaque, sealed, and stapled envelopes. Randomization sequence was created by a biostatistician using the Excel software to assign participants to the study arms using a 1:1 allocation ratio with block size of 4.

Intervention and control groups

Participants in the both intervention and control groups were informed of their metabolic syndrome conditions and its components by an e-mail and encouraged to make appropriate changes in their dietary intake and physical activity in order to manage their disease. The participants in the intervention group received the username and password for log-in to the "My Healthy Heart Profile" and encouraged to regularly visit their own profile.

My Healthy Heart Profile program

This is an interactive web-based program that includes five parts:

1. Personal page on the main page included educational materials for prevention of cardiovascular disease and metabolic syndrome. These materials are free for download and print. Nutritional recommendations were based on dietary approach to stop hypertension (DASH).
2. Personal information included name, gender, age, weight, height, phone number, and e-mail addresses.
3. Inbox as an interactive section in the profile for personal questions. Participants in intervention arm were able to send personal questions and receive answers. We sent a calorie restricted tailored diet to all participants' inbox provided by a dietitian. The calorie restricted diet was based on each participant's calorie requirement according to his/her ideal body weight (IBW) and adjusted body weight (ABW) with less than 30 % of calories derived from fat, in accordance with the National Heart, Lung, and Blood Institute guidelines [24]. IBW was calculated with Hamwi equation [25]. ABW is defined as [(IBW+

25 % (actual body weight−IBW)]. We requested the intervention group to adhere the dieting program.

4. A cardiovascular risk estimation tool for 10 years (based on the Persian online version of Framingham Risk Score—FSR). The FSR comprises from six cardiovascular risk factors, including age (≥20), sex (male and female), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP), and smoking habits. This is an interactive tool to calculate the risk score of heart attack risk score in every log-in. The estimated risk over 20 %, between 10 and 20 % and less than 10 % are considered as high, moderate, and low risk, respectively. Then users received an explanation regarding their score and were guided to educational materials on the personal homepage.
5. Anthropometric and clinical assays: in this section, user recorded periodic measurements of weight, waist circumference (WC), BMI, and blood pressure, TC, low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, and fasting serum glucose (FSG).

The flowchart of the study has been presented in Fig. 1. This study has been approved by the ethics committee of Tabriz University of Medical Sciences (5/92/1228) and Tehran University of Medical Sciences (97/130/1736). The trial protocol has also been registered in IRCT (identifier: IRCT201111198132N1).

Anthropometric assessments

Anthropometric variables which included weight, height, WC, and BMI were evaluated at the beginning and end of the study. Weight was measured while subjects wearing light clothes. WC measured in horizontal plane, midway between the lowest rib and the iliac crest with a measuring tape in centimeter [26]. Waist-to-hip ratio was calculated by waist circumference divided by hip circumference.

Biochemical measurements

Biochemical measures include serum TC, LDL-C, HDL-C, FSG, TG, insulin, adiponectin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Atherogenic index of plasma was calculated as log TG divided by HDL-C [27]. Assessment of insulin sensitivity was performed by the homeostasis model assessment of insulin resistance (HOMA-IR) based on fasting glucose and insulin measurements as follows: HOMA-IR: (glucose (mmol/l)×insulin (mU/l))/405 [28]. High HOMA-IR scores denote low insulin sensitivity. The Quantitative Insulin Check Index (QUICKI index) QUICKI was calculated as: 1/(log fasting insulin (U/l)+log fasting glucose (mg/dl)). Higher QUICKI values indicate greater insulin sensitivity [29].

Serum AST, ALT, TC, FSG, TG, HDL-C, and LDL were analyzed by enzymatic colorimetric method (Pars—Azmoon, Tehran—Iran). Serum insulin was analyzed with enzyme-linked immunosorbent assay method (ELISA—Monobind Insulin AccuBind, CA 92630, USA). The Sensitivity of this assay was 0.75 μIU/ml and mean inter- and intra-assay coefficient of variations (CV) were <9.8 and <8 %, respectively. Serum adiponectin was also analyzed by ELISA method (AviBion, Fin-01720 Vantaa, Finland) with sensitivity of <0.18 ng/ml and mean inter-and intra-assay CV of ≤12 and ≤10 %, respectively. All of the biochemical assessments were performed in Nutritional Research Center, Tabriz University of Medical Sciences and Heart Research Center, Tehran University of Medical Sciences.

Blood pressure measurements

Blood pressure was measured with mercury sphygmomanometer twice in the same arm after the individual seated at rest 10–15 min. The systolic and diastolic measurement represents the mean of two readings. All of the study measurements were performed by assessors who were blind to the group assignment.

Statistical analyses

Analysis of data was performed by SPSS software (statistical package for social analysis, version 18, SPSS Inc., Chicago, IL, USA). All comparison between treatment groups was based on intention-to-treat analysis using multiple imputation method. The normality of data was tested by Kolmogorov-Smirnov test. For parameters with normal distribution, independent Student's t test and for others the Mann-Whitney U test was used. The association between continuous variables was analyzed by Pearson correlation analysis and for categorical variables by chi-square test. The comparison of change in biochemical or anthropometric variables before and after analysis was performed by paired t test. McNemar's test was used for the comparison of matched-pair proportions. All data are expressed as means±SD. A two-sided P value less than 0.05 was considered significant. We determined the sample size according to the reference papers showing a 6-cm reduction in waist circumference after lifestyle modification [30, 31] to detect this difference with 90 % power and α-error of 5 %; a total of 80 individuals in each group were required. Primary outcome was to compare changes in anthropometric and biochemical variables between intervention and control subgroups. The secondary outcome was comparison of the difference in anthropometric and biochemical variables before and after intervention between two genders.

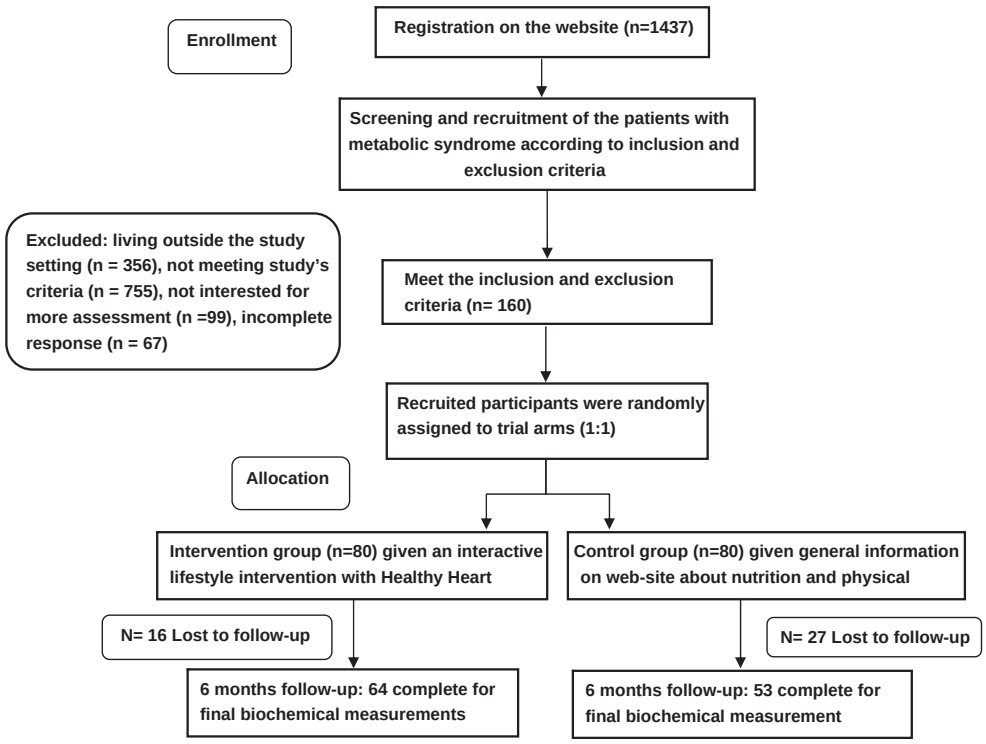

Fig. 1 Flowchart of the study for subject recruitment

Results

Baseline demographic characteristics of study participants are presented in Table 1. A total of 160 participants meeting inclusion criteria were enrolled in the study; however, there were 16 drop-outs in the intervention and 27 drop-outs in the control groups. Remaining participants include 64 participants in intervention and 53 participants in control groups who completed the study. Demographic characteristics were not different between intervention and control groups in men and women (Table 1).

Anthropometric and biochemical variables before and after intervention in overall participants are presented in Table 2. BMI; WC; and serum FSG, TC, TG, AST, and ALT; and AIP decreased and serum HDL and adiponectin concentrations increased in both intervention and control subgroups. However, decline in WHR, serum LDL, and TC were only significant in the intervention group ($P<0.05$).

In men, after a 6-month follow-up, anthropometric variables which include BMI, WC, and WHR decreased significantly in both intervention and control groups. Serum TG, AIP, AST, ALT SBP, DBP, and HOMA-IR decreased in both intervention and control groups in men. In women, like men, decrease in AIP, BMI, WC, WHR, SBP, and DBP and increase in HDL and adiponectin were in both groups; however, reduction in serum FSG, TC, LDL, and AST were only seen in the intervention group ($P=0.05$, Table 3).

We also compared mean value of change in anthropometric and biochemical variables in men and women (Table 4). The mean reduction in BMI in men and women of intervention groups were more pronounced than corresponding amounts in control groups ($P<0.001$ and $P=0.002$, respectively). The mean reduction in AIP in the intervention group was significantly higher ($P=0.05$); however, AIP reduction in female participants was not different between the intervention and control groups. There were approximately 10 and 11 mmHg reductions of DBP in men and women of intervention subgroups compared with 6.92 and 3.57 mmHg reductions in men and women of control groups ($P=0.01$ and $P=0.003$, respectively).

As shown in Table 5, higher log-in frequency was accounted for more reductions in serum insulin concentrations, HOMA-IR and more increases in QUICKI index in men of the intervention subgroup ($P<0.001$); there was no significant relationship between web site use and change in anthropometric or biochemical variables in women.

The prevalence of several components of metabolic syndrome including serum FSG≥110 mg/dl, TG≥150 mg/dl, SBP≥130, and DBP≥85 mmHg decreased significantly after a 6-month follow-up in both intervention and control groups (Table 6).

Discussion

We have found that a 6-month web-based intervention program providing nutritional recommendations for prevention of cardiovascular disease and improving

Table 1 Demographic characteristics of participants at baseline

Variable	Men			Women		
Number	Intervention 43	Control 39	*P*	Intervention 21	Control 14	*P*[a]
Age (years)	40.84±10.29	42.96±10.51	0.29	47.41±8.51	49.00±7.12	0.47
Current smoking [*n*(%)]	11 (25.58)	5 (28.62)	0.063	1 (4.76)	1 (7.14)	0.70
Educational attainment (years)						
12≤	32 (74.42)	24 (61.53)	0.079	10 (47.61)	3 (21.42)	0.79
12>	11 (25.58)	15 (38.46)		11 (52.38)	11 (78.57)	
Marital status [*n*(%)]						
Single	0	8 (20.51)	0.13	3 (14.28)	0	0.45
Married	43 (100)	29 (94.87)		18 (85.71)	13 (92.85)	
Widowed/divorced [*n*(%)]	0	2 (5.12)		0	1 (7.14)	
Family history of diabetes [*n*(%)]	8 (18.60)	8 (20.51)	0.35	6 (28.57)	5 (35.71)	0.54

Values represent percent of total individuals in each group

[a] Chi-square test results

physical activity can lead to significant reductions in WHR and reduction in serum TC and LDL-C in the intervention group compared with the control group. This study also showed that participants in intervention group experienced significantly more reductions in weight, BMI, and DBP than those in the control group. These findings confirm the study's initial hypothesis.

In the present study, we evaluated several anthropometric and biochemical factors which have not been evaluated in previous studies. Previous reports were mostly performed on patients with type 2 diabetes mellitus whereas the effect of a web-based interactive program in patients with metabolic syndrome has not been evaluated before. In the present study, patients with metabolic syndrome were recruited and the main

Table 2 Anthropometric and biochemical variables before and 6 months after intervention in overall participants

Variable	Intervention (*n*=64)			Control (*n*=53)		
	Before	After		Before	After	*P*
BMI (kg/m^2)	29.13±4.44	28.06±4.42	*<0.001*	29.48±3.29	29.10±3.07	*0.037*
WC (cm)	104.44±7.92	100.72±8.07	*<0.001*	105.64±8.31	103.35±7.35	0.29
WHR	0.93±0.08	0.91±0.05	*0.06*	0.94±0.05	0.92±0.04	*0.016*
FSG (mg/dL)	87.83±12.31	84.65±7.22	0.11	89.82±15.31	86.92±12.40	0.18
TC (mg/dL)	42.13±6.42	34.53±5.26	*0.01*	189.38±31.53	183.58±29.07	0.14
TG (mg/dL)	94.21±14.36	45.81±6.98	*<0.0001*	198.23±18.96	142.92±8.87	*0.001*
HDL (mg/dL)	39.16±6.74	44.65±5.03	*<0.0001*	38.56±8.96	43.38±10.18	*0.004*
LDL (mg/dL)	135.23±31.06	126.86±28.35	*<0.0001*	126.10±25.87	122.38±23.41	0.268
AIP	0.61±0.23	0.42±0.17	*<0.001*	0.64±0.27	0.47±0.23	*<0.001*
SBP (mmHg)	132.44±9.28	122.44±11.97	*0.0033*	133.20±16.95	124.48±7.59	*0.001*
DBP (mmHg)	88.72±6.46	78.72±7.40	*<0.0001*	88.20±8.69	81.28±6.03	*<0.001*
Insulin (μIU/mL)	16.97±2.78	8.33±3.64	0.25	23.92±3.86	18.16±9.99	0.41
HOMA-IR	3.39±0.98	1.79±0.81	0.22	5.18±0.63	3.76±4.47	*0.001*
QUICKI	0.33±0.04	0.35±0.03	0.28	0.34±0.06	0.34±0.04	0.15
AST (IU/l)	31±9.63	25.30±11.77	*0.051*	32.21±10.94	22.92±11.37	*0.02*
ALT (IU/l)	29.30±7	19±9.01	*0.008*	28.92±6.49	20.35±12.05	*0.04*
Adiponectin (ng/ml)	12.39±4.95	16.46±8.11	0.16	14.62±3.86	16.71±5.00	0.15

BMI body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio, *FSG* fasting serum glucose, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *AIP* atherogenic index of plasma, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Table 3 Anthropometric and biochemical variables before and 6 months after intervention in male and female participants

Variable	Men						Women					
	Intervention (n=43)			Control (n=39)			Intervention (n=21)			Control (n=14)		
	Before	After	P^a	Before	After	P^a	Before	After	P^a	Before	After	P^a
BMI (kg/m^2)	29.13±4.44	28.06±4.42	*<0.001*	29.48±3.29	29.10±3.07	*0.037*	31.35±4.27	29.22±4.15	*<0.001*	31.40±4.89	30.76±4.46	*0.016*
WC (cm)	104.44±7.92	100.72±8.07	*<0.001*	105.64±8.31	103.35±7.35	0.29	101.76±8.88	95.76±10.16	*<0.001*	103.00±8.62	99.35±7.69	*0.015*
WHR	0.93±0.08	0.91±0.05	*0.06*	0.94±0.05	0.92±0.04	*0.016*	0.90±0.10	0.89±0.06	0.36	0.91±0.03	0.85±0.044	*0.017*
FSG (mg/dL)	87.83±12.31	84.65±7.22	0.11	89.82±15.31	86.92±12.40	0.18	91.19±13.40	86.28±8.88	0.06	90.85±1.47	90.85±14.30	0.98
TC (mg/dL)	42.13±6.42	34.53±5.26	*0.01*	189.38±31.53	183.58±29.07	0.14	191.04±38.48	184±33.25	0.42	192.57±29.54	186.78±25.91	0.39
TG (mg/dL)	94.21±14.36	45.81±6.98	*<0.0001*	198.23±18.96	142.92±8.87	*0.001*	161.42±95.61	130.52±67.98	*0.018*	193.64±27.01	148.78±26.22	*0.009*
HDL (mg/dL)	39.16±6.74	44.65±5.03	*<0.0001*	38.56±8.96	43.38±10.18	*0.004*	42.09±8.14	49.80±10.23	*0.004*	49.35±13.12	50±11.49	0.85
LDL (mg/dL)	135.23±31.06	126.86±28.35	*<0.0001*	126.10±25.87	122.38±23.41	0.268	127.20±34.93	124.10±25.87	0.74	116.92±26.81	116.50±18.89	0.94
AIP	0.64±0.22	0.43±0.15	*<0.001*	0.66±0.27	0.49±0.21	*<0.001*	0.53±0.24	0.39±0.19	*0.004*	0.56±0.26	0.42±0.29	*0.016*
SBP (mmHg)	132.44±9.28	122.44±11.97	*0.0033*	133.20±16.95	124.48±7.59	*0.001*	130.04±6.24	118.57±11.08	*<0.001*	131.42±8.64	124.28±8.73	*0.004*
DBP (mmHg)	88.72±6.46	78.72±7.40	*<0.0001*	88.20±8.69	81.28±6.03	*<0.001*	89.47±5.89	78.09±6.20	*<0.001*	87.14±5.08	83.57±4.97	0.06
Insulin (μIU/ml)	16.97±2.78	8.33±3.64	0.25	23.92±3.86	18.16±9.99	0.41	11.13±3.77	9.86±5	0.46	14.22±8.44	12.20±7.78	0.72
HOMA-IR	3.39±0.98	1.79±0.81	0.22	5.18±0.63	3.76±4.47	*0.001*	2.23±0.97	2.19±0.89	0.71	2.66±0.98	3.18±0.70	0.68
QUICKI	0.33±0.04	0.35±0.03	0.28	0.34±0.06	0.34±0.04	0.15	0.34±0.02	0.35±0.05	0.34	0.34±0.04	0.35±0.08	0.93
AST (IU/l)	31±9.63	25.30±11.77	*0.051*	32.21±10.94	22.92±11.37	*0.02*	32.37±7.08	20.75±10.80	*0.03*	29±13.54	21.40±9.12	0.31
ALT (IU/l)	29.30±7	19±9.01	*0.008*	28.92±6.49	20.35±12.05	*0.04*	30.75±7.20	19.62±12.28	0.06	29.60±10.01	17.80±6.64	0.091
Adiponectin (ng/ml)	12.39±4.95	16.46±8.11	0.16	14.62±3.86	16.71±5.00	0.15	16.60±5.02	21.02±8.19	*0.04*	13.40±6.17	25.40±5.97	*<0.001*

Values are presented as mean±SD

BMI body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio, *FSG* fasting serum glucose, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *AIP* atherogenic index of plasma, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

[a] The comparisons were carried out by paired *t* test or Wilcoxon signed-rank test

Table 4 Comparison of the changes in anthropometric and biochemical variables between subgroups

Variable	Men			Women		
	Intervention	Control		Intervention	Control	
Number	43	39		21	14	
	Change	Change	P^a	Change	Change	P^a
BMI (kg/m^2)	−1.07±0.25	−0.37±0.08	*<0.001*	−2.12±2.5	−0.63±0.73	*0.002*
WC (cm)	−3.72±2.5	−2.28±5.14	*0.037*	−6.00±4.47	−3.64±4.84	0.11
WHR	−0.018±0.05	−0.017±0.08	0.93	−0.012±0.10	−0.05±0.05	0.28
FSG (mg/dl)	−3.18±1.29	−2.89±1.32	0.92	−4.90±1.15	0.00±1.07	0.29
TC (mg/dl)	−16.34±4.17	−5.79±2.42	0.17	−7.04±3.9	−5.78±2.48	0.72
TG (mg/dl)	−60.58±8.44	−55.30±9.09	0.83	−30.90±0.49	−44.85±5.43	0.29
HDL (mg/dl)	5.48±6.73	4.82±9.82	0.71	7.71±1.08	0.64±1.26	0.087
LDL (mg/dl)	−8.37±2.49	−3.71±2.06	0.36	−3.10±4.22	−0.42±2.46	0.83
AIP	−0.20±0.02	−0.17±0.02	*0.05*	−0.14±0.02	−0.14±0.02	0.87
SBP (mmHg)	−10.00±10.74	−8.71±1.57	0.26	−10.47±1.04	−7.14±0.75	0.23
DBP (mmHg)	−10.00±6.7	−6.92±7.03	*0.01*	−11.38±7.38	−3.57±0.63	*0.003*
Insulin (μIU/ml)	−8.63±2.36	−5.76±2.41	0.95	−1.27±0.46	2.02±0.12	0.97
HOMA-IR	−1.59±0.43	−1.41±0.54	0.65	−0.13±1.02	0.52±0.22	0.72
QUICKI	0.018±0.05	−0.006±0.07	0.49	0.01±0.003	0.003±0.08	0.76
AST (IU/l)	−5.70±8.02	−9.28±1.32	0.46	−11.62±1.24	−7.06±1.45	0.60
ALT (IU/l)	−10.30±9.63	−8.57±1.45	0.74	−11.12±1.46	−11.80±1.90	0.93
Adiponectin (ng/ml)	4.07±0.97	2.09±0.48	0.52	4.42±0.45	2.43±0.45	*0.007*

Values are presented as mean±SD, Significance level was defined as $P<0.05$

BMI body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio, *FSG* fasting serum glucose, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

[a] The comparisons were carried out by paired *t* test or Wilcoxon signed-rank test

target of the current study was prevention of cardiovascular disease and type 2 diabetes mellitus. The finding of the present study was in consistent with previous reports using web-based interventions to provide health care for patients of chronic disease [12, 32, 33].

In a study by Bond et al. [12], a 6-month web-based intervention plus usual care compared with usual care alone reduced HbA1C, weight and cholesterol, and HDL-C in patients with type 2 diabetes mellitus. In another study, Moore T et al. [34] reported that an Internet-based nutrition education program in US employees is effective to reduce weight and blood pressure and improve their eating habits after 12 months of intervention. The same results were reported by McMahon GT [32] in patients with poorly controlled diabetes mellitus. Even though there were modifications in BMI, WC, DBP, and serum adiponectin concentrations in intervention and control subgroups, it should be remembered that these changes in the intervention group were more pronounced than in the control group. This fact suggests the possible role of interactive web-based education in improving anthropometric and metabolic parameters. These findings were in accordance of the Bond et al.'s report [12]. They reported that serum cholesterol and body weight decreased in both intervention and control groups; however, mean reduction in these parameters were greater in the intervention group ($P<0.05$). They also found that reductions in SBP and DBP were only seen in the intervention group and not in the control group. This inconsistency could be derived from the different protocol administered for the control group in Bond et al.'s study who have no access to the Internet and received only their standard diabetes care from their provider and no educational or training materials associated with the intervention were provided for them. Whereas our control group received e-mails every 3 weeks to visit the web site and read the general nutritional recommendations and lifestyle changes in addition to their standard usual care advices.

In the present study, almost two thirds of participants at baseline were men and the attrition rate among men [$n=24$ (23 %)] was lower than women [$n=18$ (34 %)]. It seems that men are more interested to participate in an Internet-based educational program than women. Therefore, our study disproves the previous studies by Oenema et al. [35] and Gold

Table 5 Correlation of number of log-ins and changes in anthropometric and biochemical variables in intervention group

Variable	Men		Women	
Number	43		21	
	r	*P*	*r*	*P*[a]
Δ BMI (kg/m^2)	0.03	0.84	0.17	0.43
Δ WC (cm)	0.002	0.98	0.08	0.72
Δ WHR	−0.18	0.32	0.02	0.93
Δ FSG (mg/dl)	0.17	0.30	−0.35	0.11
Δ TC (mg/dl)	−0.19	0.24	0.005	0.98
Δ TG (mg/dl)	0.07	0.64	0.18	0.41
Δ HDL (mg/dl)	0.14	0.39	0.08	0.72
Δ LDL (mg/dl)	−0.19	0.22	−0.29	0.2
Δ AIP	0.45	0.09	0.34	0.9
Δ SBP (mmHg)	−0.02	0.89	−0.003	0.98
Δ DBP (mmHg)	0.21	0.19	−0.008	0.73
Δ Insulin (μIU/ml)	−0.99	*<0.001*	0.54	0.16
Δ HOMA -IR	−0.99	*<0.001*	0.48	0.22
Δ QUICKI	0.99	*<0.001*	−0.51	0.19
Δ AST (IU/l)	0.15	0.76	−0.31	0.45
Δ ALT (IU/l)	0.8	0.06	0.16	0.7
Δ Adiponectin (ng/ml)	−0.15	0.06	−0.35	0.44

Values are presented as mean±SD, Δ Net difference between intervention and control groups

BMI body mass index, *WC* waist circumference, *WHR* waist-to-hip ratio, *FSG* fasting serum glucose, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *SBP* systolic blood pressure *DBP* diastolic blood pressure, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Pearson correlation analysis

et al. [36] reporting the lower participation of men in health promotion behaviors. Higher familiarity with technology and higher attitude about the Internet in men are several possible reasons of their higher interest for using web-based programs [37]; moreover, this can be attributed to higher educational attainment of our male participants. We should also address the difference in the change in measured parameters between men and women, and change in anthropometric variables were not different between men and women; however, significant decrease in serum TC, FSG, and LDL-C and significant increase in serum adiponectin concentrations was only observed in women but not men. Similar to our study, previous reports also showed higher concentrations of serum adiponectin in women compared with that in men. Since higher adiponectin concentrations in serum is in close relationship with better glycemic control and favorable lipid profile [38, 39]; therefore, we can postulate that significant increase in serum adiponectin in women might be responsible in meaningful improvements in TC, LDL-C, and FSG in women.

The present study was limited to a small number of adults who have the ability to work with the Internet; also, the present study did not evaluate the long-term effectiveness of the web-based education on lifestyle change and nutritional modification. Another limitation of the current study was conducting in one of the most air polluted cities of Iran: Tehran. Air pollution is a potent factor in limiting the physical activity and walking among participants.

However, the strengths of this study should be encouraged: it was the first randomized clinical trial evaluating the effectiveness of a web-based interactive program for improving lifestyle habits in patients with metabolic syndrome; including both males and females in the current study is another potent strength which makes it more generalizable.

Table 6 The prevalence of metabolic syndrome ingredients according to the National Cholesterol Education Program's Adult Treatment Panel III Report (ATP III) criteria before and after 6 months web-based trial

Variable	Intervention (*n*=64)			Control (*n*=53)		
	Before *n* (%)	After *n* (%)	*P*[a]	Before *n* (%)	After *n* (%)	*P*[a]
WC (cm)≥90 cm	64 (100)	52 (81.25)	*0.004*	53 (100)	51 (96.22)	0.98
FSG (mg/dl)≥110 mg/dl	36 (56.25)	6 (9.37)	*<0.001*	36 (67.92)	16 (30.18)	*0.002*
TG (mg/dl)≥150 mg/dl	36 (56.25)	6 (9.37)	*<0.001*	36 (67.92)	15 (28.30)	*<0.001*
HDL (mg/dl)<40 mg/dl (male)	41 (64.02)	39 (60.93)	0.53	35 (66.03)	35 (66.03)	0.98
HDL (mg/dl)<50 mg/dl (female)	19 (29.68)	9 (14.06)	0.083	8 (15.09)	7 (13.20)	0.53
SBP (mmHg)≥130	64 (100)	19 (29.68)	*<0.001*	53 (100)	19 (35.84)	*<0.001*
DBP (mmHg)≥85	64 (100)	13 (20.31)	*<0.001*	53 (100)	22 (41.50)	*<0.001*

Values are presented as mean±SD

WC waist circumference, *FSG* fasting serum glucose, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

[a] The comparisons were carried out by McNemar's test

In conclusion, in a 6-month web-based intervention program, significant reduction in WHR, TC, and LDL cholesterol in patients receiving interactive web-based lifestyle modification program was achieved. Further studies are needed to compare the effectiveness of the web-based nutrition and physical activity intervention programs with traditional face-to-face programs.

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

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ORIGINAL ARTICLE

Individuals with diabetes are at a higher risk of asthma in India: evidence from the National Family Health Survey-3

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Abstract Asthma is a disease of the lung, and from previous studies, a decrement in lung function was observed in patients with diabetes. These results motivated us to study the prevalence of asthma among diabetics. In this study, we estimated the prevalence of asthma among men and women, and also investigated the risk factors for asthma in diabetic and non-diabetic groups in India. This analysis was based on the National Family Health Survey-3 (NFHS-3) data on 71,776 men aged 15–54 years and 115,642 non-pregnant women aged 15–49 years. X^2 test was used to check the dependency of asthma with different socioeconomic, diet-, and lifestyle-related factors. Logistic regression analysis was used to identify the risk factors for asthma. There is significant ($p<0.001$) association between diabetes and asthma. Individuals with diabetes are at a higher risk of asthma as compared to those without diabetes. The results of the study revealed that among diabetic women and men, 14.1 and 12.8 % were asthmatic, respectively. In without diabetes group, the prevalence of asthma in men and women was 1.7 and 1.6 %, respectively. Men with diabetes were 8.691 times (95 % CI: 7.154–10.558) and women with diabetes were 10.106 times (95 % CI: 8.455–12.08) more likely to have asthma than those without diabetes. Occasional/never consumption of fish, use of unclean fuel, lower age level, and not living with a partner were significantly ($p<0.05$) associated with the higher risk of asthma among diabetics.

Keywords Asthma · Diabetes · NFHS-3 · Risk factors · Logistic regression

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Introduction

The prevalence of asthma and diabetes are rapidly increasing worldwide. Asthma is a public health problem not only for developed countries but also for developing countries [1]. In fact, most asthma-related deaths occur in low- and lower-middle-income countries. According to the World Health Statistics 2012 report, 12 % of the non-communicable disease (NCD) deaths are caused by chronic respiratory diseases, and diabetes is directly responsible for 3.5 % of NCD deaths [2]. According to the World Health Organization (WHO), about 235 million people currently suffer from asthma worldwide. It is the most common chronic disease among the children. Presently, India has an estimated 15–20 million asthmatics [3]. Among the Indian states, prevalence of asthma among women is exceptionally high in the regions of West Bengal, Mizoram, Kerala, Sikkim, and Tripura, while the prevalence is highest among men in West Bengal and Tripura [4]. The WHO reports that asthma is under-diagnosed and under-treated, which critically affects the well-being of individuals and their families. Worldwide, it is estimated that the economic cost associated with asthma will exceed to those of tuberculosis and HIV/AIDS combined [3]. Thus, asthma is of major public health importance. From the review of different studies conducted between 1960 and 2011, a secular trend was observed regarding increase in diabetes prevalence in India. High prevalence of impaired glucose tolerance and impaired fasting glucose, observed in different parts of India, indicate the potential to develop diabetes [5]. According to the International Diabetes Federation, 61.3 million people in India had diabetes in 2011. This figure is projected to increase

to 101.2 million by 2030 [6]. Also, prevalence of type-2 diabetes is rapidly increasing among Indian youth [7].

For diabetic microangiopathy, the lung is one of the target organs in patients with diabetes, and a reduction in lung function was observed in diabetics from previous studies [8]. Asthma is a disease of the airways or branches of the lung that carry air in and out of the lungs. We had not found any study focused on the prevalence of asthma for the diabetics among Indians. However, using electronic records of a large health plan in northern California, a retrospective, longitudinal cohort study was conducted to evaluate and compare the risk of asthma and other pulmonary diseases in patients with and without diabetes. This study concluded that individuals with diabetes are at increased risk of several pulmonary conditions like asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, and pneumonia [8]. These findings motivated us to carry out the study for estimating the prevalence of asthma among the individuals with diabetes and to determine its risk factors, in India.

Previous studies on asthma suggested that indoor air pollution from biomass and solid fuel combustion, tobacco smoking, obesity, underweight, low education level, consumption of chicken/meat, residence in *kachha* houses, and domestic violence were significantly associated with increased risk of asthma among adult Indian population [9–14]. In the present study, we separately examined the risk factors for asthma among the men and women with diabetes and without diabetes.

Materials and methods

About sampled population

For this study, we derived data from the third round of the National Family Health Survey (NFHS-3), conducted in India during 2005–2006. NFHS-3 was India's largest demographic and health survey. NFHS-3 collected information on several socioeconomic, demographic, and health-related factors. NFHS-3 used separate sampling design for rural and urban areas. Rural sample was selected in two stages: selection of primary sampling unit (PSU) which are villages selected with probability proportionate to size (PPS) at the first stage and followed by the random selection of household within each PSU in the second stage. For urban areas, three-stage sampling was used. In the first stage, wards were selected with PPS. In the second stage, one census enumeration block was randomly selected from each sampled ward. At the third stage, households were randomly selected within each sampled census enumeration block. Of the women, 124,385 of age group 15–49 years and 74,369 men of age group 15–54 years were interviewed for NFHS-3. The eligible women response rate was 94.5 %, and for men, response rate was 87.1 % [4]. NFHS-3 used bilingual questionnaire in which the same questions were asked in the native language of the respondent along with English. In this study, we focused on 115,642 non-pregnant women aged 15–49 years and 71,776 men aged 15–54 years. As we wanted to study the risk factors separately for men and women, we have considered the respondents from all available age groups.

Response variable

As per the definition given by the Global Initiative for Asthma 2014, asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable expiratory airflow limitation [15]. But no questions related with the symptoms of asthma were asked to respondents in NFHS-3 questionnaire. The prevalence of asthma was determined by using the single question, "Do you have asthma?" included in the NFHS-3 questionnaire. The response was either "Yes" or "No". As no medical test was used to diagnose asthma in the survey, asthma was self-reported by the respondents based on their knowledge, previous diagnosis, or medication prescribed to them.

Predictor variables

Several demographic, socioeconomic, environmental, nutritional, and lifestyle-related variables were included in the present analysis. Current age (in years) of the respondent was recorded in the survey. We grouped age variable as 15–19, 20–29, 30–39, and 40–49 for women, and age group 50–54 was also added for men. Type of cooking fuel was classified as clean fuel and unclean fuel. Clean fuel included the use of kerosene, LPG/natural gas, biogas, or electricity. Unclean fuel included the use of biomass fuels such as, wood, straw/shrubs/grass, agricultural crop waste, dung cakes, or other solid fuels such as coal/lignite and charcoal. As per the revised guidelines for obesity, the obese were determined with a BMI $\geq$25 kg/m^2 [16]. Based on hemoglobin concentration(in g/dl) measured in NFHS-3, anemia level was determined as follows: mild anemia (10.0–11.9 g/dl for non-pregnant women and 12–12.9 g/dl for men), moderate anemia (7.0–9.9 g/dl for women and 9.0–11.9 g/dl for men), severe anemia (less than 7.0 g/dl for women and less than 9.0 g/dl for men), and not anemic (greater than or equal to 12 g/dl for women and greater than or equal to 13 g/dl for men).

For the variable "Tobacco use", smoking of cigarettes, pipe, snuff, or any other chewing tobacco was considered. The respondents were asked the question: "Do you have diabetes?" The response was either "Yes" or "No". Thus, here diabetes was also self-reported by the respondents. The socioeconomic and demographic variables included marital status,

Table 1 Prevalence of asthma among adult men and women with and without diabetes, by some selected characteristics

Characteristic	Women						Men					
	With diabetes			Without diabetes			With diabetes			Without diabetes		
	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value
Type of place of residence												
Urban	860(52.3)	94(9.1)	<0.001	53,348(33.2)	821(1.6)	0.421	782(51.1)	68(7.5)	<0.001	36,958(36.6)	494(1.4)	<0.001
Rural	455(47.7)	80(19.6)		62,294(66.8)	1027(1.6)		376(48.9)	60(18.5)		34,818(63.4)	577(1.8)	
Wealth status												
Poor	145(20.7)	35(28.1)	<0.001	28,924(35.8)	485(1.7)	0.336	111(20.5)	28(25.5)	<0.001	16,676(33.7)	309(2.0)	<0.001
Middle	488(40.3)	77(12.8)		50,114(41.3)	792(1.6)		378(32.3)	60(14.3)		33,180(42.8)	494(1.6)	
Rich	682(39.0)	62(8.2)		36,604(22.8)	571(1.5)		669(47.1)	40(6.2)		21,920(23.5)	268(1.3)	
Smokes tobacco												
No	1160(89.6)	136(12.1)	<0.001	101,558(89.8)	1457(1.5)	<0.001	590(46.7)	57(9.1)	0.001	32,758(42.9)	364(1.3)	<0.001
Yes	155(10.4)	38(31.5)		14,018(10.2)	389(2.5)		568(53.3)	71(16.2)		38,983(57.1)	707(2.0)	
House type[a]												
Kachha	55(6.9)	18(38.4)	<0.001	10,090(11.3)	184(1.8)	0.194	52(6.8)	13(22.4)	<0.001	5947(10.9)	135(2.2)	<0.001
Semi-*pucca*	276(25.5)	67(19.9)		39,994(37.5)	645(1.6)		243(27.8)	49(22.9)		24,621(37.0)	372(1.6)	
Pucca	945(65.6)	85(9.3)		59,874(46.0)	930(1.6)		841(64.0)	65(7.3)		39,106(49.0)	529(1.6)	
Not de jure resident	29(2.0)	4(14.3)		4879(5.2)	75(1.5)		13(1.4)	1(28.6)		1707(3.0)	30(1.7)	
Type of fuel												
Clean fuel	797(46.3)	79(7.6)	<0.001	46,284(29.5)	698(1.4)	0.007	805(52.6)	61(7.3)	<0.001	32,301(31.7)	423(1.4)	<0.001
Unclean fuel	489(53.7)	91(19.6)		64,432(70.5)	1074(1.7)		352(47.4)	66(18.9)		39,188(68.3)	646(1.8)	
Obesity												
Obese	671(41.5)	119(9.9)	0.001	16,546(12.4)	412(2.5)	<0.001	357(26.6)	17(1.6)	<0.001	7709(9.5)	133(1.9)	0.361
Not obese	601(58.5)	53(17.2)		93,865(87.6)	1384(1.5)		729(73.4)	105(17.4)		59,043(90.5)	906(1.7)	
Age group												
15–19	48(3.9)	25(45.2)	<0.001	22,614(20.1)	170(0.7)	<0.001	23(1.3)	6(38.5)	<0.001	12,779(17.7)	116(0.9)	<0.001
20–29	138(9.8)	41(26.7)		38,964(33.4)	433(1.1)		95(9.2)	27(27.5)		23,037(31.2)	203(0.8)	
30–39	395(30.7)	55(16.5)		32,335(27.7)	632(1.9)		203(18.0)	30(14.0)		18,341(25.7)	268(1.6)	
40–49	734(55.5)	53(8.4)		21,729(18.9)	613(2.9)		504(45.3)	45(11.8)		13,523(19.6)	325(2.8)	
50–54	NA	NA	NA	NA	NA	NA	333(26.3)	20(7.3)		4096(5.9)	159(4.8)	
Drinks alcohol												
No	NC	NC	NC	NC	NC	NC	684(62.4)	74(13.4)	0.467	47,146(68.1)	632(1.6)	0.001
Yes	NC	NC	NC	NC	NC	NC	474(37.6)	54(11.8)		24,628(31.9)	439(1.9)	
Consumed fruits												
Never/occasionally	601(54.2)	97(17.3)	0.001	60,522(60.0)	1079(1.7)	<0.001	403(43.0)	62(18.0)	<0.001	32,047(52.7)	613(2.0)	<0.001
Weekly	389(26.6)	46(13.0)		34,879(27.5)	510(1.5)		465(37.2)	47(9.0)		27,309(34.3)	327(1.3)	
Daily	324(19.3)	31(6.8)		20,165(12.5)	259(1.3)		290(19.8)	19(8.1)		12,395(13.0)	131(1.4)	

Table 1 (continued)

Characteristic	Women						Men					
	With diabetes			Without diabetes			With diabetes			Without diabetes		
	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value	Sample size (weighted %)	Asthmatic (weighted %)	*P* value
Consumed fish												
Never/occasionally	716(56.9)	96(15.5)	0.23	78,144(71.8)	1089(1.4)	<0.001	637(55.1)	87(15.0)	0.005	47,471(68.9)	665(1.5)	<0.001
Weekly	397(28.5)	54(13.4)		27,652(22.0)	495(1.9)		392(32.5)	33(12.7)		19,515(24.9)	303(1.9)	
Daily	202(14.6)	24(10.3)		9804(6.2)	264(2.8)		129(12.5)	8(4.0)		4765(6.2)	103(2.8)	
Anemia status												
Anemic	559(48.2)	74(15.3)	0.292	54,313(51.8)	837(1.5)	0.004	202(20.8)	26(17.9)	0.019	12,863(22.8)	236(2.0)	<0.001
Not anemic	756(51.8)	100(13.0)		61,329(48.2)	1011(1.7)		956(79.2)	102(11.4)		58,913(77.2)	835(1.6)	
Type of caste or tribe[b]												
SC	183(16.5)	25(16.0)	<0.001	18,900(19.1)	277(1.4)	<0.001	169(17.5)	21(13.9)	0.009	12,221(19.4)	206(1.7)	<0.001
ST	99(3.3)	45(52.9)		15,147(8.3)	260(1.5)		77(4.1)	16(28.2)		8610(8.4)	150(1.8)	
OBC	385(36.5)	41(10.2)		36,302(40.5)	551(1.5)		406(36.9)	41(10.0)		25,906(40.5)	338(1.3)	
General and others	578(43.6)	58(14.1)		39,947(32.1)	674(1.9)		468(41.5)	41(11.5)		22,634(31.6)	337(1.9)	
Marital status												
Never married	71(3.6)	35(42.1)	<0.001	30,231(21.8)	282(0.9)	<0.001	98(6.3)	27(34.9)	<0.001	27,852(34.6)	268(0.9)	<0.001
Currently married	1119(88.6)	127(13.4)		79,864(73.3)	1409(1.7)		1045(91.8)	99(10.7)		42,963(63.9)	766(2.0)	
Widowed/divorced/separated	125(7.9)	12(8.3)		5547(4.9)	157(2.8)		15(1.9)	2(36.8)		961(1.5)	37(5.2)	
Regional zones[c]												
North	201(11.2)	12(5.0)	<0.001	21,699(13.4)	199(1.2)	<0.001	82(9.3)	7(8.6)	0.004	8290(14.4)	83(1.4)	<0.001
Northeast	201(2.7)	61(24.1)		20,097(3.9)	417(1.9)		181(3.1)	23(6.5)		12,331(4.0)	211(1.6)	
Central	142(12.0)	18(20.3)		20,777(23.1)	210(1.0)		125(11.2)	16(19.8)		15,058(23.4)	170(1.2)	
East	218(27.8)	30(18.9)		16,556(21.9)	363(2.1)		114(26.4)	13(16.7)		6206(20.4)	135(2.3)	
West	311(23.4)	34(14.8)		27,688(29.0)	470(1.7)		497(35.0)	60(12.1)		23,353(30.0)	393(1.7)	
South	242(22.8)	19(7.4)		8825(8.6)	189(2.0)		159(15.0)	9(6.0)		6538(7.8)	79(1.6)	
Overall	1315	174(14.1)		115,642	1848(1.6)		1158	128(12.8)		71,776	1071(1.7)	

NC not considered, *NA* not applicable

[a] House type: *kachha*, semi-*pucca*, and *pucca*. The houses made from mud, thatch, or other low quality material are classified as *kachha* houses, and those made from high quality material like bricks, tiles, cement, and concrete throughout, including roof, walls, and floor are *pucca* houses. Semi-*pucca* houses are the combination of both types discussed above

[b] Type of caste or tribe: the scheduled caste (SC) or scheduled tribes (ST) are two groups of historically disadvantaged people recognized in the constitution of India. Scheduled castes and scheduled tribes are identified by the Government of India as socially and economically backward and needing protection from social injustice and exploitation. Other backward class (OBC) is a diverse collection of intermediate castes that were considered low in the traditional caste hierarchy but are clearly above scheduled castes

[c] Regional zones: north includes Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab, Rajasthan, and Uttaranchal; northeast includes Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura; central includes Chhattisgarh, Madhya Pradesh, and Uttar Pradesh; east includes Bihar, Jharkhand, West Bengal, and Orissa; west includes Maharashtra, Goa, and Gujarat; and south includes Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu

Table 2 Results of logistic regression analysis for women

Characteristic	OR for women with diabetes		OR for women without diabetes	
	Unadjusted (95 % CI)	Adjusted (95 % CI)	Unadjusted (95 % CI)	Adjusted (95 % CI)
Type of place of residence				
Urban	1.000	1.000	1.000	1.000
Rural	2.520 (1.744, 3.641)[b]	1.203 (0.702, 2.064)	1.069 (0.966, 1.183)	0.976 (0.852, 1.117)
Wealth status				
Poor	4.543 (2.846, 7.254)[b]	0.999 (0.396, 2.519)	1.122 (0.988, 1.274)[a]	1.026 (0.810, 1.300)
Middle	1.760 (1.110, 2.791)[b]	0.941 (0.480, 1.846)	1.038 (0.916, 1.177)	0.975 (0.818, 1.163)
Rich	1.000	1.000	1.000	1.000
Smokes tobacco				
No	1.000	1.000	1.000	1.000
Yes	3.297 (2.106, 5.160)[b]	2.627 (1.514, 4.558)[b]	1.698 (1.493, 1.931)[b]	1.278 (1.107, 1.475)[b]
House type				
Kachha	6.104 (3.574, 10.425)[b]	2.161 (0.879, 5.316)[a]	1.159 (1.002, 1.342)[b]	1.070 (0.867, 1.322)
Semi-*pucca*	2.447 (1.654, 3.622)[b]	0.938 (0.493, 1.784)	1.010 (0.912, 1.118)	0.998 (0.860, 1.159)
Pucca	1.000	1.000	1.000	1.000
Type of fuel				
Clean fuel	1.000	1.000	1.000	1.000
Unclean fuel	2.952 (1.989, 4.381)[b]	2.137 (1.063, 4.295)[b]	1.164 (1.046, 1.295)[b]	1.224 (1.028, 1.456)[b]
Obesity				
Obese	1.000	1.000	1.000	1.000
Not obese	0.537 (0.366, 0.788)[b]	0.709 (0.426, 1.179)	1.694 (1.499, 1.913)[b]	0.685 (0.596, 0.787)[b]
Age group				
15–19	8.002 (3.988, 16.060)[b]	5.291 (1.473, 19.004)[b]	0.251 (0.210, 0.299)[b]	0.251 (0.195, 0.323)[b]
20–29	4.047 (2.381, 6.880)[b]	2.871 (1.472, 5.601)[b]	0.358 (0.315, 0.408)[b]	0.392 (0.339, 0.452)[b]
30–39	2.164 (1.433, 3.267)[b]	1.907 (1.189, 3.059)[b]	0.660 (0.589, 0.739)[b]	0.676 (0.600, 0.762)[b]
40–49	1.000	1.000	1.000	1.000
Consumed fish				
Never/occasionally	1.518 (0.865, 2.662)	2.227 (1.113, 4.456)[b]	0.503 (0.430, 0.588)[b]	0.652 (0.542, 0.786)[b]
Weekly	1.319 (0.714, 2.436)	1.334 (0.647, 2.751)	0.687 (0.578, 0.816)[b]	0.750 (0.621, 0.905)[b]
Daily	1.000	1.000	1.000	1.000
Consumed fruits				
Never/occasionally	2.808 (1.566, 5.035)[b]	1.496 (0.748, 2.993)	1.408 (1.197, 1.657)[b]	1.526 (1.263, 1.844)[b]
Weekly	2.038 (1.070, 3.881)[b]	1.595 (0.783, 3.248)	1.203 (1.006, 1.437)[b]	1.251 (1.032, 1.515)[b]
Daily	1.000	1.000	1.000	1.000
Anemia status				
Anemic	1.275 (0.899, 1.807)	1.040 (0.690, 1.569)	0.860 (0.782, 0.945)[b]	0.799 (0.723, 0.883)[b]
Not anemic	1.000	1.000	1.000	1.000
Type of caste or tribe				
SC	1.187 (0.723, 1.948)	0.765 (0.417, 1.405)	0.759 (0.659, 0.874)[b]	0.760 (0.654, 0.883)[b]
ST	7.244 (3.481, 15.075)[b]	2.715 (1.092, 6.754)[b]	0.827 (0.686, 0.997)[b]	0.804 (0.656, 0.986)[b]
OBC	0.693 (0.449, 1.069)[a]	0.435 (0.258, 0.734)[b]	0.800 (0.716, 0.895)[b]	0.842 (0.746, 0.950)[b]
General and others	1.000	1.000	1.000	1.000
Marital status				
Never married	5.018 (2.548, 9.881)[b]	1.505 (0.394, 5.741)	0.508 (0.439, 0.586)[b]	1.196 (0.971, 1.474)[a]
Widowed/divorced/separated	0.602 (0.273, 1.328)	0.807 (0.343, 1.896)	1.630 (1.376, 1.932)[b]	1.300 (1.086, 1.557)[b]
Currently married	1.000	1.000	1.000	1.000
Regional zones				
North	0.617 (0.234, 1.627)	0.243 (0.081, 0.728)[b]	0.605 (0.492, 0.743)[b]	0.726 (0.575, 0.917)[b]
Northeast	4.119 (1.552, 10.927)[b]	1.569 (0.456, 5.404)	0.960 (0.739, 1.247)	1.038 (0.771, 1.397)
Central	2.981 (1.565, 5.678)[b]	1.108 (0.484, 2.537)	0.511 (0.422, 0.617)[b]	0.591 (0.476,0.734)[b]

Table 2 (continued)

Characteristic	OR for women with diabetes		OR for women without diabetes	
	Unadjusted (95 % CI)	Adjusted (95 % CI)	Unadjusted (95 % CI)	Adjusted (95 % CI)
East	2.781 (1.591, 4.861)[b]	1.188 (0.597, 2.364)	1.121 (0.948, 1.326)	1.134 (0.937, 1.371)
West	2.066 (1.140, 3.744)[b]	1.159 (0.568, 2.365)	0.832 (0.704, 0.983)[b]	1.004 (0.833, 1.210)
South	1.000	1.000	1.000	1.000

[a] Significant at 10 % level of significance

[b] Significant at 5 % level of significance

highest education level, age group, religion, type of caste or tribe, geographic region, and wealth index. The environmental factors included the type of cooking fuel and type of house. Diet-related factors included frequency of consumption of chicken/meat, eggs, fruits, and fish (all categorized into daily, weekly, and occasionally/never consumption). Factors related with health were obesity, anemia status, and diabetes prevalence. Variables related with lifestyle were consumption of alcohol and smoking of tobacco. For the definition of other variables, please refer to Table 1 and its footnotes.

Data analysis

We estimated the prevalence of asthma among men and women, with and without diabetes separately. The prevalence of asthma was estimated by some selected background characteristics among men and women. In univariate analysis, X^2 test was used to check association of asthma prevalence with each of the above discussed predictors. Based on the results of X^2 test, the variables which were significantly associated either in women with and without diabetes or in men with and without diabetes were considered for further analyses. The logistic regression analysis was used to assess the impact of predictors on asthma prevalence. The national sampling weights for men and women were used for the analysis. SPSS (version 17) was used for all the analyses including logistic regression modeling.

Ethical consideration

The NFHS-3 received approval from the ethical review board of the International Institute for Population Science. We presented the secondary analysis of the existing survey data without identifying the information of respondents.

Results

Prevalence of asthma among Indian men and women

In India, prevalence of asthma was 1.8 % among men and 1.7 % among non-pregnant women. The prevalence of diabetes was 0.9 % among non-pregnant women and 1.4 % among men. The prevalence of asthma among men with diabetes was 12.8 % while that among men without diabetes was 1.7 %. The prevalence of asthma among non-pregnant women with diabetes was 14.1 % while that among women without diabetes was 1.6 %. Table 1 represents the prevalence of asthma among men and women, with and without diabetes, by some selected characteristics and P value for the X^2 test of association.

From Table 1, the prevalence of asthma was higher in rural areas among both men and women, with and without diabetes. Among poor women, 28.1 % of diabetic women were prevalent to asthma while 1.7 % of women without diabetes were prevalent to asthma. Among poor men, 25.5 and 2.0 % of diabetic and non-diabetic men were prevalent to asthma, respectively. Among rich women, 8.2 % of diabetic women were prevalent to asthma. Among men who smoke tobacco, 16.2 % of diabetics were prevalent to asthma while 2.0 % men without diabetes were prevalent to asthma. Among women with diabetes, the prevalence of asthma was highest in those who were not obese while the reverse results were observed in without diabetes group. Among women without diabetes, the prevalence of asthma was highest in obese. Similar results were observed among men. Among women with diabetes, the prevalence of asthma decreased as the age level increased, while reverse effect was observed among women without diabetes. The similar results were observed among men. The factor, drinking of alcohol, was not considered for women as the number of cases was small. Among diabetic men, 37.6 % responded "yes" for the use of alcohol, and among those, 11.8 % had asthma. The percentage of men without diabetes who responded "yes" for the use of alcohol was 31.9 %, and among those, 1.9 % had asthma. Among women with diabetes, the prevalence of asthma was lowest in those who consumed fish daily while, among women without diabetes, the prevalence of asthma was highest in those who consume fish daily. The similar results were observed in men.

From the P values of the X^2 test reported in Table 1, the predictors, namely type of place of residence, wealth status and house type for women, and obesity for men, were not significantly associated with the prevalence of asthma among without diabetes group. The predictors, namely type of consumption of fish and anemia, were not significantly associated

Table 3 Results of logistic regression analysis for men

Characteristic	OR for men with diabetes		OR for men without diabetes	
	Unadjusted (95 % CI)	Adjusted (95 % CI)	Unadjusted (95 % CI)	Adjusted (95 % CI)
Type of place of residence				
Urban	1.000	1.000	1.000	1.000
Rural	3.155 (2.079, 4.789)[b]	1.302 (0.695, 2.442)	1.292 (1.140, 1.465)[b]	1.062 (0.894, 1.261)
Wealth status				
Poor	5.808 (3.496, 9.649)[b]	0.651 (0.239, 1.768)	1.526 (1.296, 1.797)[b]	1.530 (1.137, 2.059)[b]
Middle	2.798 (1.686, 4.645)[b]	1.233 (0.604, 2.515)	1.226 (1.042, 1.442)[b]	1.323 (1.063, 1.647)[b]
Rich	1.000	1.000	1.000	1.000
Smokes tobacco				
No	1.000	1.000	1.000	1.000
Yes	1.891 (1.272, 2.812)[b]	1.203 (0.714, 2.027)	1.521 (1.345, 1.721)[b]	1.025 (0.888, 1.183)
House type				
Kachha	3.735 (1.956, 7.133)[b]	1.925 (0.674, 5.499)	1.441 (1.213, 1.713)[b]	1.033 (0.801, 1.334)
Semi-*pucca*	3.786 (2.508, 5.717)[b]	1.997 (0.996, 4.006)[a]	1.045 (0.920, 1.187)	0.811 (0.673, 0.978)[b]
Pucca	1.000	1.000	1.000	1.000
Type of fuel				
Clean fuel	1.000	1.000	1.000	1.000
Unclean fuel	3.369 (2.222, 5.109)	1.558 (0.758, 3.203)	1.289 (1.130, 1.471)[b]	1.022 (0.824, 1.267)
Obesity				
Obese	1.000	1.000	1.000	1.000
Not obese	0.084 (0.031, 0.226)[b]	7.399 (2.583, 1.194)[b]	1.098 (0.905, 1.333)	1.035 (0.834, 1.285)
Age group				
15–19	5.167 (1.663, 16.055)[b]	1.301 (0.267, 6.344)	0.316 (0.255, 0.391)[b]	0.301 (0.215, 0.420)[b]
20–29	2.551 (1.437, 4.527)[b]	1.415 (0.673, 2.974)	0.292 (0.244, 0.349)[b]	0.308 (0.247, 0.384)[b]
30–39	1.232 (0.740, 2.050)	1.248 (0.659, 2.365)	0.557 (0.476, 0.650)[b]	0.561(0.476, 0.660)[b]
50–54	0.581 (0.335, 1.008)[a]	0.600 (0.317, 1.137)	1.745 (1.465, 2.079)[b]	1.732 (1.444, 2.077)[b]
40–49	1.000	1.000	1.000	1.000
Drinks alcohol				
No	1.000	1.000	1.000	1.000
Yes	0.819 (0.549, 1.221)	0.986 (0.595, 1.632)	1.184 (1.048, 1.338)[b]	0.999 (0.871, 1.145)
Consumed fish				
Never/occasionally	4.591 (1.755, 12.016)[b]	3.584 (1.164, 11.037)[b]	0.533 (0.437, 0.650)[b]	0.543 (0.424, 0.696)[b]
Weekly	3.517 (1.301, 9.506)[b]	3.366 (1.082, 10.467)[b]	0.662 (0.534, 0.821)[b]	0.672 (0.526, 0.860)[b]
Daily	1.000	1.000	1.000	1.000
Consumed fruits				
Never/occasionally	3.188 (1.708, 5.950)[b]	0.953 (0.421, 2.161)	1.517 (1.248, 1.844)[b]	1.214 (0.964, 1.529)[a]
Weekly	1.528 (0.780, 2.992)	0.878 (0.395, 1.953)	0.962 (0.777, 1.191)	0.848 (0.672, 1.070)
Daily	1.000	1.000	1.000	1.000
Anemia status				
Anemic	1.767 (1.161, 2.687)[b]	1.198 (0.706, 2.034)	1.314 (1.153, 1.497)[b]	1.087 (0.945, 1.250)
Not anemic	1.000	1.000	1.000	1.000
Type of caste or tribe				
SC	1.326 (0.767, 2.291)	0.868 (0.431, 1.747)	0.884 (0.749, 1.044)	0.823 (0.688, 0.984)[b]
ST	3.487 (1.607, 7.568)[b]	2.361 (0.916, 6.081)[a]	0.932 (0.747, 1.162)	0.799 (0.629, 1.016)[a]
OBC	0.930 (0.577, 1.497)	0.819 (0.455, 1.472)	0.710 (0.616, 0.819)[b]	0.701 (0.601, 0.818)[b]
General and others	1.000	1.000	1.000	1.000
Marital status				
Never married	3.743 (2.080, 6.736)[b]	3.037 (1.175, 7.847)	0.484 (0.418, 0.560)[b]	1.030 (0.804, 1.319)
Widowed/divorced/separated	5.204 (2.023, 13.387)[b]	4.560 (1.468, 14.163)[b]	2.815 (2.129, 3.723)[b]	1.873 (1.361, 2.577)[b]
Currently married	1.000	1.000	1.000	1.000

Table 3 (continued)

Characteristic	OR for men with diabetes		OR for men without diabetes	
	Unadjusted (95 % CI)	Adjusted (95 % CI)	Unadjusted (95 % CI)	Adjusted (95 % CI)
Regional zones				
North	1.560 (0.576, 4.224)	0.872 (0.270, 2.822)	0.931 (0.706, 1.228)	1.322 (0.954, 1.831)[a]
Northeast	1.248 (0.265, 5.870)	0.215 (0.026, 1.816)	1.107 (0.766, 1.599)	1.229 (0.803, 1.881)
Central	4.000 (1.742, 9.189)[b]	1.205 (0.449, 3.236)	0.846 (0.652, 1.097)	1.110 (0.812, 1.520)
East	3.077 (1.429, 6.625)[b]	0.859 (0.332, 2.226)	1.576 (1.232, 2.016)[b]	1.414 (1.051, 1.902)[b]
West	2.234 (1.044, 4.781)[b]	1.361 (0.572, 3.238)	1.167 (0.915, 1.488)	1.622 (1.216, 2.163)[b]
South	1.000	1.000	1.000	1.000

[a] Significant at 10 % level of significance

[b] Significant at 5 % level of significance

with the prevalence of asthma for women with diabetes. Other factors were significantly associated with asthma among men and women in both diabetic and non-diabetic groups.

In order to measure the association of asthma and diabetes prevalence, the odds ratios were calculated for the overall sample. Men with diabetes were 8.691 times (95 % CI: 7.154–10.558) more likely to have asthma as compared to those without diabetes. Women with diabetes were 10.106 times (95 % CI: 8.455–12.08) more likely to have asthma as compared to those without diabetes. Thus, diabetics were more likely to have asthma. Hence, we studied the factors associated with asthma among diabetics and non-diabetics separately.

Factors associated with asthma prevalence

Table 2 represents the results of logistic regression analysis for women. Results of both univariate and multivariate analyses are reported in Table 2. Table 3 represents the results of logistic regression analysis for men.

From Table 2, among women with diabetes, those who smoke tobacco were 2.627 times (95 % CI: 1.514–4.558) more likely to have asthma than those who do not smoke tobacco. For without diabetes group, the effect got attenuated in adjusted models (OR = 1.278, 95 % CI: 1.107–1.475). Obese women without diabetes were at a higher risk of asthma in adjusted models. In univariate model, women without diabetes who were not obese were 1.694 times (95 % CI: 1.499–1.913) significantly more likely to have asthma. Among women with diabetes, the risk of asthma decreased as the age level increased. But the reverse effect was observed in without diabetes group. Women with diabetes who consumed fish weekly and occasionally/never were 1.334 times (95 % CI: 0.647–2.751) and 2.227 times (95 % CI: 1.113–4.456) more likely to have asthma than those who consumed fish daily. But for non-diabetes group, women who consumed fish weekly and occasionally/never were significantly less likely to have asthma than those who consumed fish daily. Women of scheduled tribes (ST) having diabetes were 2.715 times (95 % CI: 1.092–6.754) more likely to have asthma as compared to women of general and other categories. But women of scheduled tribes without diabetes were significantly less likely to have asthma as compared to women of general and other categories. Never married women with diabetes were 5.018 times (95 % CI: 2.548–9.881) more likely to have asthma than married women but for without diabetes group, never married women were significantly less likely to have asthma, in univariate model. However, in multivariate model, never married women without diabetes were 1.196 times (95 % CI: 0.971–1.474) more likely to have asthma than currently married women. Women with diabetes in the central zone of India were more likely to have asthma while women without diabetes were significantly less likely to have asthma as compared to women in the south region.

From Table 3, as observed in women, among men without diabetes, age had a positive and statistically significant effect on the prevalence of asthma. But the reverse effect was observed in the diabetic group. Also, men with diabetes who consumed fish weekly and occasionally/never were significantly more likely to have asthma than those who consumed fish daily. But for non-diabetic group, men who consumed fish weekly and occasionally/never were significantly less likely to have asthma than those who consumed fish daily. Men of scheduled tribes having diabetes were 2.361 times (95 % CI: 0.916–6.086) more likely to have asthma as compared to men of general and other categories. But men of scheduled tribes without diabetes were significantly less likely to have asthma as compared to men of general and other categories.

Discussion

Based on a large-scale nationwide cross-sectional survey, NFHS-3, we separately estimated the prevalence and

determinants of self-reported asthma among men and women, with and without diabetes. We also identified the risk factors for asthma in each group of diabetics and non-diabetics. The prevalence of asthma is higher in the group having diabetes as compared to those without diabetes. Other than the factors identified in previous studies, we considered anemia status as one of the predictor variable. A cohort study conducted on children in age group 2–18 years concluded that anemia

Table 4 Risk factors for asthma

Significant risk factors for asthma					
Previous studies		Present study			
For women	For men	For women		For men	
		With diabetes	With diabetes	With diabetes	With diabetes
Study by Subramanian SV, and et al. (2007) (for sample of all adult men, women, and children) [14] • Exposure to domestic violence • Lower socioeconomic status • Smoking • Advancing age		• Use of unclean fuel for cooking • Tobacco smoking • Lower age level • Residence in *kachha* house • Never/occasional consumption of fish • Belonging to ST	• Use of unclean fuel for cooking • Tobacco smoking • Obesity • Increasing age • Residence in *kachha* house • Daily consumption of fish • Never/rare consumption of fruits • Never married and widowed/ divorced/separated/deserted	• Residence in semi-*pucca* house • Not obese • Never/occasional consumption of fish • Belonging to ST • Widowed/ divorced/ separated	• Low wealth status • Residence in semi-*pucca* house • Increasing age • Daily consumption of fish • Never/rare consumption of fruits • Widowed/ divorced/ separated
Study by Guddattu V, Swathi A, Nair NS.(2010) [10] • Biomass fuel for cooking • Low education • Overweight and obesity • Exposure to alcohol • Smoking	Men were not included in the study				
Study by Agrawal S.(2012) [11] • Biomass fuel for cooking • No separate kitchen • Tobacco smoking • Increasing age	• No separate kitchen • Tobacco smoking • Increasing age • Low wealth quintile				
Study by Jindal SK, et al. (2012) [19] • Increasing age • History of asthma in first-degree relative • Tobacco smoking • Use of solid fuel for cooking					
Study by Agrawal S, Pearce N, Ebrahim S.(2013, for men and women combined) [12] • Increasing age • Consumption of chicken/meat • Overweight and obesity • Never consumed milk/milk products, pulses and beans, green leafy vegetables, or fruits • Widowed/divorced/separated/deserted • Ever use of alcohol • Current tobacco smoking					

is a risk factor for childhood asthma [17]. Therefore, in order to study the impact of hemoglobin level on the onset of asthma among adults, we included this variable in the study. It was observed that prevalence of asthma was higher in anemic men and women both. Anemic men and women with diabetes are at a higher risk of asthma. As stressful events like marital problems, divorce or separation, and domestic violence were associated with asthma, we considered marital status as one of the predictor variable [14, 18].

From the NFHS-3, the prevalence of asthma among women was 1.9 % and among men was 1.8 % [11]. From the multicentre study (2007–2009) carried out at 12 centers located across India, the prevalence of asthma among adult (age ≥15 years) women was 1.95 % and among men was 2.10 % [19]. Table 4 shows the risk factors for asthma identified in the past studies and our present study. Different risk factors were observed for both men and women and also for with diabetes and without diabetes groups. Tobacco smoking, residence in *kachha* house, use of unclean fuel, low age level, belonging to scheduled tribes, weekly and occasionally/never consumption of fish, and not living with a partner are significant risk factors for asthma among the individuals with diabetes. Low wealth status, tobacco smoking, use of unclean fuel, weekly, occasionally/ never consumption of fruits, and not living with a partner are significant risk factors for asthma among the individuals without diabetes.

As NFHS-3 collected data from each state of India, the data was nationally representative, and thus, the estimates are reliable and nationally representative. The limitation of this study was no clinical diagnosis of asthma and diabetes. As per the definition of asthma, questions required for diagnosis of asthma were not asked to respondents. So, for this study, the diagnosis of asthma is based on a single question. Similarly for diabetes, identification of diabetics is based on a question: "Do you have diabetes?" No information on the medical history of asthma and diabetes was available which can help in determining the risk factors. So, prevalence of these chronic diseases may be underestimated due to a lack of knowledge of respondent or misunderstanding about the disease. It is also possible that the prevalence of asthma may be over-estimated, as the symptoms of asthma are similar with bronchitis or chronic obstructive pulmonary disease. So, in such cases, respondents might have wrongly reported for asthma.

In summary, we had found a significant association between asthma and diabetes prevalence. Individuals with diabetes are at a higher risk of asthma in India. For individuals with diabetes, regular consumption of fish, avoiding tobacco smoking, residence in *pucca* houses, and emotional support can help in reducing the risk of asthma. As diabetes and asthma are on rise in India, there will be a double burden of non-communicable diseases on Indian population, which can be reduced by providing effective services for prevention, diagnosis, and care of both asthma and diabetes among adults. Results of this study may provide guidelines to medical practitioners and researchers of chronic diseases, diabetes, and asthma.

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ORIGINAL ARTICLE

A study of asymptomatic bacteriuria in North Indian type 2 diabetic patients

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Abstract The prevalence of asymptomatic bacteriuria (ASB) in diabetic patients varies from 9 to 27 % in various studies which is certainly higher as compared to healthy individuals. The risk factors which lead to increased prevalence of asymptomatic bacteriuria in diabetic patients are immune system dysregulation, development of bladder dysfunction and prostatism. Studies have reported that ASB has a higher prevalence in diabetic individuals as compared to nondiabetics. Patients having type 2 diabetes mellitus along with age- and sex-matched controls who were hemodynamically stable were enrolled. A prospective case-control study was done. A total of 200 patients were enrolled, and they were divided into two groups, i.e. those with diabetes and nondiabetic patients (age- and sex-matched controls) without symptoms of UTI. Urine examination and biochemical investigations of the patients were done. In our study, the prevalence of ASB among the diabetic patients was significantly higher 28.2 % as compared to 7.5 % in the controls ($p = 0.001$). The main risk factors for asymptomatic bacteriuria in our study were female sex ($p = 0.003$), increased age ($p = 0.007$), longer duration of diabetes mellitus ($p = 0.003$), poor glycemic control ($p < 0.001$) and recent urinary tract infection ($p = 0.02$). There were no significant differences in the serum creatinine levels in the patients with asymptomatic bacteriuria among diabetics as compared to the culture-negative patients. The presence of ASB may be considered a marker of poorly controlled and long-standing diabetes.

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Keywords Asymptomatic bacteriuria · Glycemic control

Introduction

Urinary tract infection (UTI) is an important clinical problem for people with diabetes. Serious complications of urinary infection, such as emphysematous cystitis, pyelonephritis or renal and perinephric abscess, occur virtually only in diabetic patients. On a population basis, diabetic women, depending on age, are 6–24 times more likely than nondiabetic women to be admitted for acute pyelonephritis, and diabetic men are 3.4–17 times more likely than their nondiabetic counterparts to be admitted for the same condition [1] Knowledge of risk factors for UTI in diabetic patients is important to identify patients in need of therapy to prevent serious complications.

Generally, infection of urinary tract shows clinical symptoms like burning sensation during micturation, increased frequency of micturation, dysuria, increased frequency of micturation, urgency, lower abdominal/pelvic pain, pyuria, purulent discharge per urethra, fever, and strangury [2] However, in some patients, the clinical symptoms may remain unnoticed to the patient themselves despite presence of significant bacteriuria. Such symptomless infection of urinary tract is called covert or asymptomatic bacteriuria (ASB). ASB is one of the common problems seen in diabetic patients preceding symptomatic UTI. The significance of ASB is largely unknown. The prevalence of ASB in diabetic patients varies from 9 to 27 % in various studies which is certainly higher as compared to healthy individuals [3] The various risk factors which lead to increased prevalence of ASB in diabetic patients are immune system dysregulation, development of bladder dysfunction and prostatism. Many studies have reported that ASB has a higher prevalence in diabetic individuals as compared to nondiabetics [4–8].

Aims and objectives

Through this study, we aim to study the prevalence of asymptomatic bacteriuria in North Indian patients with type 2 diabetes mellitus, its effect on glycemic control in patients with diabetes mellitus and a study of clinical profile of the patients.

Methodology

Study was conducted in the Department of Medicine, King George's Medical University, Lucknow. Patients having type 2 diabetes mellitus, attending diabetic OPD and those admitted in indoor medical wards who were hemodynamically stable were enrolled. A prospective cross-sectional and comparative study was done. The patients with anatomical anomalies of the urinary tract, urolithiasis, neurological bladder dysfunction, symptoms of urinary tract infection [2] and advanced organ dysfunction were excluded from the study.

The sample size was calculated by using the following formula [9] [$n = 4pq/d^2$], where n = sample size required, p = prevalence (6 %) [5], $q = 1 - p$, d = desired precision. Assuming 80 % power and 5 % significance level with 95 % confidence interval, the total sample size (n) calculated was 90. A total of 200 patients were enrolled, and they were divided into two groups, i.e. those with diabetes and nondiabetic patients (age- and sex-matched controls) without symptoms of UTI. There were eight dropouts in the diabetic group, and seven dropouts were in the control group; thus, there were total 92 diabetic patients and 93 controls.

The participants were also inquired about the duration of diabetes, history of urinary tract infection, history of treatment for urinary tract infection (<1 year), treatment history and other clinical data. Anthropometric data including height, weight and waist circumference was also collected from each patient.

Each patient was instructed to collect a clean-catch midstream urine specimen after cleaning genital region prior to micturation. In men, glans penis was asked to be cleaned with swabs soaked in clean tap water, then asked to pass about 50 ml of urine into a toilet or bowl, but the next portion (midstream) of 5–10 ml was collected into a clean sterile bottle. In women, before collection of urine, labia were separated by patient or nurse and vulva cleaned twice in an anteroposterior direction with swabs soaked in clean tap water and then with a dry swab; whilst the labia were held still apart, urine was collected in a similar way as men. Antiseptic solutions were not used for cleaning as it may interfere with growth of bacteria during culture. About 20 ml of urine specimen was collected in a sterile screw-capped wide-mouth container from each patient. The urine sample was sent immediately to the Department of Microbiology, KGMU for routine microscopy and culture (using cysteine lactose electrolyte deficient media). Blood samples were taken from patients, and biochemical investigations including kidney function tests and HbA1c were done in the lab in the Department of Biochemistry and Pathology, King George's Medical University, Lucknow.

The diagnosis of asymptomatic bacteriuria is [10]

- For asymptomatic women, bacteriuria is defined as two consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts of greater than or equal to10^5 cfu/ml.
- For asymptomatic men, a single, clean-catch, voided urine specimen with one bacterial species isolated in a quantitative count of greater than or equal to 10^5 cfu/ml.
- In our study, we performed one urine examination and culture with colony counts $>10^5$ were labeled as bacteriuria in both men and women.

Comparisons were made in both groups using appropriate statistical tests. The statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 16.0 statistical analysis software.

Results

Both the groups were sex and age matched (Table 1). Prevalence of asymptomatic bacteriuria in group A was 28.2 % as compared to 7.5 % in group B, thus showing a significant difference between two groups ($p < 0.001$). The prevalence of females with asymptomatic bacteriuria in group A was

Table 1 Comparison of demographic profile and baseline characteristics between two groups

SN	Characteristic	Cases (n = 92) [A]		Controls (n = 93) [B]		Significance of difference
		No./mean	%/SD	No./mean	%/SD	p
1	Age	56.57	9.74	54.69	7.33	0.166
2	Gender					
	Female	46		46		0.94
	Male	46		47		
3	BMI	24.99	1.66	24.24	0.89	0.001

Table 2 Prevalence of asymptomatic bacteriuria among cases and controls

	Cases [A]	Controls [B]	*p*
Total	26	7	<0.001
Males	8	2	0.008
Females	18	5	<0.001

significantly higher (39.1 %) (Table 2) as compared to females in group B (10.8 %) ($p < 0.001$). The prevalence of males with asymptomatic bacteriuria in group A was significantly higher (17.3 %) as compared to females in group B (4.2 %) ($p = 0.008$).

The patients in group A were divided into two groups based on the presence of asymptomatic bacteriuria, i.e. ASB(+) and ASB(−).

Mean age of patients in ASB(+) was significantly higher as compared to ASB(−) ($p = 0.007$)(Table 3).The mean HbA1C levels were significantly higher in ASB(+) as compared to mean HbA1c levels of ASB(−) ($p < 0.001$). Mean duration of diabetes was significantly longer in ASB(+) as compared to ASB(−) ($p = 0.003$).There was no significant difference in the mean serum creatinine levels in ASB(+) as compared to that in ASB(−) ($p = 0.68$).

The percentage prevalence of patients in group A ASB(+) with HbA1c levels >7 (poor glycemic control) is 92.3 % is significantly higher than the percentage prevalence in ASB(−)HbA1C levels >7(poor glycemic control) is 54.5 % ($p = 0.001$)(Table 4). The patients in group A with asymptomatic bacteriuria had higher prevalence of symptomatic urinary tract infections (46.1 %) as compared to culture-negative patients (19.6 %) (Table 5).

Discussion

In our study, the prevalence of asymptomatic bacteriuria among the diabetic patients was significantly higher 28.2 % as compared to 7.5 % in the controls ($p = 0.001$). The prevalence was significantly higher in the females (39.1 %) as compared to that in controls (10.8 %) ($p = 0.003$). Geerlings S.E. et al. reported a prevalence of 26 % in female patients without diabetes as compared to 6 % in females without diabetes [5].

Table 3 Comparison of characteristics of ASB(+) and ASB(−)

	ASB(+)		ASB(−)		
	Mean	SD	Mean	SD	*p*
Age (years)	59.67	7.04	55.34	6.42	0.005
Duration of diabetes (years)	8.07	2.31	4.70	2.09	0.003
HbA1c (%)	9.27	1.76	7.79	1.39	<0.001
Serum creatinine (mg/dl)	1.26	0.28	1.21	0.16	0.68

Table 4 HbA1c levels in patients with asymptomatic bacteriuria and culture-negative patients in group A

HbA1c	Asymptomatic bacteriuria			
	ASB(+)		ASB(−)	
	n = 26	%	*n* = 66	%
>7 (poor glycemic control)	24	92.3	36	54.5
<7 (good glycemic control)	02	7.6	30	45.5
Statistical significance	$p = 0.001$			

The mean age of the patients with asymptomatic bacteriuria was significantly higher than the mean age of culture-negative patients among the diabetics ($p = 0.007$).The higher prevalence of asymptomatic bacteriuria in females in both the groups may be due to higher prevalence of these conditions in post-menopausal females, and most of the females in our study were of the post-menopausal age group. Boyko EJ et al. also observed a higher prevalence of asymptomatic bacteriuria in post-menopausal diabetic patients [4] Geerlings SE also reported increased age as a risk factor for asymptomatic bacteriuria in diabetes mellitus [5].

The mean HbA1c levels were significantly higher in the patients with asymptomatic bacteriuria as compared to mean HbA1C levels of the culture negative among the diabetic patients ($p < 0.001$). The number of diabetic patients with asymptomatic bacteriuria with HbA1c levels >7, i.e. poor glycemic control were significantly high ($p < 0.001$) as compared to the number of diabetic patients in the culture-negative patients. The high levels of glucose leads to macrophage dysfunction and improper phagocytosis [11], and this may explain the higher prevalence of asymptomatic bacteriuria in patients with poor glycemic control. Asymptomatic bacteriuria may be considered a marker of poor glycemic control.

The mean duration of diabetes in patients with asymptomatic bacteriuria was significantly higher ($p = 0.003$) than the mean duration of diabetes in culture-negative patients. Geerlings SE et al. also concluded that increased duration of

Table 5 History of symptomatic UTI in past 1 year patients with asymptomatic bacteriuria as compared to culture-negative patients in group A

History of symptomatic UTI				
	ASB(+)		ASB(−)	
Yes	12	46.1 %	13	19.6 %
No	14	53.8 %	53	80.3 %
Statistical significance	$p = 0.03$			

diabetes is a risk factor for developing asymptomatic bacteriuria [5].

There were no significant differences in the serum creatinine levels in the patients with asymptomatic bacteriuria among diabetics as compared to the culture-negative patients. Renko M et al. did not find any significant difference in the mean serum creatinine levels in patients with asymptomatic bacteriuria as compared to culture-negative diabetic patients [6].

The percentage of patients with asymptomatic bacteriuria (38.4 %) among diabetics with a history of symptomatic urinary tract infection in the last 1 year was significantly more as compared to culture-negative patients (19.6 %) ($p = 0.02$). Geerlings SE et al. also concluded that a history of symptomatic urinary tract infection in diabetic patients is a risk factor for asymptomatic bacteriuria [5].

Conclusion

The prevalence of asymptomatic bacteriuria was higher in patients with diabetes mellitus than nondiabetic patients ($p = 0.001$). The main risk factors for asymptomatic bacteriuria in our study were female sex ($p = 0.003$), increased age ($p = 0.007$), longer duration of diabetes mellitus ($p = 0.003$), poor glycemic control ($p < 0.001$) and recent urinary tract infection ($p = 0.02$). The risk factors for asymptomatic bacteriuria seem to be similar in our study as in the previous studies. The presence of asymptomatic bacteriuria may be considered a marker of poorly controlled and long-standing diabetes.

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ORIGINAL ARTICLE

The efficacy of topical phenytoin in the healing of diabetic foot ulcers: a randomized double-blinded trial

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Abstract A randomized, double-blinded, controlled clinical trial was conducted to assess the efficacy of topical phenytoin compared to conventional wound care in improving the healing process and to prove it as a relatively low-cost and easy-to-use option in the management of diabetic ulcers. Patients over 18 years of age with type 2 diabetes and foot ulcers over 1 month duration were randomized to receive daily dressings containing either powder A (test powder containing topical phenytoin and metronidazole) or powder B (control powder containing topical metronidazole) for 14 days, following which, they underwent split-skin grafting. The percentage of decrease in the ulcer surface area, rate of granulation tissue formation, graft uptake, and percentage of negative culture sensitivity were compared between the two groups using the unpaired Student's *t* test. A *p* value <0.05 was considered significant. Patient demographic and socioeconomic characteristics of the two groups were well matched. The primary outcome measured as the mean rate of decrease of size of the ulcer in patients of group A was 30.69 (±5.50 SD) and in group B was 24.43 (±5.96 SD) percent of total ulcer area ($p < 0.0001$). The mean rate of increase of granulation tissue in group A was 69 (±10.16 SD) percent of total ulcer area and in group B was 51.51 (±10.54 SD) percent of total ulcer area ($p < 0.0001$). Out of the 97 patients, 75 underwent grafting. The mean graft take up in group A was 76.57 % (±19.06 SD) and in group B was 66.48 % (±17.67 SD) ($p = 0.0082$). Forty-three percent of the study group was culture negative at day 14, of which 54.2 % belonged to group A as compared to 45.8 % in group B. Topical phenytoin is an effective, inexpensive, and easily available agent in the promotion of healing of diabetic foot ulcers.

Keywords Diabetes · Ulcer · Healing · Phenytoin · Surgery

Introduction

The management of diabetic foot ulcers is a problem of considerable magnitude. Foot ulcers eventually develop in 15 % of chronic diabetics and give rise to further complications such as infection and amputation if inadequately healed [1]. These wounds are typically resistant to healing despite meticulous wound care, control of the glucose levels, and maintenance of the nutritional status of the patient [2].

Several newer modalities have been explored to address the need for better wound healing agents. Phenytoin is one such agent that has been studied for its proliferative effect on fibrous tissue [3]. Since its introduction as an anti-convulsive agent, gingival hypertrophy has been widely noted as an adverse effect with about half the patients treated with phenytoin reporting the same [4]. This has prompted the study of this stimulatory effect on connective tissue for its potential role in improved wound healing. Its effect on the healing has been studied in trophic ulcers, decubitus ulcers, venous ulcers, diabetic foot ulcers, surgical and traumatic wounds, and burns [5].

The present study was conducted to assess the efficacy of topical phenytoin in healing diabetic foot ulcers with a view of establishing phenytoin as a low-cost, easy-to-use, effective wound healing agent.

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Method

This prospective randomized double-blinded study included 97 patients admitted to our semi-rural tertiary care teaching hospital for the care of diabetic foot ulcers during the period between October 2010 and October 2013. Patients older than 18 years of age with type II diabetes and grade I/II foot ulcers (based on Wagners wound classification) for duration of over 1 month were enrolled in the study [6]. Approval of the study design was obtained from the institutional ethics committee and informed consent was obtained from the participants.

Patients were randomized to undergo daily dressings that involved wound cleaning with saline followed by topical application of either powder A (test powder containing equal amounts of topical phenytoin and metronidazole) or powder B (control powder containing topical metronidazole) in a thin, uniform layer over which dry dressing was applied. The powdered preparations were obtained by mixture of crushed phenytoin 100 mg and metronidazole 200 mg tablets. The quantity of powdered preparation applied depended on the size of the ulcer and was maintained at 20 mg/cm^2. Metronidazole was chosen as control since its beneficial effect on wound healing has been proven in the past, and in order to enable blinding, both groups were required to receive a topical medication in powdered preparation as opposed to the use of conventional saline or dry dressings as control. Systemic antibiotics were started in both the groups according to the culture and sensitivity report.

The intervention and control drug combinations were identically packaged into opaque sealed and numbered treatment packs containing either powder A or powder B. The assignment of patients was performed in a 1:1 ratio using a computer-generated randomization sequence with a block size of four. The random allocation was done by an external reviewer. Participants, care providers, and investigators were blinded to the group assignment.

Data from all 97 patients was collected following 14 days of treatment. Forty-nine patients underwent dressing with powder A (topical phenytoin and metronidazole) and 48 patients underwent dressing with powder B (topical metronidazole). Definitive management of the ulcers was done by split-skin grafting, and the graft uptake areas were measured on postoperative day 5. The primary outcome measured was the percentage of decrease in the ulcer surface area between the two groups. Granulation tissue formation as percentage of ulcer area covered was calculated using image analysis software (Olympus, Germany). The formula used was the (original wound size − the wound size without granulation tissue/ the original wound size) × 100 %. Other variables such as graft uptake and percentage of negative culture sensitivity were also measured. The categorical variables were compared by the unpaired Student's t test. A p value <0.05 was considered significant.

Results

Patient characteristics of the two groups were well matched [Table 1]. The mean age in study group A was 45.97 (±13.54 SD) years and in control group B was 46.08 (±12.29 SD) years. The age distribution in both groups was similar. Group A constituted of 26 male and 23 female patients while group B had 30 male and 18 female patients. All the patients were socioeconomically comparable and belonged to either the middle or low income groups.

The primary outcome measured was the efficacy of the dressings in both groups and was assessed as the percentage of reduction in the ulcer surface area. The mean rate of decrease of size of the ulcer at the end of 14 days in patients of group A was 30.69 % (±5.50 SD) of total ulcer area and in group B was 24.43 % (±5.96 SD) of total ulcer area ($p < 0.0001$) [Table 2]. The wound healing efficacy of phenytoin was also assessed by the comparison of the percentage increase in granulation tissue at the end of 14 days in both groups. The mean rate of increase of granulation tissue in group A was 69 % (±10.16 SD) of total ulcer area and in group B was 51.51 % (±10.54 SD) of total ulcer area ($p < 0.0001$) [Table 3]. The foot ulcers in both groups were ultimately managed with split-thickness skin grafting. Out of the 97 patients, 75 underwent grafting. Following the procedure, the uptake of the graft was measured on the fifth postoperative day and the percentage of the graft area taken up by both groups was compared. The mean graft take up in group A was 76.57 % (±19.06 SD) and in Group B was 66.48 % (±17.67 SD) ($p = 0.0082$) [Table 4].

Patients in both groups were subjected to culture and sensitivity of their ulcers after 14 days of therapy in order to determine the effect of the topical agents on the bacterial load. Forty-three percent of the study group was culture negative, of which 54.2 % belonged to group A as compared to 45.8 % in group B. In both the groups, no complications were observed during the application of dressings, skin grafting, or in both the immediate and late postoperative period when followed up after 1 month.

Table 1 General characteristics

	Group A	Group B
Number of patients	49	48
Range of age (years)	18–65	18–70
Male to female ratio (M:F)	26:23	30:18
Range of ulcer surface (cm^2)	6–53	3–53

Table 2 Rate of decrease in size of the ulcer (expressed as % of ulcer surface area)

Group	Number of patients	Mean ± SD	*T* value	*P* value
A	49	30.69 ± 5.5	5.37	0.0001
B	48	24.43 ± 5.96		

Difference in the mean rate of decrease in the size of the ulcer of group A and group B = 6.26. 95 % confidence interval of this difference, 3.95 to 8.57

Discussion

Hyperplasia of the gums was noted as an adverse effect when phenytoin was introduced as an anti-seizure medication in the 1930s [4]. This gave way to research on the potential benefit of this proliferative effect on improved or accelerated wound healing [3]. The first controlled clinical trial involving periodontal patients reported that pretreatment with oral phenytoin prior to surgery was associated with reduced pain and inflammation and faster healing [7].

Subsequently, topical phenytoin has been studied in various types of wounds in comparison with different wound dressings. It was found superior to chlorhexidine and hydrogen peroxide in the healing of decubitus ulcers [5]. Diabetic foot ulcers healed better with phenytoin when compared to sterile occlusive dressings [8]. An increased reduction of mean-burn area of second and third degree burn wounds was observed in comparison with silver sulfadiazine [9]. Topical phenytoin healed the surgical split-skin graft donor site wounds and decubitus ulcers faster than sterile dressings and topical antibiotic applications [10]. It may also benefit other inflammatory conditions such as bullous epidermolysis [11].

Several other benefits have been described besides accelerated wound healing such as a reduction in the bacterial load of wounds, particularly Gram-negative bacteria, local pain relief, and facilitation of nerve regeneration [12–15]. The mechanism of action of phenytoin on wound healing is purported to be via proliferation of fibroblasts and enhanced granulation tissue formation. Its effect on decreasing collagenase activity and promoting the deposition of collagen also contributes to wound healing and contraction [3].

Table 3 Rate of increase in granulation tissue (expressed as % of ulcer surface area)

Group	Number of patients	Mean ± SD	*T* value	*P* value
A	49	69 ± 10.16	8.32	0.0001
B	48	51.51 ± 10.54		

Difference in the mean rate of increase in granulation tissue of the ulcers = 17.49. 95 % confidence interval of this difference, 13.32 to 21.66

Table 4 Graft uptake (expressed as % of ulcer surface area)

Group	Number of patients	Mean ± SD	*T* value	*P* value
A	49	76.57 ± 19.06	2.7024	0.0082
B	48	66.48 ± 17.67		

Difference in the mean percentage of graft uptake of Group A and Group B = 10.09. 95 % confidence interval of this difference, 2.68 to 17.50

The safety of topical phenytoin has been proven, as topical application provides direct drug access to the target site without obtaining adverse effects associated with systemic absorption [16–20]. In our study, topical metronidazole was selected as the control since studies have reported its contributory effect in wound healing through enhanced wound contraction and epithelialisation and the agent does not possess irritant properties. Enrolment of patients and obtainment of consent for their participation was made easier since they were offered one of two treatment options, both of which were potentially beneficial. The study group received both agents in order to better observe the effect of phenytoin in the group beyond the effect of metronidazole on both groups.

The limitations of the study included the extent of control of blood sugars, although optimization of blood sugar levels was attempted over the duration of hospital stay to assist the wound healing process. Incorporation of more details regarding the overall status of the patient and their comorbidities in future studies will give rise to a better understanding of the impact of their health on foot ulcer healing. Better validation and generalizability may also be obtained by studies including a larger patient population and different types of wounds.

The results of this study find that topical phenytoin is significantly more effective in healing diabetic foot ulcers when compared to topical metronidazole as control measured as a reduction in the surface area, increase in the granulation tissue, and the extent of the graft uptake as compared to control. It also results in improved elimination of bacteria causing wound infection. This confirms the findings of prior studies [3, 7, 10, 14] and supports the use of topical phenytoin in diabetic foot ulcers. Phenytoin provides a relatively inexpensive and easily available treatment option for better relieving the distress of patients with slow-healing ulcers.

Compliance with ethical standards

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

Source of funding None

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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

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ORIGINAL ARTICLE

Prevalence of yeast in diabetic foot infections

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Abstract Diabetic foot infections are a serious threat which cause a long term of hospitalization of diabetes patients and lead to amputations. Hence, prompt diagnosis is an important criterion in the treatment of these infections. This study was carried out to determine the prevalence of yeast in diabetic foot infections. The pus samples were collected from patients presenting with diabetic foot ulcer and processed for microbiological investigation. The patients investigated comprised of 82 males and 78 females. Most of the patients were grade I ulcers followed by grade II ulcers. Out of 160 samples, 138 samples were culture positive, in which yeast isolate *Candida glabrata* was the predominant isolate followed by *Candida albicans*, *Candida dubliniensis, Candida krusei* and *Candida tropicalis. C. albicans* and *C. dubliniensis* were differentiated by using tobacco agar due to the presence of hyphal fringes. The methylene blue sabouraud dextrose agar and corn meal agar were also used to differentiate *C. albicans* from non albicans. The antifungal susceptibility pattern showed 29 % of *C. albicans* was sensitive to Amphotericin B and Clotrimazole, while 75 % of *C. glabrata* was sensitive to Amphotericin B, Ketoconazole and Itraconazole. *C. dublinensis* and *C. albicans* showed the highest percentage of resistant to Ketoconazole, Fluconazole, Nystatin and Itraconazole. Thus, the results indicating that effective alternate drug of choice are required and the proper selection of antifungal agents will play an important role for the treatment of fungal foot infections. This study will also add substantial knowledge of yeast as one of the pathogenic organisms in diabetic foot infections.

Keywords Diabetic foot ulcer · Wagner's grade · *Candida* spp. · Antifungal susceptibility pattern

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Introduction

Diabetes mellitus is increasing globally and about 150–170 million populations are reported worldwide. It estimates that the prevalence of diabetes will be double by the year 2025 as per WHO reports [1]. As a consequence of this, the incidence of diabetic foot infection will also be on the rise [2]. The foot ulceration followed by subsequent invasion by the microorganism is one of the most frequent and serious complications of diabetes mellitus, resulting in frequent hospitalization. Also, infective agents retard the healing of ulcers worsening them [3]. While in some cases, if not treated properly would result in leg amputation causing severe economic burden for the patients [4]. The spectrum of microbial infection occurring in these diabetic foot infections has already been reported. While most of these studies have focused only on the bacteria, very few studies have reported the presence of filamentous fungi [5, 6]. Sometimes, the presence of low pathogenic yeast has also been reported in some studies [2, 7]. This yeast is primarily the normal mycobiota of the skin around ulcers or may colonize the diabetic foot ulcers as secondary infections thereby hindering the treatment process. Hence clinicians while treating these infection cases, suspect only polymicrobial origin and treat them with a ray of antibacterial agents [8]. Swabs taken periodically are not referred for mycological investigations due to lack of literature support. In India, the study of the prevalence of the yeast in diabetic foot ulceration is very meager [9]. The emergence of antifungal

resistance was rising as concern [10]. *C. glabrata* and *C. krusei* were more resistant to antifungal agents, particularly to Fluconazole [11]. Protracted therapy and increased use of antifungal for recurrent Candidiasis are the widespread risk factors for azoles resistant strains [12]. So studies are critical to assess the role of antifungal agents especially in diabetic foot infections in order to prevent the amputation. This study was planned to determine the prevalence of yeast infections particularly the distribution of *Candida* and non *Candida albicans* (NCA) as causative agents in diabetic foot infections. Further, to study the antifungal susceptibility pattern of these organisms to the commonly used antifungal agents.

Materials and methods

This study was carried out at the local general hospital Salem, South India over a period of 1 year. The samples were collected with the help of the diabetologists. This study was approved by the Institutional Ethical Committee for the enrollment of human subjects (IEC/PU/HR/2012/003). A total of 160 cases were assessed using the following criteria:

Inclusion criteria

1. The patients with type II diabetes and foot ulcer
2. The patients should be over 30 years of age.
3. The patients enrolled for the study should not have been on prior therapy for their clinical conditions.

Exclusion criteria

1. Individuals having superficial fungal infections other than diabetic foot ulcer
2. Pregnant women and patients on immunosuppressive drugs will be excluded from this study.

History taking and clinical examination

The study participants were given the informed consent form prior to sampling. The clinical history of the patients were recorded on a performa containing the details of duration of diabetes, types of diabetes, duration of hospital stay, site of ulcer, size and nature of ulcer, habits of the patients and other associated co-morbid conditions like hypertension, retinopathy, nephropathy, peripheral vascular disease and neuropathy [13]. After recording the details of the patients, the clinical assessment of the patients was done (i.e.,) the foot ulcers were graded according to the Wagner's grade classification [14].

Sample collection

The samples were taken from the deeper portion of the ulcer by using two sterile swabs dipped in sterile saline. The samples were collected by making a firm rotatory movement with the swabs [15]. The samples collected from foot ulcer patient were promptly transported to the laboratory and processed using aseptic techniques to avoid contamination.

Processing of the samples

The swabs were directly inoculated onto the plates of Sheep blood agar (SBA) and Sabouraud dextrose agar (SDA). The bacterial plates were incubated at 37 °C for 24 h and the fungal at 27–30 °C for 48–72 h. The yeast growth on the plates was characterized by germ tube formation, sugar fermentation and assimilation of sugars while macroscopic and microscopic appearance in slide culture. The isolated yeast cultures were speciated by inoculating on to Hi-Chrome Candida differential agar (Hi-Media, Mumbai). The species were identified based on the distinct coloration produced by the yeast colonies on the plates [16]. The isolated yeast cultures were speciated by inoculating on to Corn meal agar (CMA) for chlamydospore formation.

Sabouraud methylene blue agar

The media was prepared by adding 0.01 % methyl blue dye into Sabouraud dextrose agar (SDA) before autoclaving. A loopful of yeast colonies was transferred to the media and incubated at 37 °C for 24 h. The colonies were evaluated under UV lamp at a wavelength of 365 nm. The colonies, which do not fluorescence, were interpreted as non *C. albicans* species while the colonies that fluoresced brightly were *C. albicans* [17].

Tobacco agar

This media is used for the differentiating of *C. dublinensis* from *C. albicans*. Tobacco leaves (50 g) were weighed and mixed with 1 liter of distilled water. The mixture was boiled for 30 min and then filtered through gauze. To this filtrate, 20 g/l of agar was added (pH 5.4) and autoclaved at 121 °C for 15 min. All the test isolates were freshly subcultured on Sabouraud dextrose agar (SDA) and incubated at 28 °C for 96 h. The colony characteristics such as surface topography, formation of hyphal fringes at the periphery and color were noted. Colonies were also observed directly at low power (10X) and high power (40X) magnifications for the formation of hyphal fringes and chlamydospore [18].

Antifungal susceptibility pattern

The disc of Amphotericin B (100 units), Fluconazole (25 mcg), Clotrimazole (10 mcg), Itraconazole (10 mcg), Ketoconazole (10 mcg) and Nystatin (100 units) were used for this study. All discs were purchased from Hi - media Laboratory Pvt. Ltd., Mumbai, India. The strains of *Candida albicans* MTCC 3019 and *Candida glabrata* MTCC 227 (obtained from Microbial Type Culture Collection, Chandigarh, India) were used as control strains. The fungal inoculum was prepared by picking distinct colonies from 24 h old culture grown on Sabouraud dextrose agar (SDA) and suspended in 5 ml of sterile 0.85 % saline. The suspension was vortexed and the resulting suspension was adjusted to turbidity to yield 1–5×10^6 (i.e., 0.5 cells/ml McFarland standard). The sterile cotton swab was taken and dipped on to the standardized inoculum. The swab was rotated firmly against the upper inside wall of the tube to express excess fluid. The swab was spread on to the surface of Muller Hinton agar (MHA) plates and the disc was placed on to the plates by using aseptic technique and incubated at 37 °C for 24 h. The results of the test plates were read after the incubation period [19].

Statistical analysis

Proportions for categorical variables were compared using chi-square test and qualitative variables were expressed as percentages. The p value ≤ 0.05 was considered as significant. The data were analyzed using the SPSS software (version 17.0) statistical package.

Results

A total of 160 patients with type II diabetes were enrolled for this study. The patients investigated comprised of 82 (51 %) males and 78 (49 %) females. The numbers of patients presenting in this study were in the age range of 30–80 years. The mean age group was found to be 56.18 years. Out of 160 patients, 56 (35 %) patients who were in the age group of 51–60 years were preponderant followed by 46 (28 %) patients in the group of 61–70 years (Fig. 1). Patients in the age group of 51–60 years were predominantly reported in this study indicating that patients in this age group are more prone for diabetic foot infection due to their physical health conditions. The statistical analysis did not reveal any significant association with age group of 51–60 years. The duration of diabetes presenting in this study showed patients with 10–19 years (51 %) of diabetes were higher when compared to less than 10 years (44 %) of diabetes. In this study, 120 (75 %) patients presented with grade I ulcer (75 %), 35 (22 %) patients presented with grade II ulcer and 5 (3 %) patients presented with grade III ulcer. The results were summarized in

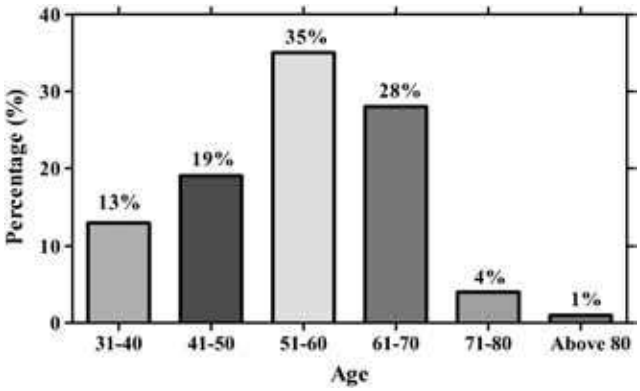

Fig. 1 Age wise distribution of patients with diabetic foot infection

Table 1. There might be a significant relationship between the yeast infections with the grade of the ulcers because when the grade II and III were found to be yeast infections. The glycemic level of the patients showed 102 (64 %) patients with >8 % poor glycemic control. Poor glycemic control (HbA1c: >8 %) was significantly found to be associated with yeast infection. Thus, a strong correlation was found between the yeast infections with the duration of diabetes, grade of the ulcer and HbA1c.

Based on the details of the clinical history collected from the patient's proforma and the clinical examination by the medical practitioner, the predominant predisposing (risk) factor identified was nephropathy in 40 (25 %) patients followed by retinopathy in 35 (22 %) patients. This result indicates that as the duration of diabetes increases there is an increased risk of diabetes-related complications like hypertension, retinopathy, nephropathy and neuropathy. Majority of the patients (38 %) reported in this study was found to have the habit of smoking followed by alcohol consumption in 35 (22 %) patients. A correlation was attempted between the genders of the patients with the predisposing factors of the patients found in this study. There was a greater proportion of male patient with hypertension (13 versus 3 %, $p = 0.003$) and nephropathy (18 versus 7 %, $p = 0.004$) compared to female patients (Table 2). This indicates that nephropathy found to be more significantly present in male patients. There was no significant difference between the gender of the patients with other predisposing factors in this study (i.e., retinopathy and neuropathy).

Out of 160 samples, 138 (86 %) samples were culture positive and 22 (14 %) were culture negative. A total of 203 organisms were isolated from 138 samples, which represent an average of 1.47 organisms per case. Out of the 203 organisms, 113 (82 %) patients grew purely bacteria alone, 5 (4 %) patients grew purely fungi alone and the remaining 20 (14 %) patients grew a combination of bacterial and fungal organisms. The patterns of mixed infection were summarized in Table 3. A total of 25 fungal isolates were isolated in this study. In these, *Candida glabrata* (48 %) was the predominant fungal isolate followed by *Candida albicans* (28 %) and

Table 1 Demographic details of patients with diabetic foot infection presenting in this study

Demographic details	Overall (%)
Age	56.18 ± 10.12
Gender	
Male	82 (51)
Female	78 (49)
Types of diabetes	
Type I	–
Type II	160 (100)
Duration of diabetics (years)	
<10	70 (44)
10–19	82 (51)
≥20	8 (5)
Duration of ulcer (months)	
≤3	111 (69)
>3	49 (31)
Size of ulcer (cm^2)	
≤4	142 (89)
>4	18 (11)
Nature of ulcer	
Necrotic	11 (7)
Non necrotic	149 (93)
Grade of ulcer (Wagner)	
Grade I	120 (75)
Grade II	35 (22)
Grade III	5 (3)
Other complications	
Hypertension	25 (16)
Retinopathy	35 (22)
Nephropathy	40 (25)
Neuropathy	29 (18)
No complications	31 (19)
HbA1c %	
<7 % (Good control)	–
7–8 % (Fair control)	58 (36)
>8 % (Poor control)	102 (64)
Habits	
Alcohol	35 (22)
Smoking	60 (37)
Tobacco chewing	10 (6)
Alcohol & smoking	30 (19)
Tobacco chewing & alcohol	8 (5)
Tobacco chewing & smoking	9 (6)
No habits	8 (5)

Candida dubliniensis (12 %) (Fig. 2). The Hi-Chrome Candida agar showed *C. albicans* with light green colonies, *C. krusei* with white, *C. glabrata* with pink, *C. tropicalis* with purple and *C. dubliniensis* with dark green colonies. Further, *C. dubliniensis* was confirmed by using corn meal agar, tobacco agar and Sabouraud methylene blue agar. In tobacco agar, the colonies of *C. dubliniensis* showed the formation of hyphal fringes and chlamydospore when observed under low power (10X) and high power (40X) magnifications. The Sabouraud methylene blue agar showed the colonies of *Candida albicans* produce fluorescence, while non *C. albicans* species does not fluorescence. In corn meal agar, the chalmydospore formation was observed in *C. albicans* and *C. dubliniensis*, whereas non *C. albicans* did not produce any chlamydospore.

Table 2 Gender differences in patients with clinical characteristics of diabetic foot infections

Clinical characteristics	Male (%)	Female (%)	*p* value
Total	82 (52)	78 (49)	0.001*
Hypertension	20 (13)	5 (3)	0.003*
Retinopathy	20 (13)	15 (9)	0.398
Nephropathy	29 (18)	11 (7)	0.004*
Neuropathy	17 (11)	12 (8)	0.353

* indicates that statistically significant

The antifungal susceptibility pattern in this study showed that 29 % of *C. albicans* was sensitive to Amphotericin B and Clotrimazole, while 75 % of *C. glabrata* were sensitive to Amphotericin B, Ketoconazole and Itraconazole (Table 4). This indicates Amphotericin B, Fluconazole, Nystatin and Ketoconazole as the effective drug of choice for treatment of *C. glabrata. C. krusei* was resistant to all antifungals tested. *C. dublinensis* and *C. albicans* showed the highest percentage of resistant to Ketoconazole, Fluconazole, Nystatin and Itraconazole (Table 5). This is indicating that effective

Table 3 Mixed infection pattern presenting in this study

Name of the organism	Total no of isolates (%)
Fungi only	
C. glabrata	3 (60)
C. albicans	1 (20)
C. tropicalis	1 (20)
Total	**5**
Mixed infections	
Staphylococcus aureus & C. glabrata	8 (40)
Staphylococcus aureus & C. albicans	4 (20)
Pseudomonas aeruginosa &C. albicans	2 (10)
Pseudomonas aeruginosa & C. dubliniensis	2 (10)
Staphylococcus aureus & C. krusei	1 (5)
Staphylococcus aureus & C. dubliniensis	1 (5)
Salmonella paratyphi & C. glabrata	1 (5)
Staphylococcus aureus, *Pseudomonas aeruginosa*, & *C. krusei*	1 (5)
Total	**20**

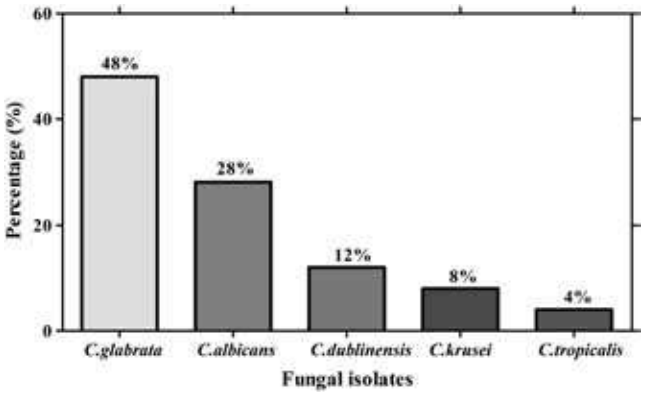

Fig. 2 Distribution of fungal isolates present in diabetic foot infections

alternate drug of choice is required in the treatment of these infections.

Discussion

The study represents a comprehensive clinical and mycological survey of patients with diabetic foot infections mainly yeast such as *Candida albicans* and non Candida species. The diabetic foot infection was significantly higher in males than females. The male preponderance was higher due to the high level of outdoor activity among males patients compared to females, in which increases their chances of getting trauma to the foot by any agents not noticed and treated properly along with other factors will lead to foot infections [20]. Ribu et al. [21] found that mens were lower self-care had a foot ulcer. Similarly, Hjelm et al. [22] reported that women were more active in self-care and preventive care when compared to men.

The patients in the age group of 51–60 years were most significantly infected with diabetic foot infections. The findings of this study are similar to the one reported by Yekta et al. [23] in which patients in the age group of above 50 years compared to less than 50 years and the mean age of the patients were 60.73 ± 11.3 years. The main reason attributed to this is mainly due to their physical conditions along with the associated other factors of the patients in this age group. Duration of diabetes showed 82 (51 %) patients for 10–19 years was high compared to patients with diabetes of less than 10 years. This result of our study correlated with the study of Gadepalli et al. [13] which reported a higher percentage of patients having diabetes for 10–19 years (54 %). Another important clinical characteristic of the patients was Wagner's grade categorization. Majority of the patients was of the grade I (75 %) ulcers followed by grade II ulcers. Thus, the patients had mild to moderate degree of ulcer severity especially grade I and II ulcers. The findings of this study were similar to the one reported by Viswanathan et al. [24] which also reported ulcers with grade I and II.

In this study, higher percentage of patients (64 %) was reported with >8 % poor glycemic control. This observation was correlated with the study of Ozer et al. [25] which also showed the higher percentage of patients with 9.7 ± 3.5 poor glycemic control. The hyperglycemic environment allows organisms to replicate at an increased rate and causes defects in leukocyte function [26]. The proportion of male patients with hypertension and nephropathy was more compared with female patients with diabetic foot infections. The results of this study correlated with the study of Oyibo et al. [27] which observed the proportion of male patients with hypertension was more compared with female patients. In contrast, Cardino et al. [28] observed that the presence of neuropathy, peripheral vascular disease and ulcer severity was associated with amputation of diabetic foot ulcers. Moulik et al. [29] and Sundresh et al. [30] reported that peripheral vascular disease, neuropathy, neuropathic, ischemic and neuroischemic ulcers were found as predisposing risk factors in patients with established foot ulcers. The other associated factors observed in this study was the habit of smoking followed by alcohol consumption in 35 (22 %) patients. The findings of the study correlated with the study of Zubair et al. [20] and Shahi et al. [31] which also showed smoking, tobacco chewing and alcoholism as the associated risk factors with diabetic foot infections. Similarly, Anderson et al. [32] reported that diabetic smokers were undergone for lower extremity amputation

Table 4 Antifungal sensitivity pattern of *Candida* spp. from cases of diabetic foot infections

Candida species	No. of isolates	Antifungal agents (No. of isolates and % of sensitive)					
		AP	FLC	NS	CC	KT	IT
C. glabrata	12	9 (75)	8 (67)	7 (58)	5 (42)	9 (75)	9 (75)
C. albicans	7	2 (29)	1 (14)	1 (14)	2 (29)	–	1 (14)
C. dubliniensis	3	–	–	–	–	–	–
C. krusei	2	–	–	–	–	–	–
C. tropicalis	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

AP Amphotericin B, *FLC* Fluconazole, *NS* Nystatin, *CC* Clotrimazole, *KT* Ketoconazole, *IT* Itraconazole

Table 5 Antifungal resistant pattern of *Candida* spp. from cases of diabetic foot infections

Candida species	No. of isolates	Antifungal agents (No. of isolates and % of resistance)					
		AP	FLC	NS	CC	KT	IT
C. glabrata	12	3 (25)	4 (33)	5 (42)	7 (58)	3 (25)	3 (25)
C. albicans	7	5 (71)	6 (86)	6 (86)	5 (71)	7 (100)	6 (85)
C. dubliniensis	3	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
C. krusei	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
C. tropicalis	1	–	–	–	–	–	–

AP Amphotericin B, *FLC* Fluconazole, *NS* Nystatin, *CC* Clotrimazole, *KT* Ketoconazole, *IT* Itraconazole

when compared to diabetic non smokers. Hence, these comorbidities of foot infections should be recognized and addressed as complicated risk factors for foot infection otherwise will finally lead to amputation of the foot.

The fungal profile showed the occurrence of non *Candida albicans* (NCA) namely *Candida glabrata* as the predominant yeast isolates followed by *Candida albicans*, *Candida dubliniensis, Candida tropicalis* and *Candida krusei*. The results of the study similar with the studies of Chincholikar and Pal [33]; Heald et al. [34] which reported non *Candida albicans* as the predominant yeast isolates from cases of diabetic foot infections. Bader et al. [35] reported that *Candida glabrata* as the emerging group of non *Candida albicans* (NCA) as it causes candidemia among diabetic patients. In this study, the mixed bacterial-fungal infection (14 %) was significantly higher than the fungal infection (4 %). The results of this study were compatible with the findings of Missoni et al. [36] which reported a higher percentage of mixed bacterial-fungal infections (68 %) and twice the percentage of pure fungal infection (32 %). The conformation of *C. dubliniensis* from *C. albicans* was by using tobacco agar which correlated with the result of Khan et al. [18]. The differentiations of *C. albicans* from non *C. albicans* were confirmed and the results were similar with the study of Yucesoy et al. [17]. *Candida albicans* and non albicans species were closely related but differentiated by epidemiology, virulence factor and susceptibility pattern. So species identification of *Candida* was essential for successful management [37]. Moreover, candidal infection in diabetic foot was a rare entity and was previously described in five reports by Heald et al. [34]; Missoni et al.[36]; Yener et al. [38]; Chellan et al. [8] and Nithyalakshmi et al. [9]. Since, our reports confirmed the prevalence of *Candida albicans* and non albicans in diabetic foot infections along with antifungal susceptibility pattern.

The antifungal susceptibility patterns of *C. krusei* and *C. dubliniensis* were resistant to all the antifungal tested. *Candida albicans* showed resistant pattern to Ketoconazole and *C. glabrata* to Clotrimazole. The observations made in this study was similar to the one by Sastry et al. [39] which showed *Candida dubliniensis* resistance to Fluconazole, while other species of *Candida* were sensitive to Itraconazole. From the results of this study, it can be suggested that Itraconazole and Amphotericin B were found to be most effective drugs of therapy for treatment of *C. glabrata. C. dublinensis* and *C. albicans* showed the highest percentage of resistance to Ketoconazole, Fluconazole, Nystatin and Itraconazole indicating that an effective alternate drug of choice is required in the treatment of these infections. Antifungal susceptibility pattern of the yeast played a major role in the proper selection of antifungal agents for the treatment of fungal infections [40]. The literature reports on systemic antifungal therapy in diabetic foot infection are very scarce and further studies are essential to clear the emerging resistant yeast isolates in diabetic foot infection. The therapeutic application of Fluconazole should be limited to high risk patients to minimize the azole resistant strains of *Candida*. Although consistent application of standard techniques, guidelines and control measures of the antifungal drugs potentially reduce the risks of drug-resistant infections [41].

Conclusion

Fungal infections constitute a significant part in diabetic foot infections. However, mycological evaluation is very essential for the selection of the drug of choice in the treatment. Currently, there is an increasing emergence of Fluconazole-resistant strains due to indiscriminate use of antibiotics. Hence, a better understanding of the pathophysiology of diabetic foot infection is very essential not only in the prevention, but also in the recognition of fungal flora and elucidating its role in these infections. Early identification and prompt treatment optimize the patient's outcome and prevent amputation of the foot.

Compliance with ethical standards

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

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

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

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ORIGINAL ARTICLE

Frequency of MRSA in diabetic foot infections

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Abstract *Staphylococcus aureus* is one of the most common bacterial pathogens isolated from diabetic foot infections (DFIs). The increasing prevalence of meticillin-resistant *S. aureus* (MRSA) in patients with diabetes is associated with complications. The aim of this study was to determine the prevalence of *S. aureus* in DFIs and antibiotic susceptibility patterns of MRSA and non-MRSA isolates. Identification of *S. aureus* and MRSA was performed by the phenotypic and molecular methods. The Kirby-Bauer and agar dilution methods were performed for determination of antibiotic susceptibility patterns. Thirty-four isolates of *S. aureus* were isolated from March 2014 to February 2015. The rate of MRSA was 38.23 % according to the disk cefoxitin and oxacillin agar dilution methods, and as by PCR method 12) 35.29 %), isolates were found to have the *mec*A gene. All MRSA and non-MRSA isolates were susceptible to linezolid and vancomycin. The resistance rate to ceftriaxone was high followed by amoxicillin-clavulanic acid, tetracycline, gentamicin, and erythromycin. The most common bacterial pathogen isolated from DFIs was *S. aureus*. To ensure effective treatment, accurate detection of MRSA is critical. Our findings showed that MRSA isolates had high-level resistance to antimicrobial agents and that appropriate antibiotic therapy, based on the antibiotic susceptibility pattern, is essential to ensure a good result.

Keywords Antibiotic susceptibility pattern · Diabetic foot infections · Minimum inhibition concentration · Meticillin-resistant *Staphylococcus aureus*

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Introduction

Diabetic foot infections (DFIs) are one of the most frequent complications in patients with diabetes mellitus [1, 2]. Overall, 15 % of all patients with diabetes develop a foot ulcer in their lives that is very sensitive to infection and spreads rapidly, leading to vast tissue destruction and subsequent amputation [3]. Regarding inappropriate antibiotic therapy, chronic course of the wound, and frequent hospital admissions, diabetic patients with foot ulcers have a high risk of infection caused by multidrug-resistant (MDR) microorganisms such as MRSA [4, 5]. Among aerobic Gram-positive bacteria, *Staphylococcus aureus* is the most common isolate from DFIs [6, 7]. Different classes of antibiotic drugs have been applied for treatment of infections [8]. Due to resistance

to most classes of antimicrobial agents, therapy of *S. aureus* infections is hard [9, 10, 8]. It is well known that the organism acquires resistance soon after initial usage of new antibiotics [9]. The most remarkable example of this phenomenon is MRSA [10]. Some studies show patients hospitalized with various types of MRSA infections compared to similar patients without MRSA infection have a fivefold increased risk of death during hospitalization [11, 12]. The emergence of MRSA, among both hospital-acquired and community-acquired infections, emphasizes that this species is a potential pathogen that is able to adapt after exposure to antimicrobial agents [8]. The recent emergence of MRSA soft tissue infections has largely originated from the community-acquired type, rather than health care-associated strains [11]. Several studies found MRSA in 15–30 % of DFIs, and these infected ulcers have thus been associated with a slower healing rate [13]. To detect the MRSA, numerous phenotypic methods have been used [14, 15]. Molecular methods are considered to be more flexible and sensitive, have a shorter turn-around time than culture methods, and more cost-effective [15, 16]. In the present study, we investigated the frequency and prevalence of *S. aureus* strains in patients with DFIs through the phenotypic and molecular methods and antibiotic susceptibility patterns of *S. aureus*.

Methods and materials

Collection of specimens

Several (80) DFI specimens were collected from March 2014 to February 2015 in Imam Reza and Sina Hospitals of Tabriz, Iran. The DFI site was first scrubbed with povidone–iodine, and then the specimens were obtained by needle aspiration of material in the depth of the infected site. The specimens were transported to the laboratory and were inoculated to culture media within 1 h [17]. This study was approved by The Ethic Commission of Tabriz University of Medical Sciences (Number: 5/4/589–23 Mar. 2014).

Bacterial strains

First, a Gram stain smear was processed for the detection of *S. aureus* in specimens. For the isolation of *S. aureus*, specimens were plated onto sheep blood (5 %) (Liofilchem Ltd., Italy) and phenyl ethyl alcohol agar (Hi Media Co., India) plates. These isolates were identified by Gram staining, catalase, coagulase, DNase activities, and mannitol salt agar tests. The plates were incubated at 37 °C under 10 % CO_2 and examined at 24 and 48 h, and standard bacteriologic techniques were used for identification of *S. aureus* [18, 19].

Antimicrobial susceptibility patterns

Kirby-Bauer method of antibacterial susceptibility test was performed on Mueller–Hinton agar (Liofilchem Ltd., Italy) as per CLSI guidelines [20]. The disks used for antibacterial susceptibility test included erythromycin (15 μg), vancomycin (30 μg), clindamycin (2 μg), gentamicin (10 μg), cefoxitin (30 μg), oxacillin (1 μg), linezolid (30 μg), amoxicillin-clavulanic acid (20/10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), and rifampicin (5 μg). All disks were provided from Mast Ltd., England. Cefoxitin disks (30 μg) (Mast Ltd., England) on Mueller–Hinton agar (Liofilchem Ltd., Italy) was used for MRSA screening according to the CLSI guideline [20]. The agar dilution assay was used to determine the oxacillin MIC according to the CLSI guideline [20]. The oxacillin concentrations used ranged from 0.25 to 256 μg/ml. The Mueller–Hinton agar (Liofilchem Ltd., Italy) plates without antimicrobial agent were used as a positive control of bacterial growth.

Detection of *mec*A gene

A loopful of *S. aureus* culture was suspended in 300 ml of TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0) and placed at 80 °C for 20 min to kill the bacteria. DNA was extracted by CTAB method [21]. For PCR reaction, specific primers of 310-bp fragments for *mec*A (forward: 5-GTAGAAATGACTGAACGTCCGATAA-3 and reverse: 5-CCAATTCCACATTGTTTCGGTCTAA-3) were used [19].

DNA amplifications was performed in 20 μl volumes that contained 10 to 100 ng of DNA, 0.5 μM of each primer, in the presence of 2 mM $MgCl_2$, 100 μM of each dNTP, 50 μm KCl, 20 mM Tris-HCL, pH 8.4, and 2.5 U recombinant DNA polymerase (Jena Bioscience, Germany). Amplification was performed in a DNA thermal cycler (Gradient Eppendorf, Germany) programmed for 94 °C (6 min) as the initial denaturation step followed by 35 cycles at 94 °C (50 s), 57 °C (50 s), 72 °C (55 s), and then 72 °C for 7 min. Gel electrophoresis was performed for 45 min on a 1.2 % agarose gel at 80 V and after staining with 0.5 μg/ml ethidium bromide visualized under UV light [21].

Control strains

A meticillin-susceptible *S. aureus* (MSSA) ATCC 29213 and MRSA ATCC 33591 strains were used as controls in the susceptibility tests and PCRs.

Results

In the current study, 119 bacterial pathogens were isolated from 80 DFIs and *S. aureus* was the predominant bacterial

Table 1 Detection of meticillin resistance among *S. aureus* isolates by presence of the mecA gene, and MIC of oxacillin

Breakpoint of oxacillin (μg/ml)[a]	PCR detection	
	mecA negative (n = 22)[b]	mecA positive (n = 12)[b]
≤0.25	2	0
0.5	4	0
1	1	0
2	13	0
4	1	0
8	1	6
16	0	3
32	0	1
64	0	2
128	0	0
≥256	0	0

[a] Oxacillin MICs were determined by an agar dilution method

[b] Presence of mecA was determined by PCR

Table 2 The frequency of *S. aureus* resistance to antibiotics according to MRSA and non-MRSA

Antibiotics	MRSA	Non-MRSA
VA	0	0
OX	100	4.76
FOX	100	0
CD	38.46	14.28
E	76.92	47.61
T	76.92	28.57
GM	76.92	28.57
RP	76.92	0
CIP	61.53	28.57
CRO	100	0
AUG	76.92	0
LZD	0	0

VA vancomycin, *LZD* linezolid, *OX* oxacillin, *CD* clindamycin, *CIP* ciprofloxacin,*T* tetracycline, *E* erythromycin, *GM* gentamicin, *FOX* cefoxitin, *AUG* amoxicillin-clavulanic acid, *CRO* ceftriaxone, *RP* rifampin, *R* resistant

pathogen (34 cases). The MRSA was detected in 13 cases (38.23 %) by cefoxitin disk, and all isolates were confirmed as an MRSA by PCR (Table 1). An amplification of the *mec*A gene by PCR showed a 310-bp fragment in 12 (34.61 %) isolates (Fig. 1). *S. aureus* isolates are characterized as resistant and susceptible with MIC ≥4 and ≤2 μg/ml, respectively. Oxacillin MIC was ≥4 μg/mL for 13 isolates, thus classified as MRSA according to CLSI recommendations. The results of MIC are shown in Table 1, and an average of MIC was 4 μg/ml. All *S. aureus* isolates were susceptible to vancomycin and linezolid (Table 2). These agents were the most effective antibiotics, and clindamycin and ciprofloxacin were modest. Nineteen *S. aureus* isolates were MDR (resistant to more than three classes of antibiotic agents).

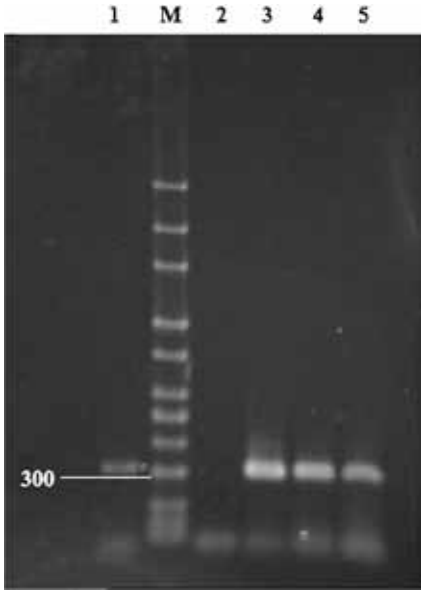

Fig. 1 The *mec*A gene PCR product detected by agarose gel electrophoresis (1.2 %). *M* (100–3000 bp DNA size marker), *lane 1* positive control strain (*mec*A), *lane 2* negative control strain (*mec*A), *lanes 3–5* clinical isolates of positive *mec*A gene

Discussion

Diabetic foot ulcers are susceptible to bacterial infections, and DFIs rapidly spread and contribute to high morbidity and mortality [22].

Aerobic Gram-positive cocci, especially *S. aureus*, are a common bacterial pathogen in DFIs [6]. In the present study, *S. aureus* was isolated as the most common bacterial pathogen (28.57 %); this is in accordance with other studies carried out by Kandemir et al. (30 %) and Zubair et al. (28 %) [4, 23]. However, the results of this study stand in contrast to two studies carried out by Shanker et al. and Ako-Nai that reported the rate of DFIs due to *S. aureus* as 14 and 13 %, respectively [1, 3].

MRSA has emerged as a serious and common problem in patients with DFIs [11]. Currently, MRSA infections have gradually been reported among groups of patients with no apparent connection to hospitals. In this research, MRSA was identified as 38.23 % by using the oxacillin agar dilution method, while Mendes et al. and Shankar et al. reported 53.1 and 10.3 % values, respectively [3, 24]. We obtained specimens from the deep tissues of the infected site; therefore, the isolated bacteria were considered the most probable causative

agents of DFIs. The conflict results could be due to an absence of strict guidelines for administration of broad-spectrum antibiotics, a prolonged antibiotic therapy, and the lack adherence to infection control measures in the hospital setting that may increase the prevalence of antibiotic resistance organism like MRSA in DFIs [6, 25].

We showed that the rate of the MDR was 55.88 %. Kandemir et al., on the other hand [23], indicated that 77.5 % of *S. aureus* isolates were MDR. Since MRSA is resistant to several antibiotics, infections with these bacteria could be treated with extended spectrum antibiotics for longer duration. Consequently, duration of hospital stay for infections with MDR can be longer and their management can be more costly [23].

All of the MRSA were sensitive to vancomycin and linezolid, which is in agreement with studies conducted by Shanmugam et al. and Raja studies [26, 27]. These antibiotics are highly effective in vitro against MRSA isolates, followed by clindamycin and ciprofloxacin which seem to be appropriate for empirical treatment of MRSA infection in DFIs. Regarding the high rates of resistance among MRSA isolated from DFIs in our study, the use of tetracycline, erythromycin, gentamicin, and rifampin might be inadequate for empirical therapy and might lead to failure. Therefore, DFIs with MRSA may require additional antimicrobial coverage.

In the present research, from all MRSA, one isolate was meticillin-resistant by cefoxitin disk diffusion and oxacillin agar dilution methods, but did not show the amplification of the *mec*A gene. Similar to other research carried out by Perez et al. and Sadeghi et al. [19, 28], discrimination between the phenotypic and genotypic assays was reported. This difference between the phenotypic and genotypic assays may be due to culture conditions (as temperature, composition of culture medium, a size of inoculums, and time of incubation). Also, an association of resistance mechanisms and genetic background (translated by interfering of other genes in the control of resistance appearance to oxacillin) hinders the standardization of methods for detecting MRSA [29]. In addition, the PCR assay is unable to detect MRSA mediated by other than the *mec*A gene, and the cefoxitin disk may fail in showing low-level or heterogeneous resistance strains [29].

The most common risk factors for MRSA diabetic foot ulcer include inappropriate use of an antimicrobial agent, long wound duration, inpatient management, and chronic kidney disease [30, 31]. The increasing prevalence of MRSA infection has further complicated the selection of an antibiotic for DFIs [32]. Correct detection of MRSA is of the utmost importance to ensure effective treatment for the affected patient and to prevent further spread [33]. Primary empirical antibiotic therapy should be based on the severity of the infection, history of recent antibiotic use, previous infection with resistant organisms, recent culture results, Gram stain findings, and patient factors (e.g., drug allergy) [34, 35]. The present study recommends that the choice of an antibiotic agent should be based on the proper coverage of MRSA in the DFIs.

Conclusion

The most common bacterial pathogen isolated from DFIs was. *S. aureus* and the frequency of MRSA was 28.6 % as well as early identification can help in the development of effective strategies to avoid the growth MRSA strains in the DFIs.

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Compliance with ethical standards This study was approved by The Ethic Commission of Tabriz University of Medical Sciences (Number: 5/4/589 -23 Mar. 2014).

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional.

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ORIGINAL ARTICLE

Cushing's syndrome in obese patients with type 2 diabetes: A single center screening study

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Abstract The frequency of Cushing's syndrome (CS) in obese patients was not properly determined and the studies focused on the frequency of occult CS and the possible improvement of diabetes and obesity with treatment of CS are needed. In this study, we aimed to investigate the frequency of CS in obese patients with type 2 diabetes. The study enrolled with 200 obese (body mass index (BMI) >30 kg/m^2), type 2 diabetes patients between 2009 and 2011 in Sisli Etfal Training and Research Hospital, Turkey. Twenty-eight males and 172 females were recruited to the study. Mean age of the study group was 51.7 ± 8.5. Nineteen patients (9.5 %) failed to suppress cortisol levels less than 1.8 μg/dL after a 1-mg overnight dexamethasone suppression test (ODST) and these patients proceeded to have a 2-day 2-mg low-dose dexamethasone suppression test. After further screening, three (%1.5) patients were diagnosed with CS in our study. Among the three patients diagnosed with CS, the tumor was located in the pituitary gland in two patients. The present study revealed that the frequency of Cushing's syndrome in obese and diabetic patients were 1.5 %, which was much higher than the general population. Occult CS should take into account as an exacerbating factor for diabetes and screening for CS should be considered in poorly controlled diabetic patients.

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Introduction

Cushing's syndrome (CS) is a rare disease with an estimated incidence of 1:50,000 to 1:100,000 inhabitants in the general population [1, 2]. Mortality rate of the patients with CS are four times higher than healthy subjects [3, 4]. Diagnosis of CS is more straightforward, when the specific signs of the disease are present. However, none of these signs or symptoms is pathognomonic and secretory activities of the tumors are variable over time [5, 6]. Subclinical hypercortisolism (SH) is a recently described entity characterized by impaired cortisol homeostasis without specific signs or symptoms of CS [7].

Type 2 diabetes and obesity are common disorders that can develop secondary to CS and approximately 80 % of patients with CS display glucose intolerance or type 2 diabetes due to insulin resistance [1, 8]. Although its epidemiological impact on diabetes and obesity development is trivial because of the low prevalence, SH which is definitely more frequent than overt CS, can play an important role in the development of these diseases in the general population [7, 9]. Moreover, it has been suggested that the patients with diabetes experienced clinical improvement after SH removal [10]. However, systematic screening for CS in patients without specific signs of hypercortisolism is not established. While a screening strategy is reasonable, if its efficacy is evident and if the pros surpass the cons, the previous studies focused on the frequency of occult CS in obese patients with diabetes, have conflicting results. Leibowitz et al. demonstrated CS in 3 % of 90 overweight patients with diabetes [11]. Chiodini et al. found that the prevalence of CS was 9.4 % in 294 diabetic patients [12]. On the other hand, any case of CS was not detected in a study with 154 diabetic patients [13]. Thereby, the frequency of CS

in obese patients was not properly determined and the studies focused on the frequency of occult CS and the possible improvement of diabetes and obesity with the treatment of CS are needed. In this study, we aimed to investigate the frequency of CS in obese patients with type 2 diabetes.

Materials and methods

Two hundred obese patients (body mass index (BMI) >30 kg/m^2) with type 2 diabetes were consecutively included to the study between 2009 and 2011 in Sisli Etfal Training and Research Hospital, Istanbul, Turkey. Twenty-eight males and 172 females were recruited to the study. The mean age of the study group was 51.7 ± 8.5. From admission to our outpatient clinic, 112 (56 %) patients were treated with at least one oral antidiabetic drug (OAD), 12 (6 %) patients were treated with insulin, and 76 (38 %) patients were treated with the combination of OAD and insulin treatment. The mean diabetes duration was 7.4 ± 5.5 years. Additionally, 136 (68 %) and 72 (36 %) patients were treated with antihypertensive drugs and statins, respectively.

All patients were subjected to a careful clinical examination and none of them displayed specific signs of hypercortisolism such as purple striae, ecchymoses, skin atrophy, or proximal muscle wasting. The patients using systemic or inhaled steroids, antidepressants, antipsychotics, or other drugs, which might interfere with dexamethasone metabolism, having a malignancy or other clinically significant disease, known or suspected abuse of alcohol were excluded from the study.

A one-milligram overnight dexamethasone suppression test (ODST) was performed as the first screen test in all patients. The patients were administered to take 1 mg of dexamethasone at 2300 and blood samples were collected on the next morning at 0800 for measurement of serum cortisol level. The patients with serum cortisol levels above 1.8 μg/dL (50 nmol/L) were considered abnormal and underwent a 2-day 2-mg low-dose DST (LDDST). The patients were advised to take 0.5 mg of dexamethasone at 0600, 1200, 1800 and 2400 for two consecutive days. The sample for serum cortisol was collected at 0800 on the third day. Patients with serum cortisol level below 1.8 μg/dL after LDDST were considered as normal. The patients with serum cortisol level above 1.8 m μg/dL were evaluated for CS. Plasma ACTH and serum cortisol concentrations were measured to evaluate diurnal rhythm. A 24-h urinary free cortisol level was assessed and high-dose DST (2 mg of dexamethasone at 0600, 1200, 1800, and 2400 for two consecutive days and blood samples were collected on the next morning at 0800) was performed.

Abdominal computed tomography (CT), pituitary magnetic resonance imagining (MRI), or inferior petrosal sinus sampling (IPSS) were performed to clarify the diagnosis if necessary.

This study was approved by the local ethics committee of Sisli Etfal Training and Research Hospital and informed consent was obtained from all individual participants included in the study.

Statistical analysis

The quantitative variables are presented as mean ± standard deviation. Mann Whitney-*U* test was used for comparison of the means of two groups. Pearson's correlation was used to evaluate the relationship between the two variables. Statistical significance was set at $p < 0.05$. Statistical analyses were performed with SPSS software, version 13.0 for windows (SPSS Inc., Chicago, IL, USA).

Results

The clinical features of the patients were shown in Table 1. Dexamethasone suppression test was performed in all patients and 19 (9.5 %) patients failed to suppress cortisol levels less than 1.8 μg/dL after ODST (Table 2). There was no significant difference in age, sex, or metabolic parameters such as BMI, glucose, HbA1c, and cholesterol levels between DST suppressors and non-suppressors. Also, cortisol level after the dexamethasone suppression test was not correlated with any of these parameters. Nineteen patients with DST non-suppression were further evaluated by LDDST. Serum cortisol levels of 16 patients were below 1.8 μg/dL and Cushing's syndrome was excluded in these patients. Physical examination considering Cushing's syndrome was carefully performed in the remaining three patients and none of them displayed specific signs of hypercortisolism such as purple striae, ecchymoses, skin atrophy, or proximal muscle wasting. Further biochemical and imaging studies were obtained in these patients (1.5 % of the whole series). Clinical features of these patients with Cushing's syndrome are shown in Table 3.

The 24-h urinary cortisol and midnight cortisol were 63 μg/24 h and 11 μg/dL in patient 1, respectively. ACTH level of this patient was under 5 pg/mL and cortisol level after high-dose DST was 12 μg/dL. These findings were compatible with ACTH-independent Cushing's syndrome and MRI revealed a 2.5-cm adenoma in the right adrenal. The patient underwent right adrenalectomy and histological investigation revealed an adrenal adenoma. Adrenocortical insufficiency developed in the postoperative period and this patient was treated with oral replacement therapy for 9 months. After cessation of replacement therapy, 7 % reduction in body weight and 1.4 % reduction in HbA1c were observed at the end of first year of surgery, while only metformin therapy was resumed.

Table 1 Clinical features of study group

	Mean ± S.D.
Age (y)	51.7 ± 8.5
BMI (kg/m^2)	37.6 ± 5.6
Waist circumference (cm)	113.4 ± 7.4
DM duration (y)	7.4 ± 5.5
Triglyceride (mg/dL)	185.1 ± 144.5
Total cholesterol (mg/dL)	196.7 ± 43.0
LDL (mg/dL)	113.6 ± 36.8
HDL (mg/dL)	49.2 ± 10.9
Fasting Blood Glucose (mg/dL)	188.1 ± 75.4
HBA1c (%)	8.4 ± 2.1
Systolic blood pressure (mmHg)	133.5 ± 7.1
Diastolic blood pressure (mmHg)	83.4 ± 4.6
ODST (μg/dL)	1.25 ± 1.6

BMI body mass index, *DM* diabetes mellitus, *LDL* low density lipoprotein

HDL high density lipoprotein, *ODST* 1 mg overnight dexamethasone suppression test

The other two patients had ACTH level above 10 pg/mL and high-dose DST suppression was observed in these patients. Although pituitary MRI of patient 2 displayed a 5-mm microadenoma in the right side of the pituitary, no adenoma was detected in patient 3 and inferior petrosal sinus sampling (IPSS) was performed to confirm Cushing's disease. The center-to-periphery ACTH ratio after CRH stimulation was 290:1 and left to right petrosal sinus ratio was 8:1. Thus, IPSS supported the diagnosis of Cushing's disease and showed left lateralization. Transsphenoidal surgery was performed and the histopathological diagnosis was ACTH-secreting adenoma in both patients. Six months after surgery, cortisol response was normal with ACTH stimulation and glucocorticoid replacement therapy was stopped in patient 2. Serum cortisol was suppressed by ODST in this patient. A better metabolic profile was attained, with 2.5 % reduction of HbA1c, 4.5 % of weight loss, and a 32-U reduction in insulin dosage, while the diabetes medications were switched from basal bolus insulin to metformin plus basal insulin therapy. However, glucocorticoid replacement therapy was not needed after surgery and no other anterior pituitary hormone deficiency was observed in patient 3. Serum cortisol was suppressed by ODST and at the sixth month of surgery, 3.3 % reduction in HbA1c and 6 % weight loss were achieved in this patient, while diabetes medications were switched from basal bolus insulin to metformin and gliclazide therapy.

Discussion

In the present study, we investigated 200 obese patients with type 2 diabetes for CS. Nineteen patients failed to suppress cortisol level after ODST and these patients proceeded to have LDDST. After further screening, three (%1.5) patients were diagnosed Cushing's syndrome in our study. Among the three patients diagnosed with CS, the tumor was located in the

Table 2 Comparison of characteristics of patients according to ODST

	Cortisol >1.8 (n = 19)	Cortisol ≤1.8 (n = 181)	P
Male (n)	2	26	0.3
Female (n)	17	155	0.4
Age (year)	54 ± 8.1	51.4 ± 8.5	0.1
BMI (kg/m^2)	35.0 ± 3.4	37.8 ± 5.8	0.056
Waist circumference (cm)	110 ± 6.6	113 ± 7.5	0.1
DM duration (year)	7.1 ± 4.4	7.4 ± 5.6	0.9
Triglyceride (mg/dL)	166 ± 66	187 ± 150	0.8
Total cholesterol (mg/dL)	200 ± 34	196 ± 43	0.4
LDL (mg/dL)	120 ± 33	113 ± 37	0.3
HDL (mg/dL)	47 ± 10	49 ± 10	0.5
Fasting blood glucose (mg/dL)	210 ± 81	185 ± 74	0.1
HBA1c (%)	9.2 ± 2.8	8.4 ± 2.0	0.3
Systolic BP (mmHg)	134 ± 5	133 ± 7	0.4
Diastolic BP (mmHg)	84 ± 4.5	83 ± 4.6	0.1
TSH (uIU/mL)	1.6 ± 0.7	1.6 ± 1.4	0.1
ODST (μg/dL)	4.9 ± 3.4	0.86 ± 0.27	0.000

BMI body mass index, *DM* diabetes mellitus, *LDL* low-density lipoprotein

HDL high density lipoprotein, *TSH* thyroid stimulating hormone, *ODST* 1-mg overnight dexamethasone suppression test

Table 3 Clinical features of the patients with Cushing's syndrome

Patient no.	1	2	3
Etiology	Adrenal	Hypophysis	Hypophysis
Sex	Female	Female	Male
Age (year)	50	55	52
BMI (kg/m^2)	39.1	34.1	32.3
Waist circumference (cm)	110	104	102
ODST (μg/dL)	7.8	8.0	14.4
LDDST (μg/dL)	4.8	2.8	5.6
Urinary cortisol (μg/24 h)	63	61	256
Basal cortisol (μg/dL)	18	23.5	29
Midnight cortisol (μg/dL)	11	17.6	16.6
High-dose DST (μg/dL)	12	3.9	1.92
IPSS	Not performed	Not performed	Left lateralization
Localization	2.5 cm adenoma, right adrenal	5 mm microadenoma. right side of pituitary	Normal
ACTH (pg/mL)	<5	26	21
Fasting blood glucose (mg/dL)	214	263	303
HbA1c (%)	8.4	10.9	9.6
Triglyceride (mg/dL)	89	123	145
Total cholesterol (mg/dL)	207	203	192
LDL (mg/dL)	128	130	124
HDL (mg/dL)	61	48	39
TSH (uIU/mL)	2.1	2.7	1.4

BMI body mass index, *ODST* 1-mg overnight dexamethasone suppression test, *LDDST* 2-day 2-mg overnight dexamethasone suppression, *IPSS* inferior petrosal sinus sampling, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TSH* thyroid stimulating hormone

pituitary gland in two patients. In the literature, there are conflicting results in the prevalence of occult CS between obese and diabetic populations. The prevalence of CS in patients with diabetes ranges from 0 to 9.3 % in different studies [14]. Leibowitz et al. demonstrated CS in 3 % of 90 overweight patients with diabetes [11]. Catergi et al. found that four of 200 overweight patients with diabetes had CS [15]. Additionally, the frequency of CS had been found 0.72 and 9.3 % in 277 and 150 obese patients in the Turkish population, respectively [16, 17]. In another Turkish study, Sahin et al. demonstrated CS in 3 % of 100 obese patients [18]. On the other hand, no CS was detected in a study of 154 diabetic patients [13]. Our center is the endocrinology clinic of a tertiary hospital in which generally, dysregulated diabetic patients are referred and the frequency of occult CS could be more likely to increase. The differences in the prevalence of CS in patients with T2DM can be explained by the selection of the patients, methodological discrepancy (preferred test, cortisol assays, etc.), and also cut-points.

ODST is the mainstay of the biochemical screening for CS [19]. However, the appropriate cut-point of ODST is still a matter of debate in the screening of CS. While cortisol suppression to ODST achieves superior diagnostic performance with the 5-μg/dL threshold at 97 % specificity, the lower 1.8-μg/dL level provides less optimal 80 % specificity in the general population [20]. On the other hand, it has been demonstrated that cortisol levels by ODST were established <2 μg/dl (<55 nmol/L) in all healthy persons using the newer immunoassays and a cut-point of 1.8 μg/dL (50 nmol/L) has been recommended [21, 22]. In a previous study, an 8 % false positive rate of a 1-mg ODST had been found in obese Turkish patients and it has been suggested that a 2-mg ODST could have more specifity compared to a 1-mg ODST [18]. In agreement with that study, the present study showed that cortisol suppression to ODST with the cut-point of 1.8 μg/dL was false positive in 16 (8 %) patients. Potential confounders including stress, simple obesity, dysregulated diabetes, and the activators of cytochrome P-450 3A4 system such as statins should be considered. However, in agreement with the previous study, no correlation was found between DST results, BMI, and glycated hemoglobin levels in our study [23]. Additionally, use of statins was not significantly different in the patients with false positive DST results. These results emphasized that high false positivity of ODST should be considered in the clinical practice and additional tools such as the 24-h urinary free cortisol or the late-night salivary cortisol test must be employed to confirm the diagnosis of CS.

Cortisol excess leads to metabolic abnormalities, such as impaired glucose tolerance and insulin resistance [24, 25]. It has been suggested that glucose metabolism and also cardiovascular risk could be improved by the removal of adrenal incidentaloma in subclinical CS [26, 27]. Leibowitz et al. in all five patients and Taniguchi et al. in all two patients had observed the improvement of diabetes after the cure of CS [11, 28]. Although assessment of the metabolic impact of the cure in occult CS is out of scope in the present study, diabetes improved and a significant weight loss was achieved after surgery. Further long-term studies are warranted to determine the improvement of diabetes and obesity in occult CS.

In conclusion, the present study revealed that the frequency of Cushing's syndrome in obese and diabetic patients were 1.5 %, which was much higher than the general population. Occult CS should be taken into account as an exacerbating factor for diabetes and screening for CS should be considered in poorly controlled diabetic patients. The results of ODST above the cut-point of 1.8 μg/dL must be carefully evaluated and additional tools should be employed to confirm the diagnosis of CS. Further prospective studies which also represent the impact of the cure of CS are warranted to determine the clinical approach for occult CS in obese patients with diabetes.

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Compliance with ethical standards

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

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ORIGINAL ARTICLE

Prevalence of 25-hydroxy vitamin D deficiency among type 2 diabetic subjects of South India

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Abstract Vitamin D levels have been documented to have significant inverse relationship with type 2 diabetes. However, data on the extent of vitamin D deficiency among type 2 diabetes subjects of India is lacking. The present study was undertaken among diabetic subjects of South India to address this lacuna. This retrospective study was conducted among patients attending a diabetes specialty hospital who had established type 2 diabetes mellitus. Demographic data and data on laboratory parameters such as vitamin D, HbA1c, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and serum calcium were obtained from the hospital information system. Vitamin D levels were classified as normal (≥30 ng/mL), insufficient (>20 to 29.9 ng/mL), and deficient (≤20 ng/mL). We included 4628 subjects with diabetes. Among them, 71.4 % were vitamin D deficient, 15 % were vitamin D insufficient, and 13.6 % were found to have normal vitamin D levels. On comparing the two genders, it was seen that the percentage of men and women with these conditions were similar. The proportion of subjects with these conditions across different age groups (30–50, 50–70, >70) were also similar. BMI, age, calcium levels, and HbA1c were found to be the major confounders for vitamin D status. Our study, done among type 2 diabetes people, show that vitamin D deficiency was highly prevalent among them. Considering such high prevalence, screening of diabetic patients for vitamin D deficiency would be beneficial in this population.

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Keywords Serum calcium · Gender · HbA1c · South India · Type 2 diabetes mellitus · Vitamin D

Introduction

Estimates show that India has the highest prevalence of type 2 diabetes in the world with over 62 million people diagnosed currently [1], and it is projected that nearly 80 million individuals would be affected by the disease by 2030 [2]. Diabetes is multifactorial in origin, and is caused by an interplay of several environmental as well as genetic factors. A steady rise in living standards, urbanization, and associated lifestyle changes are the major environmental predictors for diabetes among Indians, whereas genetic aspects emanate once environmental factors are favorable [3]. Since diabetes is associated with significant morbidity and mortality, identifying new modifiable risk factors may be beneficial in reducing its burden in a nation afflicted by it.

Vitamin D plays a major role in bone metabolism and in the regulation of intestinal absorption of minerals such as calcium and phosphorus. Various studies suggest vitamin D deficiency may play a major role in the causation of chronic diseases like hypertension, cardiovascular disease, etc. [4, 5]. Evidence generated from prospective studies in European and American populations show that a significant inverse relationship exists between vitamin D levels and risk for type 2 diabetes [6, 7]. It has been proposed that the effect of interactions between vitamin D and IGF-1 and its binding proteins on glucose metabolism could be the link between vitamin D deficiency and diabetes [8]. Population-based studies show that polymorphisms in the vitamin D receptor gene may affect the glycemic status in humans [9, 10]. These receptors are found not only in the kidneys, but in the pancreatic islets as well as other tissues [11]. Although vitamin D deficiency is

unexpected in a tropical country like India, several cross-sectional studies show that the prevalence of vitamin D deficiency is quite high even among healthy individuals of various subsets of this population [12, 13]. No prospective studies have been conducted so far looking into the association between vitamin D levels and risk for developing type 2 diabetes among Indians. There is paucity of data even on the prevalence of vitamin D deficiency among type 2 diabetes subjects of India. Hence, this study was undertaken to investigate the prevalence of vitamin D deficiency among type 2 diabetes subjects.

Material and methods

This retrospective study was conducted at a tertiary care diabetes specialty hospital in South India according to the ICMR's Ethical guidelines for biomedical research on human participants (2006). The Institutional Review Board approved the protocol of the study, and the need for informed consent from study participants was waived due to retrospective study design. All patients with established type 2 diabetes mellitus who attended the hospital from October 2011 to July 2015, and had levels of 25-hydroxyvitamin D [25(OH) D] tested, were included in the study. The Endocrine Society Task Force has recommended the estimation of circulating serum 25(OH) D as a reliable assay to evaluate vitamin D deficiency [14]. Demographic data (age, gender), clinical parameters (height, weight, BMI, duration of diabetes, history of hypertension), and laboratory parameters such as HbA1c, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and serum calcium of the enrolled subjects were obtained from the hospital information system.

Serum 25(OH) D was estimated by electrochemiluminescence binding assay (kits from Cobas, Roche Diagnostics GmbH, Mannheim, Germany). Lipid profile, serum calcium, and fasting blood glucose were analyzed in BS-400 Mindray Chemistry Analyzer, and HbA1c was estimated by HPLC (Biorad). All analytical procedures were standardized and conducted routinely in the clinical laboratory of the hospital. 25(OH) D levels were considered as normal (≥30 ng/mL), insufficient (>20 to 29.9 ng/mL), and deficient (≤20 ng/mL) as per Clinical Practice Guidelines (2011) of The Endocrine Society [14].

Data was analyzed using SPSS 19.0. The categorical variables were represented as percentages and measurable variables as mean ± standard deviation. Chi-square test, ANOVA, or *t* test were performed as applicable for comparing the variables between different groups, and a *P* value ≤0.05 was considered to be statistically significant. Bonferroni corrections were done for multiple comparisons. Pearson correlation analysis was done to find out the association between different variables, and regression model was applied to identify the confounding variables.

Results

We included 4628 subjects with diabetes in this study (2934 men and 1684 women). Their mean age was 56.64 ± 10.35 years (range 30 to 95 years). The clinical characteristics of the study population are given in Table 1. Among them, 71.4 % were vitamin D deficient, 15 % were vitamin D insufficient, and 13.6 % were found to have normal vitamin D levels. On comparing the two genders, it was seen that the percentage of subjects with vitamin D deficiency was 70.4 % among men and 73.1 % among women, those with vitamin D insufficiency were 16.1 among men and 13.1 % among women, whereas 13.5 and 13.8 % among men and women respectively had vitamin D levels in the normal range. The cumulative distribution for vitamin D levels for men and women are represented in Fig. 1. While comparing the other variables between men and women, it was found that BMI, diabetes duration, LDL, HDL, TC (P = <0.0001 for all), and calcium levels (P = 0.04) were significantly different, which could be owing to the large sample size.

The subjects were divided into three different age groups: (1) 30–50 years, (2) 50–70 years, and (3) >70 years, and the prevalence of vitamin D deficiency was assessed in each age group. Among subjects between 30 to 50 years of age (n = 1282), it was found that 76.2 % were vitamin D deficient, 14.4 % were vitamin D insufficient, and 9.4 % had normal vitamin D levels. The percentage of subjects with vitamin D deficiency was 70 %, insufficiency was 15.1 %, and normal levels was 14.9 % in the 50–70 years group (n = 2922). The percentage for deficient, insufficient, and normal conditions among those >70 years (n = 424) was 66.5, 61, and 17.5 %, respectively. The mean vitamin D level of subjects of the three

Table 1 Clinical profile of the study subjects

Male/female	2934:1694
Mean age (years)	56.64 ± 10.35
Mean duration of diabetes (years)	11.29 ± 7.89
BMI (kg/m^2)	27.76 ± 4.97
HbA1c%	8.74 ± 2.06
TC (mg/dL)	162.7 ± 46.05
TG (mg/dL)	150.11 ± 89.22
HDL (mg/dL)	40.3 ± 10.6
LDL (mg/dL)	88.35 ± 29.27
Calcium (mg/dL)	8.91 ± 0.61
Vitamin D (ng/mL)	16.83 ± 13.05

kg/m^2 kilograms per meter square, *mg/dL* milligram per deciliter

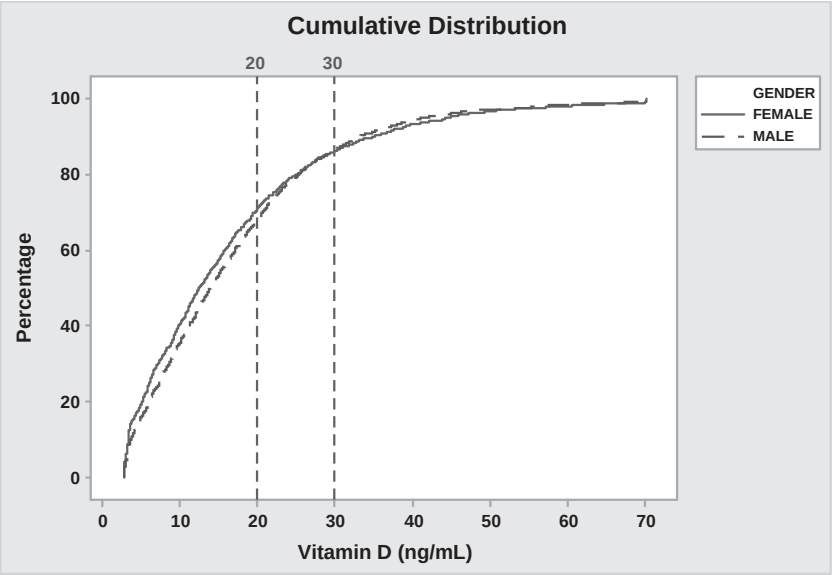

Fig. 1 Cumulative distribution for vitamin D concentrations for men and women

age groups differed significantly (P = <0.0001). While comparing the other variables between the three age groups, it was found that LDL, TG, TC, HbA1c, diabetes duration (P < 0.0001), and BMI (P = 0.044) were also significantly different. This could be because of the large sample size of this study. These results are given in Table 2.

Vitamin D levels were found to have mild positive correlation with calcium levels (r = 0.165) and mild inverse correlation with HbA1c (r = −0.112), but no significant correlation with other variables were observed. When subjects were categorized based on their HbA1c levels (<7 and ≥7 %), those with better glycemic control (<7 %) had significantly higher vitamin D levels compared to those with poorer glycemic control (mean 18.2 ± 13.98 vs. 16.5 ± 12.81, P = 0.0005). Age, gender, duration of diabetes, BMI, HbA1c, and serum calcium level have been shown as confounders of vitamin D status by earlier studies. We used these variables to evaluate their association with vitamin D levels by multinomial regression. It was found that BMI (P = 0.04), age, calcium levels, and HbA1c (P = <0.0001 for all) formed the major confounders in our study.

Discussion

The prevalence of vitamin D deficiency among the general population of India is over 70 %, irrespective of geographic or socioeconomic setting [15]. No data is available from large-scale studies on the prevalence of this condition among people with type 2 diabetes across different regions, and the current study is probably the first extensive study from this part of India. Our study, done among the type 2 diabetes population

Table 2 Comparison of anthropometric measurements and biochemical estimations between subjects of different age groups

Variable	Group 1: 30–50 years (Mean ± SD)	Group 2: 50–70 years (Mean ± SD)	Group 3: >70 years (Mean ± SD)	P value
Mean duration of diabetes (years)	7.19 ± 5.53	12.29 ± 7.64	17.03 ± 9.83	<0.0001
BMI (kg/m^2)	27.69 ± 5.01	27.88 ± 4.96	27.03 ± 4.87*	0.04
HbA1c %	8.82 ± 2.07	8.77 ± 2.08	8.31 ± 1.83$^{*\#}$	<0.0001
TC (mg/dL)	171.65 ± 45.36	160.54 ± 46.5$^{\wedge}$	150.59 ± 40.14$^{*\#}$	<0.0001
TG (mg/dL)	166.58 ± 106.83	146.41 ± 82.48$^{\wedge}$	125.75 ± 63.31$^{*\#}$	<0.0001
HDL (mg/dL)	40.09 ± 10.38	40.44 ± 10.58	40.04 ± 11.38	–
LDL (mg/dL)	94.08 ± 28.69	86.82 ± 29.42$^{\wedge}$	88.35 ± 29.27$^{*\#}$	<0.0001
Calcium (mg/dL)	8.93 ± 0.56	8.91 ± 0.62	8.87 ± 0.7	–
Vitamin D (ng/mL)	15.34 ± 11.32	17.31 ± 13.55$^{\wedge}$	18.08 ± 14.05$^{\#}$	<0.0001

SD standard deviation, *kg/m^2* kilograms per meter square, *mg/dL* milligrams per deciliter, *ng/mL* nanograms per milliliter

^$P \leq 5$ between groups 1 and 2; *$P \leq 5$ between groups 2 and 3; #$P \leq 5$ between groups 1 and 3

of Chennai, found a very high prevalence (71.4 %) of vitamin D deficiency. A small study (n = 157) among type 2 diabetic subjects of India had reported the prevalence of vitamin D deficiency to be 81.5 %, and observed that the prevalence was not significantly higher compared to normal individuals [16]. Dutta et al. had demonstrated that this condition was equally prevalent among pre-diabetics as well as normal individuals [17].

The NHANES III study had reported considerable difference in the mean vitamin D levels between men and women in the general population of the USA [18]. In an Indian study in the general population, women were found to have higher prevalence of vitamin D deficiency compared with men, both in urban and rural backgrounds [13]. The existence of gender differences in hypovitaminosis among diabetic subjects has not been investigated in an Indian population. We found that men and women had similar prevalence of this condition, and that the mean vitamin D concentrations did not differ significantly between them (men 17.04 ± 12.76 ng/mL, women 16.48 ± 13.53 ng/mL, P = 0.156). Kumar and Haria, while investigating vitamin D deficiency among diabetic Indian subjects, did not evaluate gender differences in their study [16], hence a comparison of our results with this study could not be made. Gender was not found to be a major predictor for vitamin D deficiency among our study subjects. This is in contradiction with the results from the study by Harinarayan et al. where sex was identified as a major contributor to vitamin D levels [13].

Age is an important factor affecting vitamin D levels, and a decline in vitamin D levels has been noted with advancing age [18]. On the contrary, we found that vitamin D levels increased with increasing age, and age was found to be an independent predictor for vitamin D levels in our study. The skewed results could be due to the small number of subjects in the highest age group. Interlink between vitamin D and calcium homoeostasis has been established by numerous investigators [19]. Low calcium intake invariably results in vitamin D deficiency in spite of adequate sun exposure [15]. It should be noted that a correlation between calcium and vitamin D levels was observed among the individuals in the present study. Low dietary intake of calcium in conjunction with vitamin D insufficiency could lead to secondary hyperparathyroidism, and this condition could be particularly relevant in an Indian population whose dietary intake of calcium is low [20].

Studies in an Iranian population have shown that positive correlation exists between BMI and vitamin D levels [21, 22]. On the contrary, a study by Lagunova et al. in 2126 subjects with metabolic syndrome or diabetes has demonstrated an inverse relationship between vitamin D levels and BMI; those with high BMI had lower vitamin D levels [23]. No direct correlation between these two variables was noticed in our study so as to support or negate the above observations. But our findings show that BMI was a confounder that determines vitamin D concentrations in this population.

Inverse correlation between HbA1c and vitamin D levels has been reported in an elderly population [24]. A study among African Americans with diabetes showed that vitamin D supplementation significantly improved their glycemic control [25]. But such findings are yet to be established conclusively as contradicting results have also been reported [26]. Vitamin D concentrations were significantly higher among those subjects with lower HbA1c levels in our study, and HbA1c was found to be an independent predictor of vitamin D status. A similar observation was made by Doddamani et al. in their study in a South Indian population, where HbA1c levels were higher among vitamin D-deficient diabetic subjects compared with those who had optimal levels of vitamin D [27]. Duration of diabetes was shown to predict vitamin D status in our study, but evidence from earlier investigations are lacking.

Conclusions

Our study, done among type 2 diabetes people, show that vitamin D deficiency was highly prevalent among this population. We found that hypovitaminosis was equally prevalent among both genders and across all age groups. BMI, age, calcium levels, and HbA1c were the major confounders for vitamin D concentrations. It would be beneficial to screen diabetic subjects for this condition, since hypovitaminosis is treatable, and if untreated, has been indicated as a risk factor for cardiovascular disease.

Limitations

Interference of environmental factors such as food habits, physical activity, obesity, dietary supplements, etc. were not taken into consideration. Different epidemiological studies have shown the link between vitamin D deficiency and risk of developing diabetes, but due to the retrospective nature of our study, such an association could not be investigated.

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

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

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

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ORIGINAL ARTICLE

Effect of bedtime melatonin consumption on diabetes control and lipid profile

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Abstract Hormonal change in carbohydrate metabolism through the circadian rhythm is well known. An impaired nocturnal insulin secretion and lower level melatonin coexist in diabetics at the end of the night period. Administration of melatonin may improve impaired insulin secretion and control hyperglycemia. In a randomized, double-blind study, 64 type 2 tics were treated for 12 weeks (period 1) with placebo, and then for another 12 weeks (period 2) with 6 mg of melatonin. Fasting blood glucose (FBG), total triglyceride (TG), total cholesterol (CHOL), high-density (HDL), and low-density lipoprotein (LDL) cholesterol and glycosylated hemoglobin levels (HbA1c) were measured at baseline, 12 and 24 weeks. Following 3 months of melatonin treatment, mean HbA1c (±standard error) was significantly lower than at baseline (7.65 % ± 0.086 % versus 7.1 % ± 0.111 %, respectively, $P = 0.0001$). The mean FBG level was significantly decreased at the end of the study (164 ± 5.4 versus 157 ± 5.5, respectively, $P < 0.001$). The HDL cholesterol level increased at the end of the study (42 ± 1.3 versus 45 ± 1.39, respectively, $P < 0.05$) but no significant changes in TG, CHOL, and LDL were observed. Results showed that bed time melatonin administration in patients with type 2 diabetes, improved control of diabetes. Trial registration: IRCT2012062610115N1, http://en.search.irct.ir/search?query=melatonin+and+rezvanfar

Keywords Diabetes · Bedtime · Melatonin · HbA1C · Lipid profile

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Introduction

Melatonin is a neurohormone that is normally secreted by the pineal gland. This nocturnal release mediates entrainment of endogenous circadian rhythms and influences other physiological functions. Melatonin plays an important role in sleep regulation and influences the secretion of hormones involved in carbohydrate metabolism [1, 2]. Melatonin also regulates insulin secretion from isolated islets [2]. Diabetics have increased gluconeogenesis and hyperglycemia with decreased melatonin [3]. Pinealectomy leads to glucose intolerance and insulin resistance [4]. Nocturnal melatonin administration suppresses visceral fat, plasma leptin, and plasma insulin [5] and improve glucose metabolism by restoring the insulin activity on vasculature [6]. Although studies to be contrary exist [7, 8], a growing body of evidence suggests that melatonin administration has a beneficial effect on glycemic control [9–11]. The aim of the current study was to investigate the effect of bedtime melatonin on glucose and lipid metabolism in patients with type 2 diabetes.

Method

Patients

Seventy-six patients with type 2 diabetes with normal kidney function and HbA1c ≤ 8.5 % entered a double-blinded before-after clinical trial. Diabetes mellitus was diagnosed from a previous history of diabetes according to criteria of the American Diabetes Association (ADA) [12].

Patients with confounding factors were excluded from the study. These factors included current pregnancy, breast feeding, history of epilepsy, smoking, drug addiction, alcohol or amphetamine abuse, warfarin, nifedipine, beta-blockers,

diuretics or melatonin consumption, shifting job, coagulopathy or bleeding history.

Ten patients who had arbitrarily increased or decreased their anti-lipid or anti-diabetes medication during period 1 were excluded from the study; two others withdrew from the research, and 64 patients remained and were included in the study (Fig. 1).

This research was conducted according to the Treaty of Helsinki for Human Studies; it was approved by the Ethics Committee of the Arak University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials (code: IRCT2012062610115N1). Written consent in Persian was obtained from patients before participating in the study.

Melatonin administration

After allocation, background information including records of height and weight recorded in the related checklists and all of them entered to period 1. At period 1, two placebo tablets before bedtime were added to their medication for 12 weeks. Then, during period 2 of the study, 6-mg (two 3 mg) melatonin tablets were administered at bedtime to replace the placebo.

The patients and the pharmacist were unaware of the type of prescribed medication. During the study, adherence to medication was evaluated at monthly visits, and complications recorded. Patients were advised to follow the prescribed diabetes diet and continue with their usual activities throughout the duration of the study and to avoid changes in routine.

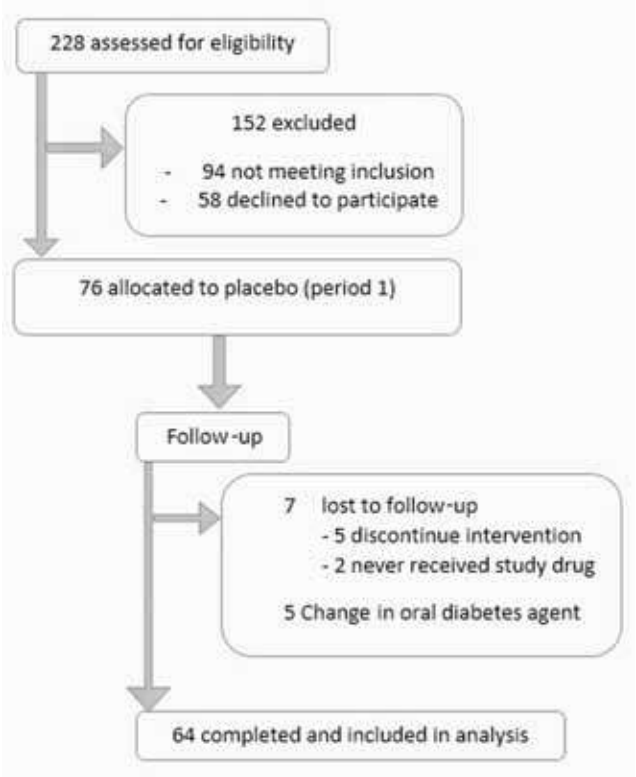

Fig. 1 Melatonin administration

Blood sampling and measurements

The HbA1c values of hemolyzed samples were measured by Cobas Integra 400 (Roche Diagnostics, Mannheim, Germany). Blood samples were obtained early morning after an overnight fast at the subject's home using heparinized tubes and then kept at 4 °C to preserve stability in samples. Serum measurements were taken for FBG, CHOL, LDL, and HDL on Cobas auto-analyser system (ABX Diagnostics, Montpellier, France) at Amir-al-Momenin Hospital. The HbA1c values of hemolyzed samples were measured by Cobas Integra 400 (Roche Diagnostics, Mannheim, Germany).

Data and statistical analyses

All variable was expressed as mean ± SE. Values for plasma concentration of FBG, TG, CHOL, HDL, LDL, and Hba1c are expressed at 0, 12, and 24th week of study.

Variables were assessed statistically using the repeated-measure method in a general linear model. Where a significant effect of time or treatment was indicated, the post hoc least significance difference (LSD) test for pairwise comparison of mean values was used to determine statistical differences. For all comparisons, statistical significance was determined at $P < 0.05$.

Results

In this study, 76.5 % of the subjects were women. The mean age of participant was 52 ± 8 years (range of 36–59 years). The mean duration of diabetes was 7 ± 2 years (range of 3–9 years). FBG at the baseline was lower than 126 mg/dl in 20.3 % and higher than 200 in 17.2 % of patients. However, HBA1C at baseline was higher than 7 % in 20.3 % and higher than 8 % in 40.6 % of patients. 21.9 % of subjects had a HDL level higher than 50 at baseline, which showed an increase of 31.3 % at the end of intervention.

Averages of diabetic and lipid control indexes are shown in Table 1. As shown in the table, the average baseline value of HBA1C was 7.65 % and it reached 7.1 % at the end of the study.

Repeated-measures ANOVA was used to examine changes in the mean values of the studied indexes in three measurements (data not shown). Results showed a significant decrease in HBA1C ($P = 0.0001$) and a significant increase in HDL ($P = 0.001$) at the end of intervention. A post hoc (LSD) test was carried out for pairwise comparison of mean values (data not shown).

Table 1 Metabolic parameters at baseline 12 and 24 weeks

Variables	Week			*p* value Between Week		
	0	12	24	0–12	0–24	12–24
	Mean ± SE	Mean ± SE	Mean ± SE			
Weight /kg	68.5 ± 1.3	69.1 ± 1.1	68 ± 0.95	NS	NS	NS
HBA1C %	7.65 ± .08	7.50 ± .09	7.16 ± .11	0.01	0.001	0.001
FBG mg/dl	164.2 ± 5.3	157.5 ± 5.5	146.8 ± 5.6	NS	0.001	0.001
CHL mg/dl	196.2 ± 5.3	205.7 ± 5.2	197.7 ± 4.8	0.03	NS	NS
TG mg/dl	157.3 ± 7	162.2 ± 7.8	169.3 ± 8.2	NS	NS	NS
LDL mg/dl	124.4 ± 4.7	125.8 ± 4.5	118.3 ± 4.5	NS	NS	NS
HDL mg/dl	42.6 ± 1.3	45 ± 1.4	45 ± 1.3	0.04	0.01	NS

Discussion

Our results show that 3 months of consumption of melatonin improves glycemic control and increased HDL cholesterol. There were no complications associated with the administration of melatonin. Hussain et al. showed improved glycemic control with melatonin use. When administered with zinc limiting a precise conclusion on the independent effect. Large data are available from the experimental animals. [13].

Hypoinsulinemia and hyperleptinemia are features in rats treated with melatonin for 30 weeks [14]. In another experiment, administration of melatonin was associated with weight loss and decreased intra-abdominal fat and leptin in rats. Moreover, increased insulin sensitivity and decreased plasma insulin levels without affecting total food take and total obesity were noted [15]. In another study, increased hepatic insulin resistance, conversion of greater amounts of pyruvate to glucose at the end of the night, and intensified gluconeogenesis were observed in rats that had undergone pinealectomy [16].

In contrast to our findings, there is evidence indicating that melatonin decreases insulin secretion and causes blockage of melatonin receptors on the pancreas, increases plasma insulin, and decreases blood glucose levels [7, 8, 17, 18]. Although insulin sensitivity or the degree of insulin secretion was not measured, it is clear that taking melatonin had a positive clinical effect on glycemia. Although improved HbA1c in patients in this study may have been due to their participation, better adherence to diet, or more careful consumption of their medication. Alternatively, it could have been due to the beneficial effects of melatonin. Patients were treated with a placebo for the first 12 weeks so a further reduction in HbA1c over the next 12 weeks seems to indicate the notable effect of melatonin on glucose metabolism.

Melatonin improves sleep quality and insomnia [19, 20]. There is evidence to support the beneficial effect of sleep quality on HbA1c in type 2 diabetes [21–23]. Increased hepatic glucose output leads to higher fasting blood glucose in type 2 diabetes. This high nocturnal gluconeogenesis is not observed in l non-diabetics. Melatonin is closely related to endogenous glucose production, and melatonin secretion is reduced in diabetics. This effect may be due to reduced ability of supra optic hypothalamus nucleus that reduces production of melatonin in patients with type 2 diabetes [3, 24]. Melatonin administration may prevent development of type 2 diabetes [25] and improve control of diabetes [26, 27].

Consumption of melatonin at night can adjust the human internal clock and is able to affect internal liver production of glucose and improve FBG and diabetes control [1]. The reduced mean FBG following melatonin consumption in our study confirms these observations. The reduction of Hba1c by 0.5 % following melatonin consumption has clinical relevance since 1 % reduction in Hba1c was been shown to have an association with a 21% reduction in any end point related to diabetes [28, 29] milder reductions will also be potentially beneficial. Another finding is the increased HDL level. In a small number of pre- and post-menopausal women, Tamura and colleagues showed similar positive result with 1 mg of melatonin l without affecting total cholesterol level [19].

This study was limited as insulin resistance, insulin sensitivity, and glucose circadian rhythms were not measured. Future studies addressing these metabolic indices in subjects given melatonin may consolidate our results.

Author contributions Mohammad Reza Rezvanfar contributed to the concept/design, drafting of the manuscript, approval of the article. Gila Heshmati contributed to the acquisition of data and drafting of the manuscript. Farshid Haghverdi contributed to the concept/design and acquisition of data. Mahnaz Edalat nejad contributed to the concept/design and approval of the article. Ali Chehreie contributed to the concept/design and data analysis/interpretation. Fatemeh rafiee contributed to the data analysis/interpretation and drafting of the manuscript. Faezeh rezvanfar contributed to the drafting of the manuscript and approval of the article.

Compliance with ethical standards

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

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

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

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ORIGINAL ARTICLE

Vitamin D deficiency and the associated factors in children with type 1 diabetes mellitus in southern Iran

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Abstract Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder caused by destruction of beta cells of the pancreas. Several reports have suggested a connection between vitamin D deficiency and T1DM and the possible role of dietary vitamin D supplementation in reducing the risk of T1DM. There is little knowledge about the prevalence of vitamin D deficiency among Iranian children with T1DM. Serum 25-hydroxy vitamin D (25OHD) was assayed by high performance liquid chromatography in 8–18-year-old diabetic patients referred to pediatric diabetes clinics in Shiraz, Iran, during a period of 14 months. The age of the onset of T1DM, daily insulin usage, weight, height, and BMI of each patient were recorded along with levels of physical activity and sun exposure. The patients' body composition was determined by DEXA and used in further analysis. This study was conducted on 39 diabetic boys and 46 diabetic girls aged 12.4 ± 4.2 years. Mean serum 25(OH)D3 was 18 ± 12.2 ng/dl. Serum levels of 25(OH)D3 were higher in boys than girls. 7.7 % of the boys and 30.4 % of the girls had severe vitamin D deficiency. There was a negative correlation between the age of the onset of T1DM and serum concentration of 25(OH)D3 (p = 0.006, r = −0.17). Girls with T1DM showed a higher prevalence of severe vitamin D deficiency than boys with T1DM. Moreover, vitamin D deficiency was more prevalent in individuals with earlier onset of the disease and in those with higher fat mass index.

Keywords Vitamin D · Children · Type 1 diabetes mellitus · Iran · Body composition

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder caused by destruction of the pancreatic beta cells [1], resulting in decreased insulin secretion and ketosis-prone hyperglycemia [2]. The prevalence of T1DM is estimated to be 35 million people worldwide with variations in different geographical and racial groups [3]. Both genetic and environmental factors combine to cause this complex disorder [4]. Previous reports have shown that hypovitaminosis D remains a major health problem worldwide [5–7]. Similar reports in Kuwait [8], India [9], and United Arab Emirates [10] show that vitamin D deficiency rickets is common in the infantile period. Prolonged breast feeding without vitamin D supplementation, maternal vitamin D deficiency, and limited sun exposure may be the contributing factors [10]. Previous studies revealed a worldwide epidemic of vitamin D deficiency and T1DM as well [3, 5, 6, 10–12]. It was also shown that dietary vitamin D reduces the risk of T1DM [13]. Some investigations indicated that vitamin D deficiency might induce autoimmune destruction of β cells and cause T1DM through loss of immune modulation of vitamin D [14, 15]. There is a highly variable prevalence of vitamin D deficiency in children with T1DM, ranging from 15 to 60 % in different countries around the world [16, 17].

Although the high prevalence of vitamin D deficiency has been documented in normal Iranian children [18], there is still limited data on Iranian children with T1DM [19]. It is essential to assess the existing status of vitamin D levels in this population, before conducting a clinical trial of supplementing vitamin D in Iranian children with T1DM. In this paper, we

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aimed to evaluate vitamin D status in Iranian children and adolescents with T1DM.

Material and methods

This cross-sectional study was performed on children with T1DM who were referred to pediatric diabetes clinics affiliated to Shiraz University of Medical Sciences from July 2013 to August 2014. We had used Med Calc software to calculate sample size in this study. Sample size was calculated as 61, considering $\alpha = 0.05$, $\beta = 0.1$(power = 1-$\beta = 0.90$), P1 = 70, and P0 = 50. To find more accurate results, 85 children with type 1 diabetes were enrolled in this study.

Inclusion criteria were age less than 18 years old, fasting blood sugar (FBS) >125 mg/dl, having clinical symptoms of T1DM (e.g., polyuria, polydipsia), insulin dependency to maintain FBS in the normal range, and affliction with TIDM for a duration of more than 2 years. Diagnosis of T1DM was confirmed by the presence of two positive autoantibody tests (glutamic acid decarboxylase antibodies, islet cell antibody and insulin autoantibody) in the patient's serum [20]. Exclusion criteria were previous diagnosis with chronic liver, kidney or heart disease, calcium and/or vitamin D supplementation, and metabolic bone disease.

Anthropometric measurements, puberty, physical activity, sun exposure, and insulin regimen

One trained physician recorded the patients' weight and height and also evaluated their tanner stages. Their weight was measured with a single standard scale (seca, Germany). Children wore light clothing and the measured weights were rounded to the nearest 0.1 kg. Their height was measured with a standard wall-mounted stadiometer when the children stood upright shoeless, and the measured heights were rounded to the nearest 0.5 cm.

Body mass index (BMI) was calculated by dividing the children's weight in kilograms by height^2 in squared meter. Pubertal stage was evaluated according to the five-stage tanner classification [21, 22]. We divided the children into two groups regarding their physical activities: Those who performed less physical activity than it was recommended by American College of Sports Medicine (which is three times per week) and those who did according to, or more than this recommendation [23]. Children were also classified according to their mean exposure to sunlight per day during autumn (<15 min/day, 15–30 min/day, and >30 min/day). Daily insulin usage was recorded as units per kilograms per day.

Biochemical studies

Five milliliters of venous blood was obtained from each participant and then centrifuged and stored at −20 °C. Serum 25-hydroxy vitamin D (25OHD) was assayed by high performance liquid chromatography (young lee 9001, South Korea). It is worth mentioning that serum 25OHD was measured in autumn, during which classification of sun exposure was recorded. Inter-assay coefficient of variation (CV) for 25OHD was 3.3 %. All the samples were checked for serum calcium, phosphorous, and alkaline phosphatase by colorimetric assay with an auto-analyzer (Biosystems SA Barcelona, Spain).

Vitamin D deficiency was defined according to the latest guideline released by the Endocrine Society for clinical practice. According to the published guidelines, vitamin D deficiency is defined as serum 25OHD below 20 ng/ml (nmol/l) and vitamin D insufficiency is defined as serum 25OHD of 21–29 ng/ml [24].

Body composition measurements

Dual-energy X-ray absorptiometry (DXA; Discovery QDR, USA) was used to check the total fat mass (g) and total lean mass (g); it was performed when the children were wearing special clothing and no shoes. Lean mass index was calculated by lean mass/height^2 (kg/m^2) and fat mass index was calculated by fat mass/height^2 (kg/m^2). The coefficient of variation (CV) in our laboratory was 0.8 % for fat mass and 1.8 % for fat percentage and lean mass.

Statistical analysis

Data were shown as mean and ± standard deviations. The Kolmogorov-Smirnov test was used to evaluate the normality of data distribution. Student's *t* test and the Mann-Whitney U test were used to compare the normal and non-normal distribution of data, respectively. Correlations between normal distributed parameters were determined using Pearson's chi-squared test and correlations between non-normal distributed ones were determined using Spearman's correlations. All variables that had a *p* value less than 0.2 in the univariate analysis were included in a multivariate binary logistic regression analysis. To assess their independent effect on 25OHD concentration, a *p* value of less than 0.05 was considered significant. Analysis was done using SPSS15.

Ethics

The study was approved by the Shiraz University of Medical Sciences Ethics Committee. Written informed consent form was signed by all the participants and their parents.

Results

To the best of our knowledge, the present study is the first to evaluate the relationship between the serum level of vitamin D and body composition in diabetic children. This study included 39 diabetic boys and 46 diabetic girls (total number 85 participants) aged 12.4 ± 4.2 years. T1DM in these children was manifested at the age of 8 ± 4 years. Mean level of serum 25(OH)D3 was 18 ± 12.2 ng/dl. We find that 78(91 %), 5(6 %), and 2(3 %) of patients with diabetes were lean, overweight, and obese, respectively. General characteristics and results of DEXA determined body composition of T1DM children who were classified by sex and summarized in Table 1. Serum level of 25(OH)D3 in boys was more than that in girls ($p < 0.001$). Total fat mass and fat mass index were greater in girls ($p = 0.046$ and 0.001), but lean mass index was greater in boys ($p = 0.001$). Vitamin D status of diabetic patients is summarized in Fig. 1. Serum concentration of 25(OH)D3 was greater in boys ($p = 0.001$) and there was a positive correlation between sun exposure and serum level of 25(OH)D3 ($p = 0.04$). 7.7 % of the boys and 30.4 % of the girls had severe vitamin D deficiency. There was a negative correlation between the age of the onset of T1DM and serum concentration of 25(OH)D3 ($p = 0.006$, $r = -0.17$). Total fat percent (%) and fat mass index had a negative correlation with serum concentration of 25(OH)D3 ($p < 0.006$, $r = -0.42$, $p = 0.003$, $r = -0.33$, respectively). Results of analysis are summarized in Table 2. There was no significant correlation between age, weight, height and BMI of diabetic children and serum level of 25(OH)D3. Moreover, the type of insulin therapy, duration from the onset of the disease, HbA1C levels, daily insulin usage, and lean body mass do not have any associations with serum levels of 25(OH)D3.

Discussion

The present study showed that severe vitamin D deficiency occurred in girls with T1DM more than the boys. 15.4 % of the boys with T1DM and 8.2 % of the girls with T1DM had a normal serum 25(OH)D3 concentration. Furthermore, this study revealed that sun exposure has a positive association with the serum levels of 25(OH)D3. However, the age of the onset of T1DM, total body fat mass, and total fat percentage (%) had negative associations with serum 25(OH)D3.

Similar to our report, 25(OH)D3 deficiency was present in patients with T1DM from Sweden, Egypt, Qatar, Saudi Arabia, and Italy [19, 25–29]. Vitamin D interacts with glycemia at multiple levels through increased autoimmunity, beta cell loss, insulin resistance, and systemic inflammation [30–37]. Vitamin D deficiency is considered to play a role in immune-mediated *ß* cell destruction and might lead to the

Table 1 General characteristics and DEXA determined body composition of children with T1DM, classified by sex

Parameter	Total		Boys		Girls		*p* value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	12.4	4.2	12.9	4.4	11.9	4	0.61
Age of disease onset (years)	8	4	8.6	4.4	7.5	3.6	0.23
Duration of disease (years)	4.4	2.8	4.6	2.1	4.4	3.4	0.23
Weight (kg)	39.7	15.3	42.7	16.6	37.3	13.9	0.23
Height (cm)	146.2	20. 1	149.6	22.6	143.3	17.6	0.08
BMI (kg/m^2)	17.8	3.2	18.2	3.2	17.4	3.1	0.91
25(oH)vit D	18	12. 2	23.8	14.8	13.1	6.3	<0.001
Albumin	4.2	0.3	4.2	0.3	4.2	0.3	0.47
FBS	228	108	207	105	246	109	0.06
Alkaline phosphatase	7.8	3.8	779	318	664	311	0.91
Calcium	9.6	1.1	9.5	1.3	9.7	0.97	0.39
Phosphorous	5	0.6	5.2	0.7	4.9	0.6	0.23
HbA1C	10.2	2.2	10.1	2.2	10.2	2.3	0.76
Total insulin (unit/day)	21.8	13	22.7	12.7	21.1	13.4	0.92
Daily insulin per kg (unit/kg/day)	0.7	0.27	0.67	0.29	0.72	0.25	0.53
Total fat (g)	11,007	5094	10,653	4752	11,310	5408	0.47
Total fat (%)	28.4	5.8	25.4	5.6	31	4.7	<0.001
Fat mass index (g/m^2)	4.9	1.6	4.5	1.6	5.3	1.6	0.046
Lean mass index (kg/m^2)	12.2	2.1	13.1	2.1	11.5	1.8	0.001
Total lean mass (g)	26,316	10,256	30,172	11,677	23,012	7544	0.002
Android/gynecoid fat ratio	0.7	0.12	0.73	0.14	0.68	0.11	0.08

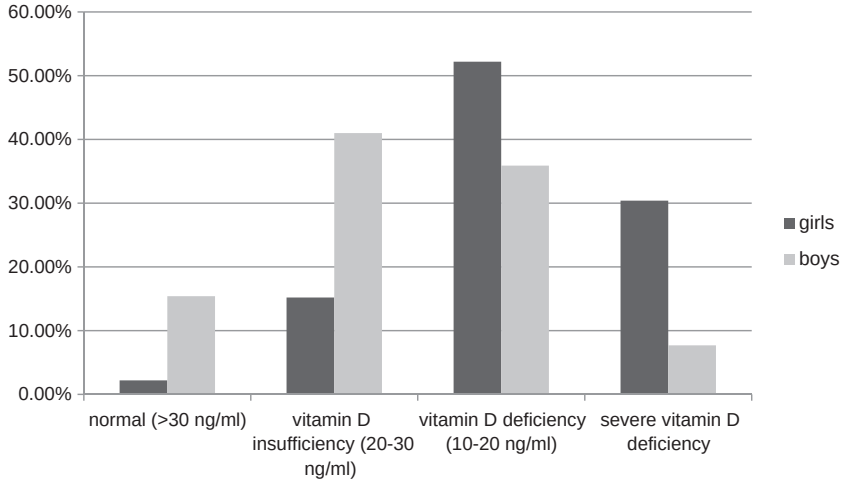

Fig. 1 Vitamin D status in T1DM children. Pearson's chi-square shows that prevalence of vitamin D deficiency in girls was more than that in boys ($p = 0.001$)

onset of clinical diabetes [30, 31]. In one study conducted to determine the effect of vitamin D supplementation during the first year of life in seven European countries, it was revealed that the risk for T1DM by the age 15 was reduced to one-third in the vitamin D supplemented group [32]. Another study revealed that vitamin D deficiency/insufficiency might have an important role in the development and worsening of insulin resistance in Indian with prediabetes who have a high cardiovascular risk. [33] Also, Dutta et al. suggested that low serum vitamin D might be associated with increased progression to diabetes, perhaps via modulation of albumin: creatinine ratio [34]. He also revealed that vitamin D supplementation in prediabetes reduced

Table 2 Association of serum level of 25 (oH) with general characteristics, laboratory data, and body composition of children with type 1 diabetes mellitus. (Factors with p value of less than 0.2 univariant analysis entered into multiple regression analysis to evaluate independent association with log of 25 (OH) vitamin D)

Parameter	Univariant (Spearman's test)	Analysis with 25(oH) D	Regression analysis with log 25 (OH) D	
	p value	Correlation coefficient	p value	Adjusted $r2$
Age (years)	0.24	−0.13	–	–
Weight (kg)	0.42	−0.08	–	–
Height (cm)	0.43	−0.08	–	–
BMI (kg/m^2)	0.89	−0.01	–	–
Sex	0.001	–	<0.001	0.36
Sun exposure	0.056	–	0.85	0.36
Physical activity	0.17	–	0.67	0.36
Tanner stage	0.14	–	0.25	0.36
Type of insulin	0.62	–	–	–
Age of onset of DM	0.11	−0.17	0.006	0.36
Duration of DM	0.15	0.15	0.34	0.36
HbA1C	0.37	−0.1	–	–
FBS	0.39	−0.09	–	–
Daily insulin per kg	0.87	−0.2	–	–
Daily total insulin	0.5	−0.07	–	–
Total fat percent (%)	<0.001	−0.42	0.006	0.36
Fat mass index	0.003	−0.33	0.056	0.36
Lean mass index	0.63	0.05	–	–
Total lean mass	0.69	−0.04	–	–
Android fat/Gynecoid fat	0.18	−0.15	–	–

progression to type 2 diabetes and was associated with low insulin resistance and systemic inflammation [35].

Significant differences in the serum levels of 25(OH)D3 between boys and girls was in agreement with our results and also with reports from previous studies [36, 37]. This fact could be explained by differences in the sun exposure and differences in clothing between males and females [19, 38]. Earlier and faster growth spurts during pubertal age in girls could be a further cause of higher prevalence of vitamin D deficiency in Iranian of female patients with diabetes [37, 38]. Previous reports in Qatar, Turkey, and Saudi Arabia also showed a high prevalence of vitamin D deficiency in females, because of wearing concealing clothes and restriction in outdoor activities [19, 26, 39, 40]. On the other hand, Saki et al. recently revealed that vitamin D deficiency is highly prevalent among normal children in the south of Iran and it was related to low physical activity, insufficient sun exposure, advanced age, and pubertal stage [41]. So, the high occurrence of vitamin D deficiency in T1DM children in Iran might be an indicator of the general high prevalence of vitamin D deficiency and insufficiency in the Iranian population.

Studies in Egypt and Iran showed a negative association between BMI and serum concentrations of 25(OH)D3 in children with type 1 diabetes mellitus [26, 38], but we did not find any associations between BMI and serum levels of vitamin D. However, we found that there is a significant negative association between the total body fat percentage or body fat mass index and serum levels of 25(OH)D3. This finding was in concordance with the results from national and heath survey in the USA in which the prevalence of metabolic syndrome was higher in obese adolescents with vitamin D deficiency [42].

The present study did not find any associations between duration from the onset of T1DM, daily insulin usage, HbA1C levels or type of insulin therapy, and serum vitamin D; however, it showed a significant negative correlation between the age of the onset of T1DM and serum levels of vitamin D after omitting the confounding factors. Lack of association between daily insulin requirement and vitamin D level in the serum of patients with T1DM was also seen in Thnc et al.'s study [43]. Daga et al. also revealed that vitamin D deficiency was common among people with youth-onset diabetes [44]. The Eurodiab's study showed that vitamin D supplementation in early years of life could reduce the risk of T1DM as much as 30 % [45].

This study revealed that fat mass index (but not lean mass index or BMI) has a negative association with serum vitamin D, and android/gynecoid fat ratio was not associated with the serum concentration of vitamin D. In a previous study, bone mineral density (BMD) of children was shown to have a greater association with lean mass index compared to fat mass index [46]. However, according to this study, the serum level of vitamin D was not associated with lean mass index and, therefore, could not be considered as the responsible factor for correlation between BMD and lean mass index. It seems that fat mass index might be a more reliable predicative factor of vitamin D status in children with T1DM. It may be explained by the fact that vitamin D is sequestered in the subcutaneous fat which could lead to reduction in its bioavailability [47]. Also, increased leptin levels released from the excess body fat could inhibit the activation of vitamin D in kidneys [48].

Conclusion

It can be concluded that vitamin D deficiency is prevalent among children with T1DM, especially in girls and in those with earlier onset of the disease. Vitamin D deficiency is also more prevalent in patients with higher fat mass index. Further studies should be done to evaluate the efficacy of prevention and early treatment of vitamin D deficiency, especially in obese girls with earlier onset of the disease.

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

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CASE REPORT

Celiac crisis in an adult type 1 diabetes mellitus patient presented with diarrhea, weight loss and hypoglycemic attacks

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Abstract Type 1 diabetes mellitus (T1DM) is an autoimmune disease, characterized by loss of the insulin-producing β cells of the islets of Langerhans in the pancreas, causing insulin deficiency. Celiac disease has been seen in 3 to 8 % of T1DM patients. Celiac crisis, an acute severe onset of celiac disease, is a rare and life-threatening manifestation. We report a 50-year-old man with type 1 diabetes mellitus who arrived at our service with a 2-month history of watery diarrhea associated with hypoglycemic attacks, abdominal pain, and weight loss of 13 kg. The diagnosis of celiac crisis was made based on diarrhea leading to dehydration, severe metabolic and electrolyte abnormalities, and subsequent improvement after introduction of a gluten-free diet.

Keywords Celiac disease · Type 1 diabetes mellitus · Hypoglycemic attacks

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease, characterized by loss of the insulin-producing β cells of the islets of Langerhans in the pancreas, causing insulin deficiency [1]. Celiac disease (CD) is characterized by impaired immunological response to ingested gluten, and 3 to 8 % of type 1 diabetes mellitus patients have CD [2]. Based on a recent metaanalysis, more than one in 20 patients with type 1 diabetes have biopsy-verified celiac disease [3]. Gluten consumption might be a shared causative factor for the development of T1DM and CD [4]. CD and T1DM have a common genetic predisposition characterized by the same HLA pattern, namely HLADQ2 and/or -DQ8 [5]. CD observed in T1DM is classified as silent in approximately half of cases due to the lack of symptoms suggestive for CD [6]. Celiac crisis is a life-threatening condition in which CD causes acute dramatic metabolic impairments. Severe diarrhea, hypoproteinemia, and metabolic and electrolyte disturbances requiring hospitalization are common manifestations of celiac crisis [7]. The diagnosis of celiac crisis needs at least two of the following in a patient with acute onset or rapid progression of gastrointestinal symptoms owing to celiac disease requiring hospitalization and/or parenteral nutrition: (1) Signs of severe dehydration including hemodynamic instability and/or orthostatic changes; (2) renal dysfunction, creatinine level >2.0 g/dL; (3) neurologic dysfunction; (4) metabolic acidosis, pH <7.35; (5) hypoproteinemia (albumin level, <3.0 g/dL); (6) abnormal electrolyte levels including hypernatremia/hyponatremia, hypokalemia, hypocalcemia, or hypomagnesemia; (7) weight loss, >10 lb. [8].

Here, we will discuss a type 1 diabetic patient presented with hypoglycemic attacks, diarrhea, and weight loss.

Case presentation

A 50-year-old man with a 9-year history of type 1 diabetes mellitus presented a 2-month history of diarrhea associated with

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loss of appetite, nausea, abdominal pain, arthralgia, and weight loss of 13 kg. Stools were in large volume, watery, without blood, or mucus, and diarrhea worsened after meals. The patient had hypoglycemic attacks, his HbA1c level at admission was 7.5 %, and his c-peptide level was <0.1 ng/ml. His anti-insulin antibody titer was 4.39 % (>18 positive, 12–18 suspicious <12 negative). His anti-GAD antibody titer was 1.71 U/mL (normal range 0–1). His diabetes was controlled by biphasic insulin aspart 30 with two times in a day, total dose of 48 IU. His mother has type 2 DM. On admission, he was afebrile and his vital signs are in normal range. His weight was 58 kg, and his height was 178 cm (BMI 18.3 kg/m^2). Initial laboratorial findings revealed hyperglycemia, metabolic acidosis with hyperchloremia, hypokalemia, hypocalcemia, and hypoalbuminemia. He had iron and folate deficiency. Hemoglobin level was 9.4 g/dl, and his mean corpuscular volume was 79.9 fL. HIV status and viral hepatitis serology were negative, and stool examination did not suggest an infectious etiology. Diarrhea was thought to cause hyperchloremic, normal anion gap metabolic acidosis, and hypokalemia. Fluid administration and electrolyte replacement were started, but the patient continued to present watery diarrhea and weight loss. His insulin regimen was changed to basal-bolus therapy. For evaluation of diarrhea, serological blood tests for celiac disease were done; antiendomysium antibodies of the immunoglobulin A (anti EA IgA) was 158.7 U/mL (N: 0–20 U/mL), tissue transglutaminase of the immunoglobulin A (tTGA IgA) was >200 U/mL (N:0–20 U/mL). Endoscopy revealed atrophic folds and scalloping at the bulbus and second part of the duodenum. Histological examination of duodenal biopsy specimens revealed lymphocyte infiltration, crypt hyperplasia, and villous atrophy to be compatible with celiac disease grade 3 according to the Marsh classification. After introduction of the gluten-free diet, he gained weight, his metabolic abnormalities resolved and he had better glycemic control (Table 1).

Discussion

Celiac disease is a gluten-sensitive enteropathy. Hyperplastic villous atrophy with hyperplasia of the crypts and an abnormal surface epithelium are characteristic, although not specific, abnormalities that are reversed by withdrawal of gluten from the diet, found in the small intestine. The diagnosis of celiac disease is confirmed when both clinical and pathological abnormalities are reversed by gluten elimination [9].

The celiac disease is classified according to the presence of gastrointestinal symptoms. Symptomatic or classical celiac disease refers to presentations with diarrhea, with or without a malabsorption syndrome, whereas in asymptomatic, atypical or silent celiac disease gastrointestinal symptoms are absent or not prominent [10]. Our patients had classical symptoms including diarrhea, abdominal pain, weight loss, and findings including hypocalcemia, hypoalbuminemia, iron deficiency, and folate deficiency, which are consistent with malabsorption.

tTGA IgA is the most sensitive test for CD (up to 97 %) whereas anti-EA IgA is used as a confirmatory test in tTGA IgA-positive cases due to their higher specificity (about 100 versus 91 % of tTGA) [11]. Both antibodies were positive with high titers in our patient. The definition of the spectrum of histological changes in celiac disease, as classified by Marsh, has provided a major advance in the diagnosis of celiac disease [12]. Our patient's histologic findings including lymphocyte infiltration, crypt hyperplasia, and villous atrophy are compatible with celiac disease grade 3 according to the Marsh classification that is now widely used in diagnosing celiac disease in clinical practice.

Patients with CD often have low cholesterol levels. High or high–normal cholesterol among patients with hypochromic anemia could possibly be used to exclude celiac disease [13]. Our patient has hypochromic anemia and low cholesterol levels. Celiac crisis had been thought of

Table 1 Labaratory values

Biochemical parameters	Normal range	Before celiac disease diagnosis	2 months after gluten-free diet
Na (mEq/L)	132–146	138	136
K (mEq/L)	3.5–5.5	2.5	5.1
Mg (mg/dL)	1.3–2.7	1.4	1.8
Cl (mEq/L)	99–109	117	100
HCO_3 (mmol/L)	21–26	11.8	29
Urea (mg/dL)	19–48	9	28
Creatinine (mg/dL)	0.7–1.3	0.56	0.6
Total calcium (mg/dL)	8.6–10.4	7.4	8.8
Phosphorus (mg/dL)	2.4–5.1	2.4	4.4
Albumin (g/dL)	3.2–5.0	3.0	4.3
Hematocrit (%)	34.35–47.72	31.0	38.3
LDL-cholesterol (mg/dL)	0–100	67	101

primarily as a childhood disease. In the literature, celiac crisis in adults have rarely been notified, and for this reason, celiac disease rarely is considered in adults presenting with acute severe diarrheal illness, even when infectious etiologies have been excluded [8].

After excluding other reasons of acute diarrhea and based on the patient's findings including metabolic acidosis, hypoproteinemia, hypocalcemia, hypokalemia, hypomagnesemia, and weight loss of 13 kg, our patient was diagnosed with celiac crisis. Advising a gluten-free diet results in prompt and dramatic improvements in the patient's symptoms.

Conclusion

Celiac crisis has high morbidity. Besides, this kind of crisis often has a clear precipitating factor and occurs in adults although it is rarely described. A type 1 diabetic patient who present with severe unexplained diarrhea and malabsorption should be tested for celiac disease.

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CASE REPORT

Alstrom syndrome—a diagnostic dilemma

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Abstract Alstrom syndrome is a rare autosomal recessive genetic disorder first described in 1959. The syndrome with an estimated prevalence of less than 1 in 1 million has about 700 cases reported worldwide and only about 20 cases have been reported from India. The features of the syndrome include progressive retinal dystrophy and sensorineural deafness with phenotypic similarity to features of metabolic syndrome. Authors report a case of a 9-year-old boy referred to tertiary care hospital for evaluation of elevated blood glucose with polyuria, polydypsia and polyphagia. The child presented with blindness since childhood with delayed motor milestones. The child has normal intellect and noted to have central obesity, hypertension, acanthosis nigricans and micropenis with atrophic testes. Investigations revealed hyperglycemia with glycated haemoglobin of 10 %, hypertriglyceridemia and microalbuminuria. DNA sequence analysis showed a homozygous mutation detected in exon 10 of *ALMS1* gene resulting in insertion of 'T' between 8150 and 8151 nucleotides of exon 10, thereby resulting in replacement of Ser by Phe at codon 2719 and development of Alstrom's syndrome. The boy was treated with insulin and metformin for elevated blood glucose and fibrates for hypertriglyceridemia.

Keywords Alstrom syndrome · Obesity · Hyperglycemia · *ALMS1* mutation

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Background

Alstrom syndrome (AS; online Mendelian inheritance in man (OMIM) 203800) is a rare autosomal recessive genetic disorder which was first described in 1959, by Carl Henry Alstrom. The syndrome with an estimated prevalence of less than 1 in 1 million has about 700 cases reported worldwide and only about 20 cases have been reported from India [1]. The distribution of AS is spread all over the world with no gender predilection. The clinical features usually start from childhood with congenital progressive cone-rod retinal dystrophy leading to blindness, sensorineural deafness and truncal obesity (Fig. 1) [2]. They also have features of insulin resistance with hyperinsulinemia, type 2 diabetes and acanthosis nigricans (Fig. 2) which develop at a median age of 16 years [3]. The other biochemical alteration is hypertriglyceridemia which may lead to pancreatitis. The endocrinal abnormalities found in these individuals are hypothyroidism and hypogonadism with gynecomastia and reduced fertility [4]. Increased incidence of serous otitis media and fluid retention has also been reported [3]. The following case presented to the authors with

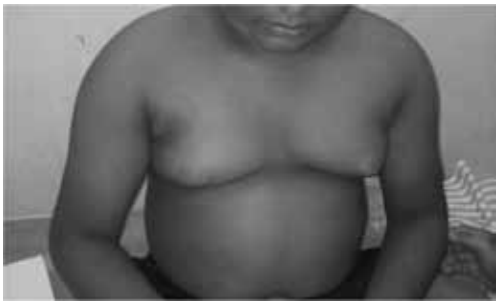

Fig. 1 Childhood obesity

all features of AS with early age of onset of insulin resistance and hypertriglyceridemia.

Case presentation

A 9-year-old boy was referred to the paediatric outpatient department of a tertiary care hospital, for evaluation of elevated blood glucose levels done at a private laboratory in his native town. Further history showed history of increased frequency of urination (>10 times), increased hunger and thirst, for the past 3 days. He also complained of drowsiness and excessive fatigability since the last few days. His past history revealed poor vision since birth and he was diagnosed to be having blindness from the age of 5 months with consequent delay in both gross and fine motor milestones. Ophthalmologic evaluation (electroretinogram) done at another centre, a year ago, suggested cone dystrophy; he was suspected to be having Bardet–Biedl syndrome. Karyotype analysis was normal. There was no history of hospitalization in the past. He has been attending special school for the blind and has no difficulty in learning or hearing. However, he had frequent episodes of serous otitis media since the age of 6 years. He is the only child of consanguineous parents, both alive and well. On physical examination, he had stable vital parameters with blood pressure of 130/70 mmHg (at 95th percentile). There was evidence of central obesity with body mass index of 24.24 kg/m^2 (above 97th percentile). The patient had a flat occiput and frontal bossing with deeply inset eyes. The fingers were short and stubby with no evidence of poly- or syndactyly as expected in Bardet–Biedl syndrome. The feet were wide and the 4th metatarsal was short (Fig. 3). The patient also had a micropenis with atrophic testes.

Investigations

Initial investigations revealed the following: fasting blood glucose—181 mg/dL, glycosylated haemoglobin (HbA1C)—10 %, fasting insulin levels—94.93 μIU/mL, homeostasis model assessment insulin resistance (HOMA-IR)—42.43, fasting triglyceride level—1183 mg/dL, high density lipoprotein cholesterol (HDL-C)—25 mg/dL, serum creatinine—1.4 mg/dL, microalbuminuria—89.8 mg/L, serum thyroid stimulating hormone (TSH)—5.16 μIU/mL (Ref range for serum TSH: 0.7–6.4 μIU/mL, for 5 months—20 years age), and free thyroxine (FT4)—0.888 ng/dL (Ref range FT4: 0.8–2.0 ng/dL). Peripheral blood smear showed microcytic hypochromic anaemia with lymphocytic leukocytosis. Ultrasound of abdomen showed hepatomegaly (19 cm) with cholestatic and fatty changes.

DNA isolation and sequence analysis Informed consent was obtained from the parent of the subject. Genomic DNA was isolated from the subject's whole blood sample at Manipal Diagnostic and Research Centre functioning under Manipal School of Life Sciences, at Mangalore. The genomic DNA was then shipped to The Jackson Laboratory, USA, for sequence analysis. On analysing the sequence by next-generation sequencing [5], a homozygous mutation was detected in exon 10 of *ALMS1* (c.8150_8151insT, p. Ser2719Phefs*7). This was a novel mutation detected and is updated in the current list of mutations related to AS in the Euro–Wabb Project Open Variation Database [6]. In this novel mutation, there was an insertion of 'T' between 8150 and 8151 nucleotides of exon 10, thereby resulting in replacement of Ser by Phe at codon 2719. The novel homozygous mutation may be due to the consanguineous union of the parents as evidenced in other similar cases reported earlier [6].

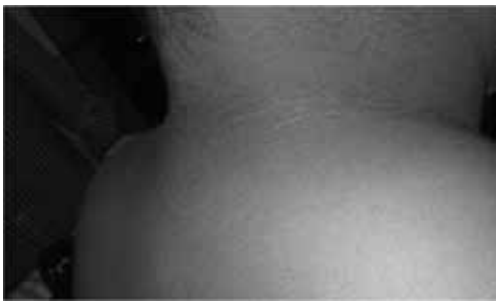

Fig. 2 Acanthosis nigricans of the neck

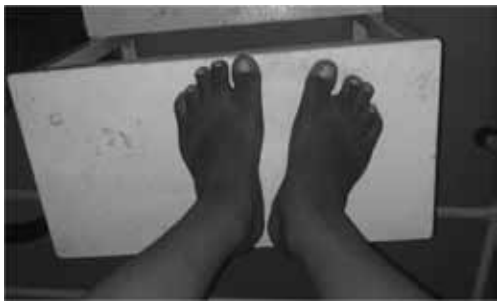

Fig. 3 Flat feet

Table 1 Differential diagnosis for Alstrom syndrome [5]

Features	Alstrom syndrome	Bardet–Biedl syndrome	Lawrence–Moon syndrome	Proband
Childhood obesity	+	+	+	+
Visual impairment	+	+	+	+
Sensorineural deafness	+	−	−	−
Short stature	+	−/+	−	+
Diabetes mellitus	+	+	−	+
Renal disease	+	+	−	+
Polydactyly/syndactyly	−	+	−	−
Mental delay	−	+	+	−
Hypogonadism	+	+	+	+
Dilated cardiomyopathy	+	−/+	−	−
Hepatic involvement	+	−	−	+
Hypertriglyceridemia	+	−	−	+

+: Present. −: Absent

Differential diagnosis

Childhood obesity and retinal dystrophy is seen in Lawrence–Moon, Bardet–Biedl and Alstrom syndromes. It is very important to distinguish AS from the others (Table 1) [7]. The absence of polydactyly and syndactyly and the normal mental health/intellect rules out Bardet–Biedl syndrome. The absence of sensorineural deafness and spastic paraparesis of limbs rules out Lawrence–Moon syndrome.

Management and follow-up

The boy was started on regular human insulin (10 units, tid), metformin (500 mg, bid) and fibrates (145 mg/day). The dietician advised a low-fat, moderate-carbohydrate diet along with adequate protein and high fibre. At the time of discharge, the insulin dosage was made 30 U/day, metformin 1000 mg/day and fibrate 145 mg/day in consultation with the paediatric endocrinologist with the plan to taper the insulin and increase the metformin dose. On follow-up, the sugar was poorly controlled hence the insulin was increased to 35 U/day. The parents were advised to review after 4 weeks and again counselled on the importance of diet.

Discussion

Alstrom syndrome presents with highly variable symptoms which progress with advancing age. As the child grows, the clinical features become evident to affect a clinical diagnosis.

The onset of the symptoms varies among individuals (Table 2). But, the most consistent finding in most cases of Alstrom syndrome is the visual problem and obesity. About one-third of the reported cases are totally blind by the age of 9 years, which is so in this case. The increased frequency of serous otitis media seen in the proband may predispose him to sensorineural impairment in the coming years as evidenced in the literature [3]. The cause for the development of sensorineural impairment may be due to the role of *ALMS1* protein in cochlear development. The control mouse models showed basal body migration of *ALMS1* protein and anchoring of the cells during final planar polarization in the hair cells of the cochlea. Loss of these proteins may lead to overactivation of the pathway and receptor accumulation in late endosomes, thereby disrupting the Notch signalling pathway leading to progressive neural deficits [8].

The proband also has facial features like round face with frontal balding and deep set eyes and short stubby fingers with thick flat foot as described by Marshall, et.al [2].

Table 2 The findings in the proband and characteristic major abnormalities in Alstrom syndrome [3, 5]

Features	% found [3, 5]	Features present in the proband
Cone-rod dystrophy	~33	+
Sensorineural deafness	80	−
Developmental milestones delay	45	+
Childhood obesity	>95	+
Hypertension	~40	− (prehypertension +)
Dilated cardiomyopathy/ congestive cardiac failure	60	−
Diabetes mellitus	80	+
Hypertriglyceridemia	~50	+
Hepatic involvement	80	+
Renal insufficiency	~50	+
Mutation at exon 10 on *ALMS1* gene		+

+: Present. −: Absent

The proband also has features of metabolic syndrome such as hyperinsulinemia, type 2 diabetes mellitus (T2DM), hypertriglyceridemia, low HDL-C, acanthosis nigricans and high HbA1C. These features are usually evident only by second or third decade, but has manifested much early in the proband [9]. However, the cause for obesity observed in all the cases of Alstrom syndrome has remained elusive.

Over the last few years, a pivotal development has been achieved in demystifying the *ALMS1* involvement in energy balance and appetite regulation, whose altered regulation leads to the development of obesity and diabetes, both features classically associated with AS patients. The mouse brain studies of the expression of *ALMS1* showed strong reduction of hypothalamic neurons with significant loss of the primary cilia involved in regulation of appetite leading to obesity, suggesting a crucial role of *ALMS1* protein in maintenance and stability of the cilia structure and function in these neurons [9, 10]. Diminished total glucose transporter 4 (GLUT4) content and altered translocation to the plasma membrane in the Alms1GT/GT mouse model suggest the role of *ALMS1* in glucose homeostasis. Furthermore, mature adipocytes from Alms1GT/GT mouse models showed reduced insulin-stimulated glucose uptake [10].

Based on the diagnostic criteria putforth by Marshall J et.al [3], the proband fulfils two major criteria (*ALMS1* gene mutation and cone dystrophy), which confirms the diagnosis of the syndrome. Obesity, insulin resistance & T2DM; hepatic dysfunction along with more supportive evidence like normal digits, flat wide feet, delayed developmental milestones and hypertriglyceridemia substantiate the diagnosis of Alstrom syndrome. The genotyping of the parents was not done, which remains a limitation of this case report; else we could have found whether the parents were compound heterozygotes carrying the affected allele.

Conclusion

Alstrom syndrome is a rare autosomal recessive disorder involving multiple organ systems at different ages with varying severity. Management of this condition includes multiple drug therapy along with lifestyle modifications and is complex and also fraught with poor compliance. Hence, regular follow-up becomes mandatory. Parents of the child need to undergo genetic counselling for future pregnancies.

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Compliance with ethical standards

Disclaimer NA.

Source of support No funding was sought for.

We contacted Dr. Jan. Marshall, at Jackson Laboratories Maine, USA, through email. She was working on Alstrom syndrome under NIH grant, and through her project, we obtained permission for DNA study for *ALMS1* mutation.

Conflict of interest The authors report no conflicts of interest in this work.

We have the patient's mother and guardian signature of approval for the tests.

No objection certificate was issued by the Medical Superintendent of KMC Hospital, Attavar, prior to sending the blood sample for DNA study at Jackson Laboratories, Maine, USA.

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LETTER TO THE EDITOR

The seasonality variation plays an important role for increasing the uncontrolled type 2 diabetes?

J. E. García de Alba-García · A. L. Salcedo-Rocha · E. Ramos-Pinzon · M. E. Milke-Najar

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Dear Sir

For México, type 2 diabetes mellitus (T2DM) represents a growing health problem. In 2000, adult's prevalence was 5.7 and 9.1 % in 2012 [1], a situation that alters the finances of our medical institutions [2].

In other hand, the seasonal variations is well known to exist within components of metabolic syndrome such as glycemias [1], which were significantly higher in winter, attributed by multiple causes: season checkups, low temperature, affective disorder, etc.

Evolution explains elude fluctuating patterns of food availability and physical activity. Adults gain weight for holiday periods or specific seasons, due to increased calories intake or decrease calories expenditure or both.

In Mexico, holidays are accumulated in the "winter season," beginning with the Day of the faithful dead persons (November 2), Mexican Revolution (November 20), Our Lady Guadalupe (December 12), "Posadas" period (December10-23), Christmas (December 25), New Year, Epiphany (January 6), and the three wise men day (February 2), periods that co-exist with food.

We collected during 2011–2012, from the largest social security family clinic in Guadalajara. City[1], 9017 routinely control glycaemias[2] of all T2DM patients (2319 people) solicited by a physician at a laboratory and the temperature monthly data from the Institute of Meteorology from the University of Guadalajara. We applied a curvilinear regression, for glycaemias and temperatures (r^2=0.567, p=0.023) and a non parametric rank sum criterion of Hewitt [3] that show significant seasonality for the winter period ($\sum$=55: p=0.004).

But, Guadalajara has not a true cold season (24.4–15.3 °C) We believe that in this significant variation, the role that the socio-cultural factors play in our developing countries has more weight, than physical factors, and are linked principally at traditional and modern holidays that integrate and give meaning at the social identity, when more food availability exist [4].

In low-income populations, the holiday-food require special focus and consideration due to the vulnerability of these populations and the specific aspects of each setting [4].

In result of this seasonality, we suggest to establish surveillance, educational programs, holistic preventive attention, and extra-institutional support, depending on the culture of population, for reversing eating habits as a problem of gastroanomie An integrative approach to the study of the mechanisms underlying responses is required for provide effective tools with which to control of diabetes during Holliday periods.

Conflict of interest The authors declare to not have any conflict of interest.

[1] Guadalajara city has 3,000,000 of inhabitants, of this 60% has social security which the institution has affiliated 100,000 insured people.

[2] The glucose levels are based on a fasting testing of a sample of venous blood, processed in a Johnson & Johnson® Vitros 5.1 FS Chemistry system, by colorimetric refractancy.

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LETTER TO EDITOR

Diabetes, diet and dental caries

V. P. Hariharavel[1] · Arun Prasad V. Rao[2] · Reddy N. Venugopal[3] · Joby Peter[4]

Received: 23 May 2013 / Accepted: 6 May 2015 / Published online: 19 May 2015

Dear Sir,

Prevalence of diabetes in India is rising in astronomical proposition, so does its complication. Oral cavity is no exception. The risk of developing complications is significantly reduced by healthy eating, regular physical activity and having well-controlled blood glucose levels. Since the consumption of simple or refined carbohydrates like sucrose increases the blood glucose levels quickly, the dietary practice of consuming complex carbohydrates has a great role in having well-controlled blood glucose levels and development of long-term complication in diabetic patient.

Therefore, from the dental perspective, one could expect that such dietary practice of lower intake of refined carbohydrates not to favour the growth of cariogenic bacteria and the subsequent development of carious lesions in diabetics [1]. But the incidence of dental caries in diabetics is higher when compared to non-diabetics. This is surprising in view of the fact that the lower intake of refined carbohydrates, especially sucrose, and high protein content of the diet make the diabetic subjects clearly less cariogenic than among non-diabetics. This difference can be attributed to the complications of diabetes which are based on microvascular and macrovascular changes.

Normal salivary function is essential to the preservation of the integrity of teeth and oral soft tissues. Salivary glands act as a filter of blood glucose that would be altered by hormonal or neural regulation [2]. But microvascular damage alters basement membrane in salivary gland, leading to increased leakage of glucose from ductal cells. This results in increased glucose levels in saliva and crevicular fluid which causes decreased fibroblastic activity which in turn produces increased plaque accumulation. The glucose released into the saliva is metabolized to lactic acid by plaque. This results in decreased salivary pH and increases acidophilic bacteria. Long-term glucose leakage into saliva is likely to increase the metabolic activity of the oral microflora. This changes the natural balance of the dental biofilm [3] and causes dental decay and periodontal diseases.

The acidic pH of saliva in diabetics may be associated either to microbial activity or to decreased levels of bicarbonate with decreased salivary flow rate. So, when treating diabetic patients, practitioners should be alert to complaints of dry mouth and signs of decreased salivary function. Hyosalivation may be indicative of poor glycemic control in some of these patients [4].

Lack of patient knowledge on the association of oral health with maintaining metabolic control of diabetes is a significant challenge to prevent the oral health complications. Hence, children and adolescents with poorly controlled or uncontrolled diabetes exhibit higher incidence of caries inspite of extensive preventive efforts.

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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.

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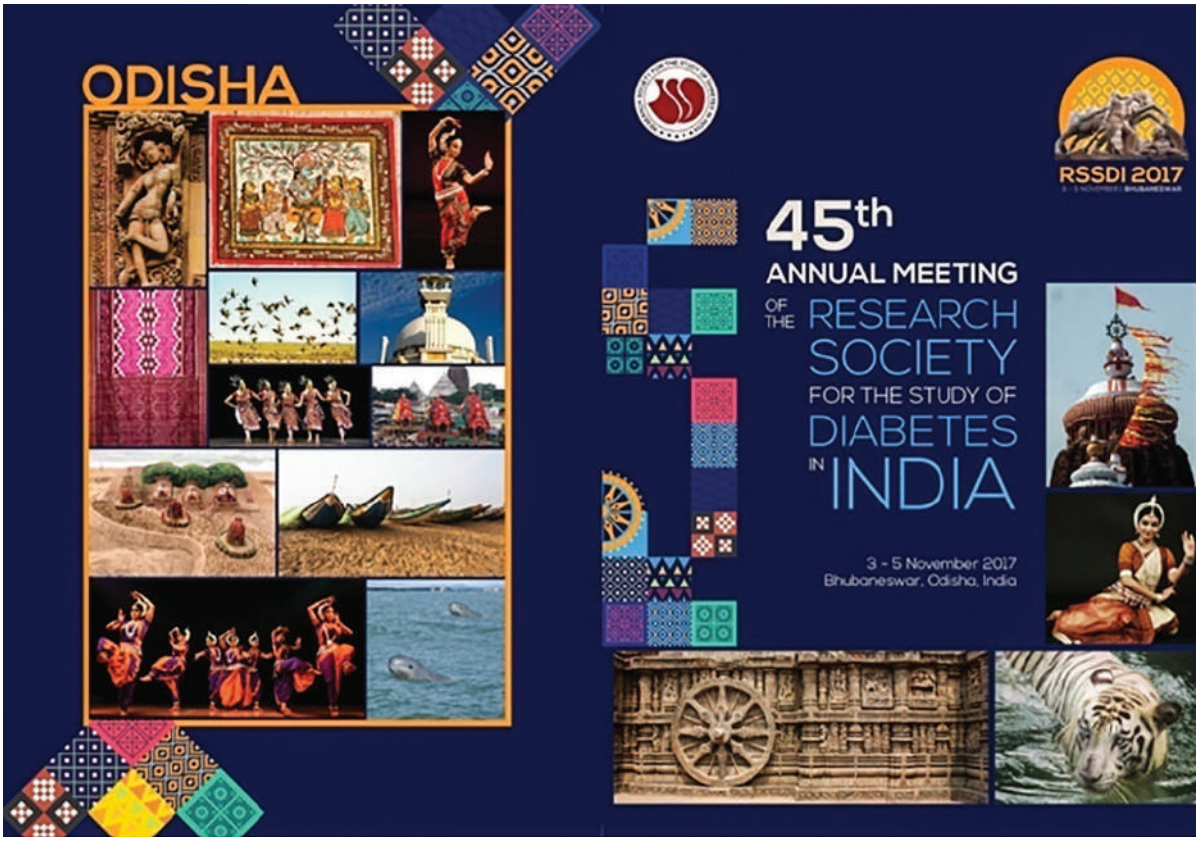

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