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

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

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Both diabetes and psychiatric disorders are common, and it is also known that each condition may worsen the other. Since they also frequently coexist, it is important to recognize the adverse impacts that each has on the other in order that both these conditions can be managed effectively and successfully. It is important to understand this two-way link between stress and psychological comorbidity on the one hand and diabetes on the other. Management of diabetes will always be incomplete and difficult without addressing the associated anxiety, depression, and diabetic distress.

The prevalence of depression and anxiety in diabetes is much higher than in normal population [1] and has been reported to be in the range of 12 and 28% in some studies. Prevalence of anxiety and depression among patients with type 2 diabetes was found to be 58 and 45% respectively from Pakistan. Another study reported major depression in 11% [2] and clinically important depressive symptoms in 31% of people with diabetes. Depression symptoms mimic diabetes symptoms and may sometimes make diagnosis difficult [3].

The risk factors for depression among diabetic patients include female sex, long duration of diabetes, presence of complications, poor glycemic control, family history of depression, and lower education levels. Depression may also be related to the complexities in management of diabetes particularly type 1 diabetes or may be secondary to diabetes-related distress. It is important that depression and its causative factors are identified early and eliminated in people with diabetes as those can lead to a far more effective control of their glycemia.

While the association of depression and diabetes is well recognized and deservedly so, the study from Pakistan and a recent study from India [4] highlight the fact that anxiety disorders may be more common in diabetic patients. The prevalence rate of generalized anxiety disorder (GAD) is three times higher than that reported in the general population. Anxiety was reported in 58% of diabetic patients from Pakistan, while the study from New Delhi reported that among diabetic women from India, anxiety (23–40%) appears more prevalent than depression (18%) [4], especially during the first 2 years after diagnosis. Anxiety disorders and hypoglycemic episodes share clinical features such as sweating, anxiety, tremor, tachycardia, and confusion, and they can sometimes cause difficulties in diagnosis [3]. It can also lead to a failure on the part of the patient to perceive the initial warning signs of hypoglycemia or to confuse these with anxiety. Anxiety disorders should receive more attention as a potential source of comorbidity with diabetes, and screening for anxiety among people with diabetes should be carried out regularly.

The current issue includes four studies which have reported on the association of various psychosocial factors including anxiety disorders and depression and their determinants in patients with diabetes. The study by Nawaz et al. [5] from Pakistan found a high percentage of type 2 diabetic patients with associated anxiety. While 66.5% of these patients were diagnosed with mild anxiety, about 21.1% were reported suffering from moderate to severe anxiety based on the Hamilton Anxiety Rating Scale. Poor glycemic control, female gender especially if they were housewives, lower education levels, higher levels of physical activity, and presence of a diabetic patient in the family were associated with anxiety among type 2 diabetic subjects. In another study being published in this issue, Atif et al. [6] from Pakistan also report high levels of depression and mild cognitive impairment among elderly diabetic subjects. Depression is reported as the most important predictor of mild cognitive impairment in these patients, reinforcing the view that it is important to recognize and treat depression in diabetic patients. Emre et al. [7] also report a high prevalence of anxiety and depression in diabetic patients who also had hypertension in their primary care settings which
adversely impacted both blood pressure and blood sugar control. The impact of psychosocial factors on glycemic control was assessed by Aghili et al. [8] who report higher rates of distress, depression, and anxiety in insulin-experienced diabetic patients compared to those who were insulin-naïve. These patients also had higher HbA1C levels suggesting poorer control of glycaemia. All these studies appearing in this issue highlight the need for early identification of psychological co-morbidity in patients with diabetes particularly if they are elderly, have coexistent hypertension, and are taking insulin.

Both depression and anxiety have been found to be associated with a negative impact on diabetes. The psychosocial impact of diabetes has been recognized as a stronger predictor of mortality in diabetic patients than many clinical and physiological variables [9]. The prognosis of both diabetes and depression (in terms of complications, treatment resistance, and mortality) is worse when the two diseases occur in the same patient. Also, higher complication rates particularly coronary heart disease have been reported among diabetic subjects with coexistent depression [10]. Similarly, children with diabetes have a higher risk of retinopathy if there is associated depression [11] and depression has been more commonly reported in diabetes patients with erectile dysfunction and diabetic foot disease [12].

Self-management is an essential component of diabetes care. The presence of comorbid psychiatric illness can make self-management difficult to implement. Depression can affect compliance with insulin and oral medications, as well as with diet and exercise and monitoring of blood glucose [1]. Specific psychological approaches to improve the therapeutic adherence may be required.

Diabetes distress is very common among patients and is distinct from other psychological disorders. It is defined as significant negative psychological reactions related to emotional burdens and worries specific to an individual’s experience in having to manage a severe, complicated, and demanding chronic disease such as diabetes. It is recommended that people with diabetes should be routinely monitored for diabetes distress, particularly when treatment targets are not met and/or at the onset of diabetes complications [13].

In a recent study, it was reported that despite living with poorly controlled diabetes, Indian women maintain participation in culturally valued roles involving the care of others. Significant biosocial stress was reported among them that could compromise diabetes self-care [14]. It is important to develop culturally sensitive diabetes management strategies that allow women to successfully manage their diabetes while being able to perform their social and family roles.

It has also been reported that depressed individuals have a 60% increased risk of developing diabetes [15]. This could be partly due to the associated reduced physical activity which increases the risk for obesity and type 2 diabetes [1] but also due to altered neuroendocrine responses.

Chronic Stress is a significant risk factor for the development of type 2 diabetes. Two cross-sectional studies [16, 17] have demonstrated the association of chronic stress at work with metabolic syndrome and diabetes while one prospective cohort study [18] has confirmed the same. It has also been shown that chronic stress and stress response markers are associated with risk of diabetes [19] and that chronic activation of HPA axis is an important link between stress and development of diabetes mellitus [19, 20]. Thus, diabetes, stress, and depression are closely linked to each other and each of these increases the risk for the other.

The close association of psychiatric disorders and diabetes throws up important management issues among diabetic patients that have an important bearing on long-term prognosis. It is therefore recommended by the American Diabetes Association that psychosocial care should be provided to all people with diabetes, with the goals of optimizing health outcomes and QOL [14]. It has also been recommended that assessment for symptoms of diabetes distress, depression, anxiety, and other psychiatric disorders should be done at the initial visit and at periodic intervals for better diabetes care.

References


CONSENSUS

RSSDI consensus on self-monitoring of blood glucose in types 1 and 2 diabetes mellitus in India

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Abstract
Maintaining a good glycemic control is crucial in the management of diabetes mellitus (DM) as it is associated with the reduction in both macro and microvascular complications of the disease. Self-monitoring of blood glucose (SMBG), which provides the day-to-day blood glucose levels, is a simple and practical tool for maintaining a good glycemic control. Although SMBG is widely practiced in other countries, its use in India is very limited. Even when used, it is not carried out in a structured manner. There seems to be a lack of education about the purpose of SMBG and the correct process and schedule to be followed. This highlights the unmet need for country-specific SMBG recommendations. In order to fulfill this need, a panel of expert endocrinologists/diabetologists came together under the aegis of Research Society for the Study of Diabetes in India (RSSDI). They reviewed the current literature, combined the evidences with their clinical knowledge and expertise, and developed consensus recommendations for SMBG practice in India. This document provides a comprehensive review of the current literature on SMBG and presents the recommendations made by the expert panel.

Keywords Diabetes Mellitus · Glucose Meters · Glycemic Control · SMBG · Self-monitoring of blood glucose · Type 1 DM · Type 2 DM

Introduction
Diabetes mellitus (DM) is a chronic illness that needs long-term multidisciplinary care. It accounts for a significant burden due to the associated morbidity, mortality, and healthcare resource utilization [1, 2]. Management is primarily targeted towards prevention of acute and chronic complications, for which constant efforts are being made to test novel interventions to improve outcomes [3]. Patient awareness and active participation in self-care to prevent both acute and long-term complications are equally important for effective management of this disorder [4].

As per the International Diabetes Federation (IDF), about 425 million people are affected with DM worldwide, and this number is estimated to reach 629 million by the year 2045. India ranks second in the world, closely following China, with almost 73 million Indians living with diabetes. With a projected prevalence of over 134 million, India is estimated to surpass China by the year 2045 [5].

Rapid increase of diabetes burden in India seems to be due to a combination of various factors including genetic predisposition, urbanization, and lifestyle changes such as sedentary lifestyle and changing nutritional habits [6–8]. Thus, diabetes is a major public health concern in India. On the brighter side, with the development of science and technology, newer methods to diagnose, monitor, and treat DM have enabled management of this condition more effectively. Nevertheless, several patients
still struggle to reach therapeutic targets and are, therefore, at an increased risk of developing complications. Long-term complications of diabetes are well known to occur, especially in patients with poor glycemic control. Hyperglycemia associated with diabetes leads to both macro- and microvascular complications. Macrovascular complications include coronary artery disease leading to angina and/or myocardial infarction and peripheral artery disease that may lead to stroke, diabetic encephalopathy, and diabetic foot [9]. Microvascular complications include nephropathy, neuropathy, and retinopathy. Unarguably, these micro- and macrovascular complications of diabetes are the cause of real burden of the disease [5]. In addition, it has been found that the cost of treatment of patients with complications is much higher than that of patients without complications [10–12]. It is, therefore, essential to put all the efforts towards preventing these complications.

Glycemic level is known to be directly associated with vascular complications of diabetes [13–15]. Moreover, there is strong evidence that good glycemic control is associated with the reduction in both macro- and microvascular complications [16–20]. Thus, maintaining a good glycemic control is of utmost importance for adequate management of diabetes. Glycated hemoglobin (HbA1c), which denotes the average level of blood glucose over about 3 months, and self-monitoring of blood glucose (SMBG), which provides the day-to-day blood glucose levels, are two important tools for monitoring of glycemic control. Fructosamine test is another tool, which denotes the blood glucose levels over the past 2 to 3 weeks. Another such tool is continuous glucose monitoring (CGM), which measures interstitial fluid glucose levels continuously for varying duration of time [21–24].

Evidence suggests that the glycemic variability or extreme changes in blood glucose (hypoglycemia or hyperglycemia) levels could have a role to play in the development of long-term complications independent of HbA1c levels, and the risk of these complications could be reduced by better daily control of blood glucose [25]. A recent study (DEVOTE 2) found that higher day-to-day fasting glycemic variability is associated with increased risks of severe hypoglycemia and all-cause mortality [26]. Evidence also indicates that blood glucose variability can have several other effects including increased cardiovascular and cerebrovascular risk, increased risk of cognitive impairment in elderly patients, and deterioration of endothelial and renal dysfunction [27–30]. All these evidences further highlight the importance of a tool that can assess the glycemic variability on a daily basis. SMBG is the simplest and possibly most practical tool to assess the effectiveness and safety of glycemic control and will be reviewed here.

What is SMBG?

SMBG refers to testing and recording of blood glucose levels by a patient and/or caretaker, at home or in hospital, at different times of the day [21, 31, 32]. The blood glucose levels obtained help patients and clinicians to make appropriate adjustments in lifestyle (diet and physical exercise) and medications [31].

SMBG technique

Before performing SMBG, hands should be washed with soap and water and dried thoroughly. The glucose meter should be prepared. Preparation may vary slightly depending on the glucose meter brand and, therefore, it is important to read the user manual carefully before using the glucose meter. A test strip should be inserted into the glucose meter. A lancet/pricking device should be used to prick the finger. It is advisable to alternate between fingers as they tend to become sensitive over time. After pricking, if required, the finger can be gently massaged in the direction of the prick to help form a drop of blood. The drop of blood should be placed on the correct spot on the test strip as indicated in the user manual. The glucose meter will display the glucose reading within a few seconds. In most glucose meters, the units can be changed from milli-moles per liter to milligrams per deciliter and vice versa. Most glucose meters store the results for weeks and can be retrieved later. These readings will enable the patient/clinician to make lifestyle/therapeutic adjustments. Used test strip and lancets should be disposed of properly as per recommendations to avoid contamination. Test strips and glucose meter should be kept away from sunlight and should also be protected from moisture. Most of the manufacturers recommend that once a bottle of test strips is opened, they should be used within 90 days of opening or the expiry date mentioned on the bottle, whichever is earlier. Some of the common sources of errors to be considered for SMBG are listed in Table 1 [33].

Structured SMBG

It is important to understand that just recording blood glucose levels on a daily basis is not enough, if not acted upon. In order to be clinically relevant and implemented successfully, SMBG must be conducted in a structured way. Structured SMBG

Disease burden of DM in India is increasing. Long-term complications, which form the main burden of disease, can be reduced by maintaining a good glycemic control.
(sSMBG) involves checking the blood glucose levels at predefined times each day [32]. It is a methodical approach to blood glucose monitoring, which enables the patients and clinicians to understand the blood glucose pattern throughout the day, so that appropriate therapeutic adjustments can be made. Along with the blood glucose levels, patients must also record their food intake and physical activity. sSMBG also involves imparting proper education and motivation to the patients and proceeding only after judging their willingness. Education should not focus just on how to conduct SMBG and how to adjust the medication based on the individual readings but should also include explaining to the patients the importance of good control [34]. The physician’s role is to regularly review the SMBG data at every follow-up visit, and to discuss the SMBG readings with the patient. Patients can be advised to make minor adjustments of insulin dosage and to incorporate appropriate lifestyle changes based on SMBG readings. The clinician himself must have proper knowledge, training, and experience to closely follow the blood glucose readings, and understand the pattern to be able to prescribe appropriate changes to diet, exercise, and/or medications. Patients must be educated about the target glucose levels as per guidelines and their importance. Patients and clinicians must agree on the target levels of blood glucose and also on the timing and frequency of testing. At each stage, proper feedback must be given to the patients including an explanation of the potential causes of low or high blood glucose levels. The action plan for maintaining blood glucose levels within target range must be also explained to the patient in a clear manner and it must be agreed upon mutually [32, 35].

In short, sSMBG occurs when the clinician and the patient both express their willingness and are motivated to perform the entire process, possess knowledge to interpret the glucose levels correctly, understand the pattern, and take appropriate actions towards achieving a good glycemic control [32, 35].

Benefits of structured over unstructured SMBG are well documented [35–42]. Also, evidence suggests that lack of knowledge about how to interpret the results of SMBG and how to adjust the dose based on those results is the main deterrent in the success of SMBG, further emphasizing the importance of sSMBG. It has been demonstrated that SMBG is of limited value when it is not applied in a structured fashion [43].

### What are the advantages of SMBG?

SMBG plays a very important role in monitoring the plasma glucose levels on a day-to-day basis. SMBG complements HbA1c testing in evaluation and monitoring of glycemic control. While HbA1c reflects the glycemic status over weeks, SMBG provides day-to-day fluctuations in blood glucose levels. Measurement of 2-h glucose level, which can be obtained with SMBG, is considered to be a stronger predictor of cardiovascular disease as compared to HbA1c. Also, in some conditions such as hemoglobinopathies, malaria, anemia, and blood loss, HbA1c level for glycemic control may not be reliable, and SMBG plays a major role here [44]. Moreover, in pregnancy, greater emphasis is placed on SMBG than on HbA1c [45].

SMBG is crucial in the management of insulin-treated patients, and its role in patients on non-insulin treatment has also been recognized [36, 37, 39, 40, 42, 46–48]. SMBG enables patients to detect acute hypoglycemia/hyperglycemia and take appropriate action in coordination with their clinicians [49]. Thus, it plays a vital role in ensuring safety of patients, especially those on intensive insulin therapies. It also helps patients feel more in control and more empowered in the management of their diabetes. They learn how their behavior, in terms of diet or physical exercise, may affect their blood glucose levels, and feel encouraged to act more responsibly and take

### Table 1 Common sources of errors while conducting SMBG

<table>
<thead>
<tr>
<th>Problem/error</th>
<th>Advice/recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test strip not fully inserted into glucose meter</td>
<td>Remove the test strip and reinsert it. Always ensure that the test strip is fully inserted in the glucose meter</td>
</tr>
<tr>
<td>Not enough blood was drawn into the test strip for measurement</td>
<td>Discard the test strip and repeat the test</td>
</tr>
<tr>
<td>Problem in patient sample site, for example the fingertip is contaminated with sugar</td>
<td>Always clean and dry the site before sampling</td>
</tr>
<tr>
<td>Not enough blood applied to strip</td>
<td>Repeat test with a new strip</td>
</tr>
<tr>
<td>Batteries low on power</td>
<td>Change batteries and repeat the test</td>
</tr>
<tr>
<td>Sites other than fingertips used</td>
<td>Results from alternative sites may not match fingerstick results</td>
</tr>
</tbody>
</table>

SMBG is an important tool for monitoring blood glucose levels. SMBG should be structured for it to be effective.
informed decisions related to their health. Patients can see positive effects of modifying their diet and exercise in real time, which further drives them to continue their efforts. Thus, in addition to controlling their blood glucose levels, sSMBG also helps weight management in these patients [50].

SMBG helps in maintaining a good glycemic control by generating data for therapeutic and lifestyle adjustments. It detects acute hypoglycemia/hyperglycemia and protects patients against extreme glucose variations.

What are the challenges associated with SMBG and how to overcome them?

While SMBG has several advantages, there are also some challenges associated with it. SMBG is a procedure that requires active participation by the patients. Patients may find SMBG inconvenient, painful, and cumbersome [51]. They may find it difficult to integrate SMBG in their daily routine [52]. Another hurdle is ignorance of patients towards the seriousness of diabetes and its complications. Cost of the test strips and needles is another concern especially for patients who have to pay for their healthcare themselves. Carrying the glucose meter with them while traveling is another barrier [51]. Undesired readings on glucose meter may also discourage patients from wanting to continue SMBG. Patients may feel that SMBG affects their quality of life [53]. Additionally, depression has also been documented in patients performing SMBG [54]. Another challenge is the unavailability of diabetes care team for titration of the doses and providing appropriate guidance to the patients.

Most of these barriers or challenges associated with SMBG can be overcome by proper communication between the patients and their clinicians/diabetes care providers. Patients may disregard the seriousness of long-term complications and therefore may display low motivation for treatment. The effects of uncontrolled blood glucose levels and day-to-day glycemic variability on long-term health should be properly explained to the patients. Patient beliefs and values must also be considered. It is of utmost importance that clinicians take sufficient time to explain the importance of SMBG to their patients so that they understand the rationale for SMBG and are encouraged to follow the instructions for conducting and recording blood glucose readings as advised. Initially, attainable targets should be set, which will give the patients a sense of achievement, and motivate them further to continue SMBG. Also, therapeutic targets recommended by guidelines should be explained to the patients and must be agreed upon by both clinicians and patients as this has been shown to improve patient outcomes [55].

Importance of accuracy of SMBG systems

Accuracy of SMBG systems is very important for the results to be reliable and safe. It has a direct effect on therapeutic decisions and may also have long-term implications. SMBG systems should comply with the International Organization for Standardization (ISO) 15197:2013 requirements [56]. Freckmann et al. conducted a study to examine the different SMBG systems and found that 7 of the 34 systems evaluated did not fulfill the minimal accuracy requirements of ISO. Regular evaluation of the blood glucose meters is therefore, of utmost importance [57].

SMBG devices have been associated with a number of user errors such as using expired test strips, inadequate storage conditions, or glucose-contaminated fingertips that compromise the analytical performance. In order to reduce potential user errors, more integrated systems (incorporation of the tests into the meter by using cassettes, discs, or drums) have been developed. Baumstark et al. carried out a study to evaluate the system accuracy of this improved system based on ISO 15197:2013, clause 6.3, for three reagent system lots. The study reported a high level of accuracy; 100% within the defined limits in the hands of trained study personnel and 99.1% in the hands of intended users [58].

Another technical challenge is that there is a difference between glucose levels in the venous and capillary blood with venous blood having a lower concentration of glucose. The difference varies between fasting and post-meal. The difference is not much at fasting but there is a larger difference after a meal [59]. The revised ISO 15197:2013 requirements specify tighter accuracy standards (when compared with ISO 15197:2003) requiring that 95% of blood glucose results should reach the following standard:

- Within ± 15 mg/dL of laboratory results at concentrations < 100 mg/dL.
Within ± 15% of laboratory results at concentrations ≥ 100 mg/dL

The 2013 guidelines also specify that 99% of the individual glucose results must fall within zones A and B of the Consensus Error Grid for type 1 DM [56]. Some glucose meters currently available in our country which conform to ISO 15197:2013 standards include Accu-Chek Performa, GluNEO Lite, Contour TS, One Touch Verio Flex, Alere G1, and SD Check Gold.

One more challenging aspect is the commonly used graphs and plots to assess the accuracy of SMBG systems, which get increasingly difficult to comprehend as the number of data points increase. Recently, a new approach of displaying SMBG measurement accuracy data has been introduced called the “rectangular target plot” (RTP), which presents data in a simple yet comprehensible manner [60]. RTP was evaluated by creating plots for 50 SMBG systems and 87 reagent system lots from 8 manufacturers. It was found that RTP remained comprehensible even when data was displayed from multiple reagent system lots or products and was completely applicable in more than 93% of the cases analyzed [61].

Also, it is important to ensure that validation and calibration of the device is carried out properly.

What is the evidence of effectiveness of SMBG?

SMBG is commonly used in developed nations as an integral part of diabetes management [62]. In a survey conducted in Canada in 2011, almost 90% of the patients with type 2 DM reported using SMBG. Further, there was no significant difference between patients using insulin only and those taking insulin plus oral medication or an oral medication only although frequency of SMBG was lower in these patients [62]. In another survey conducted in the UK, 80% of the 554 respondents reported high satisfaction with SMBG. They also reported that SMBG helped them feel more “in control” of their diabetes management [63].

Several studies have demonstrated that SMBG helps in better glycemic control and is thus essential in the management of DM [36, 40, 47, 64–70].

In type 1 DM patients

Patients with type 1 DM experience higher glucose variability leading to a greater risk of hypoglycemia. Therefore, SMBG plays a critical role in the management of these patients. The landmark Diabetes Control and Complications Trial, which was the first long-term randomized study including 1441 patients with type 1 DM, showed that intensive therapy guided by frequent blood glucose monitoring when compared with conventional therapy (with one or two daily insulin injections) was associated with delayed onset and slowed progression of microvascular complications [71]. The results of this study were published in 1993 and since then, use of SMBG gradually increased, and it is now routinely practiced in patients with type 1 DM. It has also been found that higher frequency SMBG in these patients is strongly associated with lower HbA1c levels [67, 70]. Thus, SMBG is absolutely essential for achieving and maintaining optimal blood glucose levels in all patients with type 1 DM including children, adolescents, and adults.

In type 2 DM patients on insulin therapy

As in patients with type 1 DM, there is no doubt that SMBG has a very important role to play in the management of patients with type 2 DM who are on insulin therapy. SMBG has been universally recognized as an integral part of insulin regimens. SMBG not only adds value but is crucial in patients especially on the complex insulin regimens. It ensures safety and efficacy of the insulin regimens [41, 42, 72–74].

In type 2 DM patients on non-insulin therapy

Evidence for the utility of SMBG in patients who are not on insulin therapy has been equivocal [75]. While some evidence suggests that SMBG may help in reduction of HbA1c in this group of patients, other studies have found that the advantage of SMBG in these patients is only modest, if at all [76]. A review of six randomized controlled trials (RCTs), showed that patients with type 2 diabetes on non-insulin treatment had a statistically and clinically relevant reduction of HbA1c by 0.39% with SMBG when compared with the control groups [77]. On the other hand, a meta-analysis found SMBG in type 2 patients of non-insulin therapy to be only modestly effective in reducing HbA1c [78].

Two systematic reviews, published in the year 2012, concluded that there is only limited benefit with SMBG in type 2 non-insulin-treated patients [79, 80]. The authors of one of these studies, which was a Cochrane review including 12 RCTs (N = 3259), concluded that the overall effect of SMBG in patients on non-insulin treatment was only small at short term and decreased after a duration of 1 year [79]. It is
important to note that the credibility of this Cochrane review has been questioned [81]. In the other study, which was a meta-analysis including six RCTs (N=2552), although there was a statistically significant difference in the level of HbA1c between the groups with or without SMBG, the authors concluded that individual patient data was not convincing for a clinically meaningful effect [80].

On the other hand, some individual studies have found SMBG to be useful even for patients on non-insulin therapy. In a long-term epidemiological cohort study, 3268 patients with type 2 diabetes were followed for a mean duration of 6.5 years [47]. SMBG was associated with decreased diabetes-related morbidity and all-cause mortality in overall study population and also in a subgroup of patients who were not receiving insulin therapy. In the subgroup on non-insulin therapy, SMBG was associated with a reduced risk of non-fatal (HR = 0.60, 95% CI 0.44–0.82; p < 0.001) and fatal endpoints (HR = 0.54, 95% CI 0.33–0.87; p = 0.010) [47].

Experts believe that when patients, especially those on non-insulin therapy, do not benefit from SMBG, it is mainly because the process is not conducted in a structured format. The Structured Testing Program (STeP) study was a 12-month study that compared outcomes in patients receiving enhanced usual care with those receiving structured SMBG [36]. SMBG was associated with a statistically significant reduction in HbA1c levels in both intention-to-treat analysis (−0.3%; p = 0.04) and per protocol analysis (−0.5%; p < 0.003) [36]. At the IDF 2017 congress, Parsons et al. presented the results of a 12-month multicenter RCT that assessed the efficacy of sSMBG in patients on non-insulin therapy with poor glycemic control (HbA1c ≥7.5% ≤13%). They found that use of sSMBG provided clinically and statistically significant benefits with a mean reduction in HbA1c of 0.9% (95% CI −1.18 to −0.62; p = <0.001). Levels of satisfaction with SMBG remained high throughout the course of the study and only low levels of anxiety or pain caused by SMBG were reported [82].

There have been some reports of undesirable impact of SMBG on patients such as effects on quality of life (DiGEM study) and depression (ESMON study) [53, 54]. However, this is thought to occur when the physician is not involved enough in the care and when patients are not well-educated about the procedure of SMBG [83]. This further emphasizes the importance of sSMBG. Proper education of patients is very important including the action to be taken when blood glucose levels are out of the target range [72, 73]. Additionally, some studies have found that SMBG when conducted correctly can, in fact, reduce the stress and depression associated with diabetes. A 12-month cluster-randomized trial (N = 483) was conducted on non-insulin-treated type 2 patients specifically to assess whether sSMBG reduces depressive symptoms and diabetes distress. Patients were divided into experimental (structured SMBG) and active control groups. Although both groups had significant improvement in depression and disease-related distress (p < 0.01 in both groups), experimental patients displayed significantly greater reductions in distress related to regimen adherence than controls. Further, those experimental patients who had elevated diabetes distress or depressive symptoms at baseline showed significantly greater reductions in distress and depressive symptoms than control patients at 12 months [84]. In another study sSMBG was associated with significant increases in self-confidence and autonomous motivation associated with diabetes self-management [39].

Several other studies have demonstrated clear benefit of SMBG in the management of patients with non-insulin-treated type 2 DM [50, 65, 66, 69, 85–89]. Shiraiwa et al. demonstrated that lesser frequency of SMBG (10 times per month) in addition to being cost-saving was also effective in improving glycemic control. The mean decrease in HbA1c was significantly more (p = 0.028) in the SMBG group when compared to the control group. In addition, there was a significant reduction of body weight (p < 0.001) in the SMBG group [50]. Key studies of SMBG in types 1 and 2 DM are summarized in Table 2.

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**Emerging technologies**

Although, currently, SMBG is the simplest and the most practical method of blood glucose monitoring, it is also important to consider the emerging technologies. Goals for future techniques include noninvasive monitoring and more comprehensive blood glucose data collection.

The newer technologies include real-time CGM, flash glucose monitoring, Bluetooth-enabled meter, diabetes apps, glucose-sensing contact lens and Ambulatory Glucose Profile (AGP) by Free style Libre [91, 92]. Detailed discussion of these technologies at this point of time does not appear relevant to the consensus process and therefore is not included in this document.

SMBG is essential in the management of type 1 DM patients and those patients with type 2 DM who are on insulin. Also, there is emerging evidence to support the use of SMBG in type 2 patients on non-insulin therapy.
### Table 2: Key studies of SMBG in types 1 and 2 DM

<table>
<thead>
<tr>
<th>Study</th>
<th>Summary of study</th>
<th>Number of participants</th>
<th>Duration</th>
<th>Main outcome measures</th>
<th>Results/conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parsons et al. [82]</td>
<td>RCT to assess the efficacy of sSMBG in patients on non-insulin therapy with poor glycemic control (HbA1c ≥ 7.5 ≤ 13%)</td>
<td>446</td>
<td>1 year</td>
<td>HbA1c at 12 months</td>
<td>Clinically and statistically significant benefits were obtained with sSMBG with a mean reduction in HbA1c of 0.9% (95% CI −1.18 to −0.62; p &lt; 0.001)</td>
</tr>
<tr>
<td>Miller et al. [67]</td>
<td>Large database of type 1 DM Exchange clinic registry to evaluate the relationship between the number of SMBG measurements per day and HbA1c levels</td>
<td>20,555</td>
<td></td>
<td>Association between the number of SMBG measurements per day and HbA1c levels</td>
<td>Higher number of SMBG measurements per day was strongly associated with a lower HbA1c level (p &lt; 0.001); association was present in all age groups and in both insulin pump and injection users</td>
</tr>
<tr>
<td>Kesavadev et al. [48]</td>
<td>Retrospective cohort study using electronic health records to assess the effectiveness, safety, and costs of SMBG via Diabetes Tele Management System (DTMS) in type 2 DM</td>
<td>1000</td>
<td>6 months</td>
<td>HbA1c at 6 months; hypoglycemia incidence; cost</td>
<td>The mean ± SD HbA1c value was reduced from 8.5 ± 1.4% to 6.3 ± 0.6% at 6 months (p &lt; 0.0001) The rate of SMBG values &lt; 70 mg/dL was −0.04/patient/month; 84% patients reported no hypoglycemia Extra cost to patients for DTMS was equivalent to US$9.66/month</td>
</tr>
<tr>
<td>Polonsky et al. (SteP) [36]</td>
<td>Multicenter cluster-randomized study to assess the effectiveness of structured SMBG in poorly controlled (HbA1c ≥ 7.5%), non-insulin-treated type 2 DM</td>
<td>483</td>
<td>1 year</td>
<td>Difference in HbA1c level after 12 months</td>
<td>Structured SMBG (vs. active control group) significantly improved glycemic control (per protocol analysis, reduction in mean HbA1c; 21.3 vs. 20.8%; p &lt; 0.003) without decreasing general well-being</td>
</tr>
<tr>
<td>Franciosi et al. (ROSES) [87]</td>
<td>Randomized study lead by diabetes nurses to evaluate the efficacy of SMBG in patients with type 2 DM with oral agent monotherapy</td>
<td>62</td>
<td>6 months</td>
<td>Mean change in HbA1c levels</td>
<td>Absolute mean difference in HbA1c reduction between groups (SMBG vs. usual care) was −5% (95% CI −0.9 to −0.0%; p = 0.04)</td>
</tr>
<tr>
<td>Durán et al. (St. Carlos Study) [86]</td>
<td>Newly diagnosed type 2 DM patients were randomized to either SMBG-based intervention or HbA1c-based control group</td>
<td>161</td>
<td>1 year</td>
<td></td>
<td>Significantly greater reductions in median HbA1c (6.6 to 6.1%; p &lt; 0.05) and BMI (29.6–27.9 kg/m²; p &lt; 0.001) were found in the SMBG group</td>
</tr>
<tr>
<td>Barnett et al. (DINAMIC 1 study) [64]</td>
<td>Multicenter RCT to determine whether SMBG results in greater reduction in HbA1c compared to non-use of SMBG</td>
<td>610</td>
<td>27 weeks</td>
<td>Difference between groups in HbA1c</td>
<td>HbA1c decreased from 8.12 to 6.95% in the SMBG group and from 8.12 to 7.20% in the non-SMBG group with a statistically significant difference between 2 groups (0.25%; 95% CI 0.06–0.13; p = 0.0097).</td>
</tr>
<tr>
<td>O’Kane et al. (ESMON study) [54]</td>
<td>RCT to assess the effect of SMBG on patients with newly diagnosed type 2 DM</td>
<td>184</td>
<td>1 year</td>
<td>Differences in HbA1c between groups, psychological indices, use of oral hypoglycemic drugs, BMI, and reported hypoglycemia rates</td>
<td>No significant differences between groups at any time point for any of the outcome measures SMBG was associated with a 6% higher score on the depression subscale of the well-being questionnaire (p = 0.01)</td>
</tr>
<tr>
<td>Study</td>
<td>Summary of study</td>
<td>Number of participants</td>
<td>Duration</td>
<td>Main outcome measures</td>
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<tr>
<td>Farmer et al. (DiGEM study) [90]</td>
<td>Three-arm, open, parallel group randomized trial to determine whether SMBG alone or with instruction in incorporating results into self-care, is more effective than usual care in improving glycemic control in non-insulin-treated type 2 DM</td>
<td>453</td>
<td>3 years</td>
<td>Difference in HbA1c level measured at 12 months</td>
<td>The differences in HbA1c level between the three groups were not statistically significant ($p = 0.12$)</td>
</tr>
<tr>
<td>Martin et al. (ROSSO) [47]</td>
<td>Observational study to obtain epidemiological data on SMBG in type 2 DM and to investigate the relationship of SMBG with disease-related morbidity and mortality</td>
<td>3268</td>
<td>6.5 years</td>
<td>Diabetes-related morbidity (non-fatal myocardial infarction, stroke, foot amputation, blindness, or hemodialysis) and all-cause mortality</td>
<td>SMBG group had a lower rate of non-fatal events (7.2 vs. 10.4%, $p = 0.002$) and fatal events (2.7 vs. 4.6%, $p = 0.004$) than the non-SMBG group. SMBG was an independent predictor of morbidity and mortality (hazard ratios (HR) 0.68; 95% CI 0.51–0.91, $p = 0.009$ and HR 0.49; 95% CI 0.31–0.78, $p = 0.003$, respectively)</td>
</tr>
<tr>
<td>Karter et al. [65]</td>
<td>Observational study to assess longitudinal association between SMBG and glycemic control in diabetic patients (new users and ongoing users)</td>
<td>16,091 new users + 15,347 ongoing users</td>
<td>4 years</td>
<td>Glycemic control measured by HbA1c</td>
<td>Greater SMBG frequency was associated with a graded decrease in HbA1c regardless of diabetes therapy in new users ($p &lt; 0.0001$) and only in pharmacologically treated patients in ongoing users ($p &lt; 0.0001$)</td>
</tr>
<tr>
<td>Schwedes et al. [69]</td>
<td>Multicenter RCT to evaluate the effect of meal-related SMBG on glycemic control and well-being in non-insulin-treated type 2 DM 2 groups: experimental group used SMBG device, kept a blood glucose/eating diary, and received standardized counseling; control group received non-standardized counseling on diet and lifestyle</td>
<td>250</td>
<td>6 months</td>
<td>Change in HbA1c; Changes in body weight, lipids, and microalbumin; Changes in treatment satisfaction and well-being</td>
<td>Use of SMBG significantly reduced HbA1c levels by 1.0 ± 1.08% vs. 0.54 ± 1.41% for the control group ($p = 0.0086$). SMBG also caused a marked improvement in general well-being ($p = 0.053$). There was statistically significant improvement in depression ($p = 0.032$) and lack of well-being ($p = 0.02$). No statistically significant difference in the 2 groups for other parameters</td>
</tr>
<tr>
<td>The Diabetes Control and Complications Trial Research Group [71]</td>
<td>RCT to evaluate whether intensive treatment (guided by SMBG) with the goal of maintaining blood glucose levels close to the normal range could decrease the frequency and severity of long-term microvascular and neurologic complications</td>
<td>1441</td>
<td>Appearances and progression of retinopathy, nephropathy, neuropathy</td>
<td>Risk for development of retinopathy was reduced by 76% (95% CI 62–85) in patients with no retinopathy at baseline. In patients with mild retinopathy, progression was slowed by 54% (95% CI 39–66). Occurrence of microalbuminuria albuminuria, and clinical neuropathy was reduced by 39% (95% CI 21–52), 54% (95% CI 19–74), 60% (95% CI 38–74), respectively</td>
<td></td>
</tr>
</tbody>
</table>

CI confidence interval, DM diabetes mellitus, HbA1c glycated hemoglobin, RCT randomized controlled trial, SD standard deviation, SMBG self-monitoring of blood glucose, sSMBG structured self-monitoring of blood glucose
What do the RSSDI recommendations on SMBG say?

Research Society for the Study of Diabetes in India (RSSDI) recently (2017) published the clinical practice recommendations for the management of type 2 DM. These guidelines also include a section on SMBG. RSSDI provides two levels of recommendations: “Recommended care” and “Limited care.”

As per the RSSDI, recommended care [93]:

- SMBG is useful to people with diabetes who have the required knowledge, skills, and willingness to use the information obtained through testing to actively adjust treatment with the help of the treating physician and to enhance understanding of diabetes and assess the effectiveness of the management plan on glycemic control.
- The purpose of performing SMBG and using SMBG data should be agreed between the person with diabetes and the healthcare provider.
- SMBG should be available on an ongoing basis to those using insulin.
- SMBG protocols (intensity and frequency) should be individualized to address each individual’s specific educational/behavioral/clinical requirements, specific needs, and goals (to identify/prevent/manage acute hyper- and hypoglycemia) and provider requirements for data on glycemic patterns and to monitor impact of therapeutic decision-making.
- Intensive/regular SMBG may be recommended in patients on multiple daily insulin injections, in case of pre-gestational/gestational diabetes on insulin, history of hypoglycemia unawareness, brittle diabetes, or with poor metabolic control on multiple oral antidiabetic agents (OADs) and/or basal insulin.
- SMBG should be performed at least as often as insulin is administered. Patients on intensive insulin regimens who are on multiple doses of insulin or on insulin pumps should be tested three or more times daily (all pre-meals, post-meals, bedtime, prior to exercise).
- SMBG plays an important role when low blood glucose is suspected or after treating low blood glucose until normoglycemia is achieved and prior to critical tasks such as driving. For many patients, this will require testing 6–10 (or more) times daily, although individual needs may vary.
- Pregnant women with insulin-treated diabetes should be advised to perform SMBG on a daily basis, failing which, at least weekly monitoring should be encouraged.

- Ideal SMBG is seven tests/day, i.e., three before and three after each meal and one test at 3 a.m. If this is not feasible, one fasting test and three tests each after breakfast, lunch, and dinner daily may be done, which can further be individualized to twice or thrice a week as the pregnancy advances.
- More frequent monitoring should be done in special situations like fever, vomiting, and persistent polyuria with uncontrolled blood glucose, especially if abdominal pain or rapid breathing is present. Ketone test should be performed as and when needed.
- SMBG accuracy is instrument and user-dependent, so it is important to evaluate each patient’s monitoring technique, both initially and at regular intervals thereafter. The ongoing need for and frequency of SMBG should be reevaluated at each routine visit.
- SMBG should be considered for people using oral glucose-lowering medications as an optional component of self-management and in association with HbA1c testing:
  - To provide information on, and help avoid, hypoglycemia
  - To assess changes in blood glucose control due to medications and lifestyle changes
  - To monitor the effects of foods on post-prandial glycemia
  - To monitor changes in blood glucose levels during intercurrent illness
- SMBG may be useful in type 2 DM during periods of acute illness; in patients using sulfonylureas or glinides as combination or monotherapy; to identify hypoglycemia especially in the first 3 months of starting sulfonylurea; in patients who experience episodes of hypoglycemia and who have reduced awareness of hypoglycemia; in drivers and those who fast; and in women under preconception care.
- Regular use of SMBG should not be considered part of routine care where diabetes is well-controlled by nutrition therapy or oral medications alone.
- Structured assessment of self-monitoring skills, the quality and use made of the results obtained, and of the equipment used should be made annually.

RSSDI recommendations for limited care state “SMBG using meters with strips should be considered for people with diabetes using insulin or drugs like sulfonylurea and glinides.” Table 3 shows RSSDI recommendations for target blood glucose levels in patients with DM.

<table>
<thead>
<tr>
<th>Target</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>&lt; 115</td>
</tr>
<tr>
<td>Post-prandial glucose (mg/dL)</td>
<td>&lt; 160</td>
</tr>
</tbody>
</table>

Table 3  Target blood glucose levels in patients with DM as per RSSDI recommendations [93]
Diabetes in pregnancy

Gestational DM (GDM) and pre-existing DM in pregnant women are associated with increased risk of perinatal morbidity and mortality. A common complication is macrosomia or large-for-gestational-age babies. The hyperglycemia and adverse pregnancy outcomes (HAPO) study found that there is a strong association of maternal hyperglycemia (of a level lesser than that diagnostic of diabetes) with increased birth weight and increased cord-blood serum C-peptide levels [94]. Proper management can reduce the risk of maternal and neonatal complications and improve outcomes [95].

All women with pre-existing DM should receive pre-pregnancy counseling, which should include explaining the risks and common complications and strategies to minimize them [96]. As per the IDF, women who are on insulin should be advised on maintaining HbA1c level below 6.5 or 7.0%. If HbA1c is above 8.0%, women should be discouraged from becoming pregnant until the glycemic control can be improved [96]. A meta-analysis showed that pre-pregnancy care for women with pre-gestational type 1 or 2 DM improves rates of congenital malformations, perinatal mortality, and reduces maternal HbA1c in the first trimester of pregnancy [97].

Maintaining a tight blood glucose control is essential in pregnancy and, therefore, SMBG plays an important role [98]. Government of India, in the recently published revised guidelines on diagnosis and management of GDM, has recommended target fasting blood glucose as less than 95 mg/dL and all 2-h post-prandial glucose levels as less than 120 mg/dL [99]. Women with pre-existing type 2 DM from central India have shown to have significantly higher post dinner blood glucose than post breakfast [100]. Thus, women on insulin therapy should do frequent testing including fasting, 2-h post breakfast, 2-h post lunch, and 2-h post dinner for insulin dose adjustment. The IDF guidelines also advise women with GDM to perform SMBG four times daily (fasting and 1 h after each meal) [96].

Frequency and timing of SMBG

A consensus on the frequency and timing of SMBG has not yet been established. Different SMBG regimens should be followed based on factors such as diabetes type, treatment approach (diet, oral antidiabetic medication, or insulin), glycemic control, available resources, and patient’s level of education. While patients on intensive insulin regimens may require up to 10 tests daily, patients on diet and oral medication may only need 6 to 8 tests per week [73, 93, 101].

The IDF guideline for non-insulin-treated type 2 DM describes focused and low-intensity SMBG regimens. Focused regimens include the 5- and 7-point profiles in which blood glucose is measured 5 or 7 times a day, respectively, for 3 consecutive days [102]. Another focused regimen is the staggered regimen in which blood glucose levels are measured pre- and post-meal (two tests per day) for alternating meal over a period of 1 week. Low-intensity SMBG regimens include meal-based testing (before and after selected meals), detection/assessment fasting hyperglycemia (bedtime and morning fasting SMBG), and detection of asymptomatic hypoglycemia (pre-lunch and pre-supper SMBG) [102].

In 2011, a group of experts in diabetology and endocrinology recommended two schemes for SMBG in type 2 DM, one for less intensive testing and the other for intensive testing. The less intensive testing focuses on paired testing (pre- and post-prandial) once per day. The duration of the paired testing could be 1/month, 1 week/month, 3–7 days/week, or continuous daily testing depending on individual requirement. Intensive testing involves seven tests per day over a period of 3 to 7 days. The duration could be 3 days/week to continuous daily monitoring [101].

In an Indian publication, the authors recommend blood glucose checks at least three times daily in patients with type 1 DM. They recommend a check of pre-meal blood glucose initially until the target pre-prandial levels are reached, after which post-meal levels can be checked. Thus, they divide the SMBG regimen for type 1 DM in 2 phases, “Initial phase” and “Optimization phase.” For type 2 DM, they recommend different regimens; for example, multiple tests per day regimen, and staggered regimen. For type 2 patients on intensive insulin regimens, they advise monitoring similar to patients with type 1 DM, and less intensive monitoring for other patients. For those with HbA1c above target, they advise testing at least twice daily, and for those with HbA1c on target at least 4 times per week (at different times each day) [103].

SMBG practice in India and unmet need for country-specific guidelines and tool

Burden of DM in India is very high and it is projected to get worse in the coming years. SMBG, with its potential to help in achieving good glycemic control and reducing the risk of both short-and long-term complications, can serve as an apt measure to deal with DM. While SMBG is widely used in other parts of the world, it is less commonly practiced in India. The SMBG International Working Group, in 2008, conducted a
survey to study the use of SMBG in 13 countries including India. The lowest use of SMBG was found in India (0.2%) [104]. A study conducted in Delhi to evaluate the quality of care in patients from the middle- and high-income group found that 28.4% of the patients had a home blood glucose monitoring device, and 77.4% of the patients were following the advice on SMBG [105]. Table 4 shows the estimated SMBG use in different countries.

<table>
<thead>
<tr>
<th>Country (study year)</th>
<th>SMBG use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada (2013)</td>
<td>87.8</td>
</tr>
<tr>
<td>Australia (2006)</td>
<td>70</td>
</tr>
<tr>
<td>USA (2006)</td>
<td>62.2</td>
</tr>
<tr>
<td>India (middle-/high-income population) (2006)</td>
<td>28.4</td>
</tr>
<tr>
<td>Malaysia (2007)</td>
<td>15.3</td>
</tr>
<tr>
<td>India (2004)</td>
<td>11</td>
</tr>
</tbody>
</table>

Even when used, the process of SMBG seems to be far from ideal in India. Recently, a survey was conducted in Chennai to understand the knowledge and practice of SMBG in patients with type 2 DM performing SMBG at home. Sadly, only a quarter of the survey participants had adequate knowledge of the process of SMBG and were following the procedure appropriately [109]. This could be due to lack of education about the purpose of SMBG and the correct process to be followed. The current use of SMBG in India appears to be mostly “random,” without a structured process. The importance of education and practice of “structured” SMBG cannot be overemphasized, especially in the Indian setting.

In spite of being aware of the importance of SMBG for glycemic control in patients with diabetes, primary care physicians may not have the expertise to develop an appropriate plan for their patients. Availability of an easy tool that can be applied for different clinical scenarios will be very useful in such a setting. It is, needless to say, that India has several factors such as availability of healthcare resources, spending capacity of patients, education level of patients (to understand the intricacies of SMBG), and patient beliefs, that are different from those in other parts of the world. Therefore, it is imperative to develop a tool that is easy to understand and can be implemented with ease in the Indian context.

### Consensus methodology

In order to fulfill this unmet need, a panel of expert endocrinologists/diabetologists came together under the aegis of RSSDI, reviewed the current literature, combined the evidences with their clinical knowledge and expertise, and developed the first draft for the consensus recommendations/guidelines/tools to be followed for SMBG in India. The expert panel included members of executive committee of RSSDI and invited key opinion leaders (KOLs) from across the country representing government as well as private institutions.

The first draft was circulated among the expert panel members for their critical comments and suggestions for amendments. All the relevant feedback and suggestions were included in the revised draft and it was circulated to the panel for second review and feedback was also sought for different SMBG tools which were circulated in the form of questionnaires. This was followed by the expert committee meeting held on 4 March 2018 in Mumbai where the revised consensus draft was discussed page by page and lot of important suggestions came in for the improvement of the consensus recommendations. The revised draft for SMBG consensus recommendations was circulated again to the expert panel for review and suggestions and was further circulated to extended group for critical feedback and suggestions. The final document after revision was presented at the RSSDI executive committee meeting on 7 April at Jaipur and was formally adopted by RSSDI and sent for publication to the International Journal of Diabetes in Developing Countries (IJDDC).

### Recommendations by the expert panel

The expert panel has set the following basic definitions:

- **Well-controlled diabetes**
  - Patients who are within RSSDI recommended target range of blood glucose levels and HbA1c.

- **Uncontrolled diabetes/poorly controlled diabetes**

- **Brittle diabetes**
  - Diabetes that is difficult to control, with severe instability of blood glucose levels and with frequent and unpredictable episodes of
**Table 5**  Recommended care for frequency/timing of SMBG

<table>
<thead>
<tr>
<th>Type 1 DM</th>
<th>Type 2 DM on OADs</th>
<th>Type 2 DM on insulin or insulin +OADs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Children</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New onset DM/uncontrolled DM/DM during acute illness</td>
<td>New onset DM/uncontrolled DM/DM during acute illness</td>
</tr>
<tr>
<td></td>
<td>Stable/well-controlled DM</td>
<td>Stable/well-controlled DM</td>
</tr>
</tbody>
</table>

- **2 to 8 times/day**
  - At least 4 times/day and should include pre-prandial and bedtime levels
  
- Patients on SU or meglitinides
  - At least 4 times/day and should include pre-prandial and bedtime levels
  
- Patients on other OADs
  - At least FBG on alternate days

- **At least 4 tests in a week**
  - Including an FBG and 3 post-prandial values

- **Must check whenever hypoglycemia is suspected**

**FBG fasting blood glucose, OADs oral antidiabetic agents, SU sulfonylureas**

**Table 6**  Limited care recommendations for frequency/timing of SMBG

<table>
<thead>
<tr>
<th>Type 1 DM</th>
<th>Type 2 DM on OADs</th>
<th>Type 2 DM on insulin or insulin +OADs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Children</td>
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<tr>
<td></td>
<td>New onset DM/uncontrolled DM/DM during acute illness</td>
<td>New onset DM/uncontrolled DM/DM during acute illness</td>
</tr>
<tr>
<td></td>
<td>Stable/well-controlled DM</td>
<td>Stable/well-controlled DM</td>
</tr>
</tbody>
</table>

- **At least 4 times/day**
  - At least 3 times/day
  
  - Patients on SU or meglitinides
  - At least FBG alternate days
  
  - Patients on other
  - At least FBG once a week

- **At least 4 tests in a month**
  - At least 1 test/week (including a FBG and 3 post-prandial values in a month)

- **Must check whenever hypoglycemia is suspected**

**FBG fasting blood glucose, OADs oral antidiabetic agents, SU sulfonylureas**
hypoglycemia and/or ketoacidosis, which lead to disruption of quality of life.

New onset diabetes

Recommended care

- Newly diagnosed diabetes
- Recommended care constitutes evidence-based care which is cost-effective.

Limited Care

- Limited care is the lowest level of care that seeks to achieve the major objectives of diabetes management provided in healthcare settings with very limited resources such as drugs, personnel, technologies, and procedures.

These recommendations by the expert panel include details of the SMBG regimens for different clinical scenarios. These recommendations conform with and can be considered as an extension of the recently published RSSDI recommendations, in which SMBG regimens were not discussed in detail [93].

General recommendations

- RSSDI-recommended target levels should be adequately explained to the patient/provider and mutually agreed between the patient/provider and the clinician.
- SMBG technique should be properly explained to the patient.

Recommendations for use of lancets/pricking devices

Recommended care

Single use of lancet/pricking needles (disposable injection needles are commonly used in India in place of lancets) is recommended.

Table 7 Recommended care and limited care for frequency/timing of SMBG for diabetes in pregnancy

<table>
<thead>
<tr>
<th>Patients on lifestyle modifications</th>
<th>Patients on OADs or insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended care</strong></td>
<td><strong>Limited care</strong></td>
</tr>
<tr>
<td>• A day profile once a week—FBG and 3 post-prandial values at least once a week or staggered over the week</td>
<td>• 1 FBG and one post-prandial value every week (any meal, preferably largest meal of the day)</td>
</tr>
</tbody>
</table>

FBG fasting blood glucose

- • SMBG technique of the patients should be evaluated regularly and appropriate feedback given.
- • SMBG device should comply with the ISO 15197:2013 requirements.
- • The recommended target levels that should be followed for most diabetes patients for fasting blood glucose, post-prandial blood glucose, and HbA1c are \( \leq 115 \) mg/dL, \( \leq 160 \) mg/dL, and \( < 7.0\% \), respectively [93].
- • Patients should be educated that the post-prandial blood glucose levels should be checked after 1/2 h from the start of the meal and not the end of the meal.
- • Patients may be allowed to make minor adjustments to insulin dosage and changes in diet and exercise based on the SMBG readings.
- • Annual structured assessment should be carried out to evaluate patient’s self-monitoring skills including monitoring technique, interpretation of blood glucose results, impact on patient’s quality of life, and continued benefit to the patient (a questionnaire will be developed for annual evaluation of the patients).

Table 8 Recommended schedule for patients on basal insulin

<table>
<thead>
<tr>
<th></th>
<th>Fasting/pre-breakfast</th>
<th>Post-breakfast</th>
<th>Pre-lunch</th>
<th>Post-lunch</th>
<th>Pre-dinner</th>
<th>Post-dinner</th>
<th>3 a.m.</th>
<th>SOS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>✓</td>
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</tbody>
</table>

Limited care: fasting levels twice a week or once in 3 days

After achievement of fasting target, post-prandial correction should be done

† SOS whenever hypoglycemia is suspected and during intercurrent acute illness
Limited care Although single use is recommended, it is important to also consider the cost especially in limited resource settings. It is recommended that if a patient chooses to reuse the lancet or pricking device, proper antiseptic precautions should be taken. The lancet/pricking device should be discarded when the tip goes blunt or the prick becomes painful. Also, the lancet/pricking device should be immediately discarded if it comes in contact with another individual’s blood. If a patient decides to reuse pricking needle, proper care must be taken as mentioned below:

- Cover should be placed back on the needle immediately.
- Needle should not touch any surface apart from the inside of the needle cover.
- Cleaning the needle with alcohol should be avoided as it can make the point blunt.

Recommendations based on DM type, treatment approach, and glycemic control

The expert panel recommends customizing the frequency and timing of SMBG depending on whether it is type 1 or 2 DM. In patients with type 2 DM, monitoring will further vary depending on whether the patient is on OADs or insulin and whether it is new onset DM/uncontrolled DM or well-controlled DM. The panel provides recommendations for two levels of care: recommended care and limited care (Tables 5 and 6).

All patients on multiple-dose insulin therapy should perform SMBG at least two times/day (ideally before any insulin injection). More frequent testing may be required in:

- Patients with frequent hypoglycemia or hypoglycemic symptoms
- Patients not at HbA1c target levels

In patients on intensive insulin therapy, blood glucose levels should be checked at fasting, pre-meal, at bedtime, and periodically at 3 am.

Recommendations for diabetes in pregnancy

In patients with pre-existing diabetes or GDM, target blood glucose levels should be 70 to 90 mg/dL fasting, < 140 mg/dL 1-h post-prandial, and < 120 mg/dL 2-h post-prandial. Patients on lifestyle modifications should have a day profile once a week.
This should include one fasting and three post-prandial values at least once a week or staggered over a week (this is consensus opinion, not based on published evidence) (Table 7). Patients on OADs or insulin should perform intensive monitoring.

**Recommendations by the expert panel for patients on basal insulin**

In patients on basal insulin, daily fasting levels are recommended (recommended care) (Table 8). In resource-limited settings, fasting levels can be performed twice a week or once in 3 days (limited care). Post-prandial correction should be done after correcting fasting blood glucose.

**Recommendations by the expert panel for premix insulin or basal bolus**

Patients on premix insulin or basal bolus therapy should be advised to perform three pre-prandial (including fasting) and three post-prandial tests on alternate days till target HbA1c and blood glucose levels are reached. After achievement of the target, less frequent testing can be done (Table 9).

**Recommendations by the expert panel for patients with brittle diabetes and hypoglycemia unawareness**

In patients with brittle diabetes or hypoglycemia unawareness, 7-point testing is recommended with a 3 a.m. testing at least once a week (Table 10).

### Special situations/hemodynamically unstable conditions/end stage organ disease

These patients are usually on multiple doses of insulin per day or on insulin infusions. In these patients, the frequency or timing of SMBG should be customized based on the individual case. More frequent monitoring may be required based on the clinical situation.

### Recommendations for elderly patients

In elderly patients, monitoring should be less frequent, and the target should be relaxed to avoid hypoglycemia. A consensus by the American Diabetic Association and the American Geriatrics Society recommends dividing the patients into three categories based on their health status to enable customizing the glycemic targets. Their recommendations are listed in Table 11 [110]. The expert panel endorses these recommendations for glycemic targets in the elderly.

The expert panel recommends that, in the initiation phase, the frequency of SMBG should be once daily (different time each day) and later it should be reduced further to two to three times per week (Table 12). Hypoglycemia is a special concern in the elderly and pre-prandial values are important. The family should also be educated and trained on SMBG.

The expert panel hopes that these consensus recommendations will serve as a valuable tool for the practice of SMBG in India.

### Table 11 Glycemic targets in elderly patients as per ADA and AGS consensus [110]

<table>
<thead>
<tr>
<th>Target glycemc levels</th>
<th>Healthy elderly</th>
<th>Elderly with intermediate health status</th>
<th>Elderly with poor health status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>&lt; 7.5%</td>
<td>&lt; 8.0%</td>
<td>&lt; 8.5%</td>
</tr>
<tr>
<td>Fasting or pre-prandial glucose (mg/dL)</td>
<td>90–130</td>
<td>90–150</td>
<td>100–180</td>
</tr>
<tr>
<td>Bedtime glucose (mg/dL)</td>
<td>90–150</td>
<td>100–180</td>
<td>110–200</td>
</tr>
</tbody>
</table>

### Table 12 Recommended schedule for elderly patients

<table>
<thead>
<tr>
<th>Fasting/pre-breakfast</th>
<th>Post-breakfast</th>
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</tbody>
</table>

The above regimen is for initiation phase. Once target is achieved, frequency should be reduced to 2 to 3 tests/ week

† SOS whenever hypoglycemia is suspected or during intercurrent acute illness
Acknowledgements The authors thank Turacoz Healthcare Solutions (www.Turacoz.com), Gurugram, India, for writing support.

Compliance with ethical standards

Conflict of interest This consensus was supported by restricted academic grant from Roche. However, there was no interference/influence from Roche in the consensus development. None of the authors have declared any conflict of interest.

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Association of vitamin D deficiency and type 1 diabetes mellitus: a systematic review and meta-analysis

Vanessa Rabello Lovisi Sales de Oliveira & Caroline Pereira Domingueti

Abstract
Some studies have suggested that vitamin D deficiency may be associated with autoimmune diseases such as type 1 diabetes mellitus (T1DM). The objectives of this review were to perform a systematic review and meta-analysis and to assess the association between vitamin D deficiency and T1DM. PubMed, Web of Science, Lilacs, and Scielo databases were used to search the articles. The eligibility criteria were cohort, case-control, or cross-sectional observational studies, which assessed the association between vitamin D deficiency and T1DM, comparing T1DM patients with control group. Cross-sectional studies that compared means of vitamin D levels between T1DM patients and control group were included in the first meta-analysis, and cross-sectional studies that compared frequency of vitamin D deficiency between T1DM patients and control group were included in the second meta-analysis. Thirteen studies were included in the systematic review. Most studies (n = 12) compared vitamin D levels between T1DM patients and control group and 75% of them (n = 9) found lower vitamin D levels in T1DM patients. Over half studies (n = 8) compared vitamin D deficiency frequency between T1DM patients and control group and 50% (n = 4) of them observed a higher frequency of vitamin D deficiency in T1DM patients. Meta-analysis demonstrated a significant difference of vitamin D levels between T1DM patients and control group (difference between means = 0.739 ± 0.067, p < 0.001) and that there is a significant association of vitamin D deficiency and T1DM [OR = 1.640 (1.18–1.28), p = 0.003]. There is a significant association between vitamin D deficiency and T1DM.

Keywords Diabetes mellitus, type 1 · Vitamin D deficiency · Risk factors

Introduction
Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders that is characterized by hyperglycemia, which is caused by defects in insulin action, in insulin secretion, or both [1, 2]. Hyperglycemia may increase the risk of developing microvascular injuries such as retinopathy, nephropathy and neuropathy, and macrovascular injuries such as stroke, ischemic heart disease, and peripheral vascular disease. These complications of DM, in addition to reduce the patients’ quality of life, are also associated with reduced life expectancy and significant increase in morbidity and mortality [2].

Type 1 diabetes mellitus (T1DM) comprises about 5 to 10% of all cases of DM and occurs due to destruction of pancreatic β cells mediated by autoimmune response, which involves the participation of T cells (autoimmune form) or of unknown cause (idiopathic). Autoimmune form can be confirmed by the presence of one or more autoantibodies in serum, such as anti-islet cell antigen, anti-insulin, anti-glutamic acid decarboxylase, anti-tyrosine phosphatases, anti-zinc-transporter 8 [3]. T1DM patients have autoimmune genetic susceptibility to developing such disease, so that first-degree relatives of a person affected by the disease present an increased risk of developing it [4].

Environmental factors are also reported as possible triggers of autoimmune T1DM. The most studied environmental factors can be classified into three groups: viral infections (cytomegalovirus, rubella, mumps, measles), early diet in childhood (breastfeeding versus early introduction of cow’s milk ingredients, cereals, and gluten), and toxins (e.g., derivatives of N-nitroso) [4].
Recently, some studies have suggested that vitamin D deficiency may be associated with autoimmune T1DM [5–7]. Vitamin D is a steroid hormone whose main function is the regulation of osteomineral physiology. In humans, 80 to 90% of vitamin D are synthesized endogenously, and the remaining necessary for the human body (10 to 20%) comes from the diet: vitamin D3 (cholecalciferol) found in fish such as tuna and salmon, and vitamin D2 (ergosterol) found in edible fungi [8, 9].

It has been suggested that vitamin D may act directly and indirectly in insulin secretion. Directly because the β pancreatic cells have specific receptors for vitamin D, and indirectly because the pancreatic tissue has vitamin D dependent calcium-binding proteins mediating the intracellular and extracellular calcium influx in β cells. Insulin secretion is a calcium-dependent process possibly mediated by vitamin D and parathormone. The vitamin D insufficiency leads to increased parathormone concentrations, which reduce the secretory capacity of β cells, and vitamin D deficiency hinders the ability of β cells to convert proinsulin to insulin [10].

Moreover, vitamin D consists of a potent immune system modulator. The receptor of this hormone can be found in different cells of the immune system such as lymphocytes, monocytes, macrophages, and dendritic cells. Vitamin D leads to an increased innate immunity associated with regulation of acquired immunity; thus, its deficiency may be related to the onset of certain autoimmune diseases such as T1DM, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease. Therefore, vitamin D supplementation may be used both in prevention and in treatment of such autoimmune diseases [10].

However, the association between vitamin D deficiency and T1DM is not well established, since the studies conducted to date in order to assess this issue showed conflicting results. One meta-analysis has been already conducted about this. Despite that it has analyzed the difference of vitamin D levels between T1DM patients and control group, it did not evaluate the difference of frequency of vitamin D deficiency between the groups [11]. Therefore, the aim of this study was to perform a systematic review and meta-analysis to assess the association between vitamin D deficiency and T1DM.

**Methodology**

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [12]. A search was conducted in four databases: PubMed, Web of Science, Lilacs, and Scielo until February 2016.

The selection of articles in PubMed and Web of Science databases was made using the descriptors “diabetes mellitus, type 1,” “diabetes mellitus, insulin-dependent,” “diabetes mellitus, insulin dependent,” “insulin-dependent diabetes mellitus,” “diabetes mellitus, insulin-dependent, 1,” “diabetes mellitus, juvenile-onset,” “diabetes mellitus, juvenile onset,” “juvenile-onset diabetes mellitus,” “type 1 diabetes mellitus,” “diabetes mellitus, sudden-onset,” “diabetes mellitus, sudden onset,” “mellitus, sudden-onset diabetes,” “sudden-onset diabetes mellitus,” “IDDM,” “insulin-dependent diabetes mellitus 1,” “insulin dependent diabetes mellitus 1,” “juvenile-onset diabetes mellitus, 1,” “diabetes mellitus, brittle,” “diabetes mellitus, ketosis-prone,” “diabetes mellitus, ketosis prone,” “ketosis-prone diabetes mellitus,,” “diabetes, autoimmune,” “autoimmune diabetes,” in combination to the descriptor “vitamin d,” using the connector “AND” between the terms. The Medical Subject Headings (MeSH) was used to define these descriptors.

The selection of articles in Scielo and Lilacs databases was performed using the descriptors “diabetes mellitus tipo 1,” “diabetes mellitus instável,” “diabetes mellitus insulin dependente,” “diabetes mellitus insulino-dependente,” “diabetes mellitus dependente de insulina,” “diabetes mellitus de início na juventude,” “diabetes mellitus com tendência à cetose,” “DMID,” “diabetes autoimune,” “diabetes mellitus de início súbito” in combination to the descriptor “vitamina d,” using the connector “AND” between the terms. The Descriptors em Ciências da Saúde (DeCS) was used to define these descriptors.

The eligibility criteria were cohort, case-control, or cross-sectional observational studies written in English, Portuguese, or Spanish, which assessed the association between vitamin D deficiency and T1DM, comparing T1DM patient group with control group.

The selection of articles was carried out in two steps, both performed by two researchers. In the first step, the articles were identified according to the search criteria, repeated were excluded, and a previous reading of the title and abstract of the articles was made in order to include only those that met the eligibility criteria. In the second step, the articles selected in the first step were read in full to assess their inclusion in the study according to the eligibility criteria.

The following data were extracted from the articles included in this systematic review: number of patients and number of individuals in the control group, age of the patients and control subjects, country where the study was performed, study design, vitamin D dosage method, and results obtained by each of the studies. Methodological quality of the studies was evaluated by Newcastle-Ottawa Scale (NOS) adapted for cross-sectional studies.

Meta-analysis was performed using Comprehensive Meta-Analysis software version 3. In the first meta-analysis, only cross-sectional studies that compared means of vitamin D levels between T1DM patients and control group were included, and difference between means was evaluated. In the
second meta-analysis, only cross-sectional studies that compared frequency of vitamin D deficiency, defined as vitamin D levels lower than 50 nmol/L, between T1DM patients and control group were included, and odds ratio was calculated. Heterogeneity between studies was evaluated using $I^2$ test. Studies were considered homogeneous if $I^2$ was higher than 50% and $p$ value was lower than 0.10. Difference between means and odds ratio was calculated using fixed-effect model or random-effect model in the presence of homogeneity and heterogeneity, respectively.

Results

Figure 1 shows a flowchart evidencing the study selection process. Initially, 997 potential articles were identified in the selected databases. After initial analysis, 19 were excluded because they were repeated, 812 for not evaluating the association between vitamin D deficiency and T1DM or because they were not cohort, case-control, or cross-sectional observational studies, and 137 for not comparing T1DM patients with control group. Only one study was excluded because it was unavailable. After trying to contact the related author by e-mail twice, no response was obtained. In the second step, full text of the 28 articles selected in the first step were read and 9 articles were excluded for not evaluating the association between vitamin D deficiency and T1DM and 6 for not comparing T1DM patients with control group. Therefore, 13 studies were included in this systematic review.

Characteristics of studies included in this systematic review are shown in Table 1. Chemiluminescence assay was the most widely used method for serum vitamin D determination [46.2% ($n = 6$)], followed by enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC) and radioimmunoassay [15.4% ($n = 2$ for each of them)], and tandem mass spectrophotometry [7.7% ($n = 1$)]. Among the studies, 9 (69.2%) included children and/or adolescents and 3 (23.1%) included only adults. One study (7.7%) did not report the age of patients and control group. The study design was cross-sectional in 76.9% ($n = 10$) of them, two studies were case-control (15.4%), and only one was cohort (7.7%). The number of patients and controls in selected studies ranged from 40 to 1000. Sample size of T1DM patient group was lower than 100 in 8 studies (61.5%), between 100 and 500 in 4 studies (30.8%) and higher than 500 in only one study (7.7%). Sample size of control group was lower than 100 in 5 studies (38.5%), between 100 and 500 in 7 studies (53.8%) and higher than 500 in only one study (7.7%).

Results of the studies included in this systematic review are shown in Table 2. Most studies [92.3% ($n = 12$)] compared means or medians of vitamin D levels between T1DM patients and control group. Vitamin D levels in T1DM patients were found lower compared to control group by 75.0% of them ($n = 9$) and 3 studies did not find significant difference between groups (25.0%). Over half studies [61.5% ($n = 8$)] compared frequency of vitamin D deficiency between T1DM patients and control group. A higher frequency of vitamin D deficiency was observed, defined by vitamin D levels lower than 50 nmol/L, in T1DM patients compared to control group.
by 50% of them \((n = 4)\). No significant differences in frequency of vitamin D deficiency between groups by the other 50% was verified \((n = 4)\). However, vitamin D deficiency was defined by vitamin D levels lower than 50 nmol/L by two of them and lower than 75 nmol/L by the other two.

Odds ratio (OR) was evaluated by three cross-sectional studies and all of them found a significant association between vitamin D deficiency and T1DM \([\text{OR} = 5.56 \ (1.66–18.56), \ \text{OR} = 3.11 \ (1.02–9.47), \ \text{and \ OR} = 1.385 \ (1.00–1.91)]\). The two case-control studies included in this systematic review also evaluated OR. One of them observed a higher risk of developing T1DM for the lowest quintile \(<43 \ \text{nmol/L}\) of prediagnostic vitamin D levels compared to the highest quintile \((\geq 100 \ \text{nmol/L})\) and fourth quintile \((78 \ < \ 100 \ \text{nmol/L})\) \([\text{OR} = 3.5 \ (2.0–6.0) \ \text{and \ OR} = 2.5 \ (1.5–4.2)]\). The other study found no significant association between reduced vitamin D levels at birth and the risk to develop T1DM up to 10 years of age \([\text{OR} = 1.76 \ (0.92–3.38)]\).

Methodological quality evaluation of the cross-sectional studies is presented in Table 3. Among ten studies, three (30.0%) are considered of excellent quality (received seven or more stars), seven (70.0%) have good quality (received five or six stars), and only one (10.0%) has regular quality (received four stars).

Six cross-sectional studies compared means of vitamin D levels between T1DM patients and control group (difference between means = 0.739 ± 0.067, \(p < 0.001\)).

Six cross-sectional studies compared frequency of vitamin D deficiency, defined as vitamin D levels lower than 50 nmol/L, between T1DM patients and control group and were included in the second meta-analysis (Fig. 3). Heterogeneity analysis of these studies indicated that they are homogeneous \(I^2 = 92.406, \ p < 0.001\), and therefore, odds ratio was calculated using fixed-effect model. Meta-analysis demonstrated that there is a significant association of vitamin D deficiency and T1DM \([\text{OR} = 1.640 \ (1.18–1.28), \ p = 0.003]\).

**Discussion**

Recent studies have verified a direct relationship between vitamin D deficiency and several autoimmune diseases, including T1DM \([14, 24]\). Thus, this systematic review and meta-analysis aimed to evaluate the association between vitamin D deficiency and T1DM. Most of the studies included in this systematic review have found lower vitamin D levels in T1DM patients compared to control group \([7, 14–17, 19, 20, 22, 23]\). Besides, half of the studies that evaluated differences of frequency of vitamin D deficiency between T1DM patients and control group have found a higher frequency in T1DM patient group \([6, 14, 19, 20]\). Some studies have also observed a significant association between vitamin D deficiency and T1DM evaluated by OR \([7, 14, 17, 20]\).

All the studies that have found a higher frequency of vitamin D deficiency in T1DM patients compared to the control...
The results of the studies that evaluated the association between vitamin D deficiency and T1DM 

<table>
<thead>
<tr>
<th>Author</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADARIO et al. 2015 [13]</td>
<td>There was no significant association between reduced Vit D levels (5.35 nmol/L) at birth and the risk to develop T1DM up to 10 years of age [OR = 1.76 (0.92–3.38)].</td>
</tr>
<tr>
<td>FRANCHI et al. 2014 [14]</td>
<td>Vit D levels were significantly lower in T1DM patients [36.2 (7.5–121.0) nmol/L] compared to CG [48.7 (7.5–190.2) nmol/L], p = 0.010. Vit D deficiency (&lt; 50 nmol/L) frequency was higher in T1DM patients (67.2%) compared to CG (51.8%), p = 0.046. Vit D deficiency (&lt; 50 nmol/L) was associated with T1DM [OR = 5.56 (1.66–18.56)].</td>
</tr>
<tr>
<td>AL-DAGHRI et al. 2014 [15]</td>
<td>Vit D levels were lower in T1DM patients (28.1 ± 1.4 nmol/L) compared to CG (33.4 ± 1.6 nmol/L), p = 0.03. There was no significant difference between groups with respect to vit D deficiency (&lt; 50 nmol/L) frequency (100.0% in T1DM patients vs 78.4% in CG).</td>
</tr>
<tr>
<td>AZAB et al. 2013 [6]</td>
<td>Vit D deficiency (&lt; 50 nmol/L) frequency was higher in T1DM patients (55%) compared to CG (30%), p &lt; 0.01. There was no significant difference between groups with respect to Vit D levels (61.7 ± 14 nmol/L in T1DM patients × 66.2 ± 12 nmol/L in CG).</td>
</tr>
<tr>
<td>GREER et al. 2013 [16]</td>
<td>Vit D levels were lower in T1DM patients [78.7 (71.8–85.6) nmol/L] compared to CG [91.1 (83.5–98.7) nmol/L], p = 0.01.</td>
</tr>
<tr>
<td>GORHAM et al. 2012 [17]</td>
<td>Prediagnostic Vit D levels were lower in T1DM patients (62.2 ± 31.8 nmol/L) compared to CG (72.5 ± 33.0 nmol/L), p &lt; 0.0001. Reduced Vit D levels were associated with a higher risk of developing T1DM [OR = 3.5 (2.0–6.0) for the lowest quintile of Vit D (&lt; 43 nmol/L) compared to the highest quintile (≥ 100 nmol/L) and OR = 2.5 (1.5–4.2) for the lowest quintile of Vit D (&lt; 43 nmol/L) compared to the fourth quintile (≥ 78 and &lt; 100 nmol/L)], p &lt; 0.001].</td>
</tr>
<tr>
<td>GHANDCHI et al. 2012 [18]</td>
<td>There was no significant difference between groups with respect to Vit D levels (15.3 ± 2.3 nmol/L in T1DM patients × 20.4 ± 3.3 nmol/L in CG). There was no significant difference between groups with respect to Vit D deficiency (&lt; 50 nmol/L) frequency (95.0% in T1DM patients × 95.0% in CG).</td>
</tr>
<tr>
<td>BIN-ABBAS et al. 2011 [19]</td>
<td>Vit D levels were significantly lower in T1DM patients (36.7 ± 14.3 nmol/L) compared to CG (44.8 ± 14.1 nmol/L), p = 0.001. Vit D deficiency (&lt; 50 nmol/L) frequency was higher in T1DM patients (84%) compared to CG (59%), p = 0.001.</td>
</tr>
<tr>
<td>BORKAR et al. 2010 [20]</td>
<td>Vit D levels were significantly lower in T1DM patients (50.05 ± 26.57 nmol/L) compared to CG (65.40 ± 30.70 nmol/L), p = 0.009. Vit D deficiency (&lt; 50 nmol/L) frequency was higher in T1DM patients (58%) compared to CG (32%), p = 0.035. Vit D deficiency (&lt; 50 nmol/L) was associated with T1DM [OR = 3.11 (1.02–9.47), p = 0.046].</td>
</tr>
<tr>
<td>BENER et al. 2009 [7]</td>
<td>Vit D levels were lower in T1DM patients (39.5 ± 23.0 nmol/L) compared to CG (46.2 ± 24.0 nmol/L), p = 0.009. There was no significant difference between groups with respect to Vit D deficiency (&lt; 50 nmol/L) frequency (90.6% in T1DM patients × 85.3% in CG). Vit D deficiency was associated with T1DM [OR = 1.385 (1.00–1.91), p = 0.048].</td>
</tr>
<tr>
<td>BIERSCHEK et al. 2009 [21]</td>
<td>There was no significant difference between groups with respect to Vit D levels [53.0 (30.5–75.5 nmol/L) in newly diagnosed T1DM patients × 58.0 (34.5–84.7) nmol/L in established T1DM patients × 50.2 (32.5–93.5) nmol/L in CG]. There was no significant difference between groups with respect to Vit D deficiency (≤ 75 nmol/L) frequency (76.1% in newly diagnosed T1DM patients × 68.5 in established T1DM patients × 70.1 in CG).</td>
</tr>
<tr>
<td>LITTORIN et al. 2006 [22]</td>
<td>Vit D levels were significantly lower in T1DM patients at diagnosis (82.5 ± 1.3 nmol/L) compared to CG (96.7 ± 2.0 nmol/L), p &lt; 0.0001. Vit D levels were significantly lower in patients with T1DM 18 years after diagnosis (81.5 ± 2.6 nmol/L) compared to at the time of diagnosis (86.3 ± 2.6 nmol/L), p &lt; 0.04.</td>
</tr>
<tr>
<td>POZZILLI et al. 2005 [23]</td>
<td>Vit D levels were significantly lower in T1DM patients (75 nmol/L) compared to CG (120 nmol/L), p &lt; 0.01.</td>
</tr>
</tbody>
</table>

Vit D vitamin D, T1DM type 1 diabetes mellitus, CG control group, OR odds ratio

Group [6, 14, 19, 20] and half of those that did not find significant differences between groups [15, 18] used vitamin D levels lower than 50 nmol/L as reference value for vitamin D deficiency. On the other hand, half of the studies that did not find significant differences between groups [7, 21] used vitamin D levels lower than 75 nmol/L as reference value. This divergence of reference value could interfere in the results, since a higher cut-off could increase the frequency of vitamin D deficiency in both groups, reducing the difference between them.

Furthermore, frequency of vitamin D deficiency of control group was lower in studies that have found a higher frequency of vitamin D deficiency in T1DM patients compared to the control group [6, 14, 19, 20] than in those that did not find significant differences between groups [7, 15, 18, 21]. A higher incidence of vitamin D deficiency in control group
could contribute for minimizing the difference between T1DM patient group and control group.

Only three studies did not observe significant differences between T1DM patients and control group with respect to vitamin D levels [6, 18, 21]. One of them verified that although vitamin D levels were not different between groups, frequency of vitamin D deficiency was lower in patients with T1DM compared to the control group [6]. In the other two studies, frequency of vitamin D deficiency of control group was high [70.1% of individuals with vitamin D levels lower than 75 nmol/L [21] and 95.0% with lower than 50 mol/L [18]], which could reduce the differences of vitamin D levels between T1DM patients and control group.

Only one study did not verify significant association between vitamin D deficiency and T1DM evaluated by OR [13]. This case-control study showed that reduced vitamin D levels at birth are not associated with the risk to develop T1DM up to 10 years of age. This result could be explained by the fact that vitamin D levels were determined only at birth, and vitamin D levels during childhood or adult life could be more important for T1DM development than its levels at birth. This hypothesis may be corroborated by the other case-control study included in this systematic review [17], which showed that prediagnostic vitamin D levels are associated with a higher risk of developing T1DM in adults.

Meta-analysis demonstrated a significant difference of vitamin D levels between T1DM patients and control group and a significant association of vitamin D deficiency, defined as vitamin D levels lower than 50 nmol/L, and T1DM, which suggests a protective effect of vitamin D against T1DM development. However, as all the studies included in the meta-analysis were cross-sectional, it is

<table>
<thead>
<tr>
<th>Study name</th>
<th>Std diff in means</th>
<th>Standard error</th>
<th>Variance</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Daghri et al., 2014</td>
<td>3,526</td>
<td>0,292</td>
<td>0,085</td>
<td>2,954</td>
<td>4,097</td>
<td>12,084</td>
<td>0,000</td>
</tr>
<tr>
<td>Azab et al., 2013</td>
<td>0,337</td>
<td>0,195</td>
<td>0,038</td>
<td>-0,045</td>
<td>0,718</td>
<td>1,727</td>
<td>0,084</td>
</tr>
<tr>
<td>Gandchi et al., 2012</td>
<td>1,697</td>
<td>0,182</td>
<td>0,033</td>
<td>1,341</td>
<td>2,053</td>
<td>9,341</td>
<td>0,000</td>
</tr>
<tr>
<td>Bin-Abbas et al., 2011</td>
<td>0,570</td>
<td>0,144</td>
<td>0,021</td>
<td>0,288</td>
<td>0,853</td>
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<tr>
<td>Borkar et al., 2010</td>
<td>0,535</td>
<td>0,204</td>
<td>0,041</td>
<td>0,136</td>
<td>0,934</td>
<td>2,627</td>
<td>0,009</td>
</tr>
<tr>
<td>Bener et al., 2009</td>
<td>0,285</td>
<td>0,109</td>
<td>0,012</td>
<td>0,071</td>
<td>0,499</td>
<td>2,615</td>
<td>0,009</td>
</tr>
</tbody>
</table>

Fig. 2 Meta-analysis of studies that evaluated the difference of vitamin D levels means between type 1 diabetes mellitus patients and control group.
Statistics for each study

<table>
<thead>
<tr>
<th>Study name</th>
<th>Odds ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Z-Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franchi et al., 2014</td>
<td>1.906</td>
<td>1.018</td>
<td>3.569</td>
<td>2.017</td>
<td>0.044</td>
</tr>
<tr>
<td>Al-Daghri et al., 2014</td>
<td>0.031</td>
<td>0.011</td>
<td>0.087</td>
<td>-6.547</td>
<td>0.000</td>
</tr>
<tr>
<td>Azab et al., 2013</td>
<td>2.852</td>
<td>1.272</td>
<td>6.392</td>
<td>2.546</td>
<td>0.011</td>
</tr>
<tr>
<td>Gandchhi et al., 2012</td>
<td>1.000</td>
<td>0.241</td>
<td>4.145</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Bin-Abbas et al., 2011</td>
<td>3.648</td>
<td>1.873</td>
<td>7.107</td>
<td>3.804</td>
<td>0.000</td>
</tr>
<tr>
<td>Borkar et al., 2010</td>
<td>2.935</td>
<td>1.296</td>
<td>6.647</td>
<td>2.581</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>1.640</td>
<td>1.180</td>
<td>2.280</td>
<td>2.948</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Fig. 3  Meta-analysis of studies that evaluated the difference of vitamin D deficiency frequency, defined as vitamin D levels lower than 50 nmol/L, between type 1 diabetes mellitus patients and control group.

not possible to determine whether vitamin D deficiency occurred before or after T1DM development.

A multicenter study showed that vitamin D supplementation in infancy reduces the risk of developing T1DM, thus providing a protective effect [25]. Another study demonstrated that vitamin D supplementation was associated with a decreased frequency of T1DM development. Children who received a daily dose of 2000 U of vitamin D had a lower risk of developing T1DM than children who received the lower dose [26]. These studies corroborate the association between vitamin D deficiency and T1DM demonstrated by meta-analysis.

The highest prevalence of T1DM is observed in Finland, which presented a rising incidence rate of the disease from 1980 (32.4 per 100,000 person-years) until 2006 (64.9 per 100,000 person-years) [27]. Finland presents high latitude and low solar incidence, especially during the long winters, which results in high vitamin D deficiency [28]. A recent study has demonstrated that the incidence rate of T1DM in Finland has remained stable after 2006. A significant increase in vitamin D intake after 2003 due to the introduction of vitamin D fortification of milk productions is suggested to have contributed to this stabilization of incidence rate [27].

Vitamin D seems to interact with the immune system through its action on the regulation and differentiation of cells such as lymphocytes, macrophages and natural killer cells (NK), and interfering with the production of cytokines. In general, the effects of vitamin D on the immune system lead to increased innate immunity and regulation of different forms of acquired immunity [9]. Several effector mechanisms are involved in the pathophysiology of T1DM, which lead to cell destruction, including the presence of CD8+ T lymphocytes e macrophages, which regulate the differentiation of T helper 1 cells by interleukin 1 [9]. Vitamin D acts on the inhibition of interferon-γ production and other cytokines that activate macrophages and T cytotoxic cells which, in turn, lead to destruction of pancreatic islet cells [29].

Study design varied among studies and only one study was a cohort [22]. This study verified that vitamin D levels were lower in T1DM patients 18 years after diagnosis compared to at the time of diagnosis, indicating that vitamin D levels tend to reduce over time in T1DM patients. However, this study did not evaluate vitamin D levels before the emergence of T1DM and, therefore, did not analyze the association of vitamin D deficiency with the risk to develop T1DM.

As T1DM is frequently diagnosed during infancy or adolescence [1], most studies included children and/or adolescents [6, 7, 13, 14, 16, 19–21, 23] in order to analyze differences of vitamin D deficiency between T1DM patients and control group soon after the diagnosis was made. The purpose of this strategy was trying to evaluate the association of vitamin D deficiency with the risk of developing T1DM. However, a cohort study could be better to evaluate this issue and most studies included in this systematic review have cross-sectional design [6, 7, 14–21, 23].

Furthermore, the methods used for determination of vitamin D levels were different among the studies. Mass spectrophotometry and HPLC are the most accurate methods to vitamin D dosage [8] and they were used by only 3 studies [13, 18, 20]. ELISA, radioimmunoassay, and chemiluminescent assay have less accuracy and greater variability [8], which may reduce the reliability of determination of vitamin D levels. The fact that the studies were conducted in different countries and seasons of the year is also a source of bias among them, since the incidence of sunlight is directly related to serum levels of vitamin D, because it is a hormone that has its endogenous synthesis activated by the absorption of B ultraviolet rays [8].

Conclusion

Most studies included in this systematic review found that vitamin D deficiency is associated with T1DM. Furthermore, meta-analysis demonstrated that T1DM patients have lower vitamin D levels than control group and that there is a significant association of vitamin D deficiency and T1DM. However, due to the cross-sectional design of most studies, it is not possible to establish causality between vitamin D deficiency and T1DM. It is not possible to establish if vitamin D deficiency causes the development of T1DM or if it arises after the onset of T1DM. Probably due to the critical role that vitamin D plays in regulating the immune system and
in preventing autoimmune diseases, vitamin D deficiency should consist of a risk factor for T1DM. This hypothesis can be supported by some authors who found that supplementation with vitamin D can lead to a protective effect against the development of T1DM. However, it is necessary to perform further cohort studies to confirm this hypothesis.

**Compliance with ethical standards**

**Conflict of interest** Author Vanessa Rabello Lovisi Sales de Oliveira declares that she has no conflict of interest. Author Caroline Pereira Domingueti declares that she has no conflict of interest. Author Vanessa Rabello Lovisi Sales de Oliveira

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

**References**


The purpose of this study was to compare the status of psychosocial factors and glycemic control in insulin-naïve and insulin-experienced people with type 2 diabetes (T2D). In this observational study on people with T2D, demographic, self-care behavior, resources, and affective variables as well as health-related quality of life were assessed and compared in insulin-naïve and insulin-experienced considering the number of oral glucose-lowering drugs (OGLDs). Measured variable path analysis was used to test the association among variables and their effect on HbA1c in both groups. In total, 215 insulin-naïve and 165 insulin-experienced patients were recruited in this study. The mean duration of diabetes was 11.7 ± 7.0 years in insulin-experienced and 6.8 ± 5.4 years in insulin-naïve (p < 0.001). The mean hemoglobin A1c (HbA1c) was significantly higher in insulin-experienced subjects irrespective of the number of OGLDs [68 ± 20 mmol/mol (8.4 ± 1.8%) vs. 56 ± 16 mmol/mol (7.3 ± 1.4%); p < 0.001]. Moreover, insulin-experienced subjects had significantly higher level of diabetes-related distress (2.2 ± 0.9 vs. 1.9 ± 0.8), depression (9.5 ± 5.5 vs. 8.1 ± 5.1), anxiety (18.3 ± 12.0 vs. 15.1 ± 10.5), and lower knowledge of insulin use considering the results of 9-item insulin-use subscale of Michigan diabetes knowledge test (mean 3.9 ± 1.8) compared to insulin-naïve subjects (p < 0.05). Higher levels of distress, depression, and anxiety are found in insulin-experienced people with T2D. Therefore, one should be aware that, at the time of insulin need/initiation, people with T2D have reached a more vulnerable state and this should be taken into consideration when implementing a complex insulin initiation plan.

Keywords Diabetes, type 2 · Psychosocial · Glycemic control
Introduction

The benefit of good glycemic control on diabetes-related complications is obvious in people with type 2 diabetes (T2D) [1]. Timely and effective treatment should be considered to bring patients to glycemic and other metabolic targets. However, achieving glycemic target is difficult. Several studies showed that only half of patients achieved hemoglobin A1c (HbA1c) target [2, 3], while 9% had very poor control [3]. Although lifestyle modifications and oral glucose-lowering drugs (OGLDs) are essential in management of the disease, long-term glycemic control is difficult to achieve and ultimately there is a need for initiation of insulin treatment [4]. Delay in insulin initiation or intensification [3] may be due to patient and physician reluctance [5] and/or fear of hypoglycemia [6] as well as weight gain [7] are possible causes. On the other hand, many patients remain under poor glycemic control even years after they received their first insulin prescription [4].

Considering that insulin is the most effective glucose-lowering agent, it is necessary to understand why patients with T2D who are on insulin are still unable to achieve good glycemic control [8, 9]. Hence, the aim of this study was to compare the status of psychosocial factors and glycemic control in insulin-naïve and insulin-experienced people with T2D in routine clinical practice.

Methods

We performed a non-interventional, observational study of patients with T2D, aged 30 years or above with diabetes duration of 6 months or more. A detailed description of the design, eligibility criteria, and questionnaires has been published previously [10]. Briefly stated, patients were excluded if they had severe diabetes-related complications, active psychosis, a history of substance use, dementia, or if they were pregnant.

People coming for regular clinic visits were recruited in the study. The project and its goals were explained for every eligible patient. All patients signed the informed consent. Patients were asked to complete the questionnaires during the following 7 days. Four categories of variables were assessed to explore their associations with type of treatment: 1. Demographic variables including age, gender, duration of diabetes, type of treatment, smoking, body mass index (BMI), abdominal circumference, and hip circumference. 2. Self-care behavior variables comprised of total daily calorie intake long form of international physical activity questionnaire (IPAQ) and self-management profile for type 2 diabetes (SMP-T2DM).

Calorie intake was assessed using a single 24-h recall. Detailed questions were asked about all foods and beverages consumed during the previous day.

IPAQ covers four domains of physical activity including occupational-related, transportation, household/gardening, and leisure-time activities. According to the scoring system, physical activity levels were categorized as inactive, minimally active, or health enhancing physically active [11].

SMP-T2DM consisted of 18 items on five self-care domains scoring between 0 and 7. Higher scores indicated better self-management [12].

3. Resources variables consisting of family social support questionnaire (FSSQ), brief Michigan diabetes knowledge test (DKT), and patient assessment of care for chronic conditions (PACIC).

FSSQ consisted of 79 questions with higher scores indicating higher levels of social support [13].

DKT comprised of 23 items to assess general knowledge of diabetes [14].

PACIC-5A consisting of 26 items on 6 domains was used to assess patient physician relationship [15].

4. Affective variables including WHO-5 well-being index, patient health questionnaire (PHQ-9), beck anxiety inventory, and diabetes distress scale (DDS).

WHO-5 including 5 items was used to assess emotional well-being. Higher scores was attributed to higher level of well-being [16].

PHQ-9 was comprised of 9 items to assess level of depression [17].

Beck anxiety inventory consisted of 21 multiple choice questions to assess level of anxiety [18].

DDS included 17 items. A mean item score of < 2 was interpreted as little or no distress, 2–2.9 as moderate, and ≥ 3 as high distress [19].

Health-related quality of life was also assessed using self-administered EQ-5D questionnaire [20], which contains questions about mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. In addition, patients were asked to rate a state of ill health on a visual analog scale from 0 to 100, with 0 representing being worst imaginable health and 100 representing best imaginable health.

A calibrated digital scale (Seca gmbh & co. kg. Germany) was used for weight measurement. Height measurement was done with a stadiometer (Seca gmbh & co. kg. Germany) calibrated before each measurement. A trained nurse assessed abdominal and hip circumferences using a cloth tape. The midpoint between the highest point of the iliac crest and the lowest part of the costal margin in the mid-axillary line was defined as waist, and
the hip was measured at the level of the greater femoral trochanters.

Systolic and diastolic blood pressure (BP) measurements were obtained from each patient (the right arm) in the sitting position, using a standard mercury sphygmomanometer (Erkameter 3000, ERKA, Bad Tolz, Germany) (Korotkoff I and V) with a cuff of appropriate size. Blood pressure was measured by the same trained nurse after the patient had rested for ≥ 10 min.

Blood samples were obtained after an overnight fast of at least 12 h for measurement of fasting blood sugar (FBS) using a glucose analyzer (YSI 2700 Select, YSI, Inc., Yellow Springs, OH), HbA1c using ion exchange chromatography (DS5 Analyzer, Drew Scientific limited, Cumbria, UK), Triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine (Cr), and blood urea were measured using an autoanalyzer (Liasys, AMS, Italy).

**Statistical analysis**

STATA (StataCorp. 2009. *Stata Statistical Software: Release 11.* College Station, TX: StataCorp LP.) and IBM SPSS for Windows Version 19 (IBM Corp., Armonk, NY, USA) were used to perform statistical analyses. In the first step, patients were divided into two groups, i.e., insulin-experienced and insulin-naïve. Sub-group analyses were then conducted in each group considering the number of OGLDs received (no/one, or ≥two). Percentage estimates and 95% confidence intervals (CI) were calculated for each item. Quantitative values were expressed as means ± standard deviation (SD), and qualitative values were expressed as percentages. Normal assumptions were checked by looking at the normal curve or frequency histogram as well as Kolmogorov-Smirnov test. In the case of normal distribution, we used parametric statistical test while we used non-parametric statistical, in the case of non-normal distribution or inequality in the variances of variables. Levene’s test was used to assess the equality of variances considering the number of OGLDs. When the variances were equal, the statistical significance of differences between the groups was determined using two-way analysis of variance. In the case of inequality of variances, Mann-Whitney test was used.

All statistical analyses were two-sided and a probability value of $p \leq 0.05$ was considered significant. Measured variable path analysis (MVPA), a form of structural equation modeling, was used to test the relationships among variables and their effect on HbA1c as the indicator of glycemic control using Mplus Version 6.12. The parameter estimation method was maximum likelihood. The $\chi^2$ tests were reported, but model fit was primarily evaluated with root mean square error of approximation (RMSEA) [21]. It tests how well an estimated model fits the data structure. A significant $\chi^2$ test suggests that the data fit the model well, while RMSEA values less than 0.1 indicate adequate model fit [22]. Variables included in the path model were normally distributed.

**Results**

The study population was comprised of 165 (43.5%) insulin-experienced and 215 (56.5%) insulin-naïve subjects. Baseline characteristics were the same between patient sub-groups except for diabetes duration that was longer in insulin-experienced (11.7 ± 7.0 vs. 6.8 ± 5.4 years, $p < 0.001$, Table 1). Although there was 0.76 kg/m$^2$ difference in BMI between insulin-experienced and insulin-naïve patients, the difference was not statistically significant (28.8 ± 4.6 vs. 28.0 ± 4.0 kg/m$^2$, $p = 0.11$). However, there was significant difference in waist circumference, with insulin-experienced having larger waist circumference than insulin-naïve patients (99.6 ± 11.2 vs. 97.2 ± 10.0 cm, $p = 0.01$, Table 1).

In both insulin-experienced and insulin-naïve patients, metformin was the most frequently prescribed OGLD (87.8 and 96.2%, respectively). Among 215 insulin-naïve, 83 (38.6%) were receiving one OGLD, and 132 (61.4%) were on two or more OGLDs. In the insulin-experienced group, 124 (75.1%) were under treatment with no or one OGLD, and 41 (24.8%) were receiving two or more OGLDs (Table 1). The time lag for insulin initiation was 8.1 ± 5.9 years considering the different types of insulin regimens. The mean dose of insulin was 0.56 ± 0.3 IU/kg/day. Eighty patients (48.2%) were on premixed or split-mixed insulin regimens. Basal insulin only was reported in 25.3% of the participants and basal plus rapid acting insulin in another 23.5%.

There were statistically significant differences in measures of blood glucose control (HbA1c) between insulin-naïve and insulin-experienced considering concomitant OGLD use [56 ± 16 mmol/mol (7.3 ± 1.4%) vs. 68 ± 20 mmol/mol (8.4 ± 1.8%), $p < 0.001$] (Table 2).

**Behavioral and psychosocial factors**

There were no obvious differences regarding total daily calorie intake (1890.5 ± 1275.8 vs. 1886.6 ± 510.3 Kcal, $p = 0.09$) or physical activity (median: 2448 (16702) vs. 2580 (17622) MET-min/week, $p = 0.78$) between insulin-naïve and insulin-experienced. Moreover, no statistical difference was reported in “number of days during the past week (last 7 days) missing taking diabetes medications as prescribed” between the two groups (0.4 ± 1.2 days in insulin-naïve vs. 0.5 ± 1.5 days in insulin-experienced, $p = 0.6$). However, insulin-experienced had significantly higher level of distress (2.2 ± 0.9 (moderate) vs. 1.9 ± 0.8 (little or no), $p = 0.01$), depression (9.5 ± 5.5 (moderate) vs. 8.07 ± 5.1 (mild), $p = 0.03$), and anxiety
Comparing the results of the diabetes knowledge test, although knowledge was not significantly different in insulin-experienced compared to insulin-naïve regarding the 14-item general test (7.8 ± 1.8 vs. 7.7 ± 2.02, \( p = 0.93 \)), the mean level of knowledge of insulin use was low in insulin-experienced considering the results of 9-item insulin-use subscale (3.9 ± 1.8). Among participants, no one could give correct answer to the questions on necessary action when having the flu or skipping one time of insulin injection. Only 21 (12.7%) and 24 (14.5%) of insulin-experienced patients could give a correct answer to the questions on ketoacidosis signs or time to initiate reaction of intermediate insulin.

Table 1  Baseline patient characteristics in study population

<table>
<thead>
<tr>
<th></th>
<th>Insulin-naïve</th>
<th></th>
<th>Insulin user</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No or one OGLD</td>
<td>Two or more OGLDs</td>
<td>All</td>
<td>No or one OGLD</td>
<td>Two or more OGLDs</td>
</tr>
<tr>
<td>( N ) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (female), %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since insulin initiation (months)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are shown as mean ± standard deviation

FBG fasting blood glucose, HbA1c hemoglobin A1c, TG triglyceride, Total Chol total cholesterol, LDL low-density lipoprotein, HDL high-density lipoprotein

Effects of variables on HbA1c in insulin-naïve and insulin-experienced people: a path analysis model

Duration of diabetes, BMI, total daily calorie intake, physical activity, self-management profile, family social support, diabetes knowledge, patient physician relationship, well-being index, depression, anxiety, diabetes distress, utility, and VAS score were included in the model. The estimated MVPA with parameters and statistical significance of individual paths are shown in Figs. 1 and 2.

Table 2  Metabolic control by pre-study therapy

<table>
<thead>
<tr>
<th></th>
<th>Insulin-naïve</th>
<th></th>
<th>Insulin user</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>No or one OGLD</td>
<td>Two or more OGLDs</td>
<td>All</td>
<td>No or one OGLD</td>
<td>Two or more OGLDs</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HbA1c (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Chol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are shown as mean ± standard deviation

FBG fasting blood glucose, HbA1c hemoglobin A1c, TG triglyceride, Total Chol total cholesterol, LDL low-density lipoprotein, HDL high-density lipoprotein
Insulin-naïve

The estimated model (Fig. 1) demonstrated a good fit, \( \chi^2 \) (df = 23, N = 215) = 45.32, \( P = 0.003 \), RMSEA = 0.067. As shown in Fig. 1, there was only significant positive direct effect from duration of diabetes (\( \beta = 0.19, t \text{ value} = 0.007 \)) to HbA1c.

Insulin-experienced

The estimated model in insulin-experienced people also showed a good model fit, \( \chi^2 \) (df = 12, N = 165) = 18.48, \( p = 0.10 \), RMSEA = 0.057. As demonstrated in Fig. 2, there was only significant positive direct effect from anxiety (\( \beta = 0.71, t \text{ value} < 0.001 \)) to HbA1c.

Discussion

In this study, as expected due to either duration or complexity of disease, a higher level of HbA1c was found in insulin-experienced compared to insulin-naïve independent of type and dose of concomitant OGLDs received. Moreover, the results also revealed higher level of distress, depression, and anxiety as well as low knowledge about insulin use in this group of people with T2D; therefore, the level of knowledge and distress in those eligible for insulin initiation must be considered for this treatment to be truly effective.

Insulin as the most effective glucose-lowering drug is a medical requirement in the treatment of patients with diabetes in the case of pancreatic \( \beta \) cell failure [3, 23]. However, several studies have shown that people who are on insulin still fail to achieve glycemic targets [8, 24, 25]. In the “A1chieve study,” there was no difference in relation to the glycemic control between insulin-naïve and prior insulin users; HbA1c:[80 ± 19 mmol/mol (9.5 ± 1.7%) vs. 79 ± 20 mmol/mol (9.4 ± 1.8%)] [4]. Delaying insulin initiation or intensification in real clinical practice where both patients and many physicians are reluctant to initiate insulin in the face of disease progression results in poor glycemic control in people with T2D [26–28]. Fear of hypoglycemia, needle phobia, anticipated pain, and weight gain are concerns about insulin injection [29, 30] that still continue even after patients initiate insulin use [8] resulting in insulin non-adherence [31] and overindulge in eating to prevent hypoglycemia [32].

In our study, non-adherence to treatment was not an issue as there was no difference between the two groups considering “missing taking diabetes medications.” Meanwhile, two third of insulin-experienced group were receiving basal plus at least one injection of rapid/short acting insulin; therefore, insulin therapy has been moved toward more intensified regimen. The other important finding of this study was the higher level of diabetes-related distress in the insulin-experienced group.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Behavioral and psychosocial factors in study population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin-naïve</td>
</tr>
<tr>
<td></td>
<td>No or one OGLD</td>
</tr>
<tr>
<td>Self-care behavior variables</td>
<td></td>
</tr>
<tr>
<td>Total daily calorie intake</td>
<td>2072.46 ± 1896.77</td>
</tr>
<tr>
<td>Physical activity (median)</td>
<td>2792 (16,702)</td>
</tr>
<tr>
<td>Self-management</td>
<td>5.23 ± 1.12</td>
</tr>
<tr>
<td>Resources variables</td>
<td></td>
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<tr>
<td>Diabetes knowledge (14-item general test)</td>
<td>7.60 ± 1.92</td>
</tr>
<tr>
<td>Patient-physician relationship</td>
<td>2.46 ± 1.01</td>
</tr>
<tr>
<td>Affective variables</td>
<td></td>
</tr>
<tr>
<td>Well-being</td>
<td>8.21 ± 5.54</td>
</tr>
<tr>
<td>Depression</td>
<td>14.32 ± 9.18</td>
</tr>
<tr>
<td>HRQoL</td>
<td></td>
</tr>
<tr>
<td>Utility</td>
<td>0.83 ± 0.14</td>
</tr>
<tr>
<td>VAS</td>
<td>68.91 ± 20.67</td>
</tr>
</tbody>
</table>

All data are shown as mean ± standard deviation.

Health-related quality of life, VAS, visual analog scale.
The efforts to control persistent hyperglycemia may lead to distress as a consequence of anger, disappointment, and loss of motivation affecting diabetes self-management [8]. In a study conducted by Tong et al., it was revealed that insulin-experienced subjects ascribe persistence of poor glycemic control to different factors, namely, psychosocial and emotional problems, treatment-related factors, and lack of knowledge about glycemic goals [8]. Comorbid major depression occurs in about 10–15% of patients with diabetes [33], which complicates diabetes medication non-adherence [34, 35] and increases the risk of diabetes complications [36] and hyperglycemia [37]. Consequently, depression may result in poor self-care behaviors including adherence to diet, exercise, and medication prescriptions [35]. Moreover, Makine et al. found that negative appraisal of insulin therapy is significantly associated with higher levels of depression and diabetes-related emotional distress [38]. In a study conducted by Holmes-Truscott et al., it was revealed that greater psychological resistance to insulin treatment is due to broader diabetes-related distress and its treatment. Hence, reducing diabetes-related distress by explaining disease progression and loss of β cell function in diabetes may lessen patients concern regarding insulin therapy [39].

Low knowledge of insulin use was also a reason for poor glycemic control in insulin-experienced subjects in the current study. The participants were not well informed about insulin use, more specifically the terms ketoacidosis, insulin reaction, and necessary action when having the flu or missing an insulin injection. Consistent with the results of our study, Murata et al. reported poor knowledge of ketoacidosis and insulin reaction among the majority of veterans in the USA [40]. Seemingly, poor knowledge of insulin use may increase the risk of its related complications (ketoacidosis, insulin reaction, and hypoglycemia) which may consequently result in poor glycemic control [41]. This poor level of knowledge might potentiate the burden of diabetes-related distress and poor glycemic control [42, 43]. Therefore, providing training programs to educate people with T2D, more specifically insulin-experienced subjects or those about to initiate insulin treatment, in addition to psychological counseling to overcome the fears and minimize or prevent psychological insulin resistance is warranted [24].
In this study, we also used measured variable path analysis to assess these associations and their effects on glycemic control in insulin-naïve and insulin-experienced people. The findings demonstrated that longer duration of diabetes was directly associated with worse glycemic control in the insulin-naïve group, while in insulin-experienced people anxiety was the only variable associated with poorer glycemic control. Consistent with our results, Camara et al. have shown that a higher level of anxiety is associated with a higher HbA1c level in people with T2D [44].

This was the first study in a non-western population to explore the relationships between type of treatment and key psychosocial and behavioral factors. However, there were some limitations in this study. A major limitation was the size of the study and the fact that findings might not be generalizable to a greater public, either geographically or ethically.

Moreover, any causal effect could not be shown between type of treatment and psychosocial factors due to the design of the study. In addition, we did not have enough data on timely insulin initiation and intensification, although two-thirds of insulin-experienced group were receiving basal plus rapid/short acting insulin.

**Conclusion**

Our findings demonstrated that glycemic control was not good in this group of insulin-experienced people. Psychosocial problems might directly or indirectly lead to poor glycemic control despite insulin use. In addition, both physicians and patients’ barriers to insulin therapy, namely, low knowledge of insulin use may be the other reason. Thus, health care providers should consider psychosocial factors and help patients to overcome their concerns regarding insulin treatment. Furthermore, there should be appropriate diabetes educational programs to raise patients’ knowledge and skills.

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**Compliance with ethical standards**

**Conflict of interest**  The authors declare that they have no conflict of interest.
Ethical approval  The study protocol was approved by the ethics committee of the Iran University of Medical Sciences, and the study procedures were carried out in accordance with the principles of the Declaration of Helsinki. The project and its goals were explained for every eligible patient and all patients signed the informed consent.

References


Factors associated with anxiety in type 2 diabetes mellitus patients in Pakistan

Muhammad Sarfraz Nawaz1 · Kifayat Ullah Shah1 · Haroon Ur Rashid1 · Sajid Mahmood1 · Allah Bukhsh2,3 · Inayat Ur Rehman2 · Salamat Ali1 · Tahir Mehmood Khan2

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Abstract The aim of this study was to assess the factors associated with anxiety in type 2 diabetes mellitus patients (T2DM) by using Hamilton Anxiety Rating Scale (HAM-A). A cross-sectional quantitative study was conducted after necessary ethical approval from Medicare Hospital Rawalpindi and Capital Development Authority Hospital Islamabad in accordance with Declaration of Helsinki. Three hundred thirty-eight patients with type 2 diabetes mellitus were selected by non-stratified random sampling technique from August 2016 to February 2017. Majority of the T2DM patients (n = 258, 66.5%) had mild anxiety, followed by mild to moderate anxiety (n = 82, 21.1%). A significant association (p < 0.01) was observed between anxiety and patients’ gender, education, and occupation; whereas, statistically insignificant association (p > 0.05) was observed between HAM-A score and patients’ age, family history, and life style. Linear regression analysis revealed gender (OR = −0.308, CI-0.57-0.299) as a significant predictor (p < 0.01) for anxiety. Findings of this study demonstrate that large proportion of the diabetic patients is suffering with type of anxiety. A significant association of anxiety was identified with gender, education, and occupation of type 2 diabetes patients.

Keywords Blood sugar fasting · Blood sugar random · Diabetes mellitus · Anxiety · HAM-A · Pakistan · Type 2 diabetes mellitus

Introduction

In the current era, diabetes mellitus has become a major health care problem [1]. Diabetes mellitus is a metabolic multifactorial disorder induced by either decrease in insulin production or increased insulin resistance in the body. The prevalence of type 2 diabetes is increasing rapidly. International Diabetic Federation (IDF) reports showed that around 415 million people have been diagnosed with diabetes mellitus and presumably this number would reach 642 million by 2040; there are seven million cases of diabetes in Pakistan till 2015 [2]. According to WHO diabetes prevalence list, Pakistan is on top seventh position. Diabetes doubles the risk of heart disease by increasing oxidative stress in patients. More than 8% of the US myocardial infarction cases are due to diabetes [3]. Total expenditure on diagnosed diabetes is 245 billion dollars in USA by 2012 [4]; while in Pakistan, average cost per patient per month is 5542 Pak rupees [5].

Anxiety and depression are associated with both duration as well as disease of diabetes mellitus. Anxiety disorder is about 21% more prevalent in diabetic patients than non-diabetic population [6, 7]. Anxiety and depression are more commonly observed in female than male patients. There are many factors such as unemployment, low income and poor glycemic control etc., which are associated with patient’s anxious behavior [8, 9]. There is limited data available on association of diabetes with anxiety in developing country. Mexican diabetic patients show more anxiety level than others [10]. Gois et al. reported the association of anxiety with glycemic control, a good diabetes control makes diabetic patient comfortable and poor glycemic control.
makes the diabetic patient more anxious [11]. Positive diabetes family history is another parameter involved in anxiety disorder as patients with positive family diabetes history are more vigilant about glycemic control [12]. In diabetic patients, many complications lead the patients towards depression, morbidity, and mortality. Nowadays, anxiety is one of the most frequent psychiatric disorder [13]. So, there is need to access the association of anxiety with diabetes in developing countries like Pakistan.

The aim of this study was to assess the prevalence rate of anxiety and its association with demographic characteristics of T2DM in Pakistan by using HAM-A scale.

Methodology

Study design

A cross-sectional study design was adopted for data collection for a period of 6 months (August 2016 to February 2017). The data were collected from T2DM patients visiting outpatient diabetes clinics in two tertiary care hospitals of Rawalpindi and Islamabad, Pakistan. According to latest statistics of International Diabetes Federation, there are more than seven million cases of diabetes in Pakistan. Sample size was calculated by using Raosoft sample size calculator (Raosoft, 2014) with 95% confidence interval and 5% margin of error. The calculated sample size for our study was 377 because according to Raosoft the sample size does not change for a population larger than 20,000. For this purpose, 450 diabetes patients were selected by using simple random sampling technique, from the outpatient clinics of the two tertiary care hospitals. Out of which, 388 participants fulfilled study criteria and consented to participate in our study. Data collection form was designed for patient’s demographical data collection, which included patient’s disease history, lab values (HbA1c, fasting blood glucose and random blood glucose).

Study tool

Patient’s questionnaire-based interview was conducted by using Hamilton Anxiety Rating scale (HAM-A) [14] based on 14 different questions. Hamilton anxiety scale [14] is used for patient’s anxiety level identification and it was adopted after formal permission from its relevant authors. Every query is scored on scale of 0 (not present) to 4 (severe). For a total score ranging from 0 to 56; where < 17 indicates mild severity, 18–24 mild to moderate severity, and 25–30 moderate to severe anxiety.

Participants and setting

Patients were interviewed from out patient’s diabetes clinics of Capital Development Authority Hospital Islamabad and Medicare hospital Rawalpindi.

Inclusion criteria

Adult T2DM patients with age > 28 years from both genders who were are willing to provide informed written consent for participation in the study were included. The newly diagnosed T2DM patients (diabetes patients who have been diagnosed with T2DM within the past 6 months), and patients having T2DM along with co-morbidities were also included.

Exclusion criteria

The patients who were are not willing to provide written signed informed consent to participate in the study were excluded. Type 1 diabetes patients, gestational diabetes patients, or any other type, like, LADA (latent autoimmune diabetes of adults) and MODY (maturity onset diabetes of young’s) were also excluded.

Ethics approval

The study was approved from the human research and ethics committees of Medicare Hospital Rawalpindi (Ref. no. 59) and Capital Development Authority Hospital Islamabad (Ref. no. 6/2). The current study does not involve animals for research purpose. A prior patient consent form was signed by the patients who were willing to participate in the study after describing them the nature and the objectives of the study, in accordance the Declaration of Helsinki. Patients were also communicated about their right to withdraw from the study at any time without any kind of penalization. Confidentiality of their responses and data was also assured to them.

Statistical analysis

Collected data were analyzed by SPSS 22.0 (Statistical Package for Social Sciences version 22). The results were expressed as sample number (N), percentage (%), and significant p values. Chi-square test was applied to examine the association of anxiety scores with various demographic characteristics of T2DM patients. A multiple linear regression was applied to evaluate the predicting variables for patients’ anxiety score. p value of < 0.05 (two-tailed test) was considered as criterion of statistical significance.

Results

Characteristics of 388 participants with type 2 diabetes mellitus enrolled in this study are presented in Table 1. Total number of patients included in this study was 388, including 180 male (46.4%) and 208 female (53.6%). Majority of the
patients were of age groups 41 to 50 years (39%) and 51 to 60 years (33.2%). More than half of the studied population \((n = 223; 57.5\%)\) were having education less than a primary level. The detailed demographic characteristics are shown in Table 1.

### Prevalence of anxiety

Majority of the studied patients \((n = 258; 66.5\%)\) were suffering from mild anxiety, followed by mild–moderate \((n = 82; 21.1\%)\), and moderate–severe anxiety \((n = 48; 12.4\%)\) as shown in Table 2.

### Association of demographic variables with anxiety

Cross-tabulation analysis (Table 3) showed a highly significant association \((p < 0.01)\) of anxiety with patients’ gender, education, and occupation, whereas no association was observed for life style, family history, and patients’ age, detailed results are presented in Table 3.

### Linear regression analysis

A multiple linear regression was calculated to predict anxiety based on patients’ HbA1c categories, random blood

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**Table 1** Demographic variables of type 2 diabetes patients and laboratory values. \((n = 388)\)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years)</td>
<td>49.4 ± 8.73</td>
<td></td>
</tr>
<tr>
<td>Age categories (in years)</td>
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<td></td>
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<tr>
<td>28 to 30 years</td>
<td>6</td>
<td>1.5</td>
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<tr>
<td>31 to 40 years</td>
<td>63</td>
<td>16.2</td>
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<tr>
<td>41 to 50 years</td>
<td>152</td>
<td>39.2</td>
</tr>
<tr>
<td>51 to 60 years</td>
<td>129</td>
<td>33.2</td>
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<tr>
<td>61 to 70 years</td>
<td>38</td>
<td>9.8</td>
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<tr>
<td>Male</td>
<td>180</td>
<td>46.4</td>
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<td>Female</td>
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<td>Education</td>
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<td>Private jobs</td>
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<tr>
<td>Business</td>
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<td>14.9</td>
</tr>
<tr>
<td>Co-morbid</td>
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<td></td>
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<tr>
<td>Only diabetes</td>
<td>228</td>
<td>58.7</td>
</tr>
<tr>
<td>Diabetes plus hypertension</td>
<td>126</td>
<td>32.5</td>
</tr>
<tr>
<td>Diabetes plus arthritis</td>
<td>24</td>
<td>6.2</td>
</tr>
<tr>
<td>Diabetes plus ischemic heart disease</td>
<td>4</td>
<td>1.1</td>
</tr>
<tr>
<td>Diabetes plus other diseases</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history = yes</td>
<td>278</td>
<td>71.6</td>
</tr>
<tr>
<td>Family history = no</td>
<td>110</td>
<td>28.4</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanides (B)</td>
<td>53</td>
<td>13.7</td>
</tr>
<tr>
<td>Dipeptidyl peptidase-4 inhibitors (DPP4I)</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>B + DPP4I</td>
<td>71</td>
<td>18.3</td>
</tr>
<tr>
<td>Sulfonylurea (S)</td>
<td>22</td>
<td>5.7</td>
</tr>
<tr>
<td>Human Insulin (HI)</td>
<td>56</td>
<td>14.4</td>
</tr>
<tr>
<td>S + MI (modern insulin)</td>
<td>15</td>
<td>3.9</td>
</tr>
<tr>
<td>B + DPP4I + S</td>
<td>101</td>
<td>26.2</td>
</tr>
<tr>
<td>I + DPP4I + B</td>
<td>51</td>
<td>13.1</td>
</tr>
<tr>
<td>S + B</td>
<td>13</td>
<td>3.4</td>
</tr>
<tr>
<td>S + DPP4I</td>
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<tr>
<td>Life style</td>
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<tr>
<td>Active life style</td>
<td>246</td>
<td>63.4</td>
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<tr>
<td>Sedentary life style</td>
<td>142</td>
<td>36.6</td>
</tr>
<tr>
<td>Blood sugar fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100 mg/dl</td>
<td>40</td>
<td>10.3</td>
</tr>
<tr>
<td>101–125 mg/dl</td>
<td>115</td>
<td>29.6</td>
</tr>
<tr>
<td>&gt; 126 mg/dl</td>
<td>233</td>
<td>60.1</td>
</tr>
<tr>
<td>Blood sugar random</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 140 mg/dl</td>
<td>32</td>
<td>8.2</td>
</tr>
<tr>
<td>141–199 mg/dl</td>
<td>98</td>
<td>25.3</td>
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<tr>
<td>&gt; 200 mg/dl</td>
<td>258</td>
<td>66.5</td>
</tr>
<tr>
<td>HbA1c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5.7</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>5.7–6.4</td>
<td>17</td>
<td>4.4</td>
</tr>
<tr>
<td>&gt; 6.5</td>
<td>369</td>
<td>95.1</td>
</tr>
</tbody>
</table>

---

Frequency and percentage distribution of T2DM patient’s data

*HbA1c* glycosylated hemoglobin
sugar categories, fastening blood sugar categories, age categories, and gender. A significant regression equation was found (F(5,382) = 10.282, p < 0.000), with $R^2$ of 0.12. Participants’ predicted anxiety is equal to 1.244 – 0.434 (gender), −0.021 (age categories), + 0.101 (random blood sugar categories), + 0.037 (HbA1c categories), + 0.047 (fastening blood sugar categories), where gender is coded as 0 = female, 1 = male (reference category female), HbA1c categories (Ref. < 140 mg/dl); random blood sugar categories (Ref. < 140 mg/dl); fastening blood sugar categories (Ref. < 100 mg/dl); age categories (Ref. 28–70 years). Analysis revealed gender as a highly significant predictor (OR = −0.308, 95% CI 0.57–0.299) for anxiety; whereas, HbA1c categories, BSR, FBS, and age categories proved to be poor predictors for T2DM patients anxiety. Detailed analysis is shown in Table 4.

Table 3 Chi-square association of Anxiety scores with demographic variables of T2DM patients (n = 388)

<table>
<thead>
<tr>
<th>Anxiety scores</th>
<th>Mild (&lt; 17)</th>
<th>Mild–moderate (18–24)</th>
<th>Moderate–severe (25–30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients age (years)</td>
<td></td>
<td></td>
<td></td>
<td>0.172</td>
</tr>
<tr>
<td>28–30</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>31–40</td>
<td>35</td>
<td>20</td>
<td>8</td>
<td></td>
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<tr>
<td>41–50</td>
<td>100</td>
<td>33</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>51–60</td>
<td>94</td>
<td>20</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>61–70</td>
<td>26</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Blood sugar fasting</td>
<td></td>
<td></td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>&lt;100 mg/dl</td>
<td>33</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>101–125 mg/dl</td>
<td>77</td>
<td>30</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>&gt;126 mg/dl</td>
<td>148</td>
<td>49</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Random blood sugar</td>
<td></td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td>&lt;140 md/dl</td>
<td>27</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>141–199 mg/dl</td>
<td>73</td>
<td>17</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>&gt;200 mg/dl</td>
<td>158</td>
<td>64</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
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<td>0.000</td>
</tr>
<tr>
<td>Jobless</td>
<td>12</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>99</td>
<td>59</td>
<td>39</td>
<td></td>
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<tr>
<td>Govt. employ</td>
<td>73</td>
<td>13</td>
<td>4</td>
<td></td>
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<tr>
<td>Private job</td>
<td>20</td>
<td>4</td>
<td>5</td>
<td></td>
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<tr>
<td>Business</td>
<td>54</td>
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<td>0</td>
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<tr>
<td>Education</td>
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<td></td>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td>Primary</td>
<td>133</td>
<td>54</td>
<td>36</td>
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<tr>
<td>Higher secondary</td>
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<td>7</td>
<td></td>
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<td>Secondary</td>
<td>34</td>
<td>6</td>
<td>5</td>
<td></td>
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<tr>
<td>Family history</td>
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<td></td>
<td>0.282</td>
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<tr>
<td>Yes</td>
<td>190</td>
<td>53</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>68</td>
<td>29</td>
<td>13</td>
<td></td>
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<tr>
<td>Life style</td>
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<td></td>
<td></td>
<td>0.886</td>
</tr>
<tr>
<td>Active</td>
<td>164</td>
<td>53</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>94</td>
<td>29</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Female</td>
<td>108</td>
<td>61</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>150</td>
<td>21</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square, cross-tabulation between patient’s demographic variable and total anxiety score; (p < 0.05)
Discussion

We assessed the prevalence and associated factors of anxiety in T2DM patients in Pakistani population. It was found that patients with uncontrolled fasting blood sugar suffer more anxiety than patients with controlled glycemic levels. Similarly, patients with random blood sugar level > 200 mg/dl were found to be more prone to moderate–severe anxiety than patients having blood sugar within range (fasting, 130 md/dl and random, 180 mg/dl), a very similar findings were observed by KK Hall et al. [15]. Anxiety level among female diabetic housewives was higher than other groups of population in terms of occupation. Jobless and business men showed mild type of anxiety. Education wise analysis showed that out of 388 patients, 223 (who were primary) were more prone to anxiety than higher education holding diabetes patients. Patients with secondary education showed minimum anxiety. It was observed that patients with positive diabetes family history showed more anxiety. The study results revealed that active patients are more anxious than sedentary patients. Table 3 showed that there is positive association of gender with anxiety.

The majority of studies did not disclose the potential risk factor of anxiety [16–18]. However, these studies have not described any association of age, BSF, BSR, gender, occupation, family history, and diabetes with anxiety. A similar study conducted by Palizgir et al. in Iran, to evaluate the association of age, gender, occupation, and education with anxiety [19]. It was observed that anxiety problem was high in females, late age’s diabetes, and jobless patients [20]. A study was conducted in Canada, which showed that diabetes is not only associated with anxiety but many other factors also play important role in this respect [21]. Camara et al. conducted an alike study which shows that socioeconomic impact and HbA1c are important contributing factors in anxiety and depression [22]. The present study is the first one in Pakistan, which showed the association of anxiety with age, BSF, BSR, gender, life style, education, occupation, and family history. This study was conducted on 388 type 2 diabetes patients using questionnaire-based interview. Patients with high blood sugar fasting are positively associated with anxiety (p < 0.022). Random blood sugar level also showed positive association with anxiety (p < 0.013). Anxiety score, 66.5% had mild anxiety; 21.1% had mild–moderate; and only 12.4% had moderate to severe anxiety and an alike study was reported by Khuwaja et al. [23]. Age-wise analysis showed that patients between 41 and 50 years’ age had more anxiety while no moderate–severe anxiety cases were found in 28–30 years’ age group. Anxiety association with BSF showed that patients with fasting blood sugar level < 100 mg/dl had minimum and patients with fasting blood sugar > 126 mg/dl had maximum chances of anxiety. F test result reveals that age, gender, BSF, BSR, and HbA1c are significantly associated with anxiety (F = 10.282, p < 0.05). Anxiety versus life style cross-tabulation showed that both are not significantly associated (p > 0.05). This study does not show the patient counseling effect on anxiety [23]. However, the patient counseling effect on anxiety can only be accessed in prospective studies.

The occupation wise analysis of patients was very interesting; 39 patients were housewives among total 48 patients having moderate–severe anxiety. This study showed that housewives had more chances of anxiety than others as about 50% anxiety cases of total were found in housewives. On the other hand, positive diabetes family history was also related to anxiety. Two hundred seventy-eight patients had positive diabetes family history while 110 had no diabetic family history. The statistical analysis also indicates that family history is directly linked with anxiety (p < 0.05). The statistical correlation showed a significant relation between gender, age, BSF, BSR, HbA1c, and anxiety. Almawi et al. concluded very comparable study that discovered type 2 diabetes play positive role in anxiety (p < 0.001) [24].

The life style also had an impact with anxiety and its association with anxiety showed that 63.4% patients had active while 36.6% had sedentary life style. Anxiety verses life style cross-tabulation showed non-significance association (p > 0.05). Many studies showed that anxiety is directly linked to gender, occupational status, BSF, BSR, and HbA1c. Such types of studies can play important role in patient counseling.

### Table 4

<table>
<thead>
<tr>
<th>Predictors</th>
<th>OR</th>
<th>Sig.</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c categories</td>
<td>0.013</td>
<td>0.799</td>
<td>−0.25 - 0.324</td>
</tr>
<tr>
<td>Random blood sugar categories</td>
<td>0.091</td>
<td>0.121</td>
<td>−0.027 - 0.228</td>
</tr>
<tr>
<td>Fasting blood sugar categories</td>
<td>0.045</td>
<td>0.443</td>
<td>−0.073 - 0.166</td>
</tr>
<tr>
<td>Age categories</td>
<td>−0.027</td>
<td>0.579</td>
<td>−0.095 - 0.053</td>
</tr>
<tr>
<td>Gender</td>
<td>−0.308</td>
<td>0.000</td>
<td>−0.57 - 0.299</td>
</tr>
</tbody>
</table>

For linear regression, HbA1c categories (Ref. < 140 mg/dl); random blood sugar categories (Ref. < 140 md/dl); fasting blood sugar categories (Ref. < 100 mg/dl); age categories (Ref. 28–70 years); gender (Ref. female)
Prospective studies are required for further identification of factors which are linked with anxiety [25, 26].

Conclusion

Findings of this study demonstrate a high prevalence of anxiety in large sample to T2DM patients in Pakistan, with a significant association of anxiety with female gender, low literacy, and higher random blood sugar levels. Our results support and add to the existing literature by providing the association between diabetes and anxiety. Therefore, we suggest to clinically evaluate the diabetes patients for anxiety, before the progression of anxiety into psychiatric disorder. Furthermore, the mechanism of the investigational association is not well-known and cannot be concluded from this cross-sectional design study; therefore, further studies are required to explore such association on larger population.

Acknowledgments

We wish to thank the management of Medicare Hospital Rawalpindi and Capital Development Authority Hospital Islamabad for ethics approval and facilities in this study to make this research successful.

Compliance with ethical standards

The study was approved by the human research and ethics committees of Medicare Hospital Rawalpindi (Ref. no. 59) and Capital Development Authority Hospital Islamabad (Ref. no. 6/2). The current study does not involve animals for research purpose. A prior patient consent form was signed by the patients who were willing to participate in the study after describing them the nature and the objectives of the study, in accordance with the Declaration of Helsinki. Patients were also communicated about their right to withdraw from the study at any time without any kind of penalization. Confidentiality of their responses and data was also assured to them.

Conflict of interest

The authors declare that they have no conflict of interest.

References

21. Deschenes SS, Burns RJ, Schmitz N. Associations between diabetes, major depressive disorder and generalized anxiety disorder comorbidity, and disability: findings from the 2012 Canadian


Factors affecting risk of anxiety and depression among diabetic and hypertensive patients who refer to family health centers

Nilüfer Emre¹ • Kenan Topal² • Tamer Edirne¹ • Çağdem Gereklioglu³

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Abstract This cross-sectional study was carried out to investigate the factors which influence risk of anxiety and depression among diabetic and hypertensive patients who refer to family health centers. The Hospital Anxiety and Depression Scale (HADS) was applied for assessment of emotional status of the patients and the Hypertension Compliance Assessment Scale (HCAS) was applied for assessment of adherence to anti-hypertensive therapy. Of a total of 380 patients, 170 had hypertension (HT), 83 had type 2 diabetes mellitus (T2DM), and 127 had both HT and T2DM. According to HADS, 18.7% of the patients had risk of anxiety, 24.7% had risk of depression, and 12.6% had both risk of anxiety and depression. Mean HAD-Anxiety (HADS-A) score and HADS-Depression (HADS-D) score were significantly lower in the patients who had an adequate compliance to medication therapy (5.1 ± 4.1 and 3.8 ± 3.4, respectively) compared to the patients who had a low compliance to therapy (7.6 ± 4.3 and 5.8 ± 4.0, respectively) according to the Hypertension Compliance Assessment Scale (χ² = 15.26, p < 0.01 and χ² = 13.80, p < 0.01). Mean HADS-D score was found significantly lower among the diabetic patients with good glycemic control (3.7 ± 2.9) compared to the patients with poor glycemic control (4.5 ± 3.7) (χ² = 25.00, p < 0.05). Anxiety and depression are among the most frequent disorders as hypertension and diabetes in primary care setting. We revealed that risk of anxiety and/or depression was greater among hypertensive and diabetic patients, consistently with the previous studies. Our study also revealed that this condition negatively affected treatment compliance in hypertensive patients and glycemic control in diabetic patients.

Keywords Hypertension • Type 2 diabetes mellitus • Anxiety • Depression • Medication adherence • Patient compliance

Background and objective

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by chronic hyperglycemia resulting from insulin release and related problems. Hypertension prevalence is seen to be 1.5- to 2-fold greater among patients with T2DM compared to non-diabetics. Hypertension prevalence is 40–50% at the time of diagnosis in diabetic patients [1, 2]. Mortality and morbidity increase due to cerebrovascular diseases, ischemic heart diseases, heart failure, and renal failure in these patients [3]. Chronic diseases like HT and T2DM bring psychological disorders together due to being long standing and leading to various organ dysfunctions. Depression is an independent risk factor for the onset of type 2 diabetes. It negatively affects the course of diabetes and is associated with increased risk of complications, hyperglycemia, and mortality [4]. Depression and anxiety are observed more often in diabetic and hypertensive patients [5]. While planning the treatment of hypertensive and diabetic patients, assessment of mental health status through validated scales investigating the risk of anxiety and depression would enable to provide optimal treatment and care services [6]. Severe outcomes may be decreased and prevented, and even disease progression may slow down...
through early detection of anxiety and depression [7]. Another challenging issue in follow-up of chronic diseases is medication adherence. Treatment incompliance is an important problem which leads to disease progression and emergence of complications [8]. Patients with chronic diseases may satisfy from patient care and their quality of life may improve through prevention of complications and disabilities.

In this research, we aimed to determine the risk of anxiety and depression development among diabetic and hypertensive patients who are admitted to family health centers and to investigate medication adherence for hypertensive patients.

Material and method

This cross-sectional study was conducted with 31,500 individuals who were enrolled to nine primary care units belonging to two family health centers in Denizli province between 1 April 2013 and 31 August 2013. The sample size calculation was set up at 95% confidence interval with significance level of $p < 0.05$. The estimated size of sample is 380 and sample selection was done by simple random sampling method. A structured questionnaire inquiring socio-demographic characteristics, medications, diet and exercise status, smoking status, and alcohol intake of the patients was applied with face-to-face interviews. The questionnaires were applied by the principal investigator and family doctors working in primary care units who were trained for questionnaire and the scales used in the research. The recent fasting plasma glucose (FPG) and HbA1c values were recorded and patients whose HbA1c levels $< 7\%$ were considered to have a good glycemic control and whose HbA1c $\geq 7\%$ were considered to have a poor glycemic control [9]. Body weight, height, waist circumference, and blood pressure of the patients were recorded. Adult patients who had T2DM and/or HT, who signed up written informed consent, were included in the study. The patients who had T1DM, any psychiatric diseases, and dementia who were being treated for malignancy and any participants who are unable to consent for themselves through physical or mental incapacity were excluded. All participants were applied the Hospital Anxiety Depression Scale (HADS). Hypertensive patients were additionally applied the Hypertension Compliance Assessment Scale (HCAS). General features of the scales are given above.

Hospital Anxiety Depression Scale (HADS) is a 14-item self-reported questionnaire for the assessment of symptoms of anxiety and depression with good case-finding ability and used for determination of risk, level, and severity of anxiety and depression in general medical outpatient populations. The validity and reliability study of the Turkish version of the scale has been conducted by Aydemir et al.; Cronbach’s alpha is 0.85 for the HADS-Anxiety subscale (HADS-A) and 0.77 for the HADS-Depression subscale (HADS-D). The scores of the subscales vary between 0 and 21; cut-off value is 10/11 for HADS-A and 7/7 for HADS-D. The patients whose scores are above these values are accepted as “under risk” [10, 11].

Hypertension Compliance Assessment Scale (HCAS) was used for assessment of treatment compliance of hypertensive patients. Overall score varies between 1 to 13 in this 9-item scale, scores between 1 and 7 indicate medication adherence and scores 8 and above indicate non-adherence [12]. The scale was adapted to Turkish population in 2006 and Cronbach’s alpha was found as 0.82 [13].

Statistical analysis

All statistical analyses were performed using SPSS, version 16.0 (Chicago, IL, USA). Testing for normality was done with the Kolmogorov-Smirnov test. Chi-square tests and One-way Anova, post hoc Tukey, and LSD were used for exploring the differences between groups. A two-tailed $p$ value $< 0.05$ was accepted as statistically significant.

Results

A total of 380 patients with T2DM and/or HT were included in the study and there were no missing data. Of the participants, 206 (54.2%) were female and mean age was 61.4 ± 9.7 years. Of the participants, 23 (6.1%) were illiterate, 199 (52.4%) were graduates of elementary school, 95 (25.0%) were graduates of intermediate school and high school, and 63 (16.6%) were graduates of university. Three hundred thirty-six (88.4%) of the participants stated that they were married, 217 (57.1%) had middle income, and only 28 (7.4%) were smoking. Mean systolic blood pressure was found as 130.7 ± 11.6 mmHg and mean diastolic blood pressure was found as 81.7 ± 6.3 mmHg. Mean body weight was 78.9 ± 13.0 kg and mean waist circumference was 101.6 cm, mean body mass index (BMI) was 29.5 ± 5.0 kg/m², and according to BMI, 62 (16.3%) were normal, 166 (43.7%) were overweight, 139 (36.6%) were obese, and 13 (3.4%) were morbid obese. Of the patients, 170 (44.7%) had only HT, 83 (21.8%) had only T2DM, and 127 (33.4%) had both HT and T2DM.

Table 1 presents the results of the comparison of disease groups with regard to age, anthropometric measurements, and blood pressure values in 380 participants. There were statistically significant differences between disease groups.

Mean scores of HADS-A and HADS-D were found as 5.5 ± 4.1 and 4.1 ± 3.5, respectively. While 71 (18.7%)
subjects had the risk of anxiety according to HADS-A and 93 subjects (24.5%) had the risk of depression according to HADS-D, 48 (12.6%) subjects had both the risk of anxiety and depression. The risk of anxiety was found as 20.2% (n = 60) and the risk of depression was found as 24.9% (n = 74) in hypertensive patients (n = 297). These ratios were 14.8% (n = 31) and 22.4% (n = 47), respectively in diabetic patients.

Mean HADS-A and HADS-D scores were greater among females (6.4 ± 4.3 and 4.5 ± 3.6) compared to males (4.3 ± 3.6 and 3.6 ± 3.4) (χ² = 24.576, p < 0.01 and χ² = 6.487, p < 0.01, respectively). Mean HADS-A score was greater among divorced or widowed subjects (6.1 ± 3.7) compared to the married subjects (5.4 ± 4.2), (χ² = 29.286, p < 0.05). Both HADS-A and HADS-D scores decreased as education level increased; while they were found as 6.9 ± 4.9 and 5.3 ± 4.0 among the illiterate, 5.9 ± 4.2 and 4.5 ± 3.5 among the graduates of elementary school, 5.3 ± 4.1 and 3.9 ± 3.7 among the graduates of intermediate school and high school, and they were 3.9 ± 3.1 and 3.0 ± 2.8 among the graduates of university (χ² = 13.737, p < 0.01 and χ² = 10.641, p < 0.01, respectively).

No significant difference was detected in HADS-A scores according to monthly income; however, HADS-D scores were seen to significantly decrease as monthly income increased (4.4 ± 3.7 for the subjects whose monthly income is lower; 3.8 ± 3.4 for the subjects whose monthly income is middle; 2.7 ± 2.3 for the subjects whose monthly income is upper), (χ² = 10.641, p < 0.01).

HADS-A scores were seen to significantly decrease as exercise status improved (5.8 ± 4.3 for the subjects who has no physical activity; 5.7 ± 4.1 for the subjects who has irregular physical activity; and 4.6 ± 3.8 for the subjects who has regular physical activity), (χ² = 6.627, p < 0.01); similarly, HADS-D scores were seen to significantly decrease as exercise status improved (4.7 ± 3.8 for the subjects who has no physical activity; 4.2 ± 3.5 for the subjects who has irregular physical activity; and 3.4 ± 3.1 for the subjects who has regular physical activity), (χ² = 10.641, p < 0.01).

HADS-A and HADS-D scores were seen not to change with smoking status, body mass index, and disease status.

Mean score of Hypertension Compliance Assessment Scale (HCAS) was 4.8 ± 1.9 for 297 hypertensive patients, while 244 patients (82.2%) were compliant and 53 (17.8%) were seen to be inconsistent. While mean HADS-A score was 5.1 ± 4.1 for the inconsistent patients, this was 7.6 ± 4.3 for the compliant patients and the difference was statistically significant (χ² = 15.26, p < 0.01). While mean HADS-D score was 3.8 ± 3.4 for the compliant patients, this was 5.8 ± 4.0 for the inconsistent patients and the difference was statistically significant (χ² = 13.80, p = 0.000).

Mean HbA1c value was 6.96 ± 1.1% for 210 diabetic patients. While HbA1c was ≤7.0% in 137 (65.2%) patients, it was >7.0% in 73 (34.8%). While mean HADS-A score was 4.8 ± 3.6 for the patients who had good glycemic control, this was 5.7 ± 4.1 for the patients who had poor glycemic control; however, the difference was not statistically significant (χ² = 12.94, p = 0.531). While mean HADS-D score was 3.7 ± 2.9 for the patients who had good glycemic control, this was 4.5 ± 3.7 for the patients who had poor glycemic control and the difference was statistically significant (χ² = 25.00, p < 0.05), (Table 2).

### Discussion

While anxiety and depression are observed more often in diabetic and hypertensive patients, mental status of these patients is not sufficiently addressed. In this research, we evaluated the factors affecting the risk of depression and anxiety among diabetic and hypertensive patients who refer to family health centers through the “Hospital Anxiety and Depression Scale.” HADS was administered to participants who were considered to be at risk for mental problems and it was used to assess the risk for anxiety and depression and designed to provide a simple yet reliable tool for use in medical practice. The term “hospital” in its title suggests that it is only valid in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of disease groups with regard to age, anthropometric measurements, and blood pressure values in 380 participants analyzed in two family health centers between 1 April 2013 and 31 August 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 380)</td>
</tr>
<tr>
<td></td>
<td>HT† (n = 170)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.4 ± 9.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.4 ± 12.9</td>
</tr>
<tr>
<td>BMI†</td>
<td>28.8 ± 4.6</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99.7 ± 13.2</td>
</tr>
<tr>
<td>Systolic BP** (mmHg)</td>
<td>130.7 ± 11.5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.1 ± 6.6</td>
</tr>
</tbody>
</table>

*BM/I body mass index, †BP blood pressure, †HT hypertension, ††T2DM type 2 diabetes mellitus
such a setting but many studies conducted throughout the world have confirmed that it is valid when used in community settings and primary care medical practice. It should be emphasized that self-assessment scales are only valid for screening purposes; definitive diagnosis must rest on the process of clinical examination [14].

The present study has revealed that 18.7% of 380 diabetic and/or hypertensive subjects had the risk of anxiety and 24.5% had the risk of depression and 12.6% subjects had both risk of anxiety and depression according to HADS. There is a complex relationship between physical health and mental status. Anxiety and depression are common disorders in primary care setting as T2DM and HT. These disorders which lead to significant negative outcomes even when they are alone would cause poorer outcomes when seen together [15]. Hypertension risk was found 2- to 3-folds greater among subjects with severe depression or anxiety [16]. Cheung et al. investigated anxiety and depression in 197 hypertensive patients and found that HADS-A scores were greater in HT group compared to normotensive group; however, HADS-D scores were similar between groups and concluded that HT could accompany with anxiety but not depression [17]. We found a high risk of anxiety (20.2%) and depression (24.9%) in hypertensive patients (n = 297). Depression prevalence is significantly higher among diabetic patients compared to non-diabetics. Varying degrees of depression accompanies with T2DM in the ratio of 10–30% [18]. Yarış et al. found depression in 49 out of 100 diabetic patients (49%) and in 23 patients (23%) in control group using HADS [15]. We also detected a high risk of anxiety in 31 patients (14.8%) and a high risk of depression in 47 patients (22.4%) out of 210 diabetic patients. So we may recommend the family physicians evaluating mental disease risk through HADS in hypertensive and diabetic patients followed up at primary health care settings.

We found that the mean HADS-A and HADS-D scores were greater among females compared to males. Women are affected more than men by anxiety and depression in general population and also among people with diabetes, a fact that is

<table>
<thead>
<tr>
<th>(n = 380)</th>
<th>HADS-A* (Mean ± SD)</th>
<th>χ²</th>
<th>p</th>
<th>HADS-D** (Mean ± SD)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>6.4 ± 4.3</td>
<td>24.576</td>
<td>&lt; 0.01</td>
<td>4.5 ± 3.6</td>
<td>6.487</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.3 ± 3.6</td>
<td></td>
<td></td>
<td>3.6 ± 3.4</td>
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<tr>
<td>Marital status</td>
<td>Married</td>
<td>5.4 ± 4.2</td>
<td>29.286</td>
<td>&lt; 0.05</td>
<td>4.0 ± 3.5</td>
<td>16.945</td>
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<tr>
<td></td>
<td>Divorced/widowed</td>
<td>6.1 ± 3.7</td>
<td></td>
<td></td>
<td>4.9 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Education status</td>
<td>Illiterate</td>
<td>6.9 ± 4.9</td>
<td>13.737</td>
<td>&lt; 0.01</td>
<td>5.3 ± 4.0</td>
<td>10.641</td>
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<td></td>
<td>Elementary school</td>
<td>5.9 ± 4.2</td>
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<td>4.5 ± 3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intermediate school/high school</td>
<td>5.3 ± 4.1</td>
<td>3.9 ± 3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University</td>
<td>3.9 ± 3.1</td>
<td></td>
<td></td>
<td>3.0 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Monthly income</td>
<td>Lower</td>
<td>5.8 ± 4.3</td>
<td>3.371</td>
<td>0.06</td>
<td>4.4 ± 3.7</td>
<td>5.777</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>5.3 ± 4.1</td>
<td></td>
<td></td>
<td>3.8 ± 3.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>4.1 ± 2.7</td>
<td></td>
<td></td>
<td>2.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>No</td>
<td>6.1 ± 4.4</td>
<td>6.627</td>
<td>&lt; 0.01</td>
<td>4.7 ± 3.8</td>
<td>7.181</td>
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<tr>
<td></td>
<td>Irregular</td>
<td>5.7 ± 4.1</td>
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<td></td>
<td>4.2 ± 3.5</td>
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<tr>
<td></td>
<td>Regular</td>
<td>4.6 ± 3.8</td>
<td></td>
<td></td>
<td>3.4 ± 3.1</td>
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<tr>
<td>Smoking status</td>
<td>Yes</td>
<td>4.6 ± 3.9</td>
<td>1.262</td>
<td>0.26</td>
<td>3.5 ± 3.6</td>
<td>0.969</td>
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<tr>
<td></td>
<td>No</td>
<td>5.5 ± 4.2</td>
<td></td>
<td></td>
<td>4.1 ± 3.5</td>
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<tr>
<td>Body mass index</td>
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<td>3.068</td>
<td>0.08</td>
<td>4.5 ± 3.9</td>
<td>0.277</td>
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<tr>
<td></td>
<td>Overweight</td>
<td>5.2 ± 4.1</td>
<td></td>
<td></td>
<td>3.7 ± 3.5</td>
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<tr>
<td></td>
<td>Obese/Morbid obese</td>
<td>5.9 ± 4.0</td>
<td></td>
<td></td>
<td>4.4 ± 3.4</td>
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<tr>
<td>Disease status</td>
<td>HT</td>
<td>5.9 ± 4.5</td>
<td>2.505</td>
<td>0.11</td>
<td>4.3 ± 4.0</td>
<td>0.662</td>
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<tr>
<td></td>
<td>T2DM</td>
<td>5.2 ± 3.9</td>
<td></td>
<td></td>
<td>4.0 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HT + T2DM</td>
<td>5.1 ± 3.8</td>
<td></td>
<td></td>
<td>3.9 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Compliance to anti- hypertensive treatment</td>
<td>Yes</td>
<td>5.1 ± 4.1</td>
<td>15.26</td>
<td>&lt; 0.01</td>
<td>3.8 ± 3.4</td>
<td>13.80</td>
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<tr>
<td></td>
<td>No</td>
<td>7.6 ± 4.3</td>
<td></td>
<td></td>
<td>5.8 ± 4.0</td>
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<tr>
<td>Glycemic control</td>
<td>Yes</td>
<td>4.8 ± 3.6</td>
<td>12.94</td>
<td>0.53</td>
<td>3.7 ± 2.9</td>
<td>25.00</td>
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<td></td>
<td>No</td>
<td>5.7 ± 4.1</td>
<td></td>
<td></td>
<td>4.5 ± 3.5</td>
<td></td>
</tr>
</tbody>
</table>

*HADS-A Hospital Anxiety Depression Scale-Anxiety Subscale, **HADS-D Hospital Anxiety Depression Scale-Depression Subscale
also supported by our study. The roles and the responsibilities of women in the family may make them more susceptible to mental disorders like anxiety and depression [19–21].

In our study, the mean HADS-A and HADS-D scores were greater among divorced or widowed patients compared to the married. Also, mean HADS-A and HADS-D score decreased as education level increased; while level of income does not influence HADS-A score, HADS-D score decreased as income level increased. Previous studies have revealed that depression and anxiety prevalence are influenced by socio-economic variables. It is well known that people have a greater risk for mental disorders due to poverty [22]. A study which was held in primary care clinics indicates that the contribution of the diagnosis of type 2 diabetes mellitus is associated with increased depressive and/or anxiety disorder diagnosis in a sample of low-income adults [23]. The results of our study support these findings.

Mean body weight, BMI, and waist circumference values were found high in all groups in our study. Mean values for all three measurements were significantly different in all groups and they were least in HT group followed by T2DM group and it was greatest in T2DM + HT group. The risk of anxiety and depression was investigated by Balhara et al. through HADS among patients with T2DM who are being followed up at primary care. That study has revealed a significant correlation between the HADS-A scores and BMI and also a significant correlation existed between HADS-D scores and BMI [24]. However, we found that both HADS-A and HADS-D scores were similar among normal weight, overweight, and obese/morbid obese patients. Obesity has no significant association with the risk of anxiety and depression in this study.

Exercise and physical activity have been shown to prevent or delay the onset of several mental disorders [25]. In the general population, several epidemiological studies have found significant cross-sectional correlations between mental health and physical activity levels. The overall incidence of mental disorders, such as the incidence of anxiety, somatoform, and dysthmic disorder, decreases by physical activity. It was shown that regular physical activity is associated with a significantly decreased prevalence of current major depression, panic disorder, agoraphobia, social phobia, and specific phobia [26, 27]. Balhara et al. found a significant correlation between the duration of daily physical exercise and HADS-A scores [24]. Our results revealed that both risk of anxiety and depression were significantly decreased by regular physical activity according to HADS scores.

The World Health Organization (WHO) reported compliance to therapy 50% for chronic diseases in developing countries. This is valid for also HT. Incompliance to therapy is among the main causes for not achieving the target blood pressure levels in hypertensive patients. Mert et al. found mean score of HCAS as 4.66 ± 2.23 in their study conducted with 91 hypertensive patients who referred to Nephrology Outpatient Clinic and concluded that 86.8% of the patients were compliant to therapy [12]. We have found compliance to anti-hypertensive therapy as 82.2% among 297 hypertensive patients in our study conducted at two health centers through HCAS. Anxiety and depression which accompany chronic diseases impair treatment compliance and even depression is added to the list of the treatment incompliance-related factors for hypertensive patients [3]. A significant relationship was shown between incompliance to anti-hypertensive therapy and depression by Wang et al. and the authors recommended adding depression to the list of factors which influence patient incompliance to therapy [28]. We have found that mean HADS-A score and HADS-D score were significantly lower among the patients who were compliant to anti-hypertensive treatment according to HCAS. Our study indicated that both risk of anxiety and depression were significantly lower among the compliant hypertensive patients compared to the noncompliant patients. We consider that using the HCAS for assessment of treatment compliance of hypertensive patients has potential to improve treatment adherence and to identify risk groups in primary health care.

Balhara et al. found a significant correlation between the HADS-A scores and HbA1c levels [24]. Another study conducted using different assessment tools has revealed that anxiety and depression symptoms were more frequent among type 2 diabetic patients with poor glycemic control [29]. Similarly, we have found that mean HADS-Anxiety score was lower among the patients who had good glycemic control; however, the difference was not statistically significant and mean HADS-Depression score was found significantly lower among the subjects who had good glycemic control. We suggested that poor glycemic control might be a predictor for the risk of anxiety and/or depression development among patients with type 2 diabetes.

**Limitations**

This study has some limitations which have to be pointed out. First is the small sample size to generalize the results as the study has been conducted at two health centers in Denizli province. Absence of a healthy control group, including only patient groups, is another limitation. While some other validated instruments are available for evaluating and measuring the risk of anxiety and depression separately [30, 31], they were not used in our study due to taking a longer time and not being used in primary care settings.

**Conclusion**

Anxiety and depression are common disorders in primary care setting as T2DM and HT. We found that the risk of anxiety
and depression is greater among hypertensive and diabetic patients, similarly with the previous studies. We also found that this condition is negatively affected by female gender, being divorced or widowed, having a low education and income level, treatment incompliance in hypertensive patients, and poor glycemic control in diabetic patients. Our results confirm that diabetic and/or hypertensive patients who refer to family health centers have a high risk to develop anxiety and/or depression. Determination of the risk factors for mental disorders through proper assessment tools among hypertensive and diabetic patients who are being followed up at primary health care settings and taking measures have the potential to increase the effectiveness of treatment.

Compliance with ethical standards

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

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

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. The study protocol was approved by Pamukkale University Medical School Non-Interventional Clinical Research Ethics Committee, April 04, 2013, number: 2013/06.

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 Springer


Depression and mild cognitive impairment (MCI) among elderly patients with type 2 diabetes mellitus in Pakistan: possible determinants

Muhammad Atif1 · Quratulain Saleem1 · Shane Scahill2

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Abstract
This descriptive, cross-sectional, questionnaire-based study was designed to assess the extent of depression and mild cognitive impairment (MCI) and their possible determinants among the elderly with type 2 diabetes mellitus in Pakistan. The study was carried out at the diabetes outpatient clinics of two tertiary care hospitals in Lahore, district of Punjab in Pakistan using convenience sampling techniques between December 1, 2015 and February 28, 2016. All consenting patients were interviewed to complete the Geriatric Depression Scale (GDS-15) and the Montreal Cognitive Assessment (MoCA). Standard scoring schemes were used for these scales. Multiple logistic regression analysis was carried out to identify any statistically significant variables from the univariate analysis, to segregate independent factors. A \( p \) value < .05 was taken as the mark of statistical significance for tests. A total of 490 elderly patients with type 2 diabetes mellitus were approached. Of those, 400 patients agreed to participate, resulting in a response rate of 81.6%. GDS-15 scores suggest that only 130 (32.5%) patients were not depressed to some degree. High HbA\(_1\)C and MCI were significant predictors of depression. MoCA scores indicated that 269 (67.3%) patients had MCI, and depression was the only predictor of MCI among the study patients. The implications of the findings include the need by healthcare providers to consider the assessment and management of depression and MCI as part of diabetes treatment protocols so that elderly patients can achieve positive diabetes outcomes and have improved health-related quality of life.

Keywords Health-related quality of life · Type 2 diabetes mellitus · Elderly · Depression · Mild cognitive impairment

Introduction
Diabetes is one of the most common chronic diseases and it threatens the health and well-being of a global community [1]. With a patient population exceeding 0.4 billion, it is expected to be the seventh most prevalent chronic disease by 2030 [2]. Type 2 diabetes mellitus makes up 90–95% of diabetic patients, and the elderly are especially prone [2]. Globally, the severity and prevalence of diabetes is at epidemic proportions in the elderly [3] with a reported prevalence as high as 15% [4]. In Pakistan, the prevalence is 15% in 60–69-year-olds and 15–20% in 70–79-year-olds and this poses enormous health and economic burdens for the nation [4].

In addition to numerous complications, diabetes has been known to cause deleterious effects to mental health and function in elderly patients. This is seen in the associated prevalence of depression and cognitive decline among this cohort of patients [5]. Depression is a mental disorder marked by feeling of sadness, tiredness, guilt, loss of interest, and concentration for a period of 2 weeks or more [6]. A high prevalence of depression has been reported among elderly diabetes patients [7]. A review of the literature reveals that almost one third (30%) of diabetic patients suffer from some level of depression, while 5–10% are afflicted with severe depression [5] which has been attributed in several studies to their uncontrolled glycemic state [8]. Persistent depression has been reported to hamper cognitive functioning of the brain [5]. Moreover, depressed diabetic patients can also present with poor motivation and self-care, poor adherence to therapy, and consequently poor clinical outcomes [9], which aggravates...
their diabetes further and poses long term threats to their health-related quality of life (HRQoL).

Cognition involves mental processes which incorporate multiple brain functions including; memory, attention, retention, perception, reasoning, problem solving, planning, and carrying out planned work [10]. Mild cognitive impairment (MCI) is considered the primary stage of cognitive decline [11] while dementia and Alzheimer’s disease (AD) present as more severe and advanced forms of decline [12]. Although cognitive decline is accepted as part of the normal aging process, diabetes is known to accelerate this cognitive decline in elderly diabetic patients [13]. An increase in the risk of vascular dementia (1.3–3.4-fold) and AD is an established clinical picture among diabetic patients [5]. One systematic review of the literature reports the incidence of cognitive decline increasing from 1.2- to 1.7-fold among diabetes patients [14]. Another study has reported 13.5 and 2.34% prevalence of MCI and dementia, respectively, among elderly patients with type 2 diabetes mellitus [15]. Association between MCI and depression warrants consideration as depression has also been reported among 30–85% of elderly with MCI [16]. MCI may result in poor clinical outcomes by effecting the extent to which patients with diabetes can carry out their activities of daily living, as well as those tasks that relate specifically to their diabetes management [17].

In Pakistan, the implications of neuropsychological disorders specifically depression and MCI have been understudied and underestimated to date. This is despite the fact that this group of disorders constitutes approximately 14% of the burden of disease worldwide [18]. Part of the problem is that mental ailments of this nature carry significant personal stigma and are merely considered public health problems in Pakistan [19]. Internationally there is scarce literature which addresses the predominant causes of depression and MCI among elderly diabetes patients and this is certainly the case in Pakistan. With these multiple co-morbidities—both neurological and metabolic—it is difficult for the patients to maintain a good glycemic state and is a challenge for healthcare workers who are wanting to achieve positive clinical outcomes for these often complex patients [5].

In light of this, the present study investigates the extent and possible determinants of depression and MCI among elderly patients with diabetes in Pakistan. This study is expected to inform both healthcare providers and policy-makers and health managers of the significance of depression and MCI in this patient group in Pakistan. They will be able to take account of predictors and make assessment of parameters appropriate to diabetic mental function as part of their treatment protocols. The study is also expected to reveal the causes of treatment failure among elderly Pakistani diabetic patients and promote the use of assessment techniques for both depression and MCI in this group. Early detection of these co-morbidities and identifying the likely determinants should prevent Pakistani diabetic patients from suffering reduced HRQoL precipitated by depression and MCI.

**Methods**

**Study setting and population**

The study was conducted at the diabetes outpatient clinics of two tertiary care hospitals in Lahore, district of Punjab in Pakistan. Lahore is the capital city of the province of Punjab and is the second most populated city having a population in the vicinity of 9 million people. There are over 50 public and private sector hospitals in Lahore, of which 10 are major public sector hospitals and one is a semi-government hospital (i.e., Gulab Devi Chest hospital).

The two tertiary care hospitals in Lahore that contributed data were Jinnah Hospital and the Sir Ganga Ram Hospital. Jinnah Hospital, named after the founder of Pakistan, Muhammad Ali Jinnah, is ranked the second largest teaching hospital in Punjab and has been operational since February 2, 1996. Jinnah Hospital has a separate and well established outpatient diabetes clinic named the Jinnah-Allama Iqbal Institute of Diabetes and Endocrinology, which is the second accredited fellowship clinic in Pakistan. This clinic has been open since November 14, 2009 and provides diabetes and endocrinology care to more than 200 patients per month. Patient counseling and free blood glucose testing is also performed here, along with diabetic medical care. The second hospital, named after the founder—Sir Ganga Ram—is much older and was established in 1921. This hospital provides medical care to the majority of low and middle-income class populations in Lahore. The diabetes outpatient clinic is located in the Department of Internal Medicine and provides diabetes care to a large number of patient visitors each day. Patient counseling and free blood glucose monitoring is part of the medical care provided.

The sample population comprised of elderly patients diagnosed with type 2 diabetes mellitus for 6 months or longer, age ≥ 60 years, visiting the outpatient diabetes clinics to have their HbA1C test performed. Both male and female patients from all walks of life were included in the study. Those excluded from the study were: patients suffering from accidental physical disabilities apart from the natural degenerative process, those who were health illiterate (native language) and unable to comprehend medical instructions, those with terminal or life threatening illness such as HIV/AIDS or epidemics such as, malaria, or cholera. Institutionalized patients and those suffering from severe dementia, AD, or other severe neuropsychological disorders were also excluded.
Study design and data collection

This was a descriptive, non-experimental, cross-sectional, questionnaire-based study. It was conducted in the Punjab province of Pakistan from December 1, 2015 to February 28, 2016. A representative sample was drawn from the total population (9 million) of Lahore. The Raosoft sample size calculator was used to determine the number of participants required [20]. With a margin of error of 5% and confidence interval (CI) set at 95%, the minimum sample size was 385. However, a target of 475 patients was set with the aim of having an adequate final sample size based on the possibility of outliers or incomplete responses. Using a convenience sampling technique, the data collection was carried out from the diabetes clinics outlined previously. Information about the study was provided to patients visiting the diabetes clinics. Following consent, an interview questionnaire was completed. A detailed description of the questionnaire and its development is provided as follows:

Survey instrument

The survey instrument/questionnaire comprised of four parts. The first and second parts contained information about the socio-demographic and clinical characteristics of the patients. The third and fourth parts were the instruments for measurement of depression and MCI, namely the Geriatric Depression Scale (GDS) and the Montreal Cognitive Assessment (MoCA) [21]. The socio-demographic characteristics of the patients included information about education, employment and living. The clinical characteristics of the patients included information about co-morbidities, diabetes complications, glycemic levels, and drug therapy.

Geriatric depression scale and its scoring

GDS-15 is a reliable, valid, and widely used scale to measure depression in the elderly population [22]. It was developed in 1986 by Yesavage, et al. and is free to use for educational and research purposes [23]. The approximate administration time is 5–7 min and it can be used for both ill and healthy populations [24]. The original questionnaire comprised of 30 items. A Short Form with 15 questions was then developed. Each of the 15 items is attributed a score of one, making a maximum possible score of 15. A score of less than five is considered normal, whereas scores ranging from 5 to 8 show mild depression, 9–11 moderate depression, and 12–15 indicate severe depression in the respondent. The questionnaire was translated into Urdu by the standard method of forward backward translation [25]. The translated version was administered to 10% of the target population for pilot testing. The internal consistency of the Urdu version of the GDS-15 questionnaire was .89.

Montreal cognitive assessment tool and its scoring

MoCA is one of the most reliable and valid tools used for the assessment of early dementia and specifically MCI [21, 26]. It was developed in 1996 by Ziad Nasreddine in Montreal, Quebec and it takes 10 min to administer [27]. The tool has been translated into many languages and the Urdu version of the tool was obtained for use from the developer. The MoCA has been designed to measure various mental functions namely, visuospatial and executive abilities (five points), naming (three points), memory (no points), attention (six points), language (three points), abstraction (two points), delayed recall (five points), and orientation (six points). The total score achievable for all domains combined is 30. A score of less than 26 indicates MCI is present. An extra point is awarded to the participants with 12 years or less of education.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (IBM, SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp.). Categorical variables were described by counts (n) and proportions (%), whereas the continuous variables were described by means (x) and standard deviations (SD). Simple logistic regression analysis was used to assess the association between the categorical dependent variables and the independent variables. The crude odds ratio (OR), 95% CI, beta, p value and standard error were reported for each predictor. Multiple logistic regression analysis was carried out to identify any statistically significant variables from the univariate analysis, to segregate true predictors or independent factors. The adjusted odds ratio (AOR), 95% CI, beta, standard error, and p value were described for each predictor, in a similar manner as for the univariate analysis. The model fit was assessed using chi-square, degrees of freedom and p value. The model was assessed by Hosmer-Lameshow goodness of fit test. Pseudo R square values were included to describe the percentage of variance given by the model. A p value < .05 was taken as the mark of statistical significance for tests.

Results

Socio-demographic characteristics

A total of 490 elderly patients with type 2 diabetes mellitus were approached. Of those, 400 patients agreed to participate resulting in a response rate of 81.6%. From the 400 patients, 185 (46.3%) were male and 215 (53.8%) were female. More than half of patients (n = 226, 56.5%) were ≥ 62 years old and 286 (71.5%) patients were married.
Table 1 shows a detailed description of socio-demographic characteristics of the patients.

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>Patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>185 (46.3)</td>
</tr>
<tr>
<td>Female</td>
<td>215 (53.8)</td>
</tr>
<tr>
<td>Mean age</td>
<td>64 (SD = 5.5)</td>
</tr>
<tr>
<td>Age ≥ 62 years</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>226 (56.5)</td>
</tr>
<tr>
<td>No</td>
<td>174 (43.5)</td>
</tr>
<tr>
<td>Mean years of education</td>
<td>7.4 (SD = 4)</td>
</tr>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>178 (44.5)</td>
</tr>
<tr>
<td>No</td>
<td>222 (55.5)</td>
</tr>
<tr>
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<tr>
<td>Secondary</td>
<td>73 (18.3)</td>
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<tr>
<td>Tertiary</td>
<td>73 (18.3)</td>
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<tr>
<td>Illiterate</td>
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</tr>
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<td>286 (71.5)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>6 (1.5)</td>
</tr>
<tr>
<td>Divorced</td>
<td>6 (1.5)</td>
</tr>
<tr>
<td>Widowed</td>
<td>102 (25.5)</td>
</tr>
<tr>
<td>Employment</td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>47 (11.8)</td>
</tr>
<tr>
<td>Self-employed</td>
<td>54 (13.5)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>220 (55)</td>
</tr>
<tr>
<td>Pensioner</td>
<td>79 (19.6)</td>
</tr>
<tr>
<td>Living</td>
<td></td>
</tr>
<tr>
<td>With family</td>
<td>381 (95.3)</td>
</tr>
<tr>
<td>Solitary</td>
<td>19 (4.8)</td>
</tr>
</tbody>
</table>

Of the 400 study patients, 340 (85%) had co-morbidities and 261 (65.3%) suffered from cardiovascular (CVD) disorders. With regard to diabetes complications, 309 (77.3%) patients had diabetes complications, and 239 (59.8%) were suffering from peripheral neuropathy. Furthermore, 252 (63%) patients had a duration of diabetes ≤10 years and two-thirds of the patients (n = 271; 67.8%) did not have their HbA1C at target levels. Table 2 provides a detailed description of the clinical characteristics of the study cohort.

Clinical characteristics

Of the 400 study patients, 340 (85%) had co-morbidities and 261 (65.3%) suffered from cardiovascular (CVD) disorders. With regard to diabetes complications, 309 (77.3%) patients had diabetes complications, and 239 (59.8%) were suffering from peripheral neuropathy. Furthermore, 252 (63%) patients had a duration of diabetes ≤10 years and two-thirds of the patients (n = 271; 67.8%) did not have their HbA1C at target levels. Table 2 provides a detailed description of the clinical characteristics of the study cohort.


Depletion

Of the 400 study patients, only 130 (32.5%) did not have some level of depression, whereas 85 (21.3%) patients had severe depression (Table 3).

Depression score: Normal/No depression= 0 – 4, Mild depression= 5 – 8, Moderate depression= 9 – 11, Severe depression= 12 – 15

Regarding the predictors of depression; multiple logistic regression analysis showed that having high HbA1C levels (AOR 2.67; 95% CI 1.35, 5.29) and MCI (AOR 33.49; 95% CI 16.04, 69.92) were significant predictors of depression in the study cohort (Table 4).

Cognitive function

Of the 400 diabetics in this study, 269 (67.3%) had MCI, whereas 131 (32.8%) had normal cognitive function. The final predictor of MCI in the multiple logistic regression analysis was depression (AOR 42.50; 95% CI 20.99, 86.06) (Table 5).

Discussion

Depression and MCI are the co-morbidities that when coupled with diabetes, compromise the mental capacity of these
patients. Once mental health deteriorates it leads to chronic disability and may contribute to poor clinical outcomes [18]. Although it is difficult to quantify the degree of mental and psychological stress and to identify the pre-eminent causes of depression and MCI, these are the areas that need early detection and immediate clinical intervention to prevent their intensification. Depression and MCI impact on HRQoL and patients’ abilities to manage their disease [5, 28]. Despite this, insufficient attention is paid by Pakistani healthcare providers to the timely detection and management of these important maladies, among elderly Pakistani diabetes patients. Consequently, patients have to endure not only the health-related burden but also have to bear extra economic burden due to depression and MCI in addition to conventional diabetes treatment costs [29]. The present study was conducted to ascertain the degree to which depression and MCI are associated with elderly diabetes patients in Pakistan. The study was also aimed to draw attention to the importance of diagnosis and management of depression and MCI in elderly with diabetes, among healthcare providers and healthcare authorities who formulate diabetes treatment protocols.

Depression at some level has affected the majority of patients participating in this study with only one-third of patients...
being free from depression. Previous studies have also reported a high prevalence of depression among elderly patients with diabetes [29, 30]. A systematic review supports this with a significant prevalence of depression for diabetes patients compared with non-diabetics [31]. This study has revealed two predictors of depression; firstly, high levels of HbA1C and secondly, MCI. High HbA1C or impaired glycemic control has been significantly associated with depression due to its directly leading to the development of diabetes complications and conditions of poor health [32] resulting in low self-esteem and depression [33]. The findings of this study are consistent with another showing that poor glycemic control could cause depression [34]. However, the literature also supports the notion that a high prevalence of depression could result in suboptimal poor health outcomes due to poor adherence to therapy, which could further aggravate depression [35].

MCI was the second predictor of depression among the patients in this study. There could be multiple reasons for this. First, MCI could have led to poor glycemic control, as indicated by a previous study [17], which could cause depression [35]. Second, a forgetful patient may become frustrated by this and find it difficult to adhere to therapy and subsequently become depressed [36]. Third, patients with MCI might also be depressed as they face the consequences of hyper or hypoglycemia as a result of under or over dosing due to cognitive deficiency [37]. Fourth, patient’s inability to carry out complex diabetes self-care tasks as a result of MCI could also be linked to stress and accompanying depression [38]. Fifth, the dependence and need of assistance by those with cognitive impairment for their regular diabetes management might also be a significant reason for the development of depression [28]. In contrast to the findings of this study, a previous study has suggested the opposite, i.e., depression could be a risk factor for cognitive decline and early dementia among the elderly [39]; however, a study conducted in stroke patients has confirmed that cognitive decline could be a determinant of depression among elderly patients [40]. Moreover, a review of the literature also supports this study by reporting that patients with premature cognitive decline often become sad and depressed. The reason provided was feeling of loss of autonomy of their disease management and thoughts associated with adverse health outcomes upon progression of MCI to full-blown dementia [41].

MCI has affected a significant number of participants in our study. Such findings have also been suggested by several studies which report a high prevalence of MCI among elderly diabetes patients who have transitioned to dementia as the disease has progressed [42, 43]. In this study, depression

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>p value</th>
<th>AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of education</td>
<td>−0.024</td>
<td>0.048</td>
<td>0.612</td>
<td>0.976 (0.888, 1.073)</td>
</tr>
<tr>
<td>Being single</td>
<td>0.072</td>
<td>0.412</td>
<td>0.861</td>
<td>1.075 (0.480, 2.408)</td>
</tr>
<tr>
<td>Economic dependence</td>
<td>0.115</td>
<td>0.395</td>
<td>0.771</td>
<td>1.122 (.517, 2.434)</td>
</tr>
<tr>
<td>Diabetes complications present</td>
<td>0.182</td>
<td>0.647</td>
<td>0.779</td>
<td>1.199 (.338, 4.260)</td>
</tr>
<tr>
<td>Number of diabetes complications</td>
<td>−0.169</td>
<td>0.441</td>
<td>0.701</td>
<td>0.84 (0.36, 2.00)</td>
</tr>
<tr>
<td>HbA1C above target level</td>
<td>0.982</td>
<td>0.349</td>
<td>0.05</td>
<td>2.67 (1.35, 5.29)</td>
</tr>
<tr>
<td>Not on OHA</td>
<td>0.043</td>
<td>0.454</td>
<td>0.925</td>
<td>1.04 (0.43, 2.54)</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.013</td>
<td>0.033</td>
<td>0.684</td>
<td>1.01 (0.95, 1.08)</td>
</tr>
<tr>
<td>Duration of insulin therapy</td>
<td>0.036</td>
<td>0.061</td>
<td>0.562</td>
<td>1.04 (0.92, 1.17)</td>
</tr>
<tr>
<td>MCI</td>
<td>3.511</td>
<td>0.376</td>
<td>&lt;.0005</td>
<td>33.49 (16.04, 69.92)</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.013</td>
<td>0.033</td>
<td>0.684</td>
<td>1.01 (0.95, 1.08)</td>
</tr>
</tbody>
</table>

Table 5 Predictors of mild cognitive impairment: multiple logistic regression analysis
was an independent predictor of MCI. As depression is a negative psychological state [6], it may harm the normal functioning of the brain [44] by damaging the hippocampus [45] and compromising the memory processing ability of the brain [44]. In line with this study, others have also identified depression as an independent predictor of cognitive decline, and as a factor that could aggravate cognitive decline among the elderly [46, 47]. Likewise, another published study has reported an association between chronic depression and MCI, illustrating that chronic depression can damage the cortical region of the brain, resulting in the progression of MCI to dementia and AD [16]. According to one systematic review, the prevalence of cognitive decline and dementia was twice as high among depressed patients as compared to those patients who were not depressed [48]. Similarly, a study conducted with depressed elderly patients reported a high prevalence of MCI among those with a positive history of depression [49]. Despite a strong association between depression and MCI, studies specifically dealing with depression and MCI among elderly patients with type 2 diabetes mellitus are lacking.

The findings of our study have shown that a bi-directional relationship prevails between depression and MCI where MCI is a determinant of depression and vice versa. The authors are not aware of such findings having been presented in a single published study; however, a number of studies have explored these associations. Some studies have reported depression to be a causative agent of MCI, which manifests as a comorbidity with depression [45–47], whereas others have supported the notion that MCI can indirectly lead to depression through compromising a patient’s executive functioning [40, 41]. Figure 1 shows the summary of the regression analysis relating to depression and MCI from this study.

**Conclusion**

In our study, impaired glycemic control and MCI were the independent predictors of depression which in turn was the sole determinant of MCI. These findings indicate a bi-directional relationship between depression and MCI in type 2 diabetes patients in Pakistan.

**Implications**

In this scenario, there is a dire need of a shift in clinical practice with the requirement to make the assessment of depression and MCI a mandatory part of patient’s routine examinations at diabetes outpatient clinics in Pakistan. In addition to this, healthcare professionals (physician, pharmacist and nurse) and family members should play a supportive role with the aim of tackling depression and MCI openly in type 2 diabetes patients. Prescribing antidepressants and keeping glycemic levels on target will be beneficial in mitigating depression in at-risk patients. Similarly, prescribing drugs that reduce the risk of cerebrovascular disorders would be expected to prevent cognitive decline. Improving glycemic control and efficient diabetes management may indirectly avert the transition from MCI to severe dementia thereby reducing the incidence of depression, a strong predictor of cognitive dysfunction [44]. Healthcare professionals, including pharmacists need to devise ways to increase patient compliance with prescribed regimens. Patients’ families and caregivers can also play a vital role in assisting patients to be more adherent to their treatment regimens with the long-term aim of achieving positive clinical outcomes.

**Limitations of the study and directions for future research**

Inclusion of only elderly diabetic patients is the major limitation of our study. This subgroup of diabetes patients was recruited as they are the most vulnerable group likely to develop depression and MCI. In addition to the management of diabetes, this age group also needs a comprehensive strategy for the management of neuropsychological problems and another limitation of this study is the short duration when trying to understand depression and MCI and the relationship with diabetes. It is suggested that future researchers should perform longitudinal studies to describe the complex phenomenon of depression and MCI in elderly diabetes patients.

**Compliance with ethical standards**

**Conflict of interest** MA, QS, and SS declare that they have no conflict of interest.

**Ethical approval** Code of Ethics of the Declaration of Helsinki was considered at every step of the study. Ethical approval was gained from the Pharmacy Research Ethics Committee (PREC) at the Islamia University Bahawalpur (Reference: 11–2015/PREC, dated October 20, 2015). The purpose of the study was explained to each respondent before the start of the study. Written informed consent was obtained from respondents following reading of the “respondent information pack”, before administration of the questionnaire.

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


**Bacillus**-produced surfactin for intranasal delivery of insulin in diabetic mice

Qin Yu1 · Shihong Dong1 · Dan Yang1 · Xiaoying Xing1 · Xiuyun Zhao1 · Gaofu Qi1

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**Abstract** Previous studies have shown that **Bacillus**-produced surfactin (SFN) can be used for oral delivery of insulin (INS). To improve the bioavailability of INS, we determined the effects of SFN on intranasal delivery of INS in diabetic mice. Combinations of SFN and INS at different doses were used for intranasal administration of diabetic mice. The plasma levels of glucose and INS were determined at various time intervals after intranasal administration, and then, the hypoglycemic effects and relative bioavailability of INS were calculated. Glucose tolerance test was performed to determine the effects of intranasal delivery of INS plus SFN on the control of glucose levels. Diabetic mice were also intranasally administered with the INS and SFN combo for 7 days to determine the short-term stability of this formulation for controlling blood glucose levels. A combination of 20 IU/kg INS and 1.6 mg/kg SFN achieved the best hypoglycemic effects for intranasal administration, with a maximal hypoglycemic rate of 29.59% and a maximal blood INS concentration of 45.47 μIU/ml 2 h after administration. As a result, a relatively increased bioavailability of 8.55% was achieved. Glucose tolerance test showed that intranasal delivery of INS plus SFN could effectively control the blood glucose levels after the influx of glucose. Furthermore, intranasal INS plus SFN could be used for controlling blood glucose daily for a short term. Histological evaluation showed no changes in the morphology of the nasal mucosa after exposure to SFN plus INS. SFN is potentially useful for intranasal delivery of INS to control blood glucose levels.

**Keywords** Diabetes · Insulin · Surfactin · Intranasal delivery · Bioavailability

**Introduction**

Diabetes is one of the most common diseases that heavily threaten human health in the world. Patients with diabetes need daily insulin (INS) injections to control blood glucose. The intranasal administration has attracted a lot of attention as an alternative route for the parenteral injection of INS. This can be attributed to the relatively large surface area of nasal mucosa available for INS absorption, the rich vascularity of the nasal mucosa that facilitates drugs to directly enter the systemic circulation, a porous endothelial membrane, and a lower enzymatic activity than that in the gastrointestinal tract [1]. The ease of intranasal administration also allows patients to self-medicate on long-term therapy. Furthermore, INS can enter directly into the brain tissue or cerebrospinal fluid through olfactory neurons after intranasal administration for controlling nervous system diseases such as Alzheimer’s disease, cognitive impairment, and neurodegeneration [2–4].

Although nasal delivery of INS has many advantages, this formulation gave poor bioavailability (generally less than 1%) and local irritation [1]. Patton and Platz firstly tested intranasal delivery of INS in animals, but no notable hypoglycemic effects were observed [5]. INS is not well absorbed through nasal mucosa because of its large molecular size and low permeability through the membrane. Therefore, low permeability of the nasal mucosa to INS is becoming one of the major limitations for achieving high nasal bioavailability. Additionally, possible enzymatic degradation may contribute to the low bioavailability of nasal INS. Many methods have been studied to overcome the absorption barriers and achieve efficient INS absorption via the nasal route, including the use...
of absorption enhancers and enzyme inhibitors, as well as designing mucoadhesive systems to prevent rapid mucociliary clearance from the nasal cavity [1]. However, most absorption enhancers (such as surfactants, bile salts, and fatty acids) can cause damage to epithelial cells that accompanies their absorption-enhancing effects. Although enzyme inhibitors can prevent the hydrolysis of INS in the nasal cavity, they cannot themselves facilitate the penetration of INS across the nasal mucosa [6]. A biomaterial with the activity both as absorption enhancers and enzyme inhibitors that can overcome mucosal barriers and improve the nasal delivery of protein and peptide drugs would, therefore, be of interest.

Surfactin (SFN) has received a lot of attention owing to its unique properties. SFN, a bacterial lipopeptide produced by *Bacillus*, consists of a cyclic heptapeptide headgroup (Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu) linked to a C13–15 β-hydroxy fatty acid by lactone bond. This structure endows SFN with a powerful biosurfactant activity by its amphiphilic nature, a polar amino acid head, and a hydrocarbon chain [7, 8]. Recently, SFN is gaining attention for potential biomedical and pharmaceutical applications. SFN can bind to proteins (e.g., hemoglobin) to form a fractal structure representing a “necklace model” of micelle-like clusters randomly distributed along the protein polypeptide chain [9]. SFN can also penetrate through the cell membrane. The penetration process is mainly governed by the hydrophobic interactions between the fatty acid chain of SFN and phospholipid chains of the cell membrane [8]. Moreover, SFN has generally been known as a biosurfactant with low toxicity [10], and its safety for oral administration has already been verified in animals [11]. Recently, our group found that SFN can enhance oral delivery of INS in mice. After oral administration of INS (90 IU/kg) plus SFN, a maximal hypoglycemic rate of 49% and a relative bioavailability of 12.48% were found in the diabetic mice [12]. Compared to the gastrointestinal tract, the nasal mucosa lacks proteases to digest INS and more efficiently facilitate absorption of peptide/protein drugs. Therefore, we tended to determine whether SFN could promote INS absorption by the nasal mucosa for a higher relative bioavailability after intranasal administration in diabetic mice.

**Materials and methods**

**Materials**

SFN (C15) was purified from *Bacillus amyloliquefaciens* WH1 culture as described previously [13]. Recombinant human INS was a gift from JS Bioway Pharmaceutical Co., Ltd. (Changzhou City, China). Streptozotocin was purchased from Sigma (St. Louis, MO, USA). Glucose fluid monoreagents were purchased from Baoding Great Wall Clinical Reagent Co Ltd. (Hebei, China). Human INS ELISA kit was purchased from RayBiotech (USA). Ketamine and xylazine were purchased from Shengda Animal Pharmaceutical Co., Ltd. (Dunhua, Jilin, China). All other chemicals were of analytical grade and supplied by Sinopharm Chemical Reagent (China).

**Animals**

Balb/C mice (5–6 weeks old, 18–20-g body weight) were purchased from the Center for Disease Prevention and Control of Hubei Province, China, following the University Ethics Committee’s guidelines.

**Induction of experimental diabetes**

Type 1 diabetes mellitus was induced in mice by intraperitoneal injection with streptozotocin dissolved in 0.1 M citrate buffer (pH 4.0) at a dose of 70 mg/kg bodyweight. The development of diabetes was determined by the observation of two serious symptoms, polyuria and polydypsia; then, the blood glucose levels were measured using glucose fluid monoreagents by the directions of manufacture. Only mice with fasting blood glucose levels greater than 25 mmol/l were regarded as diabetic and then were included in this study.

**Determining hypoglycemic effects of insulin plus surfactin for intranasal administration**

The diabetic mice (n = 8 per group) were anesthetized by intraperitoneal injection with ketamine (150 mg/kg body weight)/xylazine (10 mg/kg body weight) combination. After anesthetization, the diabetic mice were intranasally administered with 50 μl solution of normal saline, SFN (0.4 mg/kg), INS (10 IU/kg), or INS (10 IU/kg) plus SFN (0.4 mg/kg) at 25 μl per nostril. One group of mice was subcutaneously (s.c.) injected with INS (1 IU/kg) as the positive control. Blood samples were collected for detecting blood glucose before intranasal administration as fasting glucose levels and at different time intervals (0.5, 1, 1.5, 2, 3, 4, and 5 h, respectively) post-administration. Results are presented as the % reduction from the initial value. The decrease in blood glucose concentration was obtained from the blood glucose concentration-time curves (% change of initial) of each mouse by Eq. 1:

\[
\% \text{ change} = \left( \frac{F - Pr}{F} \right) \times 100
\]

where \(F\) is the fasting blood glucose level and \(Pr\) is the blood glucose level at time \(t\) post-administration [14–17].
**Determination of optimal doses of insulin and surfactin for intranasal delivery**

Initially, SFN (0.8 mg/kg) was mixed with a selected dose of INS (10, 20, or 30 IU/kg) in 50 μl normal saline solution for intranasal administration of diabetic mice as described earlier. After the dose of INS was selected, selected doses of SFN (0.4, 0.8, or 1.6 mg/kg, respectively) were mixed with INS in 50 μl normal saline solution for intranasal administration of mice; then, the blood samples were collected at following time points for detecting the blood glucose. The reduction in blood glucose concentration was obtained from the blood glucose concentration-time curves (% change of initial) of each mouse by Eq. 1.

**Determination of bioavailability of intranasal insulin plus surfactin**

For determination of bioavailability of INS plus SFN delivered through intranasal route, 24 diabetic mice were randomly divided into two groups of 12 mice per group. One group was intranasally administered with 20 IU/kg of INS plus 1.6 mg/kg SFN, and another group was subcutaneously (s.c.) injected with INS (1 IU/kg) as the positive control. Blood samples were collected before intranasal administration as the fasting glucose levels and at different time intervals post-intranasal administration. The samples were detected for blood glucose and for serum INS concentrations by human INS ELISA kits. The relative intranasal bioavailability (BA) of INS formulated with SFN was calculated by Eq. 2:

\[
BA = \frac{\text{AUC}_{0-6\text{h intranasal}} \times \left( \frac{\text{weight}}{\text{dose}} \right)_{\text{intranasal}}}{\text{AUC}_{0-6\text{h s.c.}} \times \left( \frac{\text{weight}}{\text{dose}} \right)_{\text{s.c.}}} \times 100\% \quad (2)
\]

The AUC\(_{0-6}\) is the area under the reduction of blood glucose levels from 0 to 6 h; weight (kg) is the body weight of mice, and dose (IU) is the amount of INS administered to the animals [18–21].

**Oral glucose tolerance test**

According to the optimal dose of INS and SFN, the oral glucose tolerance test (OGTT) was conducted in diabetic mice. Sixteen diabetic mice were randomly divided into two groups for eight mice per group. One group was intranasally administered with 20 IU/kg of INS plus 1.6 mg/kg of SFN, and another group was intranasally treated with 50 μl normal saline solution as a placebo control. One hour after intranasal administration, all mice were orally given 200 μl glucose solution at a dose of 2 g/kg body weight. Then the blood glucose was determined at each time interval [18].

**Determination of hypoglycemic effects of insulin plus surfactin for practical use**

To assess whether the intranasal delivery of INS formulated with SFN has potential to be used in the control of glucose levels in the face of the continuous influx of glucose and possibly other carbohydrates, we determined the hypoglycemic effects of intranasal administration with INS plus SFN in diabetic mice for 7 days. The diabetic mice were allowed access to food and water prior to and throughout the course of the experiment and intranasally given 20 IU/kg INS plus 1.6 mg/kg SFN once a day for 7 days. The blood glucose was determined everyday post-intranasal administration, and the reduction in blood glucose concentration was obtained from the blood glucose concentration-time curves (% change of initial) of each mouse by Eq. 1.

**Toxicity analysis**

The possible toxicity of SFN or INS plus SFN was tested using the optimal doses described earlier. After intranasal administration, the diabetic mice were observed for any physical signs of toxicity such as writhing, gasping, salivation, diarrhea, cyanosis, pupil size, any nervous manifestations, or mortality. After intranasal administration once a day for 7 days, the nasal mucosa was collected from mice, fixed in formalin, sectioned into slides, stained by hematoxylin-eosin, and then examined with a microscope for any possible pathological changes due to SFN or INS plus SFN [22].

**Statistical analysis**

Results are presented as the means ± SD. Statistical differences were analyzed by one-way ANOVA followed by Tukey’s comparison test with LSD. A value of \( p < 0.05 \) (*) was considered significant, and that of \( p < 0.01 \) (**) was considered highly significant.

**Results**

**Intranasal administration with insulin plus surfactin showing hypoglycemic effects**

As illustrated in Fig. 1a, INS (10 IU/kg) plus SFN (0.4 mg/kg) resulted in significant hypoglycemic effects when compared...
to SFN alone or normal saline control. Although INS alone (10 IU/kg) also showed hypoglycemic effects, it only worked for 1 h while INS plus SFN worked for 2 h. Moreover, the blood glucose was maximally decreased to 58.27% of the initial level at 2-h post-administration for INS plus SFN, while the blood glucose was only maximally reduced to 72.27% of the initial level at 1.5-h post-administration for INS alone.

Different from s.c. administration, the inter-individual response to intranasal INS was variable in a broad range. Here, we found that intranasal delivery of INS plus SFN could induce obvious hypoglycemic effects in most of the diabetic mice tested. The effective rate of intranasal INS plus SFN was 91.13%, while it was only 22.22% for the group intranasally treated with INS alone (Fig. 1b). This result indicates that SFN can help to reduce the barriers to bioavailability and enhance the effect of intranasal INS in vivo.

Fig. 1 INS formulated with SFN showing significant hypoglycemic effects after intranasal administration. a Hypoglycemic effects of intranasal INS (10 IU/kg) plus SFN (0.4 mg/kg). Compared to INS alone, intranasal INS plus SFN showed higher hypoglycemic effects. b Effective rate of intranasal INS plus SFN. Compared to INS alone, intranasal INS plus SFN showed much higher effective rates, indicating that SFN is favorable for overcoming the individual difference for intranasal INS. INS insulin, SFN surfactin, NS normal saline solution, s.c. subcutaneous administration. Values are presented as means ± SD (n = 8)

Optimal doses of surfactin and insulin for intranasal administration

SFN (1.6 mg/kg) plus different doses of INS (10, 20, or 30 IU/kg, respectively) all resulted in significant hypoglycemic effects when compared to normal saline control. The relative glucose concentration for 30 IU/kg INS plus SFN (1.6 mg/kg) was 74.25, 48.24, 38.26, 37.18, and 36.60% post-administration for 0.5, 1, 1.5, 2, and 3 h, respectively (Fig. 2a). For the dose of 20 IU/kg, the relative glucose concentration was 71.40, 42.35, 24.16, 23.41, and 27.57% post-administration for 0.5, 1, 1.5, 2, and 3 h, respectively (Fig. 2a). The INS dose of 20 IU/kg showed the highest hypoglycemic effects among all three doses. Therefore, we selected this dose for the following studies.

INS was given as 20 IU/kg and then mixed with different doses of SFN for intranasal administration. It was found that 20 IU/kg INS plus 1.6 mg/kg SFN resulted in the best hypoglycemic effects when compared to other two doses (0.4 and 0.8 mg/kg, respectively), with the relative glucose concentration of 70.83, 34.18, 36.30, 40.11, and 48.78% post-administration for 0.5, 1, 1.5, 2, and 3 h, respectively (Fig. 2b). Collectively, the dose of 20 IU/kg INS plus 1.6 mg/kg SFN was selected for the following studies.

High bioavailability of intranasal insulin plus surfactin

The combination of 20 IU/kg INS and 1.6 mg/kg SFN was used for intranasal administration of diabetic mice, and the blood glucose levels and INS concentrations were determined subsequently. After intranasal administration, the blood glucose levels began to fall after 30 min,
reached a nadir 60 min after dosing, and waned to baseline levels approximately 3 h post-dosing. In detail, the relative glucose concentration was respectively 49.25, 38.60, 31.59, 38.28, and 45.62% post-administration for
0.5, 1, 1.5, 2, and 3 h, similar to that of s.c. administration (1 IU/kg) in this study (Fig. 3a).

We further determined the bioavailability of intranasal INS plus SFN. Figure 3b shows the serum INS concentrations (μIU/ml) versus time curves. For the intranasal dose, the peak INS level was attained in 1.5–2.0 h and remained elevated for approximately 2 h, with the maximum INS concentration achieved at 45.47 μIU/ml and the biggest AUC (0–6 h) at 118.79 μIU h/ml. The serum INS concentrations versus time curves were used to calculate the relative intranasal bioavailability of INS, which was 8.55% according to Eq. 2.

**Oral glucose tolerance test**

To determine whether the intranasal delivery of INS formulated with SFN is useful in the control of glucose levels, OGTT was performed. Figure 4 shows the comparison of the blood glucose levels in diabetic mice fed with glucose. The blood glucose levels of mice intranasally treated with INS (20 IU/kg) plus SFN (1.6 mg/kg) were significantly lower than the control animals at all time points (0.5, 1, 1.5, 2, 3, and 4 h postoral administration with glucose, respectively). The results suggest that intranasal INS formulated with SFN may be useful for the control of blood glucose to normal levels in the face of the continuous influx of glucose and possibly other carbohydrates.

**Intranasal administration with insulin plus surfactin showing hypoglycemic effects for potential practical use**

To determine the effects of intranasal INS plus SFN for controlling blood glucose in the short term, we measured the hypoglycemic effects of intranasal INS plus SFN in diabetic mice for 7 days. The results show that significant hypoglycemic effects were observed post-administration in the diabetic mice intranasally administered with INS plus SFN from day 1 to day 7, which were similar to the hypoglycemic effects of INS administered by subcutaneous injection (Fig. 5). This result indicates that intranasal INS formulated with SFN may be useful for controlling the patients’ blood glucose levels at least for short-term use.

**Intranasal insulin plus surfactin not inducing apparent injury in mice**

After intranasal administration with SFN or SFN plus INS for 7 days, no apparent drug-induced physical signs of toxicity were found during the whole experimental period, and no animal death was observed. Intranasal administration with SFN or SFN plus INS did not cause any inflammatory injury to the nasal mucosa. The nasal mucosa tissue was relatively intact, without irritation or damage to the nasal epithelial cell membrane (Fig. 6).

**Discussion**

The nasal route provides an attractive needle-free alternative for currently injectable drugs which may improve patient compliance and allow extended use of self-medication for many chronic diseases including diabetes. However, although it is well known that the intranasal route is highly efficient, the success to effectively deliver INS via this route is still difficult due to the large molecular size and low permeability of INS [23]. Buccal spray has benefits such as the relatively large area of coverage, resulting in better bioavailability [24]. However, it is limited in promoting absorption from buccal mucosa and the permeability varies greatly among the different areas of the oral mucosa [25]. Compared with the buccal spray of INS which has been approved for clinical use, nasal delivery has advantages in drug delivery because nasal mucosa has higher blood vessel density than oral mucosa and it has a porous endothelial membrane allowing the passage of medications directly into the systemic circulation. To increase the bioavailability of INS absorption across the nasal mucosa, an extensive range of enhancers has been used such as bile salts and derivatives, sodium lauryl sulfate, phospholipids, cyclodextrins, and chitosan [1, 23]. Although it is difficult to achieve
accurate drug administration through both nasal spray and dripping delivery, nasal dripping is easier to handle within our animal model than nasal spray. Other than the natural biosurfactant of SFN, other chemically synthesized surfactants were also used for delivery of INS. For instance, sodium deoxycholate (SDC) in combination with cyclodextrins (CD) can be used to enhance nasal INS absorption. Through this method, the minimal the relative glucose concentration was (72.6 ± 2.1) % of baseline, which lasted for 3 h [26]. Hydrophobic bile salts can be used to increase nasal INS absorption. This approach achieved 50% decrease in blood glucose concentration [27]. Phospholipids and cyclodextrins are also reported to act as an enhancer for nasal INS absorption. Cyclodextrins increased INS bioavailability from a negligible value (approximately 0.06%) to 5.63% [28]. However, even following the addition of absorption enhancers to the formulation, the absolute bioavailability remains low in most studies [29]. Moreover, many of the enhancers that are useful in improving the nasal absorption of INS caused severe irritation and damage to the nasal mucosa at the concentrations required to effectively promote nasal absorption [1].

SFN with an appropriate hydrophilic/hydrophobic balance can produce a transient increase in the mucosa permeability to a wide variety of both peptide and proteins [12, 30]. Earlier studies have shown that SFN can act as a protease inhibitor and an absorption enhancer [12, 30]. SFN serves a dual role, to both inhibit protease activity in the nasal cavity and act as an absorption enhancer. This remarkable duality of actions provides an important and unique role for SFN in the development of an effective, stable nasal INS formulation.

In this study, despite the nearly absolute impermeability of the nasal passage to INS under basal conditions, there is a remarkable increase in INS absorption when SFN is added to the formulation. The INS formulated with SFN for intranasal administration is relatively well absorbed. This is illustrated by an increase in serum INS levels and concomitant suppression of plasma glucose levels. Both INS data ($C_{\text{max}} = 45.47 \, \mu \text{IU/ml}$ and $\text{AUC} = 118.79 \, \mu \text{IU h/ml}$) and glucose data (% change of initial) support a trend toward better absorption of INS after intranasal administration in the presence of SFN. As a result, the intranasal administration of INS in the presence of SFN resulted in 8.55% relative bioavailability when compared to the subcutaneous administration. In many studies, the bioavailability was calculated with a high value according to the detected INS concentration in the blood [18–21]. From the Fig. 3b, we found that the blood INS

![Fig. 5 Intranasal administration with INS plus SFN showing hypoglycemic effects for potential practical use. Diabetic mice with access to food and water were intranasally administered with INS (20 IU/kg) plus SFN (1.6 mg/kg) once a day for 7 days; then, the hypoglycemic effects were measured from day 1 to day 7. The results show that intranasal administration with INS plus SFN could control the blood glucose to normal levels for 7 days. s.c. subcutaneous administration of INS, NS normal saline solution. Values are presented as means ± SD ($n = 8$)](image)

![Fig. 6 Micrographs showing the intro tissue of mouse nasal mucosa. a: Diabetic mice intranasally administered with INS. b: Diabetic mice intranasally administered with SFN. c: Diabetic mice intranasally administered with INS plus SFN. The intro tissue of nasal mucosa was indicated by arrows. Magnification, ×40)](image)
concentration of intranasal group was much higher than the s.c. injection group in most of the time points. However, the hypoglycemic effects were similar between these two groups. It is possible that SFN may cause irreversible denaturation of INS and the denatured INS also contributes to the ELISA measurements. The method used to detect INS may also contribute to the discrepancy. Generally, ELISA was used to detect INS by many researchers, but the antibody for reacting with INS could recognize the intact INS as well as the digested INS fragments if they contain the epitope that can be recognized by the antibody. Therefore, some digested INS fragments without hypoglycemic activity were also detected by ELISA and used for calculation of bioavailability. Accordingly, the calculated bioavailability was a little higher than the effective bioavailability. As for the experimental design, there are concerns about the subcutaneous and nasal delivery of human INS into mice. Since there is a four amino acid difference between human INS and mice INS, it is possible that this could cause immune rejection in mice. However, researchers found that human INS secreted from insulinogenic xenograft restores normoglycemia in type 1 diabetic mice without immunosuppression [31]. That means that human INS could be used in diabetic mice without immune rejection. The profile of plasma glucose levels obtained after intranasal application of the formulation (20 IU/kg INS plus 1.6 mg/kg SFN) was similar to the subcutaneous profile of 1 IU/kg INS. A dose of intranasal INS plus SFN could maintain the blood glucose at a relatively low level for about 2 h in diabetic mice. INS alone could barely reduce blood glucose after intranasal treatment. The results of this study clearly showed that the hypoglycemic effects in the absence of SFN were only maintained for 1 h, much shorter than those in the presence of SFN. Moreover, about 91.13% of diabetic mice responded to the nasal administration with INS plus SFN, while only 22.22% responded to the nasal INS alone. This result clearly showed that SFN, as a biosurfactant, can improve the absorption of INS by the nasal mucosa.

In this study, the high dose of INS (30 IU/kg) did not show higher hypoglycemic effects than the low dose of 20 IU/kg in the formulation (Fig. 2a). This is also observed in the formulation of INS plus SFN for oral administration [12]. We deduced that INS could efficiently bind to SFN at an appropriate ratio. If too many INS molecules were used, some of them could not be bound with SFN. Therefore, these INS molecules cannot be efficiently absorbed by the nasal mucosa. As a result, the 30 IU/kg of INS did not show higher hypoglycemic effects than the 20 IU/kg dose.

In the glucose tolerance test, the results showed that SFN plus INS could control the blood glucose to normal levels, indicating that intranasal INS formulated with SFN is useful in the control of glucose levels in the face of the continuous influx of glucose and possibly other carbohydrates. In a constant delivery assay, intranasal INS formulated with SFN could control the blood glucose levels for a short term. Furthermore, in this study, the intranasal INS was administered in the form of liquid nose drops, applied by pipettes. It is anticipated that intranasal delivery of INS formulations using a spray device, which instills small droplets of the solution throughout the nasal cavity in human, will increase the surface area contacted by INS and increase the overall amount of INS absorbed.

A flaw of intranasal administration is that it may possibly induce injury of the nasal mucosa [1]. In this study, we intranasally administered diabetic mice with SFN or SFN plus INS for 7 days and found no apparent side effects resulting from the intranasal treatment, indicating that INS formulated with SFN might be safe for intranasal administration for controlling the blood glucose levels in diabetic patients. SFN was previously reported to be safe to be used in the animal model [11]. It has low toxicity and shows non-toxicological effects at the dose of 2500 mg/kg after single oral administration [11]. Moreover, it has not been reported that SFN formulated with INS in the nasal administration is toxic or causes serious side effects or immune response in the long term. Based on previous experiments in our lab, our results indicate that oral SFN might be a novel therapeutic approach for the treatment of type 1 diabetes mellitus [30]. However, it remains unclear whether there is any immunoadjuvant and immunosuppressive effect when treated with SFN through nasal delivery.

Conclusions

SFN is very effective for improving the absorption of INS by the nasal mucosa without apparent side effects, which can notably decrease the blood glucose levels in diabetic mice.

Author contribution  G Qi designed the study. Q Yu, S Dong, and X Xing executed the experimental work. D Yang analyzed the data. X Zhao contributed reagents and materials. Q Yu and G Qi wrote and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Human and animal rights  All applicable University Ethics Committee’s guidelines for the care and use of animals were followed.

This article does not contain any studies with human participants performed by any of the authors.
References

SNP in microRNA sequences or binding sites of miRNAs: association with type 2 diabetes mellitus susceptibility and in silico analysis

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Abstract Type 2 diabetes mellitus (T2DM) is characterized with defects in insulin secretion, increased hepatic glucose production, and resistance to the action of insulin. The Iranian population is genetically predisposed to T2DM similar to other Asian populations. Therefore, it is imperative to search the genetic variations in T2DM susceptibility unique to the Iranian population. Three hundred patients with type 2 diabetes mellitus and 300 healthy subjects were selected. Peripheral venous blood samples were collected from all subjects. SNPs of mir-605 and the 3′-UTR of ESR1 gene were genotyped by PCR-RFLP. Also, in silico analysis was performed to evaluate potential effects of two SNPs. A significant association was found between all genotypes of ESR1 and T2DM. The data analysis identified an increased risk of T2DM associated with rs9341070 CT genotype among case subjects in relation to control subjects (p value = 0.005; OR = 2.05; 95% CI = 1.24–3.28). The rs2043556 genotypes in mir-605 were significantly correlated with T2DM and there were correlations between GG genotypes of mir-605 rs2043556 with decreased risk for T2DM (p value = 0.01; OR = 0.38; 95% CI = 0.18–0.80). The rs9341070 CT acted as a non-protective factor to increase the risk of T2DM, while the rs2043556 GG genotype is identified as protective factor in diabetes mellitus type 2. According to bioinformatics analysis, it was predicted that rs9341070 in 3′-UTR of ESR1 gene and rs2043556 in mir-605 possibly participate in the regulation of mature miRNA and mRNA targets.

Keywords mir-605 · 3′-UTR of ESR1 · Type 2 diabetes mellitus (T2DM) · Binding site of microRNA

Introduction

Type 2 diabetes mellitus (T2DM), a disease with a rising prevalence worldwide, is a complex metabolic condition with high blood glucose level [1]. It is currently estimated that the prevalence of type 2 diabetes mellitus is 5.5%, and the prevalence in males and females is 5.1 and 5.8%, respectively in Iranian population [2]. Age, gender, obesity, race, and family history are known as the risk factors involved in the disease [3]. It is widely demonstrated that both genetic susceptibility and environment exposure contribute to type 2 diabetes mellitus, and a lot of SNPs are associated with T2DM [4]. Recently, it has been reported that the functional SNPs located in microRNA (miRNA) sequences or binding sites of miRNAs (3-UTR of miRNA target mRNAs) were associated with susceptibility to type 2 diabetes mellitus [5–7].

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression post-transcriptionally. They bind to their site in 3′-untranslated region of target mRNA, leading to the mRNA degradation or the suppression of translation. miRNAs regulate various biological processes, such as tumorigenesis, proliferation, apoptosis, and metabolism [8]. Each miRNA acts on hundred different target sites, and all human miRNAs together may regulate, at least, 30% of protein coding genes [9]. The functional SNPs located in miRNA sequences can affect miRNA function through change in the processing and binding ability of mature
miRNAs. In addition to SNP of microRNA sequence, SNPs in 3′-UTR of genes also can alter the binding site of target microRNA [7]. It was identified that SNP rs9341070 in 3′-UTR of ESR1 gene was associated with diabetes mellitus type 2 in African population [10]. Also, several studies have investigated the correlation between mir-605 and P53 in the pathogenesis of diabetic nephropathy and also, it is determined that P53 protein is involved in pancreatic cells-related apoptosis [11–13]. Hence, the current study aimed to evaluate the association between polymorphism of mir-605 sequence (rs2043556) and polymorphism in the 3′-UTR of ESR1 gene (rs9341070) and diabetes mellitus type 2 risk in 300 cases and 300 controls in southeast Iranian population.

Methods and materials

Sample collection

In this case-control study, 300 diabetic type 2 patients and 300 healthy subjects from the diabetic care clinics and the routine laboratory of Ali Asghar pediatric hospital in Zahedan, Iran were selected. The institutional ethics committee approved the study procedure; written informed consent was obtained from all participants. Patients with T2DM were diagnosed with clinical criteria and then were confirmed in accordance with the criteria of American Diabetes Association [14]. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article. T2DM was determined by a fasting plasma glucose level of more than 126 mg/dl after a minimum fast of 12 h or a hemoglobin A1c (HbA1c) level of > 7%. Control individuals were diagnosed by fasting plasma glucose of < 100 mg/dl or an HbA1c level of < 6%. Factors including age, sex, gender, and body mass index (BMI) were recorded. Fasting blood was examined for biochemical parameters including total cholesterol, triglyceride, HDL, LDL, plasma glucose, and HbA1c values using the manual method.

Genotyping

A total of 2 ml of venous blood was collected from each patient in EDTA coated tubes and stored at −80 °C. DNA was extracted from the peripheral blood sample using routine salting out procedure.

Two SNPs were determined using PCR-RFLPs method: the variant of 3′-UTR in the estrogen receptor 1 (rs9341070) described as banding site of miR-206 and SNP of mir-605 (rs2043556). The oligonucleotides and the restriction enzymes used for the PCR-RFLPs are shown in Table 1. DNA amplification was carried out in a final volume of 20 μl, using 1 μl of genomic DNA and 1 nmol of the primers and 10 μl of 2x master mix PCR. Annealing, extension, and denaturing were carried out using an automatic thermal cycler (DNA Engine®, Bio-RAD, Japan). The amplified products were digested at 37 °C for 16 h using 20 μl of each PCR-amplified product with 1 unit of the restriction enzyme. The amplification parameters for the rs9341070 comprised an initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 30s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 5 min. Amplification for the mir-605 (rs2043556) SNP was identical, except that annealing was carried out at 61 °C. The digested products were loaded into 2% agarose gel containing ethidium bromide for electrophoresis.

Statistical analysis

Allelic and genotypes frequencies in the case and control groups were compared with Hardy-Weinberg expectations using χ2 analysis. Contribution of BMI, age, gender, LDL, HDL, FBS, and HbA1c was also evaluated by independent Student’ s t test or Fisher’s exact test whenever appropriate. To determine the effect of polymorphisms on the risk of type 2 diabetes mellitus, Logistic regression analysis was used. For significant allelic and genotypic associations, odds ratios (OR) and 95% confidence interval (CI) were considered.

In silico analysis

In silico analysis was performed to evaluate potential biological functions of two SNPs, located in mir-605 sequence (rs2043556) and SNP (rs9341070) in the 3′-UTR of ESR1 gene. Nucleotide sequence of the mir-605 and the 3′-UTR of ESR1 genes with accession no. M10003618 and NM_001122740 were deduced from miRBase database and National Center for Biotechnology Information (NCBI) data bank. MicroSNiPer database was used for prediction of SNP rs9341070 effects in 3′-UTR of ESR1 gene on putative microRNA targets [15]. The functional effect of rs2043556 SNP on mir-605 functions was evaluated by miRmut2GO tool of miR2GO server. PolymiRTS database was used to predict the effect of SNPs in the ESR1 mRNA 3′-UTR on mRNA–microRNA interaction [16]. To explore the effect of SNPs on the transcriptional regulation of microRNA genes, dPORE–miRNA database (Dragon database on Polymorphic Regulation of human miRNA Genes) was used [17].

Results

Demographic characteristics

Table 1 shows the clinical and demographic characteristics of the case and control groups. From 300 patients with type 2 diabetes mellitus, 79 (47.3%) were males and 221 (51%) were
females. From 300 healthy controls, 88 (52.7%) were males and 212 (49%) were females. There was no significant difference in gender between the case and control groups (p > 0.05). In contrast, there were obvious significant differences regarding BMI, HbA1c, TG, cholesterol, FBS, LDL, and HDL (p < 0.0001) (Table 2).

Associations between alleles and genotypes of rs9341070 and rs2043556 and type 2 diabetes mellitus

The results showed significant association between all genotypes of ESR1 and T2DM risk (p value < 0.05). The data analysis identified an increased risk of T2DM associated with rs9341070 CT genotype among case subjects related to control subjects (p value = 0.005; OR = 2.05; 95% CI = 1.24–3.28). Also, rs2043556 in miR-605 was significantly correlated with T2DM, and there were correlations between GG genotypes of mir-605 rs2043556 with the risk for T2DM (p value = 0.01; OR = 0.38; 95% CI = 0.18–0.80). The two SNPs had significant associations with T2DM in allelic frequency (Table 3).

In silico analyses to predict the effect of ESR 3′-UTR SNPs in the miRNA target sites

To predict the effect of ESR 3′-UTR SNPs on the miRNA target sites, the algorithm of PolymiRTS database was applied. The algorithm of PolymiRTS database predicted that one miRNA target site was destroyed by C variant and one new miRNA target site was created by T variant (Table 4).

The assessment of the effect of SNPs on the transcriptional regulation of miRNA genes

To explore the effect of SNPs on the transcriptional regulation of miRNA genes, dPORE–miRNA database was utilized. The output of dPORE–miRNA database revealed that the SNP of mir-605 sequence was due to the loss of the transcription factor binding sites (TFBS) (Table 5).

Table 1 Primers Sequences for rs9341070 and rs2043556 polymorphisms

<table>
<thead>
<tr>
<th>Primer 5′ - 3′</th>
<th>Product</th>
<th>Method</th>
<th>Allele (bp)</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9341070 F:CCCACACGGTTCAAGATAATC</td>
<td>252</td>
<td>RFLP</td>
<td>C:83, 169, A:252</td>
<td>HpaII</td>
</tr>
<tr>
<td>R:ATCCCTGGCAATTAGTACATAG</td>
<td>rs2043556 F:GAAAGAATAGTACCAGGAAATG</td>
<td>369</td>
<td>RFLP</td>
<td>A:26, 93, 166, 84, G:26, 93, 250</td>
</tr>
<tr>
<td>R: TATGTCTCTAGCCCTAGCTTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The assessment of the functional effect of rs2043556 SNP on mir-605 functions

The functional effect of rs2043556 SNP on mir-605 functions is evaluated by miRmut2GO tool of miR2GO server. The output of miR2GO sever predicted low similarity score for biological process between the reference and derived alleles of mir-605 (Table 6) and (Fig. 1).

Prediction of of rs9341070 SNP effects on putative microRNA targets

MicroSNiPer database was used for prediction of SNP effects in 3′-UTR of ESR1 gene on putative microRNA targets, and output of the software predicted that SNP within the target site could change the microRNA binding site (Table 7).

Association analysis of SNPs: rs2043556 and rs9341070 with other biochemical parameters

We stratified the association analysis of SNPs: rs2043556 and rs9341070 with BMI < and > 25 kg/m² because BMI > 25 kg/m² is defined as overweight. We compared the T2DM risk in subjects who were overweight and those who were underweight. Data showed that all genotypes are not associated with T2DM with BMI < 25 kg/m² and BMI > 25 kg/m². Also, to...
predict the association of these polymorphisms with phenotypes, such as fasting plasma glucose levels, total cholesterol, triglyceride, HDL, and LDL, comparison analyses among T2DM patients carrying three different genotypes GG, GA, and AA were conducted. No statistically significant difference of phenotypes among the carriers was observed (data not shown). Following logistic regression analysis by adjusting BMI was performed to exclude false positives (Table 8).

**Discussion**

The present study assessed the association between rs9341070 alleles and genotypes with T2DM susceptibility. Our result showed that rs9341070 in 3′-UTR of ESR1 gene was significantly associated with T2DM susceptibility. The genotype CT of rs9341070 in 3′-UTR of ESR1 gene was associated with an increased risk of T2DM.

Estrogen receptor-α, encoded by the ESR1 gene on chromosome 6q24, acts as a transcription factor although no transcriptional effects have been identified [18]. Estrogen receptors (ESR) are as important as molecules involved in the adaptation of beta cells to insulin resistance [19, 20]. In addition to the insulin secretory dysfunction and the decreased beta-cell mass is marked in the onset of type 2 diabetes mellitus [21]. Therefore, the alterations of estrogen receptor affect on insulin resistance, which subsequently affect the T2DM risk. Several studies have been performed to investigate the relationship between ESR polymorphisms and the diabetes risk including type 2 diabetes mellitus [5, 7, 22, 23]. Some GWAS (genome-wide association study) studies for susceptibility to T2DM indicated the evidence of linkage (LOD = 2.26) to 6q24, containing the gene encoding estrogen receptor-α (ESR1) [22]. Similarly, In 2008, Keene et al. identified a novel C > T transition within 3′-UTR of ESR1 associated with type 2 diabetes and/or nephropathy in an African-American population [10]. Although there are several reports about the possible link between ESR polymorphism and T2DM, the published report on the association between this polymorphism and T2DM in Iranian population is limited.

Moreover, the present study assessed the association between rs2043556 alleles and genotypes with T2DM

### Table 3  Genotype and allele frequency of mir-605 and ESR1

<table>
<thead>
<tr>
<th></th>
<th>Diabetes mellitus (DM)</th>
<th>Control</th>
<th>p value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3′-UTR of ESR1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC, n (%)</td>
<td>239 (79.7)</td>
<td>265 (88.3)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CT, n (%)</td>
<td>50 (16.7)</td>
<td>27 (9)</td>
<td>0.005</td>
<td>2.05 (1.24–3.28)</td>
</tr>
<tr>
<td>TT, n (%)</td>
<td>11 (3.6)</td>
<td>8 (2.7)</td>
<td>0.37</td>
<td>1.5 (0.6–3.8)</td>
</tr>
<tr>
<td>CT + TT, n (%)</td>
<td>61 (20.3)</td>
<td>35 (11.7)</td>
<td>0.005</td>
<td>0.5 (0.3–0.8)</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, n (%)</td>
<td>528 (88)</td>
<td>557 (93)</td>
<td>0.005</td>
<td>1.76 (1.18–2.6)</td>
</tr>
<tr>
<td>T, n (%)</td>
<td>72 (12)</td>
<td>43 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>mir-605</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA, n (%)</td>
<td>263 (87.7)</td>
<td>242 (80.7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AG, n (%)</td>
<td>26 (8.6)</td>
<td>32 (10.7)</td>
<td>0.29</td>
<td>0.74 (0.43–1.32)</td>
</tr>
<tr>
<td>GG, n (%)</td>
<td>11 (3.7)</td>
<td>26 (8.6)</td>
<td>0.01</td>
<td>0.38 (0.18–0.80)</td>
</tr>
<tr>
<td>AG + GG, n (%)</td>
<td>37 (12.3)</td>
<td>58 (19.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, n (%)</td>
<td>552 (92)</td>
<td>516 (86)</td>
<td>0.001</td>
<td>1.87 (1.2–2.7)</td>
</tr>
<tr>
<td>G, n (%)</td>
<td>48 (8)</td>
<td>84 (14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4  ESR 3′-UTR SNP predicted to destroy or create a miRNA target site

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Wobble base pair</th>
<th>Ancestral allele</th>
<th>Allele</th>
<th>miR ID</th>
<th>MiRSite</th>
<th>Function class</th>
<th>Exp support</th>
<th>Context + score change</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9341070</td>
<td>N^d</td>
<td>C</td>
<td>C</td>
<td>hsa-miR-1225p</td>
<td>A(_{\text{ABC}})C_ggcatg</td>
<td>D(_{\text{ABC}})</td>
<td>N^a</td>
<td>−0.178</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>hsa-miR-759</td>
<td>A(_{\text{ABC}})C_ggcatg</td>
<td>A(_{\text{ABC}})C_ggcatg</td>
<td>C(_{\text{ABC}})</td>
<td>N</td>
<td>−0.182</td>
</tr>
</tbody>
</table>

^a Predicted target site with no experimental support
^b The derived allele disrupts a conserved miRNA site (ancestral allele with support ≥ 2)
^c The derived allele creates a new miRNA site
^d The SNP cannot form a G:U wobble basepair with the miRNA
susceptibility; however, genotype GG of rs2043556 in mir-605 was less frequent in the patients with T2DM than in the controls, and this genotype could decrease T2DM risk. miR-605 is an important microRNA in the p53 gene network, which directly decreased the Mdm2 expression at the post-transcriptional level and upregulated via interaction between the p53 and the promoter region of the mir-605 gene [12]. Similarly, it is proved that there is a link between DNA double-strand breaks and p53 activity in β cells in type 2 diabetes mellitus [24]. Previous studies showed that functional polymorphisms in mir-605 sequence may affect its functions [5, 7, 22]. In 2009, Duan et al. performed a genome-wide association study for identification of SNPs and CNVs with potential effects on microRNA sequence using data mining public resources and they identified 187 SNPs with potential effects on processing and structure of microRNA in the pre-miRNAs sequence, including the SNP (rs2043556) of mir-605 sequence [25]. There are limited studies about the probable effect of rs2043556 polymorphism on lung cancer and breast cancer and other multifactorial diseases [26–28]; however, there is no published report about the association between A > G transition in mir-605 gene and T2DM susceptibility.

The SNPs located in regulatory regions may alter expression and translation level of genes, which highlighted a new paradigm for genetics [29, 30]. Some studies reported that SNPs in 3′-UTR of genes are deleterious for the binding aspects of microRNAs in which they occur [31]. The effect of C > T transition in 3′-UTR of ESR1 gene on expression of reporter gene were assessed in previous studies [32]. In current study, we evaluated the effect of SNP on the binding sites of microRNAs via bioinformatics tools. Our bioinformatics results showed that rs9341070 SNP has significant effect on the binding sites of several miRNA (Table 6). Our data from different bioinformatics tools revealed that C > T variation changed the binding sites of targeted microRNAs (Table 4). The functional SNPs located in miRNA sequences can affect miRNA

**Table 5** The effect of SNP on the transcriptional regulation of miRNA gene

<table>
<thead>
<tr>
<th>SNP</th>
<th>SNP class</th>
<th>UCSC reference</th>
<th>Absolute position on chromosome</th>
<th>Effect (s) on TFBS (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2043556</td>
<td>Single</td>
<td>T</td>
<td>52729412</td>
<td>Loss*</td>
</tr>
</tbody>
</table>

*The introduced change in the DNA sequence due to the observed state of the SNP causes the loss of TFBS

**Table 6** The functional effect of rs2043556 SNP on mir-605 functions

<table>
<thead>
<tr>
<th>miRNA ID</th>
<th>Sequence</th>
<th>Biological process similarity score</th>
<th>Molecular function similarity score</th>
<th>Cellular component similarity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-605</td>
<td>CUCCUUGGGAAAAACAGAGAAGGCACUAUGAGAUUAGA[U/C]CAAGUUAGG</td>
<td>0.174a</td>
<td>NAb</td>
<td>NAb</td>
</tr>
</tbody>
</table>

*a Similarity score: a score (ranging from 0 to 1) for the semantic similarity between the enriched GO terms associated with target gene sets for the reference and derived alleles. A score close to 1 indicates high similarity. A score close to 0 indicates low similarity

b Undetermined

![Gene ontology figure for biological process](image)
function through change in the processing and binding ability of mature miRNAs [33]. Because of the rs2043556 polymorphism, was located in mir-605 gene, a novel in silico analysis performed to predict the effects of this variation on the transcriptional regulation of mir-605 [17] as well as, a novel in silico analysis was performed to evaluate effect of the variation on mir-605 function using bioinformatics tools [16]. Bioinformatics analysis showed that existence or absence of this polymorphism in mir-605 gene would lead to the loss of the transcription factor binding sites (Table 5). Moreover, they illustrated the low similarity score for biological process between the reference and derived alleles of mir-605 (Table 6 and Fig. 1). So, it is speculated that the genetic variation in miR-605 sequence may change the processing and the binding ability of mature miRNA.

Finally, to predict the association of SNPs: rs2043556 and rs9341070 with phenotypes, such as BMI, fasting plasma glucose levels, total cholesterol, triglyceride, HDL, and LDL, comparison analyses among T2DM patients carrying three different genotypes were conducted. No statistically significant difference of phenotypes among the carriers was observed. So, our results did not show significant association between lipid profile and T2DM susceptibility.

Although sample size was relatively sufficient, the present study had several limitations including different ethnic groups living in southeast of Iran, different environmental conditions.

### Conclusion

In conclusion, the findings indicated that rs9341070 in 3′-UTR of ESR1 gene was significantly associated with T2DM risk and the genotype CT of rs9341070 in 3′-UTR of ESR1 gene was associated with an increased risk of T2DM. Moreover, the present study assessed the association between rs2043556 alleles and genotypes with T2DM susceptibility; however, genotype GG of rs2043556 in mir-605 was less frequent in the patients with T2DM than in the controls, and this genotype could decrease T2DM risk.

### Acknowledgments

We thank the technical directors of the routine laboratory and the diabetes clinics of Ali Asghar pediatric hospital who provided the patients and their medical information and also the healthy controls.

### Funding Information

This study was funded by Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

### Compliance with ethical standards

**Informed consent**

Informed Consent Form to Participate in Research Projects

**Subject of research project**

Diabetes is a multi-factorial disease that the genetic and environmental factors have the impact on it. The blood is used as an affordable diagnostic tool to investigate polymorphism in patients with diabetes.

**Name of executor/executors**

Mahdiyeh Moudi

**Faculty or unit**

Diabetes Clinic of Ali Asghar Hospital

**Introducing the research**

Diabetes is a multi-factorial disease that the genetic and environmental factors have the impact on it. The blood is used as an affordable diagnostic tool to investigate polymorphism in patients with diabetes.

**Bloodletting**

Yes

**Advantages**

Providing the blood is an easy and affordable way and the investigation of polymorphism can be screened for diabetes patients

**Side effects**

No

**Compensation of possible effects**

None

**Costs related to the research project**

No cost for the patient

**Alternative methods**

None

**Confidentiality**

All patient information remains confidential

---

**Table 8**  Genotype distributions and allele frequencies in BMI > 25 and BMI < 25 groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BMI</th>
<th>No</th>
<th>Yes</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>144 (48%)</td>
<td>67 (22.4%)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; 25</td>
<td>156 (52%)</td>
<td>232 (77.5%)</td>
<td>3.20 (2.24–4.55)</td>
<td>3.33 (2.32–4.78)*</td>
<td></td>
</tr>
<tr>
<td>Genotype ES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>265 (88.3%)</td>
<td>239 (79.7%)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>27 (9)</td>
<td>50 (16.7%)</td>
<td>2.05 (1.24–3.28)*</td>
<td>2.15 (1.26–3.66)*</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>8 (2.7)</td>
<td>11 (3.6%)</td>
<td>1.5 (0.6–3.8)</td>
<td>1.86 (0.38–9.22)</td>
<td></td>
</tr>
<tr>
<td>Genotype cGMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>242 (80.7%)</td>
<td>263 (87.6%)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>32 (10.7)</td>
<td>26 (8.6%)</td>
<td>0.74 (0.43–1.32)</td>
<td>0.75 (0.42–1.33)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>26 (8.6)</td>
<td>11 (3.7%)</td>
<td>0.38 (0.18–0.80)</td>
<td>0.34 (0.16–0.73)*</td>
<td></td>
</tr>
</tbody>
</table>

*P value < 0.05
Respond to questions Dr. Mostafa Montazer Zohour, address of Zahedan University-Genetics faculty, Tel: +98 54-33295789

The right of refusal or withdrawal My participation in this study is completely optional and I am free not to participate in this study and I can exit from the study at any time without any change in physician behavior or therapist or the treatment way or disease care

Satisfaction (it has been achieved when the patient is in full consciousness and situations without stress and pain and if the patient is in emergency situations, the satisfaction is obtained from his legal guardian) According to information in this form and the verbal explanations of the executers or staffs in this project, I agree to participate in this study. I was given a copy of the form and I have the opportunity to read it.

Name of surname of the patient/healthy volunteer (or the legal guardian) and signature:
Date: 2016/15/10
Signature of the patient/healthy volunteer (or the legal guardian)

I as the executer/executers of the research project, Dr. Miss/Mr. Mahdiyeh Moudi, be aware of all provisions of the human subject protection codes in the researches in medical sciences with 26 paragraphs. I recommend the mental retardation or the patients with permanent or recurrent changes in consciousness and situations, the satisfaction is obtained from his legal guardian)

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

References


Patterns of finger sepsis and predictors of its outcome in patients with diabetic septic hand

Mugahid A. Salih1 · Shadad M. Mahmoud1 · Mohamed E. Ahmed1

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Abstract The objectives of this study were to determine the different patterns of finger sepsis among diabetic patients, to know its different outcomes, and to detect the predictors of poor functional outcome (using the Sollerman hand function test). One hundred diabetic patients with finger sepsis were assessed using a prospective analytical hospital-based study, conducted in Khartoum Teaching Hospital and Jabir Abu Eliz Diabetic Centre (JADC) from October 2015 to October 2016. The most common pattern of finger sepsis was a superficial abscess (27.9%) followed by necrosis (27.1%), gangrene (14%), ulcers (11.5%), and cellulitis (6.3%). Finger sepsis spread to involve the hand in 39 patients (39%). Eighteen patients (18%) had an amputation of one or more fingers, and four patients (4%) had an amputation of the whole hand. Complete healing was the outcome in 82 (82%) patients. Twelve patients (12%) required reconstructive surgery and grafting. Forty-two patients (42%) presented with late complications such as stiffness, contractures, and deformities. Thirty-three patients (33%) had a Sollerman functional score below 80%. Poor control of diabetes, presence of neuropathy, low educational level, delayed presentation to hospital, and severe necrosis requiring surgical reconstruction were significant predictors of poor functional results of the hand (p value < 0.05). The etiology of sepsis, use of self-monitoring of blood glucose devices, duration of diabetes, positive family history, and presence of comorbidities were not related significantly to the functional outcome (p value > 0.05). The loss of more than one finger or the loss of the thumb resulted markedly in reduced hand function, compared to the loss of the middle or ring finger. Stiffness was an influencing factor in reducing hand function. It developed from severe tissue damage and prolonged periods of immobilization in a dressing which covered the whole hand. Finger sepsis among diabetics has different patterns and different outcomes. Better control of diabetes, early presentation to diabetic centers, and proper management of hand wounds will be in favor of good functional outcomes.

Keywords Fingers · Diabetics · Sepsis · Pattern · Outcome · Sollerman hand function test

Introduction and background

Diabetes mellitus is a chronic systemic disease with lots of morbidities and mortalities [1]. One of the most common complications is wound infection [1]. Hand sepsis particularly is not uncommon among diabetic patients; however, there are few reports in the literature [2], first described in the USA in 1977 and in Nigeria in 1984 [3]. In 1999, western countries pointed that the incidence of diabetes in patients who require hospital admission for significant hand infection was more than 10% [1]. More figures were reported from Africa and tropical countries [4]. Some local studies regarding hand sepsis were likewise reported in Sudan [5, 6]. Incidence of finger sepsis is more common in patients with diabetes mellitus than in non-diabetic peers. Authors revealed higher incidence of finger abscess, cellulitis, paronychia, and tenosynovitis among diabetic patients [7–10]. Many studies and case reports in the literature demonstrated that initial finger infection can be the provoking factor for more serious hand infections in diabetic patients [11–13]. Severe consequences of these infections may occur due to destruction of the tendon sheath,
scarring, ischemic changes, and gangrene of the fingers [14–17]; thus, improper response from the patient and delayed treatment can lead to finger amputation and long-term functional disability [18]. On the other hand, it can also progress to involve the whole hand and forearm with serious outcomes.

Inquiries about hand function in disabled people due to sepsis, trauma, surgery, and arthritic and neurological disorders highlighted the need for standard hand function tests to evaluate the effectiveness of treatment and in research [19]. The ideal hand function test must be valid in terms of broad general hand function used in daily activities. It must be quick to administer and easy to score, with readily available test materials. The Sollerman hand function test is a standardized hand function test based on seven of the eight most common handgrips. The test consists of 20 subtests, each comprising a task considered to be an activity of daily living, the performance of which could be easily scored [20].

Awareness of the predictors of progression of finger sepsis in diabetics, to involve the whole hand and/or to transform into gangrene, is crucial for early surgical and antimicrobial control, thus preventing poor functional outcomes. Since there is no enough data regarding finger sepsis among Sudanese diabetic patients and the subsequent disability, this study investigated the pattern and outcome of finger sepsis in patients with diabetes mellitus presenting to Khartoum Teaching Hospital (KTH) and Jabir Abu Eliz Diabetic Centre (JADC). Results could be compared with studies in other parts of the world and may be useful to the general population and health workers.

Patients and methods

This is a prospective analytical hospital-based study conducted in Khartoum Teaching Hospital and Jabir Abu Eliz Diabetic Centre (JADC) in the period from October 2015 to October 2016. It included 100 diabetic patients who presented with finger sepsis to the study areas. Informed consent was signed by the patients after being told the objectives of the study. Privacy was ensured, and they were able to stop their participation at any time. One close-ended, pretested, coded questionnaire was used to collect the data. The questions focused on determination of the level of patient’s education, the affected hand, the presence of pain, swelling, fever, the cause of finger sepsis with special concern regarding the history of trauma, and the type of trauma including self-monitoring of blood glucose (SMBG). Also, any delay of presentation to the hospital, the duration of the delay, the cause of the delay, the use of traditional therapy at home, and the progression of finger sepsis to involve the hand were included. Other questions included the past medical history and the control of diabetes. The hands were then examined to determine the involved finger(s), the pattern of finger sepsis (cellulitis, abscess, felon, paronychia, tenosynovitis, gangrene), the proximal involvement of the hand or forearm, the presence of intact sensations, and any amputated finger or hands. Data on glycemic control and patient’s surgical management were collected from their records. Follow-up was done to note the following: the spontaneous healing of wounds, the duration of healing, any further operations done to the patient like reconstructive surgery (graft/flare), any physiotherapy sessions received by the patient, and any complications, e.g., stiffness, contractures, deformities, or death.

Following complete recovery, another interview with the patients was arranged to evaluate hand function using the objective standardized Sollerman hand function test. Table 1 shows the guidelines for scoring of the subtests, and Table 2 shows the 20 subtests comprising the Sollerman grip function test [21]. Results of the test were calculated as percentages out of 80 in the dominant hand and out of 79 in the non-dominant hand (according to the guidelines of the test, these figures represent the total score of the test and are equivalent to 100%). The data collected was analyzed using the Statistical Package for the Social Sciences (SPSS) computer software and using both descriptive and analytical statistical methods. Poor hand function was defined as Sollerman score below 80%. The predictors of hand function were considered significant if the p value was < 0.05.

Results

The age of the patients ranged between 14 and 86 years with a mean age of 49 ± 15.314 years SD. Forty-four (44%) were males, and fifty-six (56%) were females with a male-to-female ratio of 1:1.27. Thirty-nine patients were housewives (70% of the affected females). The right hand was affected more than the left hand. Pain was the main complaint in 95% of the patients, and swelling was present in almost all the patients (99%). The first presentation for most of the patients (35%) was to diabetic centers, followed by local health centers in 33%, peripheral (rural) hospitals in 31%, and a pharmacy in 1%. In 50% of the cases, finger sepsis developed spontaneously (without obvious cause). This is in contrast to 42% of the patients who admitted trauma as an etiology. Only 15 patients (15%) acknowledged the use of SMBG devices regularly, but it was not the cause of sepsis in all of them. Delay of presentation to doctors occurred in 71 patients (71%); thus, only 29 patients (29%) presented immediately to a diabetic center or a hospital. The duration of the delay ranged from 2 days up to 1 month with a mean of 8.92 ± 5.6 days SD. 78.9% of the delayed patients spent more than 5 days at home before they searched for a medical advice. Most of them (66.2%) preferred to use traditional therapy at home, and all of them had proximal spread of infection from the fingers to involve the hand (p < 0.05). The low level of education was
related significantly to the delay of presentation ($p = 0.01$) (Table 3).

The most commonly encountered pattern of finger sepsis was superficial abscess (27.9%), followed by necrosis (27.1%), frank gangrene (14%), and ulcers (11.5%). The less common patterns included paronychia (7.2%), cellulitis (6.3%), felon (2%), tenosynovitis (2%), and osteomyelitis (2%). The most commonly involved digit was the middle finger (31.6%) followed by the index finger (21.4%) and the ring finger (20.5%). The thumb and little finger were affected in 14.5 and 12%, respectively. Figure 1 shows the different patterns of finger sepsis. Eighteen patients (18%) had amputation of one or more fingers. Out of them, two patients (11.1%) lost their thumb, four patients (22.2%) lost the index, and another four (22.2%) lost the middle finger. One patient (5.5%) has lost his ring finger while three (16.6%) lost the little finger. Four patients (22.2%) lost more than one finger. Another four (4%) had amputation of the whole hand due to severe sepsis and necrosis. The decision of amputation was made by consultants. The association between finger amputation and high levels of HBA1C (above 8) was statistically non-significant ($p = 0.28$). Complete healing was the outcome in 82 patients (82%). Healing duration ranged from 1 to 5 months with a mean duration of 1.87 ± 1.482 months SD. The outcome in the remaining patients was reconstructive surgery (grafting) in 12 patients (12%), amputation of the whole hand in 4 patients (4%), and death before complete healing in 2 patients (2%). The cause of death was not related to hand sepsis; rather, it was related to the associated comorbidities. Following complete healing and cessation of dressing, 42 patients suffered from complications, which included stiffness in 12 patients (12%), contractures in 8 patients (8%), and deformities and disability of the finger(s) or hand loss in 22 patients (22%). Figure 2 shows some of the patients who suffered from painful contractures of the fingers.

The Sollerman hand function test was applied on the patients in order to assess the functional outcome. Sixty-seven patients (67%) gained a score above 80. The remaining 33 had a lower score. The mean score was 78.48 ± 33.19% SD.

The patients, according to their scores, were categorized as follows:

1. Patients who scored 100% (49 patients): This group included patients with complete and rapid healing (usually in less than a month) without residual contractures or deformities and patients who lost a central finger (middle or ring) without residual contracture or stiffness. Figure 2 shows a patient who gained a fully functioning hand despite the loss of his middle finger (100%).

2. Patients who scored 80–99% (18 patients): This group included those with residual low-grade stiffness or minimal contractures without any amputated finger(s).

### Table 1 The guidelines for scoring of Sollerman subtests

<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>The task is completed without any difficulty within 20 seconds and with the prescribed hand-grip of normal quality.</td>
<td>4</td>
</tr>
<tr>
<td>The task is completed, but with slight difficulty, or the task is not completed within 20 seconds, but within 40 seconds, or the task is completed with the prescribed hand-grip with slight divergence from normal.</td>
<td>3</td>
</tr>
<tr>
<td>The task is completed, but with great difficulty, or the task is not completed within 40 seconds, but within 60 seconds, or the task is not performed with the prescribed hand-grip.</td>
<td>2</td>
</tr>
<tr>
<td>The task is only partially performed within 60 seconds.</td>
<td>1</td>
</tr>
<tr>
<td>The task cannot be performed at all.</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: [20]

### Table 2 The 20 subtests comprising the Sollerman grip function test

| 1. Put key into Yale lock, turn 90 | 11. Cut Play-Doh with knife and fork |
| 2. Pick coins up from flat surface, put into purses mounted on wall | 12. Put on Tubigrip stocking on the other hand |
| 3. Open/close zip | 13. Write with pen |
| 4. Pick up coins from purses | 14. Fold paper, put into envelope |
| 5. Lift wooden cubes over edge 5 cm in height | 15. Put paper-clip on envelope |
| 6. Lift iron over edge 5 cm in height | 16. Lift telephone receiver, put to ear |
| 7. Turn screw with screwdriver | 17. Turn door-handle 30° |
| 8. Pick up nuts | 18. Pour water from Pure-pak |
| 9. Unscrew lid of jars | 19. Pour water from jug |
| 10. Do up buttons | 20. Pour water from cup |

Source: [20]
3. Patients who scored 50–79% (14 patients): This group included those with peripheral finger amputation (index or little finger, not including the thumb) and patients with contractures and/or moderate degree of stiffness.
4. Patients who scored below 50% (12 patients): This group included patients who lost more than two fingers or lost the thumb with marked stiffness in the other fingers. Figure 2 shows a patient with severe sepsis and proximal involvement of the right hand. He has lost his thumb. He underwent reconstructive surgery, after which he developed severe hand stiffness together with a functional score of 25%. About 6 weeks later, his score has improved to 50% following 15 sessions of physiotherapy.
5. Patients who scored 0% (7 patients): This group included all the patients who lost the whole hand or had a proximal spread of the finger sepsis to involve the palm and/or the dorsum of the hand together with severe necrosis and damage to the tendons, superficial nerves, and vessels. They had reconstructive surgery and prolonged periods of dressings which covered the whole fingers. Ultimately, severe stiffness developed; however, it has improved by 20 to 40% following 15 sessions of physiotherapy. Figure 3 shows the hand of a patient before and after reconstruction. She had severe stiffness as well as a functional score of 0. Marked improvement in her hand function was noticed following physiotherapy. Table 4 shows the significant predictors of poor functional outcomes (Sollerman test < 80%), and Table 5 shows the factors that were non-significantly related to poor hand functions.

<table>
<thead>
<tr>
<th>Delay of presentation to the hospital</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Education level</td>
<td>Illiterate</td>
</tr>
<tr>
<td>School</td>
<td>40</td>
</tr>
<tr>
<td>Higher degree</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
</tr>
</tbody>
</table>

$p$ value 0.01

Fig. 1 Diabetic patients with different patterns of fingers sepsis. a A patient with severe hand necrosis from an initial little finger sepsis. b A patient with osteomyelitis of the ring finger. c A patient with an ulcer in the index finger. d A patient with gangrene of the index finger together with proximal hand involvement.

Fig. 2 The outcome of different diabetic patients with finger sepsis. a, b represent patients who had painful contractures. Their functional score of the hand was above 80%. c The normal grip of a patient with an amputated middle finger who scored 100%. d Twenty-year-old male patient with type 1 IDDM, who had partial thumb amputation and reconstructive surgery. His initial score was 25%, and it improved to 50% after physiotherapy. The figure demonstrates his failure to perform a pinch grip.
Discussion

Hand sepsis among diabetics is not uncommon. The increase in the incidence and prevalence of diabetes explains the relatively large number of diabetic septic hand cases that were met in this study. A number of authors favor to name it tropical diabetic hand syndrome (TDHS) [20]. Since most of the serious septic events of the hands may start from initial finger sepsis, finger sepsis had special considerations in this study. It is generally agreed that proper management of early finger sepsis among diabetics can prevent serious devastating outcomes and can preserve the functions of the hand.

The predominance of females with finger sepsis in this study does not match most of the literature studies, which usually reveals a predominance of finger sepsis in males [1, 2, 5, 10, 23]. This discrepancy may not be significant because of the close male-to-female ratio in this study. On the other hand, the involvement of the right hand more than the left in this study agrees with most of those in the literature [5, 10, 24] which is probably related to the common use of the right hand in the daily activities and thus its exposure to trauma.

Most of the cases of diabetic hand are idiopathic in origin [1, 10]. This study confirms the same fact; however, Mohamed et al. [5] has reported trauma as the commonest cause.

The level of patients’ education played an important role in the control of diabetes and moreover in the early management of finger sepsis. Low educational level was associated with negligence of diabetes control and delayed presentation to hospitals. Patients seldom went to hospitals initially, and most stayed at home adopting traditional therapy. This has been reported in many studies [12, 25], and it is considered to be an important provoking factor for the proximal spread of hand sepsis as well as for the development of severe necrosis associated with poor functional outcomes. Health education remains a fundamental element in the management of diabetes.

Comorbidities such as neuropathy and poor glycemic control of diabetes were significant predictors of unfavorable outcomes, matching the results of many authors [2, 4, 5, 18, 24, 26, 27]. This confirms a substantial evidence that these factors have an important role in the development of complications of diabetes. On the other hand, factors, like the duration of diabetes and positive family history, were not related significantly to the outcome of diabetic hand, and this matches the results of a local study [7].

Seif et al. [28], in a local study in Khartoum, highlighted that superficial abscess occurs commonly in the hands of diabetics. Similarly, it was the most common pathological pattern in the current study. Although superficial abscess can be small and simple, every effort should be made to educate the

### Table 4 The significant predictors of poor hand function (Sollerman test < 80%)

<table>
<thead>
<tr>
<th>The variable</th>
<th>Hand function &lt; 80%</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spread of finger sepsis proximally to involve the hand</td>
<td>31 out of 39</td>
<td>0.00</td>
</tr>
<tr>
<td>Development of complications (stiffness and contractures)</td>
<td>32 out of 42</td>
<td>0.00</td>
</tr>
<tr>
<td>Amputated finger(s)</td>
<td>14 out of 18</td>
<td>0.00</td>
</tr>
<tr>
<td>No regular follow-up and monitoring of diabetes</td>
<td>30 out of 57</td>
<td>0.00</td>
</tr>
<tr>
<td>HBA1C levels above 8 (poor control)</td>
<td>30 out of 67</td>
<td>0.01</td>
</tr>
<tr>
<td>The need for reconstructive surgery (graft, flap, etc.)</td>
<td>9 out of 12</td>
<td>0.00</td>
</tr>
<tr>
<td>Presence of neuropathy</td>
<td>19 out of 44</td>
<td>0.05</td>
</tr>
<tr>
<td>Presence of necrosis (not simple abscess)</td>
<td>17 out of 30</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### Table 5 The variables which are non-significantly related to poor hand function (Sollerman < 80%)

<table>
<thead>
<tr>
<th>The variable</th>
<th>Hand function &lt; 80%</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of diabetes more than 5 years</td>
<td>24 out of 67</td>
<td>0.39</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>16 out of 53</td>
<td>0.53</td>
</tr>
<tr>
<td>Spontaneous cases</td>
<td>16 out of 50</td>
<td>0.62</td>
</tr>
<tr>
<td>Traumatic cases</td>
<td>14 out of 42</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Fig. 3 a Before and b after reconstruction in a 70-year-old left-handed female. She had necrosis which started at the base of the middle finger and spread proximally. The patient was poorly controlling her diabetes, and neuropathy was present. Her hand performance was 0% due to severe tissue loss as well as stiffness that resulted from prolonged periods of dressing and immobilization. This figure then has improved to 40% after 15 sessions of physiotherapy.
patients about the importance of early presentation and drainage of it, in order to avoid subsequent change into more morbid pathologies. The development of necrosis in this study had a significant association with poor outcomes in many patients. Necrosis in the hands is mainly related to the accumulation of purulent material in closed anatomical spaces which leads to an element of compartment syndrome causing necrosis that becomes aggravated again by infection (vicious circle). The infection may then spread proximally through the pathway of decreased resistance to involve the hand [21].

On one hand, finger amputation in this study was unavailable in 18 patients (18%); on the other hand, hand amputation was rare and occurred only in 4 patients (4%). Such results were comparable to those of Mohamed et al. [5] who had 17 patients (14.3%) who ended with digit amputation and 2 patients (1.7%) who ended with hand amputation. The absence of the element of peripheral vascular disease and hence the good vascularity of the hand would explain the rarity of hand amputation. Nonetheless, some authors reported higher figures of amputation rates [12, 29]. Fortunately, complete spontaneous healing was the outcome in most of the patients (82%) and it occurred in a relatively short period of time. A close figure (80%) was found in a local study [6]. This indicates that chronic non-healing wound usually is not a worrisome complication of diabetic septic hand unlike the cases of diabetic foot. The absence of pressure effect and good blood supply in the hand can explain this. Those with large defects in the hands after debridement required reconstructive surgery. Unfortunately, most of them had poor functional outcomes. This can be explained by the loss of the superficially situated digital tendons during debridement [19] and thus the loss of the ability to use the hands properly. The involvement of plastic/hand surgeons early in the management at the initial interventions or before extensive necrosis and major soft tissue loss will ensure maximum post-healing functional recovery. Understanding basic principles of hand surgery and applied anatomy remains a vital requirement before embarking into managing hand infections.

Small percentages of patients ended with residual deformities and complications in the hands, matching the results of Sabir et al. [22].

The Sollerman hand function test is a standardized test that was used mainly to assess and compare the results of reconstructive hand surgery [20]. Although no one reported its use in assessing the outcome of diabetic septic hands, it still provides a simple objective way to assess the hand function in the form of a specific score. In this study, the final score was represented as a percentage of the total. It gives an important clue regarding the use of the hands in the daily activities by testing the most common handgrips [20]. This was the justification to use this test in the current study. The results of this test in this study can be compared with other studies. Significant number of patients got 100% on the assessment of hand function. Those were the patients who presented early to a diabetic center, and their infection was limited to the fingers. Moreover, these patients used to follow their disease and they have been compliant with medications. Eventually, most of them had absent or minimal neuropathy and reasonable figures of HBA1C.

On the other hand, poor control of diabetes, presence of neuropathy, and delay of management of finger sepsis have led to the proximal spread of the necrosis and/ or transformation into gangrene and amputation, with resultant poor functional outcomes. Gaining 100% hand function after loss of the middle or ring finger indicates the marked ability of the rest of the fingers to compensate for this loss and provided no stiffness in the remaining parts of the hand. Hand function was reduced more in those who lost the index or ring finger; however, the loss of more than one finger or the loss of the thumb has major implications in hand function and is a cause of great morbidity. According to this, attempts of preserving the thumb during debridement are of paramount importance. This agrees with many other studies [12]. In this study, the stiffness of the hand joints and contractures occurred rapidly following treatment. They are important factors that mandate attention because they increase the likelihood of poor hand function. Prolonged periods of dressing, edema, improper placement of hand incisions, lack of splinting, and early physiotherapy, together with wrapping all the fingers and the wrist, resulted in marked stiffness and deterioration of the whole hand function even from a local focus of sepsis. This indicates the need for applying standardized methods of surgical approach, splinting, elevation, and rehabilitation.

Conclusion

Neglected finger sepsis can progress to involve the hand causing severe damage or can lead to finger gangrene and amputation. This can result in poor functional outcomes. Factors like poor control of diabetes, presence of neuropathy, low educational level, delay of presentation to hospital, and severe necrosis which requires surgical reconstruction are significantly associated with poor functional outcome. The loss of more than one finger or the loss of the thumb can reduce hand function markedly, compared to the loss of the middle or ring finger. Stiffness is a significant predictive factor for reduced hand function. It develops from severe tissue damage demanding reconstruction and from prolonged periods of immobilization in a dressing which covers the whole hand.

Compliance with ethical standards

Conflict of interest  The first author (Mugahid A Salih) declares that he has no conflict of interest. The second author (Shadad M Mahmoud) declares that he has no conflict of interest. The third author (Mohamed
E. Ahmed) is a member of the ethical committee Sudan Medical Specialization Board.

Ethical approval All procedures performed in human participants in this study were in accordance with the ethical standards of the ethical committee of the Sudan Medical Specialization Board and with standards equivalent to the 1964 Helsinki Declaration and its later amendments. This article does not contain studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

References

A standardized approach based on dietary and multiple daily insulin injections for the management of diabetes in infants

Hakan Doneray1,2 · Ayse Ozden1 · Remziye Seda Yesilcibik3

Abstract Plasma glucose control in the neonates and infants with diabetes based on dietary and multiple daily insulin injections is highly difficult. In this study, the infants were fed at 3- to 4-h intervals. All patients were started on insulin therapy with 0.6 U/kg/day divided equally into four doses. The morning, noon, and evening doses were given as premixed insulin (25% insulin lispro and 75% insulin lispro protamin sulphate), while the night insulin dose was given as premixed or neutral protamin Hagedorn (NPH) insulin according to the feeding frequency during the night. Nine-point capillary blood glucose profiles, HbA1c, and anthropometric measurements were evaluated. The study had 15 infants diagnosed with neonatal diabetes (ND) or type 1 diabetes mellitus (T1DM). The mean duration of the follow-up was 22.9 ± 8.7 months. During the follow-up, all patients had experienced sufficient weight and length gains and had experienced neither any episode of ketoacidosis nor wide fluctuations in blood glucose levels. Hypoglycemia, normoglycemia, and hyperglycemia were reported in 9.1, 79.9, and 11% of all the blood glucose measurements, respectively. Severe hypoglycemia was not experienced by any patients. The mean HbA1c levels in the patients with ND and T1DM at the last visit were 7.0 ± 0.2 and 7.6 ± 0.56%, respectively. The findings suggest that our approach is useable for plasma glucose control without wide fluctuations in infants with diabetes.

Keywords Neonatal diabetes · Infant · Diabetes · Management

Introduction

Neonatal diabetes (ND) and infantile type 1 diabetes mellitus (T1DM) constitute two important groups of diabetes in infants. ND is extensively defined as insulin-requiring diabetes mellitus that occurs in the first six months of life [1, 2]. However, the age limit has revised from six months to nine months recently [3]. ND is a genetically heterogeneous disorder, with at least 20 different causal genes identified to date [4]. The impaired development or function of β cells is the basic mechanism for insulin deficiency.

Type 1 diabetes mellitus in infants is very rare [5]. It is a T cell-mediated autoimmune disease that results from a selective destruction of the pancreatic insulin-producing β cells. Pancreatic antibodies in the plasma are a hallmark of the underlying autoimmune process. The disease results from a combination of genetic predisposition and a number of potential environmental factors. The earlier the clinical onset of the disease, the stronger the genetic susceptibility, especially when diabetes presents before 2 years of age [6]. Infants diagnosed with diabetes before 6 months of age are less likely to be antibody-positive than infants diagnosed later [4].

Insulin therapy is essential for achieving adequate weight gain and growth in the management of diabetes in infants and can be delivered by insulin syringes, insulin pen, and insulin pump [7]. Insulin pumps have been used successfully in developed countries. However, insulin pens are the major treatment option for infants from developing countries because pump therapy is expensive. The neonates and infants under multiple daily insulin injections are more likely to have high blood glucose levels immediately after a feeding. However,
they are also more likely to have low glucose dips because they tend to be quite sensitive to insulin. This “roller coaster” effect is called fluctuation in blood glucose, and the dramatic symptoms it causes may be dangerous. The wide fluctuations in blood glucose levels occur frequently in diabetic infants. In the literature, the management based on dietary and multiple daily insulin injections in diabetic infants has not been precisely determined because there has been no prospective clinical study. In this paper, we describe a standardized approach to the management of diabetes in infants and share our clinical experience.

Material and methods

Male and female infants younger than 36 months, who had ND or T1DM, were enrolled in the study. The protocol was approved by local institutional review boards. The study was conducted in accordance with the Declaration of Helsinki [8] and Good Clinical Practice guidelines [9]. Written informed consent was obtained from all parents.

Therapy protocol

The patients with T1DM who presented with ketoacidosis were given fluid and intravenous insulin infusion therapies. The fluid mixing was modulated based on the patients’ electrolyte and blood glucose levels. Insulin was administered at 0.05 IU/kg/h, and the rates of insulin infusion were reduced according to the infants’ glucose levels.

Infant feeding recommendations and practices of the World Health Organization (WHO) were carried out [10, 11]. The infants under the age of six months were allowed to drink only breast milk or formula (if there was no breast milk) until they were full at each feeding time. An interval of 3 h was left between feedings (Fig. 1). However, if the infants wanted to feed more than one time during the night (between 00:00 and 06:00), they were allowed to prevent nocturnal hypoglycemia. During the period of complementary feeding (after the first 6 months of life), breastfeeding was permitted on a decreasing basis for the remaining of the first year of life or longer. If breastfeeding was desired by the mother and infant, it was done only at snack time after the three main meals were constituted. Daily calorie requirement was calculated according to ideal weight. Carbohydrates accounted for 55% of daily calorie intake. Three main meals and three snacks were organized. Each snack contained six g of carbohydrates for infants younger than 12 months and 12 g of carbohydrates for those older than 12 months. The remaining carbohydrates were equally divided into three main meals. An interval of 3–4 h was left between feeding times (main meal or snack). However, the feeding times were flexible. If the interval between main meals (breakfast-lunch and lunch-dinner) was planned as 3–4 h, for example, the snack between the main meals was skipped because the interval between them would be too short (Fig. 2). In addition, the last snack was mandatory for the infants. The most important point for the parents was to keep every feeding time in their mind. According to the last feeding time, the next one could be determined by preserving an interval of 3–4 h. In addition to breast milk, formula, fresh fruit or its juice, yogurt and crackers were offered as the snack.

The patients were started on insulin therapy with 0.6 U/kg/day divided equally into four doses. Premixed insulin (Humalog® Mix25 cartridge, Eli Lilly and Co.; 25% insulin lispro and 75% insulin lispro protamin sulphate and NPH insulin (Humulin N® cartridge, Eli Lilly and Co.) were used. The insulin doses in the infants under the age of six months were given at certain hours (Fig. 1). If the infants wanted to feed more than one time during the night, the last insulin dose was given as premixed insulin, whereas if the feeding at 03:00 AM was sufficient for the infant, the last insulin dose was given as NPH insulin (Fig. 1). During the period of complementary feeding, premixed insulin doses were given at the main meal times, while the last insulin dose as NPH insulin was carried out at the last snack time (Fig. 2). All insulin doses were performed subcutaneously after a feeding because the food intake in infants was unpredictable. Insulin doses and the amount of carbohydrate in the diet were adjusted according to each patient’s food demands and blood glucose levels. The fasting and postprandial (120 min) capillary blood glucose levels were measured at each insulin injection time. At 03:00 AM, one more capillary blood glucose level was obtained. Medisense Optium® (Abbott Laboratories) glucose meters were given to all the families for capillary blood glucose measurements, and all measurements were recorded. The blood sugar normal ranges for the fasting and postprandial measurements were determined as 70–180 mg/dl (3.9–10 mmol/l) and 70–200 mg/dl (3.9–11.1 mmol/l), respectively. The targets for the fasting and postprandial blood glucose levels were 180 and 200 mg/dl, respectively. The required insulin doses to cover blood sugar deviations from the targets were determined by insulin sensitivity factor (blood sugar correction factor), which was calculated by dividing 1800 by the total daily dose. The insulin correction dosage was given as insulin lispro (Humalog® cartridge, Eli Lilly and Co.). If there was a high blood glucose level above the target for the fasting blood glucose level, insulin lispro was given separately in addition to the premixed insulin, while it was given by itself if there was a high blood glucose level above the target for postprandial blood glucose level.

Parents received training by a certified diabetes educator on basic information related to hypoglycemia, capillary blood glucose monitoring, and treatment medications. The study staff monitored the subjects’ glucose records, insulin doses, hypoglycemic events, and medication adherence and compliance. Office visits occurred at weeks 1 and 2 and then every
3 months. Adverse events were assessed at each visit. Parents recorded blood glucose meter values and associated symptoms of hypoglycemia. Mild hypoglycemia was defined as any symptom of hypoglycemia (i.e., palpitations, muscular weakness, sweating, tremor, jitteriness, irritability) with a confirmed blood glucose meter reading < 70 mg/dl (3.9 mmol/l) or any asymptomatic blood glucose meter reading < 70 mg/dl (3.9 mmol/l) and which was handled by the parents. Severe hypoglycemia was defined as symptoms (semiconsciousness or unconsciousness, coma, convulsions) that might have required parenteral therapy (glucagon or IV glucose) were associated with a blood glucose meter reading < 70 mg/dl (3.9 mmol/l) and required third-party assistance. A glucose level higher than 180 mg/dl for fasting and 200 mg/dl for postprandial measurement was considered hyperglycemic [12].

**Laboratory studies and measurements**

If the patient was less than one year old and the antibodies (glutamic acid decarboxylase-65 antibody, insulin antibody, and islet cell autoantibody) for T1DM were negative, genetic analysis for neonatal diabetes was done. If the patient was

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**Fig. 1** Insulin and feeding times before the period of complementary feeding. The fasting and postprandial capillary blood glucose levels were measured at insulin injection times. One more blood glucose level was obtained at 03:00 AM

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**Fig. 2** Flexible insulin and the main meal and snack times in the period of complementary feeding. a, b are the examples of our method. They can be diversified. Asterisk represents the preferred feeding time. According to it, the next feeding time could be determined by preserving an interval of 3–4 h (a, b). If the interval between main meals was planned as 3–4 h, for example, the snack between these main meals was skipped (b). Premixed insulin was given in main meals while NPH insulin was used at the last snack time. The fasting and postprandial capillary blood glucose levels were measured at insulin injection times. One more blood glucose level was obtained at 03:00 AM.
younger than six months old, HbA1c level was not measured because it could interact with HbF. HbA1c was measured every three months. The mean HbA1c levels were calculated for every patient at the last visit.

Anthropometric measurements, including body weight and height, were performed and recorded at the diagnosis and every visit. Weight was measured using an electronic scale (Seca Model 770, Hamburg, Germany). Length (± 0.1 cm) was measured using a body length measurer.

Plasma HbA1c was determined in serum using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, D-68298, Germany). Coefficients of variations for both interassay and intra-assay determinations for all the parameters were from 2.8 to 7.6% in our laboratory.

The primary end points included nine-point capillary blood glucose profiles (four fasting and four postprandial at insulin dose time and one more at 03:00 AM) and sufficient weight gain. Secondary efficacy end point was the change in the mean HbA1c at the last visit.

Results

Of the 15 patients who entered the study, cases 1 and 5 had mutant genes for neonatal diabetes, while cases 2, 3, and 4 did not have any genetic abnormality in terms of genes, including KCNJ11, ABCC8, INS, and EIF2AK3. Additionally, cases 3 and 4 had no mutation for SLC19A2, FOXP3, ZFP57, and BCS1L genes either. However, since these patients were younger than nine months and did not have antibodies for T1DM, they were accepted as ND having undefined genes. Thus, the study was completed with five patients with ND and T1DM.

Seven-point capillary blood glucose profiles (four fasting and four postprandial at insulin dose time and one more at 03:00 AM) and sufficient weight gain were performed and recorded at every visit. Weight was measured using an electronic scale (Seca Model 770, Hamburg, Germany). Length (± 0.1 cm) was measured using a body length measurer.

Plasma HbA1c was determined in serum using an electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, D-68298, Germany). Coefficients of variations for both interassay and intra-assay determinations for all the parameters were from 2.8 to 7.6% in our laboratory.

The primary end points included nine-point capillary blood glucose profiles (four fasting and four postprandial at insulin dose time and one more at 03:00 AM) and sufficient weight gain. Secondary efficacy end point was the change in the mean HbA1c at the last visit.

Statistical analysis

Descriptive calculations were made using the SPSS (version 15.0 for Windows) software program (SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD).

Discussion

Our protocol was intended to implement a standardized approach based on dietary and insulin guidelines. It was applied to the infants under 36 months through practical education, during which the parents acquired techniques for self-management. Our method showed effectiveness and methodical convenience with more flexibility, especially in toddlers with variable meal times.

There are four important goals for the management of infants with diabetes: the maintenance of regular blood sugar levels without acute metabolic deteriorations and fluctuations in the blood sugar levels, sufficient weight gain, parental self-confidence, and active monitoring and evaluation of the disease. To achieve these goals, we believe that the key is to offer a clear and simple method to parents in terms of diet and insulin therapy. Generally, in diabetic infants, caloric and glucose restrictions to avoid or delay insulin therapy should be strongly discouraged [4]. Thus, we focused first on essential knowledge related to feeding suggested by WHO and allowed the infants under the age of six months to take as much breast milk as they wanted at each feeding time, whereas we...
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Chronological age at diagnosis</th>
<th>Gestational age (weeks)</th>
<th>Birth weight (g)</th>
<th>KA at diagnosis</th>
<th>Blood glucose at diagnosis (mg/dl)</th>
<th>HbA1c at diagnosis (%)</th>
<th>Antibodies for diabetes</th>
<th>Mutant genes</th>
<th>Insulin dose at discharge (U/kg/day)</th>
<th>Chronological age at the last visit (month)</th>
<th>The duration of follow-up (month)</th>
<th>Insulin dose at the last visit (U/kg/day)</th>
<th>Mean HbA1c at the last visit (%)</th>
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<td>ND</td>
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<td>KCNJ11</td>
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<td>562</td>
<td>–</td>
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<td>NF</td>
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<td>–</td>
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<td>NF</td>
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<td>–</td>
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</table>

*All values are means ± SD

ND neonatal diabetes, T1DM type 1 diabetes mellitus, NF not found, KA ketoacidosis, GAD65 glutamic acid decarboxylase 65 antibody, SU sulfonylurea
composed a more flexible feeding program with main meals and snacks in the complementary feeding period. Thus, the elevations in the blood sugar before the next feeding were also prevented by the maintenance of intervals of 3 or 4 h between feedings. We believe that these intervals are also important to arrange the insulin doses because they standardize the plan in terms of feeding time. Therefore, the next step will be which or how the insulin therapy is given. However, there has been no consensus with regards to insulin therapy in infantile-onset diabetes in literature, although it has been said that insulin can be provided by multiple daily injections or continuous subcutaneous infusion (insulin pump) [13]. Some authors suggest intermediate-acting insulin (NPH insulin) should be given as a once- or twice-a-day therapy because short-acting (regular insulin) and rapid-acting insulins (insulin lispro, aspart, and glulisine) may sometimes cause hypoglycemia that is difficult to control in infants [14]. Similarly, one review, updated in 2014 [7], contained a recommendation for NPH insulin or the longer-acting insulins (insulin glargine and insulin detemir), as well as the suggestion that rapid-acting and short-acting insulins (except when used as a continuous intravenous or subcutaneous infusion) should be avoided due to the risk of severe hypoglycemic events. In that approach, the intervals between the feedings have not been certain, and one disadvantage of this may be an inability to control the post-prandial glucose levels. For this reason, in another review [4], a high basal insulin substitution and very low mealtime boluses were recommended for infants nursing more than six times a day. Additionally, when more intermittent oral feeding is started, it is suggested that basal insulin is reduced to 30–50% of the total daily dose, while the doses of rapid-acting preparations are increased. In that approach, however, the main concern is giving very low rapid-acting doses. As a solution, the diluted insulin may be used [7]. However, insulin-diluting mediums for rapid-acting insulins are not available in Turkey or many other developing countries. Insulin pump therapy, as an alternative method, can be used for insulin delivery in this age group. The recommended basal insulin is 10–30% of total daily insulin, and a very low bolus should be given for the requirement associated with meals [4]. The insulin pump for neonates and infants is preferable to multiple daily injections. Insulin pumps have been used successfully in developed countries [13, 15], but this is expensive, and subsequent maintenance is a major issue for infants from developing countries. Again, getting the diluted insulin for the insulin pump may be a concern in most developing countries. To our knowledge, all these recommendations mentioned above are based on clinical observations obtained from case reports, because no previous longitudinal clinical study has resulted in the development of insulin and diet regimes in infantile-onset diabetes. Additionally, the wide fluctuations in blood glucose levels in infants under insulin therapy, regardless of insulin type and its application method, are a major challenge.

We first used our method on case 1 and determined that the fasting and postprandial blood glucose levels measured at insulin injection times were in acceptable limits [16]. In that patient, HbA1c, at the last visit when premixed insulin was

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Nine-point mean capillary blood glucose (mg/dl) measurements from the diagnosis to the last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>MB</td>
</tr>
<tr>
<td>1</td>
<td>92 ± 25</td>
</tr>
<tr>
<td>2</td>
<td>138 ± 36</td>
</tr>
<tr>
<td>3</td>
<td>104 ± 41</td>
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<tr>
<td>4</td>
<td>131 ± 66</td>
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<td>5</td>
<td>151 ± 81</td>
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<tr>
<td>6</td>
<td>165 ± 95</td>
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<tr>
<td>7</td>
<td>160 ± 16</td>
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<tr>
<td>8</td>
<td>128 ± 69</td>
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<tr>
<td>9</td>
<td>151 ± 83</td>
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<tr>
<td>13</td>
<td>164 ± 97</td>
</tr>
<tr>
<td>14</td>
<td>182 ± 123</td>
</tr>
<tr>
<td>15</td>
<td>165 ± 13</td>
</tr>
</tbody>
</table>

All values are means ± SD

MB morning before insulin, M120 2 h after insulin, NB noon before insulin, N120 2 h after insulin, EB evening before insulin, E120 2 h after insulin, NGB night before insulin, NG120 2 h after insulin
transitioned to sulfonylurea, was 5.4. During premixed insulin therapy, there was sufficient weight gain and no episode of ketoacidosis or severe hypoglycemia. We used that experience for other neonates and infants with diabetes. We preferred Eli Lilly and Co.’s insulin because only its pen in Turkey can deliver insulin in 0.5-unit increments. We regulated postprandial glucose levels with insulin lispro in premixed insulin. Additionally, we did not need the dilution of insulin because insulin lispro in premixed insulin comprises 25% of the total dose, and a very small dose of insulin lispro at 0.125 unit could be given when premixed insulin was carried out at 0.5 unit. Insulin lispro protamin sulphate, with a proportion of 75% in the total dose, also provided us with the advantageous ability to regulate fasting glucose levels because it is a kind of intermediate-acting insulin. During the follow-up, all the patients had sufficient weight and length gains but no episode of ketoacidosis. The mean total daily insulin dose of all the patients decreased from 0.79 ± 0.9 to 0.56 ± 0.03 U/kg/day, and there was a substantial reduction in mean HbA1c for the patients with T1DM (10.6 ± 0.9 vs 7.9 ± 0.5%). However, none of the patients had severe hypoglycemia or wide fluctuations in the recorded blood glucose measurements. Although we defined a high-threshold blood glucose level for hypoglycemia (70 mg/dl), only 8% of all the blood glucose measurements were lower than that limit. Ninety-six percent of those measurements were between 62 and 70 mg/dl, and 92% of the patients were asymptomatic. As observed in the nine-point blood glucose profiles (Table 2), our therapy protocol provides convenient meal time dosing and may mostly regulate both fasting and postprandial blood glucose levels in acceptable limits.

In this study, we were unable to compare our method due to the fact that no other groups were given NPH insulin or long-acting insulins nor were any using insulin pumps. Therefore, we cannot claim our method is superior. However, we could not find any study in the literature to compare our results either. Additional, we did not need the dilution of insulin because insulin lispro in premixed insulin comprises 25% of the total dose, and a very small dose of insulin lispro at 0.125 unit could be given when premixed insulin was carried out at 0.5 unit. Insulin lispro protamin sulphate, with a proportion of 75% in the total dose, also provided us with the advantageous ability to regulate fasting glucose levels because it is a kind of intermediate-acting insulin. During the follow-up, all the patients had sufficient weight and length gains but no episode of ketoacidosis. The mean total daily insulin dose of all the patients decreased from 0.79 ± 0.9 to 0.56 ± 0.03 U/kg/day, and there was a substantial reduction in mean HbA1c for the patients with T1DM (10.6 ± 0.9 vs 7.9 ± 0.5%). However, none of the patients had severe hypoglycemia or wide fluctuations in the recorded blood glucose measurements. Although we defined a high-threshold blood glucose level for hypoglycemia (70 mg/dl), only 8% of all the blood glucose measurements were lower than that limit. Ninety-six percent of those measurements were between 62 and 70 mg/dl, and 92% of the patients were asymptomatic. As observed in the nine-point blood glucose profiles (Table 2), our therapy protocol provides convenient meal time dosing and may mostly regulate both fasting and postprandial blood glucose levels in acceptable limits.

In this study, we were unable to compare our method due to the fact that no other groups were given NPH insulin or long-acting insulins nor were any using insulin pumps. Therefore, we cannot claim our method is superior. However, we could not find any study in the literature to compare our results either. Therefore, we think our study may be only a small step forward for the insulin and feeding management of diabetes in infants, especially in some situations where the access to diluted insulin and to an insulin pump is a concern.

Our study has several limitations: (1) The number of subjects was relatively small, and the ages were variable. Therefore, the power of the study is relatively limited. However, given the rarity of diabetes in infants, with an overall estimated incidence of less than 2 cases per 100,000 infants [17, 18], our study represents an alternative opportunity in diabetic infant care in terms of providing reasonable HbA1c measures and promoting weight gain and protecting infants from wide fluctuations in blood glucose and severe hypoglycemia. (2) We used the capillary blood glucose measurements. We think that it would be better if continuous glucose monitoring system could be used to see whole fluctuations in blood glucose levels.

**Conclusion**

The overall results from our study suggest that our approach is useable for the management of diabetes in infants from developing countries. However, prospective and controlled studies that include a large number of subjects are required.

**Acknowledgements** The authors are grateful to Andrew Hattersley and Sian Ellard (Peninsula Medical School, Molecular Genetics Royal Devon & Exeter NHS Hospital, Exeter, United Kingdom), Jukka Kalljarvi (Folkhälsan Research Center, University of Helsinki, Helsinki, Finland), and Roger Colobran (Hospital University Vall d’Hebron, Barcelona, Spain) for the molecular genetic tests in cases 1–5. All genetic tests were done free of charge. They also thank Ayhan Tastekin (Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey) for the cooperation and participation in case 5.

**Compliance with ethical standards** The study was approved by the Atatürk University of Medical Sciences ethics committee. Written informed consent form was signed by parents.

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

**References**

Is the CANVAS of canaglifozin wide?

Anil Pareek1 · Nitin Chandurkar2 · Kumar Naidu2 · Ravi Tejraj Mehta3

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Dear Editor,

- The CANVAS Program has set a different precedence, as for the first time, data from two different studies (CANVAS and CANVAS-R) have been allowed to be pooled together to assess CV outcomes [1]. However, in the pooled analysis, the authors have not reported statistical similarity of the baseline characteristics between the canagliflozin and placebo arms. The baseline characteristics of participants in CANVAS Program reveal history of CVD in 64.8% participants in the canagliflozin arm and in 66.7% participants in the placebo arm. Statistical calculations show significantly more participants with history of CVD in the placebo arm ($p = 0.046$ and chi square = 3.967). The effect of this unequal distribution on the reported CV outcome data cannot be excluded.

- Participants in CANVAS were randomized to receive canagliflozin in different doses (100 and 300 mg), while in CANVAS-R, participants initially received 100 mg daily with an optional increase to 300 mg from week 13. However, in the CANVAS Program, the authors have not reported the effects of the two doses of canagliflozin separately. It is important from safety and efficacy point of view that the data of these two different doses of canagliflozin are examined.

- In sub-group analysis, effect of canagliflozin on the primary CV outcome appears to be better in patients receiving diuretics and beta-blockers, and not in participants not receiving these cardio-protective drugs. As SGLT-2 inhibitors have an osmotic diuretic effect due to glycosuria, the lack of benefit in participants not receiving diuretics is surprising. Further, canagliflozin was not found to be better in patients without history of CVD, without long-standing diabetes or with eGFR > 60 ml/min/1.73m2. Thus, the effect of canagliflozin may not be similar across varied patient profiles.

- The CANVAS study was unblinded due to an adverse effect on the LDL cholesterol [2]. This effect persisted even in the CANVAS Program. The LDL cholesterol elevation may have attenuated the positive effects on CV outcomes.

- Canagliflozin has been shown to lower both glycated hemoglobin levels and blood pressure. The investigators should have made efforts to achieve similar glycemic and blood-pressure control in the placebo group [3]; in the absence of which, the beneficial renoprotective effect cannot be attributed solely to canagliflozin.

In conclusion, the pooling of two separate studies done for the first time in CANVAS Program needs to be carefully interpreted and adjudicated before application in clinical practice.
Authors' contributions All authors have contributed equally to the manuscript.

Compliance with ethical standards

Conflict of interest The authors are affiliated to Ipca Laboratories Limited and are involved in research studies on anti-diabetic drugs.

References


VISION STATEMENT
To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

MISSION STATEMENT
1. Promotion of excellence in diabetes care to make India the Diabetes Care Capital
2. Empowerment of persons living with diabetes
3. Support for diabetes research
4. Dissemination of information and knowledge in diabetes care
5. Advocacy for the cause of diabetology

RSSDI Research Grants
• For providing research grants, RSSDI invites proposals from Indian scientists, interested in conducting original research in the field of diabetes mellitus. Furthermore, limited grants are also available for the students of medical colleges for smaller projects.
• There is no deadline for submission of the proposals, which can be sent throughout the year. These proposals may fall into one of the following three categories:
  1. Projects involving funding up to Rs 40,000 per project (preference will be given to young scientists <40 years).
  2. Projects involving funding up to 10 lakhs.
  3. We also invite proposals for more than 10 Lakhs as major projects but these have to be preferably multicentric.
• The detailed proposals should include the following:
  ◊ Title, names of principal and co-investigators, summary, introduction/background, review of literature, aims, methodology, study design, and detailed plan of work and bibliography. Brief biodata of principal investigator and other co-investigators
  ◊ Importance of work in the context of national priorities. Detailed budget sought along with full justification/proposed utilization, of funding sought from RSSDI
  ◊ Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.
  ◊ Ethical committee clearance of the institution or other bona fide body.

Travel grants for young diabetes researchers to attend International Conferences
Criteria’s for the travel grant are as follows:
• Applicant should apply 2 months in advance.
• Travel Grant is open only to the RSSDI members.
• Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.

• Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY
(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)
Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential “Advanced Certificate Course in Diabetology”. This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 16 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Institute Name</th>
<th>Institute Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diacon Hospital</td>
<td>Bengaluru, Karnataka</td>
</tr>
<tr>
<td>2</td>
<td>North Delhi Diabetes Centre</td>
<td>New Delhi, Delhi</td>
</tr>
<tr>
<td>3</td>
<td>Prithvi Hospital</td>
<td>Tumkur, Karnataka</td>
</tr>
<tr>
<td>4</td>
<td>Totall Diabetes Hormone Institute</td>
<td>Indore, Madhya Pradesh</td>
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<td>5</td>
<td>Dia Care A Complete Diabetes Care Centre</td>
<td>Ahmedabad, Gujarat</td>
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<tr>
<td>6</td>
<td>Sonal Diabetes Hospital</td>
<td>Surat, Gujarat</td>
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<td>7</td>
<td>Jothydev’s Diabetes and Research Center</td>
<td>Trivandrum, Kerala</td>
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<td>8</td>
<td>Advanced Endocrine &amp; Diabetes Hospital</td>
<td>Hyderabad, Telangana</td>
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<td>G D Hospitals and Diabetes Institute</td>
<td>Kolkata, West Bengal</td>
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<td>10</td>
<td>Aditya Diagnostics &amp; Hospital</td>
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<td>Sunil’s Diabetes Care N’ Research Centre Pvt Ltd.</td>
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<td>Marwari Hospital and Research Centre</td>
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<td>13</td>
<td>Down Town Hospital</td>
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<td>St.Theresa’s Hospital</td>
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<td>15</td>
<td>Aegle Clinic</td>
<td>Pune, Maharashtra</td>
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<tr>
<td>16</td>
<td>Tulip Hospital</td>
<td>Sonipat, Haryana</td>
</tr>
<tr>
<td>17</td>
<td>Lilavati Hospital &amp; Research Centre</td>
<td>Bandra West, Mumbai</td>
</tr>
</tbody>
</table>
COURSE DETAILS

**Name of the Course:** Advanced Certificate Course in Diabetology

**Duration:** 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine) * (Full Time) Educational.

**Qualification:** A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

**Number of seats:** 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

**Selection of Candidates:** Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given preference.

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

**Session:** Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

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