World Journal of
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Contents

Contents

Contents

ABOUT COVER

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AIMS AND SCOPE

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Do we need to screen every patient in intensive care unit for diabetes in community with high prevalence of diabetes?

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Abstract

Diabetes mellitus (DM) is marked as global health care challenge with almost 10% of the United States population being diagnosed with DM. A sizeable percentage of patients are oblivious of their disease, in spite of easily accessibility knowledge about its early signs and symptoms and rapid diagnostic modalities. Critically ill patients with undiagnosed DM are likely to have an increased mortality as compared to intensive care unit (ICU) patients with diagnosed DM. DM may have adverse effect on ICU patients causing organ failure and complications. Early Screening of patients at the risk of developing disease may prevent long term complications. Early screening and management may be beneficial as controlled DM patients have similar morbidity as non DM patients in ICU. An intense glycaemic and blood pressure control improves retinopathy and albuminuria, but may not affect the macrovascular outcomes.

Key words: Diabetes mellitus; Intensive care unit; Microvascular; Macrovascular; Diabetes screening

Core tip: Undiagnosed diabetes mellitus (DM) predisposes critically ill patients to DM complications, which may affect their morbidity and mortality during intensive care unit stay.

Citation: Dutt T, Kashyap R, Surani S. Do we need to screen every patient in intensive care

INTRODUCTION

An estimated 30.3 million people of all ages, or 9.4% of the United States population had diabetes mellitus (DM) in 2015. This included 30.2 million adults aged 18 years or older (12.2% of all United States adults), of which 7.2 million (23.8%) were unaware of or did not report having DM. The percentage of adults with DM increased with age, reaching a high of 25.2% among those aged 65 years or older[1]. In spite of the wide accessibility of knowledge about the early signs and symptoms of DM and ease of diagnostic modalities, many patients are oblivious of their disease[2]. Worldwide approximately 193 million diabetic patients remain undiagnosed predisposing them to the development of several long-term complications of untreated chronic hyperglycaemia, making this a global health care challenge[3].

PREVALENCE OF DM IN AMONG CRITICALLY ILL PATIENTS

The complications of DM include both microvascular and macrovascular pathologies and comprise of retinopathy, neuropathy, renal failure, cardiovascular complications and increased risk of death. A study conducted by Tancredi *et al*[4] concluded that there is 15 fold increases on all-cause mortality in patients with Type-2 DM. These complications have profound physical as well as psychological burden on the patient, the family, and the care givers and on a larger scale they affect the health of the society.

On the other hand, intensive care unit (ICU) admissions with undiagnosed DM have been showing a steady increase in the past few years[2]. Carpenter *et al*[2] studies the impact of undiagnosed DM in 9 ICU's. The study reported that patients with undiagnosed DM had an increased mortality as compared to ICU patients with diagnosed DM; and also showed increased trend for higher average blood glucose level and insulin infusion. Thus need for DM screening amongst critically ill patients is paramount.

BENEFIT OF DM SCREENING

A study conducted by Kunthi *et al*[5] suggested that screening of subpopulations using risk scores can rule in high risk patients and the diagnosis can be confirmed by measurements of fasting plasma glucose or HbA1c concentrations or tests for oral glucose tolerance. Screening of the individuals who are at the risk of developing disease will prevent the long term microvascular as well as macrovascular complications. Early detection also helps in optimal disease management by practicing lifestyle modifications such as weight reduction, quitting smoking and alcohol, increased physical activity and healthy diet[6]. However, various methods of screening may have used in the different studies including risk score, fasting plasma glucose, HbA1c concentrations or tests for oral glucose tolerance. This questions the applicability of a universal operational definition for DM diagnosis.

CONTROVERSIES AND COST EFFECTIVENESS OF DM SCREENING

Alongside, the various large multicentre studies concluded that macrovascular complications do not show any significant change[7]. The risk of cardiovascular disease and other macrovascular complications does not improve with intensive management of the screened population; hence the application of universal screening method is not promoted[7]. The UKPDS researchers[8] showed that despite an intense glycaemic and blood pressure control macrovascular outcomes were not improved but there was a

significant improvement in retinopathy and albuminuria. Krinsley *et al*[9] have shown that hyper-glycemia not only affects the morbidity in critically ill patients but also the patients admitted to the general medicine wards. They noted that high glucose variability (CV > 20%) increased mortality in non DM patients in both ICU as well as the floor settings but for the DM patients it was restricted only for ICU. Patients with DM having low HbA$_{1c}$ levels and patients without DM have equal mortality and morbidity risks and hyperglycaemia increases mortality. Siegelaar *et al*[10], in their meta-analysis showed that the diabetic patients have higher chances of developing complications like sepsis or organ failure and these in turn have increased mortality rate compared to non-diabetic population. However, Diabetes does not serve as an independent factor for ICU mortality and after acquiring complications the morality rate would be same in diabetic as well as non-diabetic patients[10].

CONCLUSION

Despite DM being widely prevalent in United States, still substantial numbers of patients in older age are undiagnosed. This predisposes them to micro and macrovascular complications, which in turn may affect their morbidity and mortality during ICU stay. Universal screening of DM has been proved beneficial to prevent microvascular compilations but not much difference is seen in the macrovascular maladies. Early screening and management may be beneficial as controlled DM patients have similar morbidity as non DM patients in ICU. DM may be associated with increased mortality in ICU patients. However, how DM intrinsically affects the ICU mortality, is still open for discussion.

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World Journal of Diabetes

REVIEW

Cataract in diabetes mellitus

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Abstract

Diabetes mellitus (DM) is a chronic systemic disease that has increases in prevalence over time. DM can affect all ocular structures, with cataract being the most common ocular complication. Cataract is the leading cause of blindness worldwide. Due to several mechanisms, there is an increased incidence of cataract formation in the diabetic population. Advancements in technology have now made cataract surgery a common and safe procedure. However, the diabetic population is still at risk of vision-threatening complications, such as diabetic macular edema (ME), postoperative ME, diabetic retinopathy progression, and posterior capsular opacification.

Key words: Diabetes; Cataract; Complications; Surgery

Core tip: Because the number of people with diabetes mellitus is predicted to increase in the future, cataract surgery will remain an important procedure for diabetic patients. Patients with diabetes have multiple issues which should be evaluated preoperatively, perioperatively, and in the postoperative period. The preoperative, intraoperative, and postoperative factors are of paramount importance in the management of such complications and in improving visual outcomes. This article aims to review diabetic cataracts and related complications, and to outline important management strategies.

INTRODUCTION

The prevalence of diabetes mellitus (DM) is increasing on a daily basis, with the International Diabetes Federation estimating that there will be 439 million DM patients by 2030[1]. An aging population and longer patient life expectancy also means that the prevalence of DM will exceed 33% by 2050[2]. DM can lead to pathologies in many tissues in the eye structure, with both a systemic chronic metabolic disease and a microangiopathic character[3]. Cataract is one of the major causes of visual impairment in diabetic patients[4]. Patients with DM are reported to be up to five times more likely to develop cataract, in particular at an early age[5-8]. Due to the increasing prevalence of DM, the incidence of diabetic cataracts has also risen. Cataract extraction is one of the most common surgical procedures among the general population, and the number of cataract surgeries each year also continues to increase. Recent technological advancements in cataract surgery have improved surgical outcomes. However, in diabetic individuals, the scale of improvement is still a matter of debate, and many studies have revealed both the results and complications of cataract surgery in diabetic patients. In the light of these findings, this study will review related articles in order to highlight current developments and controversies regarding cataract surgery management in patients with DM.

BIOCHEMICAL MECHANISMS FOR CATARACT IN DIABETES

Different types of mechanisms have been proposed for the pathogenesis of cataract in cases of DM.

Polyol pathway

It has been suggested that the polyol pathway-*via* which the enzyme aldose reductase (AR) catalyzes the reduction of glucose into sorbitol-is a central part of the mechanism of cataract development[9-11]. Multiple studies have been conducted to explain the AR pathway's role in this process. The increased intracellular accumulation of sorbitol leads to a hyperosmotic effect, resulting in hydropic lens fibers that degenerate and form cataract[9,12]. The production of sorbitol in diabetic patients (as compared to nondiabetic patients) takes place more quickly than it can be converted into fructose by the enzyme sorbitol dehydrogenase. Intracellular removal of sorbitol through diffusion is also prevented because of its polar character. A hyperosmotic effect is created when an accumulation of sorbitol results in an infusion of fluid. Finally, animal studies have shown that the intracellular accumulation of polyols causes liquefaction of lens fibers resulting in the formation of lens opacities[9,10,12,13]. In the study of Oishi *et al*[13], it was found that AR levels in red blood cells of patients under the age of 60 and with short duration of DM had a positive correlation with the prevalence of posterior subcapsular cataract. Moreover, a negative correlation was reported between the level of AR in erythrocytes and the density of lens epithelial cells, which is known to be lower in diabetics than in nondiabetics. These findings suggest that AR may play a role in this pathomechanism.

Osmotic and oxidative stress

Osmotic stress as a result of extensive swelling of the cortical lens fibers is another compounding mechanism in the rapid development of cataracts, especially in young patients with type 1 DM[14-16]. Osmotic stress resulting from the accumulation of sorbitol induces stress in the endoplasmic reticulum (ER), the main site of protein synthesis, resulting in the formation of free radicals[17]. Stress in the ER can also be caused by fluctuation of glucose levels that initiate an unfolded protein response producing reactive oxygen species and cause oxidative stress damage to lens fibers. Moreover, increased glucose levels in the aqueous humor may lead to glycation of lens proteins, a process that results in the formation of advanced glycation end products[18]. Fenton reactions resulting from elevated levels of hydrogen peroxide (H_2O_2) in the aqueous humor of diabetics also induces the generation of hydroxyl radicals (OH^-) after entering the lens[19]. Another factor that is elevated in the lens and aqueous humor of diabetic patients is free radical nitric oxide (NO•), which may cause an increase in peroxynitrite formation, which contributes to cell damage due to oxidizing properties[20,21]. However, diabetic lenses have increased susceptibility to oxidative stress due to their impaired antioxidant capacity. Superoxide dismutase (SOD) is the most predominant antioxidant enzyme in the lens that degrades superoxide radicals ($O2^-$) into H_2O_2 and oxygen. Several *in vitro* and *in vivo* animal studies have shown that SOD has protective properties against cataract development

in the presence of DM[22-24].

Some studies have shown that osmotic stress in the lens resulting from sorbitol accumulation causes apoptosis in lens epithelial cells and leads to cataract formation[25]. Rapid glycemic control can also increase these effects in the lens by creating a hypoxic environment that reduces protective enzymes and increases oxidative radicals. High AR expression could constitute a risk factor that predisposes the lens to distortions in signaling through the extracellular signal-regulated kinase and c-Jun N-terminal kinase pathways-involved in cell growth and apoptosis, respectively-thereby altering the balance required for lens homoeostasis[11,26]. These findings show that impairments in osmoregulation may render the lens susceptible to even the smallest increase in AR-mediated osmotic stress, potentially leading to progressive cataract formation.

Autoimmunity

Another recently proposed mechanism is autoimmune hypothesis in acute bilateral type 1 diabetic cataracts[26]. The authors reported that insulin autoantibodies became positive within three months of beginning insulin treatment, and that this period coincided with cataract formation. Their suggestion that there could be an autoimmune process behind acute bilateral cataract in DM warrants further investigation[26].

The type of cataract seen in diabetic patients has also been investigated. The most common is the senile type[10]. However, snowflake cataracts, which are characteristic for DM, are very common in type 1 diabetics. Posterior subcapsular cataracts have also been shown to be significantly associated with diabetes. Increased levels of glycated hemoglobin were demonstrably associated with an increased risk of nuclear and cortical cataracts[6]. Further analysis revealed that diabetic patients were prone to developing cortical cataracts and that this process was associated with the duration of diabetes[5,7].

Finally, the initiating mechanism in diabetic cataract formation is the generation of polyols from glucose by AR. However, osmotic stress, apoptosis of the lens epithelial cells, and the autoimmune theories may be confounding mechanisms in the development of the cataract formation in DM.

CATARACT INCIDENCE IN DIABETIC PATIENTS

Several clinical studies have reported that cataract formation occurs more frequently and at an earlier age in diabetic patients than in nondiabetic patients[7,27-29]. Some studies indicate that cataracts are three to four times more prevalent in patients with diabetes under the age of 65. In patients over 65, cataracts are twice as prevalent[27,30]. The main risk factors are longer duration of diabetes and poor metabolic control. Although older patients suffer from irreversible cataract formation, good metabolic control may reverse cataract in young diabetics.

Several important study groups have investigated cataract incidence in diabetic patients. The Wisconsin Epidemiologic Study of Diabetic Retinopathy investigated the incidence of cataract and factors associated with a higher risk of cataract surgery[7]. They found 8.3% of patients suffering from type 1 diabetes and 24.9% of those with type 2 diabetes had a 10-year cumulative incidence of cataract surgery. For type 1 diabetics, they found some risk factors, including age, severity of diabetic retinopathy (DR), and proteinuria; for Type 2 diabetics, risk factors included age and use of insulin[7].

The Beaver Dam Eye Study also reported an association between DM and cataract formation[5]. The study took place over five years and consisted of 3684 participants aged 43 and older. It showed an increased incidence and progression of cortical and posterior subcapsular cataracts for DM patients. It also found an increased risk of nuclear and cortical cataracts with increased levels of glycated hemoglobin. Further analysis of the study showed that diabetics had a higher rate of cortical lens opacities and previous cataract surgery than nondiabetics[6]. A longer duration of diabetes was also associated with increased frequency of both cortical cataracts and cataract surgery.

The Blue Mountains Eye Study aimed to examine the relationship between nuclear, cortical, and posterior subcapsular cataracts[31]. The study supported the findings of previous research, but also found an association between posterior subcapsular cataracts and DM. In contrast to the Beaver Dam Eye Study, nuclear cataracts showed a weak association with DM.

The Barbados Eye Study evaluated the relationship between diabetes and lens opacities among 4314 black participants[32]. The authors found that a history of DM

(18% prevalence) was related to all lens changes, especially at younger ages. Another study by Srinivasan *et al*[33] found, for diabetics, the cumulative incidence of cataracts is much higher than that of progression. Moreover, they indicated that the main risk factor for cumulative incidence and progression of most types of cataract is age, with higher rates of both in older patients.

TIMING OF SURGERY

Approaches to the timing of cataract surgery in diabetic patients seem to be changing worldwide. Where once a more conservative approach was applied, now there is a growing tendency toward early surgery. Pollack *et al*[34] reported that the main cause of poor visual outcomes is macular edema (ME). For this reason, they do not recommend cataract extraction for eyes with DR until visual acuity has deteriorated to 20/100–20/200. Similarly, Schatz *et al*[35] stated that diabetic patients with cataracts might wish to postpone surgery, especially if there is any retinopathy present preoperatively.

The growing tendency toward earlier cataract surgery in patients with diabetes has contributed to improved visual outcomes[36]. This approach facilitates panretinal photocoagulation (PRP) and also allows for the identification and adequate treatment of diabetic macular edema (DME) before cataract surgery. In addition, if surgery is undertaken before lens opacities make it more difficult to detect retinal thickening using macular assessment, then risk of ME decreases and visual outcomes may be considerably improved[37].

PREOPERATIVE EVALUATION

Preoperative counseling is crucial for diabetic patients. Before surgery, patients should have good glycemic control and no evidence of ocular or periocular infection. Transient refractive changes related to morphologic and functional changes in the crystalline lens should be observed during periods of unstable blood sugar[38]. Hyperglycemia induces myopia and, when intensive medical therapy is applied, patients tend to become more hyperopic as opposed to hyperglycemia. Changes in corneal topographic parameters during periods of glycemic changes can be a potential source of error in keratorefractive and biometric calculations[39].

A thorough and comprehensive ophthalmologic examination-including an assessment of bestcorrected visual acuity (BCVA) and relative afferent pupillary defect; using slitlamp biomicroscopy to assess the corneal health and neovascularization of the iris (NVI); and using tonometry, dilated fundoscopy, and gonioscopy for the evaluation of neovascularization at the angle-is mandatory. In select cases, advanced diagnostic evaluations such as fluorescein angiography, optical coherence tomography (OCT), and Bscan ultrasonography may be helpful. Due to the range of diabetic anterior segment changes, an experienced surgeon will perform better[40].

Consultation with vitreoretinal subspecialists is recommended by some authors, especially in complicated cases[41]. PRP is recommended preoperatively in patients with pre-existing proliferative diabetic retinopathy (PDR), because of its possible rapid progression after cataract surgery. In situations where lens opacity precludes PRP, it can be performed after surgery. Another approach is preoperative panretinal cryopexy or combined cataract surgery with vitrectomy and endolaser photocoagulation, particularly in cases with posterior pole tractional retinal detachment (TRD). ME should be efficiently treated preoperatively, since pre-existing maculopathy may worsen postoperatively and is strongly associated with a poor visual outcome[42].

Treatment options for ME are laser photocoagulation, pharmacotherapy with intravitreal injections of antivascular endothelial growth factor (anti-VEGF) agents, or steroids[43,44]. Because preexisting DME can increase the risk of ME progression by 20%–50%, intravitreal antiVEGF agents are recommended perioperatively[45,46]. Steroids, on the other hand, have been shown to be effective for persistent or refractory DME[47]. Dexamethasone implants and fluocinolone implants resulted in significant improvement in clinically significant ME and visual outcomes[48,49]. It has also been shown that dexametason has a potentially lower risk of intraocular pressure elevation and cataract formation compared to fluocinolone acetonide and triamcinolone acetate[50]. Recently, preoperative use of nonsteroidal antiinflammatory drugs, such as diclofenac and nepafenac, has been examined. Most studies suggested that they did not reduce the chances of postoperative ME in patients with DR[51-54].

Patients with NVI also need prompt treatment, including PRP. In patients who develop neovascular glaucoma (NVG), medical therapy is the first line of defense, however, it is usually ineffective. Eyes with active NVI are at greater risk for intraoperative and postoperative complications. Anti-VEGF agents such as bevacizumab showed dramatic short-term responses in terms of intraocular pressure reduction and regression of neovascularization in the treatment of NVG[55,56]. Cataract surgery after administering anti-VEGF agents should be done with or without vitrectomy as early as possible to enable treatment of the posterior segment. When NVG is a problem, a combination of trabeculectomy with phacoemulsification may also be considered after regression of NVI. Despite all these options, the visual outcomes following phacoemulsification in eyes with NVG are generally poor.

CATARACT SURGERY IN DIABETIC PATIENTS

Cataract surgery in diabetic patients yields better results since the introduction of phacoemulsification, when compared to extracapsular or intracapsular cataract surgery[57,58]. Different options are available during surgery that can lead to better surgical results and improved postoperative retinopathy evaluation. As anterior capsular phimosis is more common in diabetic eyes, capsulorhexis size should be larger than normal but smaller than the intraocular lense (IOL) optic diameter, in order to prevent anterior IOL displacement and posterior capsular opacification (PCO)[59-61]. However, a large diameter optic is also important for the postoperative diagnosis and treatment of peripheral retinal pathology[8].

Progression of retinopathy after cataract surgery is another problem in diabetic patients[62]. The duration and complexity of cataract surgery are the main risk factors for progression of retinopathy[63]; it is therefore important to reduce the time and complexity of the surgery. Poor pupillary dilatation can be seen in diabetic patients as the result of damage to pupillary parasympathetic supply and elevated prostoglandin levels[64,65]. This means that pupil dilation is also a problem for these patients. As such, iris hooks, malyugin rings, or other iris expanders should be considered for intraoperative use. In cases with NVI, bleeding in the anterior chamber during or after surgery should also be kept in mind. Photic retinopathy during cataract surgery, especially surgeries of a longer duration, was also more prevalent in diabetic patients than nondiabetics[66].

While the presence of DM does not increase complications such as posterior capsular rupture, zonular dehiscence, or vitreous loss, the effect of DM on the entire eye can result in other problems. The effects of DM on the ocular surface include neurogenic effects (subbasal nerve abnormalities) and impaired corneal stem cell and epithelial cell division, which can result in keratoepitelyopathy and lead to corneal epithelial defects/abrasions, which may heal slowly[40,67]. It has also been shown that corneal endothelial cell loss is higher in people with diabetes than in nondiabetics[68-70]; this means that routine evaluation of diabetic patients using specular microscopy is recommended. Moreover, surgeons should take greater care in order to reduce endothelial stress during surgery.

INTRAOCULAR LENS CHOICE

The most common problem for diabetic patients is DR. For this reason, optimal visualization and treatment of the retina should be kept in mind during cataract surgery. As the diameter of the lens increases, it will provide a larger optical area-a difference that may be crucial for optimal management of DR.

PCO is another concern following cataract extraction. It has been reported that the development and severity of PCO is increased in DM patients as compared to non-diabetic patients[60,71]. Several studies have shown a relationship between the development of PCO and lens material type, and that the shape of the lens[72]. A square edge design seems to inhibit lens epithelial cell proliferation and may therefore prevent PCO formation[72].

Several studies have evaluated the biocompatibility of three common materials used to manufacture foldable IOLs with diabetic patients. One performed a comparison between hydrophobic acrylic and plate-haptic silicone IOLs in diabetic patients; although PCO developed less frequently with hydrophobic acrylic IOLs, it was demonstrated that this material was associated with a higher risk of anterior chamber flare in the early postoperative period[72]. In addition, hydrophobic acrylic lenses have the lowest propensity for silicone oil adhesion, meaning that they may be the IOL of choice for diabetic patients. Because diabetic patients may need

vitreoretinal surgery during the course of managing their disease, silicone IOLs that develop condensation during pars plana vitrectomy may be relatively contraindicated in such individuals[73]. Hydrophilic acrylic IOLs are prone to opacification, particularly in patients with PDR, since elevated levels of phosphorus in the serum combined with the aqueous humor of diabetic patients may lead to opacification. Several reports have proved progressive calcific opacification of hydrophilic acrylic IOLs in diabetic patients[74-77]. Rodríguez-Galietero *et al*[78] evaluated contrast sensitivity and color discrimination in diabetic patients and suggested that blue-light filtering IOLs do not cause chromatic discrimination defects, but that they may even improve color vision in the blue-yellow chromatic axis. Multifocal and accommodative IOLs in people with diabetes are controversial. Postoperative laser treatment and fundus visualization during vitrectomy are difficult because of the optics of these types of lenses[79]. Additionally, the design of multifocal IOLs reduces contrast sensitivity and could be a cause of visual dissatisfaction for patients with preexisting maculopathy[79].

The implantation site in diabetic patients is also important. For DM patients, the ideal site is the capsular bag, as usual. The use of anterior chamber angle-fixated lenses and sulcus fixated posterior chamber IOLs in diabetic patients is controversial. It is recommended that iris claw lenses be avoided in patients with DM, due to the increased risk of iris neovascularization. The theoretical risk of cystoid ME, ovalization of the pupil, and poor mydriasis are other risk factors for diabetic patients after iris claw IOL implantation.

POSTOPERATIVE MANAGEMENT AND INDICATORS OF POOR VISUAL OUTCOMES

Carefully performed cataract surgery in diabetic patients should yield optimal postoperative results. Patient follow-up should also be done carefully. Preoperatively, patients diagnosed with NPDR who have adequate retinal view should undergo detailed retinal examination within three months of cataract extraction. Patients with PDR or those with inadequate retinal view prior to cataract extraction should be examined closely after surgery in order to evaluate their DR status[80].

Endophthalmitis is the most serious complication of cataract surgery. The risk of postoperative endophthalmitis in diabetic patients has increased and is associated with a poor visual prognosis.

As previously mentioned, as a patient's age and duration of diabetes increases, there is greater prevalence of corneal epithelial defects and persistent erosions due to impaired corneal innervation[40,68]. Corneal endothelial cell damage and persistent corneal edema in diabetic patients following cataract surgery have also increased[81,82]. Specular microscopy should therefore be used to evaluate DM patients and all the necessary precautions should be taken intraoperatively. Also more frequently observed in diabetic patients are severe iritis, posterior synechiae, pupillary block, and pigmented precipitates on the IOL[83].

The Early Treatment Diabetic Retinopathy Study (ETDRS) outlines the prognostic factors after cataract surgery. The presence of clinically significant macular edema (CSME) at the time of surgery was found to be a predictor of poor final BCVA in cases of uncomplicated phacoemulsification[84]. Another determinant of poor postoperative BCVA was the severity of DR at the time of surgery. As the severity of retinopathy increased, the risk of macular ischemia or edema also increased[36,58,85]. More severe retinopathy also correlated with a reduced tendency for spontaneous resolution of postoperative ME, which is itself associated with poor postoperative BCVA. PDR without any treatment prior to cataract surgery is another factor-one which comes with an increased risk of vitreous hemorrhage and TRD following surgery[86].

COMPLICATIONS

Despite the advancement in phacoemulsification technology, poor visual acuity following cataract extraction is still common in patients with DM. PCO, postoperative cystoid macular edema (CME), DME, and worsening of the DR are the main complications seen in diabetic patients[87].

PCO formation
PCO is one of the most common causes of decreased vision after cataract extraction. Although modifications in surgical technique and improvements in IOL technology have reduced the incidence of PCO, it is still a problem for these patients. Proliferation of lens epithelial cells and the degree of postoperative inflammation are

associated with development of PCO. Proliferation of lens epithelial cells is affected by several factors, including optic edge design, optic-haptic junction, and IOL material. However, surgical trauma and contact with the IOL can induce inflammation and cause epithelial cells to produce cytokines, which induce collagen production and fibrous metaplasia[88].

While some studies revealed a higher incidence of PCO in diabetic patients[60,89], others showed fewer cases of PCO in diabetic eyes, regardless of the retinopathy stage, over the course of two years[90]. In a study by Hyashi *et al*[91], the development of PCO was significantly higher in diabetic patients 18 mo after surgery, even though it was similar to the control group for the first 12 mo. Severity of retinopathy did not have an impact on the development of PCO, according to some studies[92]. Figure 1 demonstrates PCO development in a diabetic patient six months after cataract surgery.
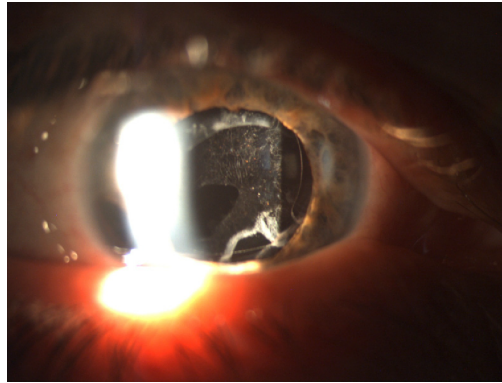
Macular edema

The development of DME, pseudophakic macular edema (PCME), CME, or Irvine-Gass syndrome are other frequent causes of postoperative vision deterioration among the general population[93,94]. Altered concentrations of angiogenic factors after cataract surgery may aggravate maculopathy[95]. OCT imaging has also revealed increased retinal thickness following an uneventful cataract surgery in diabetic eyes without retinopathy as compared to non-diabetic eyes[96]. Chu *et al*[93] reviewed 81,984 eyes and reported that, even in the absence of retinopathy, diabetic patients' eyes had an increased relative risk of ME after surgery. In addition, patients with preexisting DR had a higher relative risk of ME, with this risk being proportional to the increasing severity of retinopathy[93]. Figure 2 shows the development of CME in a diabetic patient after cataract surgery.

The incidence of CME varied between 0.2% and 20% in older studies. However, recent studies report lower rates of CME, ranging from less than 1% to 2%-3%[97]. The methods of detection used in these studies have a significant effect on the rate of CME detection. Fluorescein angiography and OCT were more sensitive, for example, reporting higher rates of CME than clinical detection[97]. It is also important to differentiate DME from PCME (Irvine-Gass syndrome), since the pathogenesis, treatment, natural course, and outcomes for both are very different. While the underlying presence of DR, exudates, and ME point toward DME, if there is minimal or no DR and there are no exudates in the posterior pole, this suggests PCME. When in doubt, fluorescein angiography can help to distinguish; if the angiography shows a petaloid pattern associated with hyperfluorescence of optic disc and there is no retinopathy or microaneurysms, edema may be considered as a result of Irvine-Gass syndrome[36].

According to Medicare data, the cost of cataract surgery and related patient care in the United States can be doubled due to ME[98,99]. Therefore, the prevention of CME in diabetic patients is very important. Recently, both prophylactic and therapeutic usage of both topical steroidal and non-steroidal anti-inflammatory eye drops (NSAIDs) has become central to perioperative management of CME in diabetic patients. Especially NSAIDs have been shown to decrease the incidence of CME in the general population. In a systematic review of 15 randomized trials, Kessel *et al*[100] showed that topical NSAIDs are more efficient in preventing CME than topical steroids. However, the use of NSAIDs did not change the incidence of CME in patients with DR[101].

In addition to facing a higher risk of CME, diabetic patients with preexisting DME are at an increased risk of worsening edema following cataract surgery[29,36]. In ETDRS Report 25, the presence of preexisting, CSME-though it showed no statistically significant difference in the prevalence of ME before and one year after surgery-was associated with worse visual outcomes[36]. Although ME is commonly seen after cataract surgery, it can follow a benign course. The development of postoperative CSME may be the result of the natural progression of the disease rather than a direct effect of surgery on many patients. On the other hand, the clinical course was quite different in eyes with CSME at the time of surgery. None of them resolved spontaneously within a year and the majority showed clinical and angiographic signs of deterioration. Dowler *et al*[37] have shown that CSME at the time of cataract surgery is associated with worse visual acuity outcomes at one year post-surgery. It seems possible that severe ME after cataract surgery represents a postoperative deterioration of pre-existing ME that was previously untreated because of lens opacity[36].

Attempts to stabilize and resolve DME will help improve outcomes, if DME is present prior to cataract surgery. Many strategies for the preoperative medical management of DME are available. Postoperative laser photocoagulation for diabetic ME is controversial. The ETDRS established the utility of focal/grid laser photo-coagulation for the treatment of ME[102]. Focal/grid laser treatment (as described in the ETDRS) was considered as first line treatment for CSME, prior to the use of anti-VEGF

Figure 1 Anterior segment photograph of a diabetic patient who developed posterior capsular opacification six months after phacoemulsification surgery.

agents for central involved DME. It remains an alternative treatment in cases in which anti-VEGFs are not applicable or the center of the macula is not involved[103]. On the other hand, Pollack *et al*[34] and Dowler *et al*[37] showed that ME resolves spontaneously if it arises postoperatively but not when it is present preoperatively. They suggested that early laser treatment is unnecessary for all cases of postoperative DME. Generally, experts do not perform argon laser treatment until six months after cataract surgery.

The advent of anti-VEGF injections has shifted the paradigm in the treatment of DME. Many studies performed on anti-VEGF agents in diabetic patients have shown their effectiveness at preventing and treating CSME[104-111]. Current opinion supports that anti-VEGF agents are first-line therapy in preoperative treatments, perioperative stabilization of DME, and postoperative management and that they show great success in anatomic recovery and visual function. Focal laser treatment and steroid injections still provide significant additional support.

Progression of retinopathy
Numerous studies have evaluated the effect of cataract surgery on the progression of DR. The progression of DR after intracapsular (ICCE) and extracapsular (ECCE) cataract extraction has been extensively studied[80,112,113]. Sebestyen *et al*[112] and Alpar *et al*[113] demonstrated the progression of retinopathy after ICCE and ECCE, with ICCE showing worse results than ECCE. However, the effect of phacoemulsification is controversial. Modern phacoemulsification procedures are considered faster, safer, and more cost-effective than ICCE and ECCE[114]. Even with the advances in modern phacoemulsification techniques, some studies have demonstrated a similar trend of DR progression after phacoemulsification surgery; others have reported no significant change[37,42,115]. Figure 3 shows the progression of DR in a diabetic patient's right eye after phacoemulsification surgery.

Prospective studies by Dowler *et al*[37] and Squirrell *et al*[42] have reported that uncomplicated cataract extraction using phacoemulsification have no effect on the progression of DR. However, Squirrell *et al*[42] have shown an increased risk of DR progression following cataract surgery in patients with elevated hemoglobin A1c. These studies included one eye that underwent phacoemulsification surgery and one eye as a control. Conversely, some studies that included diabetic patients undergoing phacoemulsification cataract surgery showed a retinopathy progression rate that had nearly doubled at the 12-mo period as compared to unoperated eyes[84]. Similarly, ETDRS Report 25, which enrolled 3711 patients with a nine-year follow-up period, also showed increased rates of retinopathy progression in cases of phacoemulsification than in unoperated eyes[36]. A recent study by Denniston *et al*[116] reported significant postoperative progression of center involving DME, which was associated with the preoperative grade of DR. Shah *et al*[41] found that recent studies do not support the generalized conclusion that phacoemulsification causes progression of retinopathy and ME in all diabetic patients.

The risk factors for DR progression have also been investigated. In a retrospective study by Krepler *et al*[115], these included being male, the disease duration, and poor glycemic control. Dowler *et al*[37] reported that a smaller incision size and shorter surgical duration for phacoemulsification decreased inflammation and may induce less breakdown of the blood–ocular barrier, meaning that uncomplicated phacoemulsification cataract surgery does not accelerate DR progression. Additionally, recent studies suggest that anti-VEGF injections may also affect the incidence of DR

Figure 2 Horizontal optical coherence tomography scan of a diabetic patient, showing the development of cystoid macular edema and serous macular detachment after cataract surgery.

progression[117]. Despite no current consensus on the prophylactic use of anti-VEGF, their use for patients with more advanced NPDR or PDR and DME should be considered. Other ocular co-morbidities such as vitreous hemorrhage, epiretinal membranes, or TRD may benefit from a combined pars plana vitrectomy and cataract surgery[118].

CONCLUSION

As the number of people with DM is estimated to continue to increase, cataract surgery will remain important for diabetic patients. Patients with diabetes have multiple issues to be evaluated preoperatively, perioperatively, and in the postoperative period. With the advent of modern surgical and pharmacologic therapies, these patients can, like other cataract patients without diabetes, recover excellent vision. Postoperative monitoring and management of surgical complications will also help to alleviate the risk of vision loss in these patients.

Figure 3 Colored fundus photographs of a diabetic patient reveal the progression of diabetic retinopathy in the right eye after surgery. A, B: Both eyes have dot-blot hemorrhages and hard exudates; two months after surgery, massive preretinal hemorrhages occurred in the right eye (A). A: Right eye; B: Left eye.

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Crosstalk between gut microbiota and antidiabetic drug action

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Abstract

Type 2 diabetes (T2D) is a disorder characterized by chronic inflated blood glucose levels (hyperglycemia), at first due to insulin resistance and unregulated insulin secretion but with tendency towards global spreading. The gut microbiota is recognized to have an influence on T2D, although surveys have not formed a clear overview to date. Because of the interactions between gut microbiota and host homeostasis, intestinal bacteria are believed to play a large role in various diseases, including metabolic syndrome, obesity and associated disease. In this review, we highlight the animal and human studies which have elucidated the roles of metformin, α-glucosidase inhibitors, glucagon-like peptide-1 agonists, peroxisome proliferator-activated receptors γ agonists, inhibitors of dipeptidyl peptidase-4, sodium/glucose cotransporter inhibitors, and other less studied medications on gut microbiota. This review is dedicated to one of the most widespread diseases, T2D, and the currently used antidiabetic drugs and most promising new findings. In general, the gut microbiota has been shown to have an influence on host metabolism, food consumption, satiety, glucose homoeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in cardiovascular diseases, colon cancer, rheumatoid arthritis, T2D, and obesity. Therefore, the main effect of antidiabetic drugs is on the microbiome composition, basically increasing the short-chain fatty acids-producing bacteria, responsible for losing weight and suppressing inflammation.

Key words: Type 2 diabetes; Gut microbiota; Metformin; α-glucosidase inhibitors; Glucagon-like peptide-1 agonists; Peroxisome proliferator-activated receptors γ agonists; Dipeptidyl peptidase-4 inhibitors; Sodium/glucose cotransporter inhibitors

Core tip: Gut microbiota was found to have an influence on host metabolism, food consumption, satiety, glucose homoeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in cardiovascular diseases, colon cancer, rheumatoid arthritis, type 2 diabetes, and obesity. Therefore, the main effect of antidiabetic drugs is on the microbiome composition, basically increasing the short-chain fatty acids-producing bacteria, responsible for losing weight and suppressing the inflammation.

INTRODUCTION

Over the last few decades, diseases related to metabolic processes, such as type 2 diabetes mellitus (T2D), obesity, dyslipidemia, hypertension and cardiovascular diseases (CVD) have become the main health problems around the world[1]. T2D is a disorder characterized by chronic inflated blood glucose levels (hyperglycemia), at first due to insulin resistance and unregulated insulin secretion but with a tendency towards global spreading. Environmental and genetic factors' contribution are known, but sedentary life style and dietary habits are not the least constituents. Influence of gut microbiota on T2D is also recognized[2-4], although findings differ between surveys. Because of the interactions between gut microbiota and host homeostasis, gut bacteria are thought to play a great role in diseases, including metabolic syndrome[5-7].

The composition and richness of the gut microbiota is modulated by diet, host health, age, ethnicity and genetics, and thus are unique and highly variable among individuals[3,4]. Turnbaugh *et al*[8] suggest that there is a "core gut microbiome" that could be responsible for proper gut functioning. That core gut microbial profile predominantly consists of bacteria, which belong to the Gram-positive Firmicutes and the Gram-negative Bacteroidetes[5]. Nevertheless, the increase of intestinal Firmicutes/Bacteroidetes ratio is observed in both obesity and during consumption of energy-rich diets in humans and animal models[5,9,10]. Similar to obesity outcomes, T2D induces a dysbiosis, mainly by reduction in butyrate-producing bacteria[11,12] and in *Akkermansia muciniphila*, which is now considered a biomarker for glucose intolerance[4].

The bacterial phylotypes found to be correlated with weight are associated with the phyla Firmicutes (2 families and 11 genera), Bacteroidetes (1 family and 2 genera) and Tenericutes (1 family and 1 genus)[3,13]. Among them are five genera affiliated with an increase in weight, including *Erysipelotrichaceae incertae sedis*, *Marvinbryantia*, *Roseburia*, *Candidatus arthromitus*, and *Parabacteroides*[3,13]. The phylotypes associated with weight loss were of the genera *Lactobacillus*, *Turicibacter*, *Anaerostipes*, *Coprococcus*, *Blautia*, *Oscillibacter*, and *Clostridium*[3,13]. For instance, Vrieze *et al*[14] showed that obesity was associated with modifications in the abundance, diversity, and metabolic function of the gut microbiota, mostly represented as a higher quantity of Firmicutes and a reduced abundance of Bacteroidetes in animal experiments.

Furthermore, one main function of the gut microbiota is to devastate nondigestible carbohydrates into short-chain fatty acids (SCFAs), mostly propionate, acetate and butyrate[15]. Lines of evidence have suggested that intestinal microbiota and SCFAs exert positive effects on glucose-lowering agents in T2D. Glucose-lowering agents can also alter gut microbiota[16], thus meliorating glucose metabolism and energy balance; they also have an influence on the production of SCFAs, thereby providing beneficial effects[12]. Perhaps mechanisms may also affect gene expression, levels of inflammatory cytokines, and the regulation of SCFA synthesis. Furthermore, gut microbiota may attenuate side effects caused by glucose-lowering agents, which is an advantage for diabetic patients. It has even been suggested that human gut microbiota express some enzymes which are capable of binding to and transforming a wide spectrum of bioactive substances[17,18].

Orally-taken medicines reach the gastrointestinal tract and encounter the intestinal microbiota. It has been shown that microbiota-encoded enzymes have the ability to metabolize xenobiotics and to impact the pharmacogenetics of drugs and their

bioavailability[19,20]. Accordingly, the gut microbiota may have an influence on drug effectiveness.

Moreover, supplementation with probiotic strains and their combination with nutraceuticals has been demonstrated to provide health benefits in obesity and associated diseases in both animal[21,22] and human studies[23-25].

In this review, we will focus on gut microbiota alterations in obese and T2D patients and its response to currently used antidiabetic drugs (Figure 1). Below, we highlight the animal and human research that has begun to elucidate the role of metformin (1,1-dimethylbiguanide hydrochloride), alpha-glucosidase inhibitors (α-GIs), glucagon-like peptide-1 (GLP-1) agonists, peroxisome proliferator-activated receptors (PPARs) activators, inhibitors of dipeptidyl peptidase-4 (DPP-4) and sodium/glucose cotransporter (SGLT-2), and other less studied medications on gut microbiota.

GUT MICROBIOTA AND METFORMIN

Metformin is the most used nonmetabolizable compound from the biguanide class that patients take orally. It is currently the drug of choice recommended by the American Diabetes Association and the European Association for the Study of Diabetes. Metformin has blood glucose-lowering and insulin sensitizing effects and inhibits liver glucose production. Also, this drug modulates the incretin pathway by improving the expression of GLP-1 receptor in the pancreatic islets and raising plasma levels of GLP-1[5,26,27]. A recent study suggested that inhibition of mitochondrial glycerophosphate dehydrogenase, an enzyme in the glycerophosphate shuttle, could be the main system involved in the metformin-induced inhibition of gluconeogenesis[28].

Treatment with metformin also alters bile acid recirculation[6], suggesting that the primary actions of metformin could be in the gut[11]; however, the absorption of metformin mainly occurs in the small intestine. Moreover, T2D patient treated with metformin can experience improvement in their lipid levels, which would contribute to the reduction of chronic micro- and macrovascular complications. Most of metformin's pleiotropic effects are predetermined by adenosine monophosphate–activated protein kinase (AMPK) activation in the skeletal muscle and liver[29]. In addition, AMPK activation is known to upregulate autophagic activity through direct phosphorylation of unc-51-like kinase and Beclin 1, key molecules involved in the initiation of autophagy; consequently, metformin can magnify autophagy[30]. Autophagy is valuable for nutrient supply in the case of energy deficiency, has a significant impact on body metabolism, and is also essential for the proper turnover of organelles, such as mitochondria and the endoplasmic reticulum[28]. These organelles play critical roles in pancreatic β-cell physiology and insulin sensitivity. Although, a global increase in autophagic activity is likely to improve the metabolic profile under metabolic stress conditions[28,31], which might be related to attenuation of the chronic low-grade tissue inflammation associated with obesity[28,32].

Metformin treatment is accompanied by the enrichment of SCFA-producing bacteria, such as *Blautia*, *Bacteroides*, *Butyricoccus*, *Bifidobacterium*, *Prevotella*, *Megasphaera*, *Butyrivibrio*[33] or *Phascolarctobacterium*, and has positive effects on the Proteobacteria phylum as well as the Allobaculum and Lactobacillus genera[5,32]. Additionally, the composition of the phylum Verrucomicrobia in a high-fat diet (HFD)-Met-treated experimental group was notably raised. Metformin treatment was also shown to have an effect on the gut microbiota in mice on normal diet. Furthermore, the families *Rikenellaceae*, *Ruminococcaceae*, and *Verrucomicrobiaceae*, as well as *Alistipes spp.*, *Akkermansia spp.*, and *Clostridium spp.*, were found to be more abundant with normal diet plus metformin treatment than in the control experimental group[34].

At the genera level, an increase of Escherichia and a decrease of Intestinibacter has been detected in a metformin-treated group[33]. The number of positive connections among microbial genera, especially those within Proteobacteria and Firmicutes, was also found to be increased after 2 mo of metformin treatment[33]. After 4 mo of treatment with metformin, there were significantly larger increases in fecal concentrations of lactate and a trend toward a larger increase in fecal concentrations of succinate[33]. In addition, Shin *et al*[35] showed significant differences in the abundance of Firmicutes and Bacteroidetes and gut microbiota composition between metformin-treated and non-treated mice but only under HFD conditions. Correspondingly, Lee *et al*[34] observed that metformin caused a decrease in bacterial diversity in mice on HFD.

Recently, the degradation of mucin was reported in mice on HFD[36] and suggested

Figure 1 Crosstalk between metformin action and gut microbiota. GLP1: Glucagon-like peptide-1; GLP2: Glucagon-like peptide-2; LPS: Lipopolysaccharide; SCFA: Short-chain fatty acid.

as possibly related to metabolic disorders. After metformin treatment in female mice, but not in male mice, expression of the genes *MUC2* and *MUC5* in the small intestine became remarkably increased[34]. This is a beneficial effect of the drug, because gastrointestinal mucins produced by goblet cells protect the underlying epithelium from pathogens. Moreover, female hormones are known to exert a protective effect against metabolic disorders[37] and to be involved in lipid and glucose metabolism. Therefore, the observed differences in the gut microbiota between male and female mice during metformin treatment might be caused by differences in hormone levels, which might be associated with metabolic phenotypes[34].

Nevertheless, the abundance of *Akkermansia*, which are mucin-degrading bacteria, is positively correlated with the quantity of goblet cells. Studies have highlighted that metformin multiplies the number of goblet cells, irrespective of diet[5,35]. Another study showed that treatment with *A. muciniphila*, which was identified from enterotype gut microbiota, improved metabolic parameters[34,36]. Albeit, approximately 30% of patients report experiencing side effects with metformin, including diarrhea, nausea, vomiting, bloating, and lactic acidosis.

GUT MICROBIOTA AND ALPHA-GIs

The α-GIs are oral hypoglycemic antidiabetic drugs that postpone the digestion of carbohydrates such as disaccharides and starch in the small intestine, and which reduce postprandial hyperglycemia[5] and delay the absorption of glucose, thereby managing blood glucose levels and related complications. To this class belong acarbose, voglibose (also naturally occurring in Streptomyces) and miglitol. Thus, α-GIs alter the nutrient sources of bacteria by segregating complex carbohydrates.

Acarbose delays the enzymatic carbohydrates decaying in the small intestine and thereby diminish postprandial hyperglycemia. Clinical studies have shown that acarbose significantly enhances glycemic control and lowers known CVD risk factors, including triglycerides' levels, body mass index, insulin levels, and systolic blood pressure[38-40]. The prominent mechanisms for this cardiovascular protective function are only partially understood, but they can be attributed to the ability of acarbose to neutralize oxidative stress by increasing H2 production in the gastrointestinal tract[38,41]. Panwar *et al*[42] found that Lactobacillus strains exert effects of glucosidase-inhibitors and regulate blood glucose responses to carbohydrates *in vivo*. As mentioned above, SCFAs play a crucial role in diabetes. In individuals with impaired glucose tolerance, acarbose was found to increase serum butyrate levels. The underlying mechanism for this effect might be that acarbose increases the fermentation of insoluble fibers in the colon. Interestingly, oral supplementation of

butyrate was found to improve insulin sensitivity and increase energy expenditure by enhancing mitochondrial work in mice[38,43]. To minimize the known gastrointestinal side effects of acarbose (*e.g.*, flatulence, diarrhea, or abdominal cramps), the drug was administered in small proportions. Zhang *et al*[39] compared treatment for T2D with acarbose and metformin and showed that both treatments notably increased GLP-1 concentration and decreased glucagon after 24 wk. However, additional benefits of acarbose besides its antidiabetic effect remain unknown.

Acarbose is effective in lowering blood glucose level in patients with T2D by delaying the digestion of complex carbohydrates through the inhibition of pancreatic α-amylase and a variety of α-glucosides[38]. Later, microbiota will ferment these carbohydrates, which will alter the composition of the intestinal microbiota. The features of gut microbiota in patients with prediabetes (before treatment) are genera abundance of Bacteroides (belonging to Bacteroidetes) and Faecalibacterium (belonging to Firmicutes). The most plentiful phyla include Firmicutes (68.53% of all reads), Bacteroidetes (27.85% of all reads), Proteobacteria (1.98% of all reads), and Actinobacteria (0.98% of all reads)[38].

After the treatment with acarbose, five genera, including Lactobacillus and Dialister, flourished. In response to acarbose, *Lactobacillaceae*, *Ruminococcaceae* and *Veillonellaceae* increased and six genera, including *Butyricicoccus*, *Phascolarctobacterium* and *Ruminococcus*, decreased. Likewise, many of the operational taxonomic units that greatly increased in response to acarbose belong to SCFA-producing taxa, such as *Faecalibacterium*, *Prevotella*, and *Lactobacillus*[38]. Some species of *Megasphaera* also thrived following acarbose treatment. They can transform carbohydrates into SCFAs, including butyrate, formate, acetate, valerate, and caproate, a process which is valuable for Lactobacillus development[38]. Consequently, the effect of acarbose on body weight might be related to reorganized microbiota structure. It is supposed that SCFAs such as acetate, butyrate and propionate and their concentrations are prognostic of lifespan[38]. Thus, the increased levels of SCFAs in acarbose-treated mice may lead to the beneficial effect on the lifespan[44]. However, studies have shown that the weight loss in female mice on acarbose was more dramatic than in males, while the longevity effect is much stronger in males[45].

Another α-GI, voglibose, changes dysbiosis in diet-induced obese mice[46]. These changes could increase the production of bile acid metabolites and have an advantageous systemic outcome. Specifically, scientists have found favorable effects of voglibose on several cardiovascular end-points, as it improves glycemic control in mice with cardiac overpressure[47]. Voglibose has anti-obesity effects on diet-induced obese mice. Possibly, the effects of incretins in voglibose, activation of neuroendocrine linked to leptin, and inducement of the genes responsible for magnified energy metabolism cause the reduction in energy intake and improvement of mitochondrial function[48]. The reduction in food intake is possibly derived from increased GLP-1 levels due to voglibose supplementation or from direct modulation of hypothalamic genes which lead to the satiety response.

Miglitol shortens the intestinal transit time and suppresses histological and molecular markers of inflammation, for which concentrations are elevated by a high-fat and high-glucose diet and which shifts with the increases in *Erysipelotrichaceae* and *Coriobacteriaceae* induced by the energy-rich diet[49]. Miglitol is able to alter human gut microbiota because of the transit time reduction.

The development of nonalcoholic steatohepatitis (NASH) could be dependent on the gut environment as well. As α-GIs change the gut environment, they might also protect against NASH development, because of its sensibility to changes in the gut environment[49]. NASH is characterized by hepatocellular lipid accumulation along with inflammation and fibrosis that is a precondition for oxidative stress, inflammatory cytokines, and endotoxins. There is an essential need for therapeutic interventions considering that NASH can lead to cirrhosis and liver cancer. In addition, acarbose was also demonstrated to have a protective effect against NASH development in HFD-induced obese rats[50]. However, the underlying mechanisms should be further investigated.

Miglitol was shown to restrain the accumulation of lipid droplets and inflammatory cell infiltration, and to lead to a decrease in the numbers of ballooning hepatocytes as well as to stoppage of the activation of stellate cells, which plays a role in liver fibrosis[49].

The administration of an α-GI has been found to increase the levels of butyric acid in the intestines of healthy individuals. Indeed, the administration of butyric acid was demonstrated to suppress intestinal inflammation in mice[51]. These findings suggest that miglitol administration increases the butyric acid level in the intestine and suppresses colon inflammation.

Human gut bacterium *Blaubia* (*Ruminococcus*) *obeum* expresses enzymes, such as α-glucosidases (Ro-αG1), which have specific crystal structures with free active site(s) to

bind and interact with volatile substrates. Therefore, the proposed theory is that α-GIs (acarbose, voglibose, miglitol) can affect the bacterial Ro-αG1 in human gut and exert positive effects or create adverse gastrointestinal symptoms[52]. The α-GIs bind to the active site of Ro-αG1 and change the enzyme's activity. Acarbose was found to slightly inhibit the gut bacterial α-glucosidases as well as other currently used α-GIs.

GUT MICROBIOTA AND GLP-1 AGONIST

Intestinal endocrine cells (L cells) respond to food ingestion by secreting GLP-1, an incretin hormone[53]. This hormone can intensify glucose-induced insulin from pancreatic β-cells and suppress glucagon secretion; in addition, it can protect pancreatic β-cells from apoptosis and promote β-cell proliferation. Together, incretins are liable for 50%–60% of postprandial insulin secretion. In addition, GLP-1 plays critical roles in gastrointestinal motility as well as in metabolism; moreover, GLP-1 can possibly suppress gastrointestinal motility, thus affecting the absorption of digested food[54]. However, the natural GLP-1 is degraded rapidly, primarily through enzymatic destruction by DPP-4. Therefore, another pharmaceutical approach to treat T2D is to increase GLP-1 function, either by the administration of GLP-1 peptide mimetics or suppressing its degradation by DPP-4. GLP-1 expression could be stimulated by binding of SCFAs and secondary bile acids (lithocholic acid and deoxycholic acid) with the G-coupled protein receptor FFAR2 (formerly GPR43)[53]. Many studies have shown that satiety and glucose homoeostasis are modulated by the gut microbiota that induces the secretion of GLP-1[55,56].

The body weight control induced by GLP-1 is maintained by reduced food intake and inhibition of appetite and gastric emptying[57]. However, restricted dietary intake supplemented with GLP-1 results in more significant weight loss. Interestingly, the microbial diversity after GLP-1 increment seemed to be dependent on the glycemic state of the mice studied. In normoglycemic mice treated with liraglutide and saxagliptin, bacterial variety significantly decreased, while in transiently hyperglycemic mice it rose to the normal level[13]. Of late, scientists have proposed liraglutide as a prospective anti-obesity drug because of its additional impact on weight loss in obese and diabetic individuals. The daily injection of liraglutide has been shown to significantly improve glucose tolerance and insulin tolerance in diabetic rats rather than in nondiabetic rats. Although, it was found to alter the microbial composition in both simple obese and diabetic obese rats. The genera *Candidatus*, *Roseburia*, *Arthromitus* and *Marvinbryantia* may promote weight gain, while the genera *Coprococcus* and *Lactobacillus* are associated with weight loss[58].

Liraglutide administration has been shown to decrease the relative abundance of all of the obesity-related phylotypes (such as *Romboutsia*, *Ruminiclostridium*, and *Erysipelotrichaceae*) and to enrich the lean-related genera *Blautia* and *Coprococcus*[59]. After liraglutide intervention, the abundance of Firmicutes was also found to tend towards decrease in obese rats, whereas the finding was contrary when the study was carried out in human volunteers under field conditions without restriction[60]. Patients with long duration of T2D show a significantly reduced *Akkermensia* variety. After comparison of the gut microbiota of subjects receiving a GLP-1 agonist and metformin, higher *Akkermansia* abundances were detected in the liraglutide-treated patients[61]. At first, the genus *Akkermansia* and some genera in the family *Christensenellaceae* increased prominently under liraglutide, unlike that seen under metformin, with the latter of which leading to a greater expansion of *Dorea* and *Sutterella* genera.

GLP-1 receptors are placed on neurons innervating the portal vein, on β cells of the pancreas, and the central nervous system[62]. GLP-1 after its release can affect afferent neurons innervating the gastrointestinal tract which signal to the caudal brainstem or enteric neurons, and/or they can enter the circulation to functionate centrally, or on peripheral targets to regulate metabolic disorders[59,63]. Thus, the weight-loss and glucose-controlling effects of liraglutide are possibly mediated by the gut–brain axis. In addition, the GLP-1 analog liraglutide reduces visceral hypersensitivity and acts as a sort of pain-killer[64]. ROSE-010, another GLP-1 analog, has been shown to diminish visceral pain in patients suffering from irritable bowel syndrome[65].

Long-term HFD intake has been shown to result in a lack of energy substrates, reduced acetylcholine synthesis, membrane deterioration and oxidative stress, and consequently is valid in intestinal myenteric neurons loss in mice[66]. Grasset *et al*[67] revealed that gut microbiota dysbiosis causes the loss of enteric neurons, attenuated nitric oxide production and following GLP-1 resistance. Nitric oxide produced by nuclear nitric oxide synthase has been generally expected to have a protective effect on enteric neurons that is greater than its damaging effect. The GLP-1 secretagogue l-

arginine oral administration has also been shown to improve glucose tolerance by influencing GLP-1R signaling[68]. L-arginine is a substrate of nuclear nitric oxide synthase which improves GLP-1 sensitivity in HFD-fed mice and increases the glucose-induced insulin secretion. In consequence, l-arginine supplementation could have beneficial effects on postprandial GLP-1 response and GLP-1 sensitivity in patients with T2D.

GUT MICROBIOTA AND DPP-4 INHIBITORS

Protein CD26 present on the lymphocyte cell surface was first described to have a proteolytic activity in 1966 and was later named as DPP-4. The potential substrates of DPP-4 activity are gut hormones, like incretins, GLP-1 and gastric inhibitory polypeptide, neuropeptides, chemokines, and dietary proteins[69]. Through the cleavage of key hormones and peptides, the DPP-4 activity influences behavioral, intestinal and metabolic disorders[70]. DPP-4 can enhance the agonistic activity of gut hormones, like neuropeptide Y and peptide YY (PYY), by cleaving off the N-terminal dipeptide. As PYY has an influence on the ileal and colonic brake of digestion and on the induction of satiety *via* activation of hypothalamic Y2 receptors, these effects could be enhanced by DPP-4[71]. However, the capacity of DPP-4 in the regulation of satiety has not yet been fully elucidated.

An experiment on Dpp-4 knockout mice showed an increased GLP-1 level and improved glucose tolerance associated with it. This effect was achieved in humans by administration of DPP-4 inhibitors, antidiabetic agents that maintain incretins in their active form[72]. Moreover, Ahmed *et al*[73] revealed that overweight and obese patients, in comparison to normal-weight patients, have increased DPP-4 activity, which decreases the activity of GLP-1. The administration of prebiotics led to decreased DPP-4 activity, which was explained by double concentration in the active form of GLP-1[74].

Some studies have acknowledged that some commensal bacteria, such as *Prevotella* or *Lactobacillus*, can express human DPP-4 homologs[70,75]. Two groups of scientists published findings of DPP-4 expression being higher in gnotobiotic mice colonized with feces of a lean subject that in germ-free mice, which strongly indicates that their intestinal microbiota produced DPP-4-like activity[70,76]. The overall literature affirms that DPP-4 encoded by the gut microbiota could compose an innovative mechanism to alter protein digestion, host metabolism, and behavior.

The novel type of DPP-4 inhibitors are sitagliptin, saxagliptin and vildagliptin, which are administrated orally. Sitagliptin increases insulin and suppresses glucagon secretion. In patients with T2D, therapies which include DPP-4 inhibitors promote healing of colitis and diminish depression symptoms. DPP-4 was proposed as a possible target for treating autoimmune diseases, including inflammatory bowel disease as it has an influence on the immune system, particularly on T cell function[77]. Obese dams with adverse pregnancy outcomes have been reported as having decreased *Lactobacillus spp*. compared with dams with normal litters. Secondly, maternal obesity and reduced fertility are related to bad pregnancy outcomes, gestational diabetes, and preeclampsia[78]. Sitagliptin or prebiotic consumption during pregnancy could normalize gestational weight gain, increase *Bifidobacterium spp*., reduce fasting glucose levels, and possibly alleviate pregnancy termination associated with maternal obesity while improving offspring metabolic health and composition of the intestinal microbiome[79].

Saxagliptin, another DPP-4 inhibitor, appears to act only on a small target group of gut microbes, mainly on the Firmicutes/Bacteroides ratio. It has been shown to enhance the development of the genus Lactobacillus within the class *Lactobacillaceae*, the genera *Allobaculum* and *Turicibacter* within class *Erysipelotrichaceae* and suppresses the genus Bacteroides within the class *Bacteroidaceae*, and the genus *Prevotella* within the class *Prevotellaceae*. As compared to the GLP-1 agonist liraglutide, saxagliptin reduced the enrichment of the genus Blautia and of the genus Coprococcus. Moreover, despite the fact of similar food intake reduction, saxagliptin had a neutral effect on body weight, with subjects on liraglutide having significantly lower weight regardless of their glycemic control[13].

Another DPP-4 inhibitor, vildagliptin, affects the gut microbiota composition and its metabolic activity. Vildagliptin has been shown to reduce the fasting blood glucose and HbA1c levels. In obese murine models, vildagliptin reduced *Ruminococcaceae*, such as the genera *Oscillibacter*, *Ruminiclostridium_6*, *Anaerotruncus*, and *Ruminococcaceae_UCG_007*, as well as the families *Planococcaceae*, *Christensenellaceae*, and *Prevotellaceae*. The enriched phylotypes were of the Streptococcaceae family, the genera Bacteroides, and the family Bacteroidaceae. In general, it modified the

Firmicutes/Bacteroides ratio, reduced DPP-4 activity in the portal vein and increased the concentration of active GLP-1, improving gastrointestinal function according to AMPs' expression restoration and the depth of the crypts in the ileum[80]. Vildagliptin also has a potential effect on inflammation, due to reduction of TLR and cytokine expression. Zhang *et al*[80] suggested that vildagliptin enriches SCFA-producing bacteria and ameliorates gastrointestinal health and could ultimately mediate their beneficial effects on the host, especially in diabetes.

The DPP-4 inhibitor PKF-275-055 improves glucose/cholesterol metabolism, decreases Firmicutes/Bacteroidetes ratio, and drops mass gain and mesenteric adipose accumulation. Moreover, mice on HFD with PKF-275-055 treatment showed enriched butyrate-producing *Rumminococcus* and of the acetogen *Dorea* compared to the control group[81].

GUT MICROBIOTA AND SGLT-2 INHIBITORS

The sodium/glucose cotransporters SGLT1 and SGLT2 are generally expressed in the small intestine and are regulated by a sodium gradient created by Na+/K+ ATPase. SGLT1 transports glucose and galactose across the apical membrane of enterocytes, whereas SGLT2, and at some extent SGLT1, reabsorbs glucose in the renal tubule. SGLT2-selective inhibitors are a new class of treatment for T2D[82]. SGLT2 differs from other antidiabetic medications because it ameliorates vascular function and thus has advantageous effects on CVD. Among the numerous T2D consequences, CVD is the most widespread and dramatic. Data characterize the gut microbiota as an important regulator of vascular function[83]. Indeed, people with diabetes have vascular dysfunction and heightened risk of CVD. Distinct signs of diabetes-related CVD are arterial stiffness, endothelial dysfunction, and vascular smooth muscle functional disorder. Reduction in endothelium-independent dilation is present in T2D patients and is a marker of cardiovascular complications.

After 8 wk of treatment with a selective SGLT2 inhibitor dapagliflozin, diabetic mice showed lower arterial stiffness and blood glucose level, and improvements in endothelial and vascular smooth muscle dysfunctions compared to nontreated diabetic mice. In addition, reductions in circulating inflammatory markers, such as MCP-1, IL-1β and IL-6, and hyperglycemia improvement were detected[84]. Animals with diabetes treated with dapagliflozin showed decreased Firmicutes/Bacteroidetes ratio and Oscillospira, and increased *Akkermansia muciniphila*.

The dual SGLT1/2 inhibitor has been shown to reduce blood glucose levels and HbA1c and to increase total GLP1 in mice fed a high-sucrose diet[85]. The higher doses of SGLT1/2 inhibitor accelerated body weight gain and increased Bacteroidetes and decreased Firmicutes quantity, but the *Akkermansia spp*. was not modified[82]. SGLT1/2 inhibitors or SGLT2-selective drugs like canagliflozin guarantee intestinal SGLT1 inhibition in T2D[86]. They enhance GLP-1 and PYY secretion and delay the glucose excursion after carbohydrate intake. The dual SGLT1/2 inhibitor LX4211/sotagliflozin, in clinical testing, has the same effects as the medicine described directly above[87]. However, SGLT1 cannot be completely inhibited as long as changed Na+ homeostasis and elevated colonic carbohydrates can lead to the opposite gastrointestinal effects and diarrhea.

GUT MICROBIOTA AND PPAR

The PPARs belong to the nuclear receptor family of regulatory factors that are ligand-activated transcription factors. There are two PPARγ isoforms: PPARγ1 and PPARγ2. They form a heterodimeric complex with the retinoid X receptor, which binds to PPAR-responsive elements and then regulates transcription. Its main functions are linked to maintaining homeostasis in the intestine, inducing adipocyte growth and differentiation[88], cellular apoptosis[89], regulation of genes involved in glucose and lipid metabolism[90], and inflammatory responses. PPARγ is expressed mainly in several tissues such as of the lungs, breast, ovaries, placenta and at most in the colon, where it regulates colonocyte metabolism and cell cycle[91].

PPARγ is considered to have anti-inflammatory effects and to be a molecular target for cancer chemoprevention[92]. It also enhances insulin sensitivity and regulates the genes involved in hypertension and contributing to atherosclerosis. There are lines of evidence indicating reduction of intestinal inflammation and colon cancer development and T2D[93] by specific PPARγ agonists.

Nepelska *et al*[91] engineered a colonic epithelial HT-29-PPARγ reporter cell line to control the influence of bacterial metabolites on transcriptional activity of PPARγ.

Two main metabolites of intestinal bacteria, butyrate and propionate, were linked to activation of PPARγ transcriptional activity. Notwithstanding, phylogenetic affiliation of the strains were found to not rigorously correspond to reporter gene activities, among them the most general stimulating effect was noticed for Firmicutes and Fusobacteria, while Actinobacteria exerted moderate or no modulation.

The strongest potential of PPARγ activation is exerted on *Roseburia hominis, Roseburia intestinalis* ,and *Fusobacterium naviforme*. These are well-known producers of butyrate, therefore the response pattern of PPARγ reporter cells is exposed to the composition of organic acids of conditioned media. Gene regulation in intestinal epithelial cells is known to be regulated by the SCFAs, especially butyrate[94]. Acetate negatively affected the PPARγ reporter system, demonstrating a reverse correlation. Moreover, butyrate and propionate stimulated PPARγ activity, even at such low concentrations as 0.5mM. Acetate, however, showed an insignificant activation starting from 8 mM, and lactate did not affect the activity but was cytotoxic from 2 mM, which lead to cell detachment[95]. In general, high concentrations of all organic acids, especially acetic and lactic, have a deleterious effect on the viability of cells, possibly because of decrease in pH.

Some species can activate the expression of PPARγ target genes even without presence of butyrate and propionate in their conditioned media. To them belong *Atopobium parvulum* and *Prevotella copri*. These bacteria were shown to increase ANGPTL4 and ADRP expression in HT-29 cells. Their underlying mechanisms are probably different, because stimulation with conditioned media of *A. parvulum* showed its influence after 6 h and *P. copri* in 12 h[91]. The induction of ANGPTL4 by the PPARγ-specific ligand troglitazone was weaker in either case. In addition, *A. parvulum* and *P. copri* promote PPARγ phosphorylation *via* ERK1/2[91]. Studies confirmed that bacterial upregulation of PPARγ in enteral epithelial cells occurs by phosphorylation[96]. Though, high levels of *P. copri* and *A. parvulum* in the intestine has been connected to arthritis[97] and linked to periodontitis[98] accordingly.

However, the elevated risk of cardiovascular ischemic events is affiliated with the use of such PPARγ ligands as rosiglitazone. PPARγ agonists are used in clinics despite the fact that they have serious adverse effects, such as heart failure, weight gain, and increased bone fracture[99]. At present, natural products could be found as a source of drugs[100].

Fish oil has established favorable effects in diabetes, CVDs, autoimmune inflammatory diseases, and inflammatory bowel diseases. Neschen *et al*[101] revealed advantageous effects of fish oil on glucose and lipid metabolism, improvement of insulin sensitivity, and reduction of triglycerides. Additionally, the n-3 polyunsaturated fatty acids of fish oil, eicosapentaenoic acid and docosahexaenoic acid, are endogenous ligands for PPAR; consequently, even small changes in their structures affect PPAR activation. These fatty acids adjust the insulin-sensitizing, anti-inflammatory and lipid-lowering properties of fish oil[102].

GUT MICROBIOTA AS A TREATMENT OPTION FOR T2D: FUTURE PERSPECTIVES

Over the past 10 yr, an increasing body of literature has suggested that the gut microbiota plays a crucial role in the host immune system, modulation of inflammatory processes, extraction of energy from the host diet, and alterations of human gene expression, and is considered to make an important impact on obesity/insulin resistance development. Several mechanisms that contribute to explaining the link between altered gut microbiota and pathogenesis of insulin resistance have been described[7]. They control the fermentation and absorption of dietary polysaccharides to produce SCFAs, which may explain their importance in the regulation of fat accumulation. SCFAs can stimulate the secretion of GLP-1 and GLP-2, thus increasing insulin and adiponectin expression, which might contribute to enhanced insulin sensitivity and pancreatic ß-cells proliferation[103,104]. Another mechanism by which the microbiome may contribute to insulin resistance is compromised gut barrier function with an increased intestinal permeability, accumulation of lipopolysaccharide, and metabolic endotoxemia development[105].

Lactobacillus and Bifidobacterium are commonly used as probiotics and are the most studied strains in the treatment and prevention of obesity-associated disorders[15]. Moreover, several potential bacterial candidates, such as *Saccharomyces cerevisiae var. boulardii*, *Parabacteroides goldsteinii*, *Enterobacter halii* or *Akkermansia muciniphila*, have been identified and innovative mechanisms of action overriding their beneficial effects for insulin resistance/obesity have been elucidated[106,107].

The abundance of *Akkermansia muciniphila*, which is a mucin-degrading bacterium

that resides in the mucus layer, has been found to be decreased in obese/T2D and inversely correlated with body weight in both rodents and humans[34]. Metformin treatment[35,36], consumption of oligofructose[108], dietary concord grape polyphenols[109], and gastric bypass surgery in humans[110] and mice[111] leads to a marked increase in *A. muciniphila* abundance with subsequent weight loss and reversed metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance[34].

F. prausnitzii plays an important role in preserving the gut barrier and controlling inflammation and T2D progression[112]. A traditional Chinese berberine-containing herbal formula given to T2D patients[112,113] changed the gut microbiota by increasing *F. prausnitzii*, which was negatively correlated with fasting blood glucose, HbA1c and postprandial blood glucose levels, and positively correlated with homoeostasis model assessment of β-cell function (commonly known as the HOMA-B).

Parabacteroides goldsteinii is a commensal bacterium with reduced level in HFD-fed mice. Oral treatment of HFD-fed mice with live *P. goldsteinii* reduced obesity and was found to be associated with increased adipose tissue thermogenesis, enhanced intestinal integrity, and reduced levels of inflammation and insulin resistance[106].

Identifying the most important microbiota-related metabolic pathways could lead to the development of integrated strategies using new prebiotics or beneficial bacterial strains to prevent and treat these metabolic disorders in the near future[112].

CONCLUSION

This review is dedicated to one of the most widespread diseases, diabetes, and the currently used antidiabetic drugs and possible promising new findings in this field. The gut microbiota has been found to have an influence on host metabolism, food consumption, satiety, glucose homoeostasis, and weight gain. Altered intestinal microbiota composition has been noticed in CVDs, colon cancer, rheumatoid arthritis, diabetes, and obesity. Therefore, the main effect of antidiabetic drugs is thought to be on the microbiome composition, basically increasing the SCFA-producing bacteria responsible for losing weight and suppressing inflammation. Scientists have found that some drugs for T2D also elicit favorably effects on several cardiovascular end points and have anticancer as well as anti-aging effects. However, further detailed experimental and clinical investigations should be conducted to gain a deeper understanding of antidiabetic drugs' functions.

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Antidiabetic treatment on memory and spatial learning: From the pancreas to the neuron

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Abstract

The detrimental effects of constant hyperglycemia on neural function have been quantitatively and qualitatively evaluated in the setting of diabetes mellitus. Some of the hallmark features of diabetic encephalopathy (DE) are impaired synaptic adaptation and diminished spatial learning capacity. Chronic and progressive cognitive dysfunction, perpetuated by several positive feedback mechanisms in diabetic subjects, facilitates the development of early-onset dementia and Alzheimer's disease. Despite the numerous clinical manifestations of DE having been described in detail and their pathophysiological substrate having been elucidated in both type 1 and type 2 diabetes mellitus, an effective therapeutic approach is yet to be proposed. Therefore, the aim of this review is to summarize the growing body of evidence concerning the effect of current antidiabetic treatment options on diabetic and non-DE.

Key words: Memory; Spatial learning; Cognitive; Neural remodeling; Type 2 diabetes mellitus; Antidiabetic drugs

Core tip: In this review, we aim to create a concise overview of the effects exerted by hyperglycemia on neural tissue, while describing the potential of each antidiabetic drug to improve functional and cognitive capacity in subjects with diabetic encephalopathy.

INTRODUCTION

Diabetic encephalopathy (DE) is defined as a complex combination of central nervous system (CNS) structural and functional changes, stemming mostly from oxidative stress and chronic inflammation of the neural tissue in the setting of long-standing hyperglycemia. While several mechanisms have been proposed for the explanation of cognitive decline in diabetic subjects, the intricate interplay of various signaling pathways along with the numerous co-morbidities of patients with diabetes do not allow for a definite pathogenetic model to be proposed. Moreover, the patho-physiological substrate of type 2 diabetes mellitus (T2DM) encephalopathy appears to be different from that of T1DM DE[1]. Currently, despite the abundance of evidence of the subject, the molecular mechanisms implicated in the development of DE and its rate of progression have not been clarified, resulting in a subsequent lack of treatment options for interruption or reversal of the cumulative neuronal damage and functional decline of patients. The purpose of our review is to summarize and describe the interaction between the various antidiabetic substances and DE, in order to facilitate the possible development of a therapeutic algorithm for affected patients.

ENCEPHALOPATHY IN T2DM

Several studies in T2DM subjects have confirmed the dysfunction of cognitive capacity, both in executive and processing tasks, when compared to healthy controls[2-5]. While the decline of neural capacity in T2DM is described as a multi-factorial process, it is evident that tissue insulin resistance (IR) plays a pivotal role in the pathogenetic process. Insulin receptors are expressed in all major components of the CNS (neurons, microglia, astrocytes, oligodendrocytes and vascular system) in varying degrees. The downstream effects of insulin signaling in neural tissue include neurogenesis, apoptosis inhibition, cytokine release, attenuation of inflammatory response, vasodilation and glucogen uptake and storage[6]. While some researchers have proposed the possibility of de novo insulin synthesis in the CNS, current experimental data support the fact that the majority of centrally-acting hormone is produced at the pancreatic β-cells and subsequently transported through the blood brain barrier *via* the systemic circulation, with vascular endothelium significantly affecting the process[7]. The role of other peripherally-acting hormones such as glucagon-like peptide-1 (GLP-1), leptin or ghrelin on insulin transport and potency in the CNS has not been described so far. IR, defined as a dysfunction on any of the several stages preceding or during the signaling cascade activated by the insulin-receptor complex formation, can affect the homeostasis of all the processes described above that are mediated by the hormone.

ANTIDIABETIC TREATMENT AND NEURAL FUNCTION

Biguanides

The information surrounding metformin and its effect on cognitive impairment is contradictory and highly complex, varying between different types of test subjects and changing in accordance to different treatment dosages and pathophysiological substrates studied. On a cellular level, metformin exhibits pleiotropic effects, including interaction with multiple signaling pathways such as those of mitogen-activated protein kinases (MAPK) and mammalian target of rapamycin complex 1, that are closely linked to proliferation and apoptosis. Given the relative safety of the substance and its role in cellular turnover, the possibility of repurposing it for use in neurofunctional disorders is currently being investigated[8]. Chemical derivatives of metformin, such as HL271, induce comparable neuromodulatory effects, without any metabolic action, an indication that the drug effects may be only partially related to glucose homeostasis as is suggested in most of the experimental studies discussed on the following paragraphs[9].

Ou *et al*[10] designed an Alzheimer's disease (AD) model in an effort to elucidate the anti-neuroinflammatory properties of metformin. APPswe/PS1ΔE9 mice underwent treatment with the biguanide, resulting into overall neuroprotective effects, with attenuation of spatial memory impairment, neural cellular proliferation, decreased local inflammation (both inflammatory cells and cytokines) of the brain cortex and the hippocampal region, as well as, reduced amyloid-β plaque deposition. The study results were attributed to drug-induced altered regulation of AMPK, mTOR, ribosomal protein S6 kinase, p65 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways[10].

Type 1 and 2 diabetes, induced in animal models through streptozotocin and high-fat diet respectively, have been linked to aberrant hippocampal neuroarchitecture with accompanying inflammation. Long-term metformin administration was shown to have a positive effect on hippocampal neural proliferation and memory function, despite the achieved hypoglycemic effect, a pathway mediated through interaction with insulin receptor substrate-1 *via* adenosine monophosphate (AMP) -kinase phosphorylation cascade activation[11].

Following a similar pattern of beneficial neural effects, on a diabetic rodent model where both memory and spatial recognition where evaluated with passive avoidance tasks and Y maze spontaneous alternation tests, metformin administration appeared to reverse the diabetes-induced functional decline[12]. Passive avoidance assesses the capacity of test subjects to avoid certain choices linked to painful stimuli, by use of their previous memory of similar situations, while the Y maze trial recruits several neural compartments and reviews the tendency of a subject for exploring new pathways, a process inherently linked to cognition. The treatment-mediated effects were attributed to numerous metabolic effects including achievement of normoglycemia, upregulation of vascular endothelial nitric oxide production, attenuation of oxidative damage and increased anti-apoptotic potential.

On a study including subjects with non-dementia vascular cognitive decline with impaired glucose homeostasis, the efficacy of donepezil when combined with either metformin or acarbose was evaluated as to the possible achievement of functional improvement. Carotid artery intima-media thickness (CA-IMT), cognitive capacity and IR where assessed at baseline and at 12 mo. The metformin-donepezil group showed superiority in the functional tests administered, a fact that can be attributed to the slower CA-IMT increase and decreased IR indexes when compared to the acarbose group, allowing for better neural tissue perfusion and metabolic signaling, respectively[13].

One of the several pathogenetic mechanisms explored in relation to DE, among other neurodegenerative processes, is autophagy dysfunction, leading to tissue-accumulation of non-functional peptides, in the form of aggravates. Chen *et al*[14] attempted to elucidate the effect of metformin administration on the regulation misfolded polypeptide clearance, by treating diabetic mice with an eight-week regimen of intraperitoneal metformin and/or chloroquine. Neural capacity was evaluated by the Morris water maze (MWM) test, while the presence of aggravates or abnormal tissue architecture were examined by histological preparations and immunochemistry. Biguanide treatment had a positive overall effect with enhancement of autophagy, reduction of hyperphosphorylated tau proteins and improved cognitive functionality, when compared to the control group[14].

Different treatment regimens comprising of metformin and ursolic acid combined or as monotherapy, as well as gliclazide were used by Mourya *et al*[15] in rodents with metabolic and cognitive impairment due to chronic restraint stress (containment for 2 h/d for 30 d). A total of 60 subjects were subdivided into 10 groups according to treatment protocol, with several metabolic parameters relative to cardiovascular function and IRs were observed. Behavioral and neurological performances were assessed by the MWM test. While insulin sensitivity and cognition were improved in all treatment groups, the most marked anti-inflammatory and neuroprotective effects were produced by the combination of metformin with ursolic acid, suggesting the existence of a synergistic effect between the two[15].

Metformin-induced neuromodulation has been studied on non-diabetic subjects as well, as is the case in the study of Fatemi *et al*[16], including a population of ovariectomized mice as the treatment group. Post-surgical subjects presented with cognitive impairment, anxiety disorders and reduced brain-derived neurotrophic factor (BDNF). Treatment with metformin had beneficial effects on behavioral dysfunction and BDNF reduction on both of the treatment groups (Group A: 7 mg/kg and Group B: 15 mg/kg). Reinforcing the idea that the neuroprotective effects of the biguanide class are not solely the result of metabolic normalization of glucose homeostasis[16].

As opposed to the aforementioned studies, Wennberg *et al*[17] found no correlation between metformin or other anti-diabetic treatments and cognitive capacity. T2DM subjects with lack of functional impairment at baseline (*n* = 508) were followed-up for a mean duration of 3.7 years. Mild cognitive impairment (MCI) diagnosis was defined as difference equal to or greater than 1 standard deviation than the age-specific mean score of the general population on each test administered. The study population was divided into 4 groups according to treatment type as following: insulin monotherapy, metformin monotherapy, other oral agents as monotherapy or diet and exercise without pharmacological intervention. A universal lack of positive effect on cognition was observed among all groups, with patients on metformin treatment having higher rates of MCI diagnosis at follow-up. The latter was attributed to vitamin B12

reduction secondary to long-standing metformin administration. While the results are validated by the size of the study population and the numerous validated cognitive tests performed, notable limitations such as partial correction of treatment group differences despite covariate consideration and propensity score utilization and lack of B12 measurement should be taken into consideration when evaluating the research conclusions[17].

Correspondingly, a study conducted on C57BL/6 mice of different age groups yielded neutral results concerning the effect of metformin on metabolic parameters while a negative, age-dependent, impact was observed on both spatial memory and visual acuity of the test subjects. Treatment regimen comprised of 2 mg metformin /mL of drinking water, which is analogous to a human dose of 1500-2000 mg/d (when converted in a body-weight dependent manner), for three months[18]. While, the contradicting results could be partially attributed to the short study duration there is further research with similar conclusions, in which metformin attenuated memory dysfunction in female subjects and amplified it in males, on an experimental model of AD[19].

The relationship between the class of biguanides and functional neural capacity remains unclear due to several relevant research projects with controversial results. At the same time, the underlying pathophysiological mechanisms by which metformin exerts its effects on neural tissue have not been, as of yet, entirely elucidated. While there appears to be a positive predilection towards the exploration of metformin administration as a form of neuroprotection, mainly due to its potency in altering a multitude of signaling pathways in the cell cycle, further research is needed in order to clarify whether it is truly efficacious in the clinical setting on patients with diabetes-induced cognitive decline.

Alpha-glucosidase inhibitors

Some of the main representors of the class of alpha-glucosidase inhibitors (α-GIs) are acarbose, miglitol and voglibose. Yan *et al*[20] administered acarbose to SAMP8 mice for a period of 6 mo. The study population was divided into 3 groups, including the acarbose group (*n* = 9, 9-mo old), young (*n* = 11, 3-mo old) and old controls (*n* = 8, 9-mo old). An age-dependent cognitive decline was observed when the control groups were compared, while the acarbose group showed attenuation of this decline, accompanied by higher levels of insulin, insulin receptors and acetylated histone H4 lysine 8 (H4K8ac). The altered functional phenotype of the acarbose group (less memory impairment, improved spatial recognition) was attributed to both the changes in the concentration of insulin and its receptor and the H4K8ac increase. Higher levels of the latter have been linked to ameliorated long-term memory formation[20].

Since the data concerning the neurological effect of α-GIs is scarce, with no relevant research including miglitol or voglibose, safe conclusions cannot be currently drawn for their possible actions on neural tissue.

Sulphonylureas

As far as the class of sulphonylureas (SUs) is concerned, there appears to be a lack of relevant clinical studies discussing their effects on the homeostatic regulation of the nervous system. Given their mode of action, through binding on adenosine triphosphate-sensitive potassium channels and the subsequent activation of voltage-gated calcium channels, their possible use for inducing and regulating neuroexcitatory potentials is an interesting perspective. Currently available research discussing the role of SUs in the setting of cognitive decline is centered on the use of glimepiride and glibenclamide.

Ishola *et al*[21] administered glimepiride on a rodent model of paraquat-induced Parkinsonism with subsequent functional and molecular assessment of the treatment-induced changes. Sulfonylurea treatment attenuated oxidative stress and activation of inflammatory cascades in the neural tissue, while, simultaneously, improving the paraquat-induced memory dysfunction and cognitive performance on the rotarod, open field and Y-maze trials[21].

Glibenclamide has been shown to exert long-term protective properties on the hippocampal cortex in the setting of traumatic brain injury (TBI)[22]. Moreover, the aforementioned exerted a beneficial effect when used on an experimental AD model, *via* regulating the activity of the hypothalamic-pituitary-adrenal axis and alleviating AD-related mood-disorders[23].

Thiazolidinediones

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor (PPAR) agonists, also widely known as glitazones, have been established to interact with the cell cycle and inflammatory cascade.

Pioglitazone was administered as monotherapy and in combination with simvastatin, on a model of lipopolysaccharide (LPS)-induced cognitive dysfunction secondary to amyloid deposition and inflammation. While LPS exacerbated neural oxidative stress, amyloid Aβ deposition, glutamate tissue-levels and memory impairment, both simvastatin and pioglitazone mitigated the changes. The subjects performance on both the neurobehavioral tests chosen (Y-maze and novel object recognition) did not differ significantly between the combination therapy or the monotherapy group for each treatment alone, a fact possibly explained by both the substances exerting their anti-inflammatory properties on the same pathway of NF-κB signaling[24]. In a different study, pioglitazone was administered on subjects with LPS-induced febrile seizures and subsequent memory deficits. On the treatment groups, proinflammatory markers, such as tumor necrosis factor alpha (TNF-α) and interleukine-1β (IL-1β), along with oxidative stress were reduced in the hippocampal neural tissue, with accompanying partial resolution of memory impairment and cognitive dysfunction[25]. Moreover, a meta-analysis performed by Cao *et al*[26], on the efficacy and tolerance of antidiabetic treatment as adjunct therapy on AD indicated that pioglitazone (15 to 30 mg) was the most beneficial agent (when compared to placebo) in improving cognitive capacity.

Kushwaha *et al*[27] have indicated the existence of a rosiglitazone-induced anti-apoptotic effect on cerebral cortical tissue of high-fat-diet diabetic mice, for which the underlying mechanisms have not been clearly established. PPAR-γ mediated epidermal growth factor signaling appears to be the most probable pathway by which both glial and neural cells are affected. In a similar fashion, on a model of spontaneously hypertensive rats with consequent brain damage, rosiglitazone exerted a neuroprotective effect by mediating oxidative stress and affecting the levels of apoptotic cellular pathway mediators, independent of blood pressure correction[28].

Although the anti-apoptotic effects of TZDs on neural tissue are both supported by their mode of action and have been recreated in the experimental setting, there is a current lack of clinical correlation with the molecular findings. In order to establish the possible treatment benefits of this class in DE or other neuropathologic states, there is a definite need for further studying the performance of TZD-treated subjects on functional tests assessing both cognitive capacity and memory impairment.

Incretins

The two antidiabetic drug classes acting on the metabolic pathway of incretin hormones are glucagon-like peptide-1 receptor agonists (GLP-1 RA) and dipeptidyl peptidase-4 inhibitors (DPP-4i). GLP-1 is a hormone with multiple effects in the gut, pancreas and neural tissues, affecting processes such as gastric motility, appetite, insulin and glucagon secretion, while DPP-4 is the enzyme that deactivates it.

DPP-4 inhibitors

Sitagliptin, vilagliptin, saxagliptin, linagliptin and alogliptin are the current DPP-4is being used for treatment of T2DM[29].

APP/PS1 mice having been treated with sitagliptin (20 mg/kg for an 8-wk period) underwent neurofunctional assessment with the MWM test. The treatment group presented with ameliorated functional potential attributed to upregulation of BDNF and activation of tyrosine receptor kinase B (TrkB) signaling[30]. Through similar mechanisms of BDNF and tyrosine hydroxylase upregulation, sitagliptin administered on a model of Parkinson's disease moderated memory deficits, in addition to cellular density increase of dendritic spines in the CA1 region of the hippocampus[31]. Male Wistar rats with cisplatin-induced neurotoxicity further confirmed the neuroprotective effect of sitagliptin on both the molecular level and motor-cognitive performance, accredited to attenuation of drug-induced cerebellar damage[32].

As far as vildagliptin is concerned, upon administration in an Alzheimer's experimental model, the substance exhibited anti-apoptotic action in the hippocampal tissue with accompanying attenuation of memory deficits, changes associated with reduced tau phosphorylation and increased expression of neurotrophic proteins. An important mediator pathway and possible treatment target, identified in the above study, was that of phosphorylated protein kinase B/p-glycogen synthase kinase 3β (Akt/GSK3β)[33]. The exact same treatment signature was observed when vildagliptin was used on a model of streptozotocin-induced T2DM with diabetes-related cognitive decline[34]. Fibroblast Growth Factor 21 (FGF21) has shown superiority when compared to vildagliptin with the study therapeutic end-points being improvement of metabolic function and neuroprotection. Despite both the substances having insulin-sensitizing, anti-apoptotic, mitochondrial and cognition-sparing properties, they differed on several other measurements. FGF21 was a more potent regulator of metabolic parameters and synaptic plasticity in the hippocampus[35].

Saxagliptin (0.25/0.5/1 mg/kg for 60 ds) has shown neuroprotective properties on streptozoticin-induced AD rats by increase of hippocampal GLP-1 levels, decrease of amyloid plaque formation and deposition[36]. A slightly different rat model of AD disease, with cognitive deficits produced by D-galactose treatment, was used as grounds for comparing the efficacy of saxagliptin and metformin on learning and memory impairment secondary to aberrant insulin signalling. Several parameters on the MWM test were improved by antidiabetic treatment, along with oxidative biomarkers, tau phosphorylation products being normalized and insulin levels dropping with concurrent insulin receptor elevation[37]. On the contrary, saxagliptin in an experimental model of Parkinson's (produced by 6-hydroxydopamine administration) showed no cognitive- or motor-sparing properties but produced an interesting functional deterioration in the sham group, deeming it a possible candidate as post-traumatic stress disorder adjunct treatment[38].

Similar to other members of the DPP-4i class, linagliptin treatment has a beneficial role in ameliorating the progression of neural dysfunction on models of AD disease *via* numerous mechanisms such as amyloid plaque clearance, down-regulation of tau hyperphosphorylation, reduction of oxidative stress and mitochondrial dysfunction[39-41]. In T2DM test subjects, the neuroprotective attributes of the substances have been linked to changes in cerebral perfusion. In one study, linagliptin treatment post-carotid inclusion related transient cerebral ischemia attenuated cerebral damage unrelated to glucose homeostatic regulation, by mediating oxidative stress and blood brain barrier permeability[42]. Further, Hardigan *et al*[43] studied the effects of a 4-wk treatment regimen with linagliptin on vascular remodeling and flow properties of the middle cerebral arteries with beneficial effects being observed on the treatment group. The neuromodulatory role of linagliptin when compared to glimepiride is being studied by use of a composite 3-trial score (Mini-Mental State Examination, Trail Making Test, Verbal Fluency Test) in the cognition sub-study of double-blind, randomized Cardiovascular Safety of Linagliptin (CAROLINA) trial, including 4335 participants with T2DM[44].

Much like linagliptin, alogliptin has been shown to exert an effect on the architectural and functional integrity of cerebral vasculature. In a mice model of middle cerebral artery occlusion, the treatment group mediated the results of tissue ischemia and restored the defects of the blood brain barrier *via* altering the expression patterns of metalloproteinases and their inhibitors along with occludin and zona occludens-1 proteins[45]. On another model of diabetic nephropathy with silent cerebral infracts, the combination of alogliptin and hyperbaric oxygen treatment had a beneficial restorative effect on neural function[46]. Additionally, on high-fat fed doubly-negative apolipoprotein E mice with resultant cognitive decline, alogliptin up-regulated BDNF and calcineurin hippocampal production with accompanying higher performance on MWM and novel object recognition test than controls[47].

GLP-1 receptor agonists
The currently approved GLP-1 agonists are exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide and semaglutide.

In three studies (two included subjects with AD and one with T2DM) where the long-lasting GLP-1 analogue exenatide was used, restored BDNF signaling resulted into improved neurocognitive capacity by inhibiting neural apoptosis, in a manner analogous to that discussed on previous segments[48-50]. Bader *et al*[51] used a sustained-release preparation of the substance, named PT302, in order to study the role of exenatide treatment in TBI. Subjects in the treatment group presented with a down-regulation of pro-inflammatory markers in the neural tissue, prolonged cellular survival and reversal of functional impairment[51]. Similarly, on the topic of TBI, Rachmany *et al*[52] administered exenatide on a similar study population of mice with mild TBI, measuring both the neurofunctional changes and levels of synaptophysin (a biomarker for the viability of presynaptic neurons), pre- and post-trauma, with treatment ultimately attenuating the effects of the injury. Other changes following exenatide treatment include remodeling of hippocampal tissue architecture and diabetes-related deficits reversal, reduction of cortical TNF-α levels, preservation of brain choline acetyltransferase activity and improved amyloid oligomer clearance with subsequent decreased deposition[53,54]. A novel dual incretin agonist with combined gastric intestinal peptide and GLP-1 activity, the latter in the form of exenatide, has shown similar neuroprotective actions like memory refinement and hippocampal neurogenesis and synaptic remodeling along with a positive metabolic profile[55].

While liraglutide has been shown to effectively attenuate memory and functional deficits in subjects with various AD or similar pathology patterns in neural tissue, through mediating tau hyperphosphorylation and amyloid deposition[56-58], contradicting research does exist, in which 12-wk liraglutide treatment was not

superior in cognitive function improvement when compared to placebo[59]. In the setting of cognitive decline following mood disorders, the GLP-1 RA improved performance in the Trail Making Test-B and composite Z-score of several neuropsychiatric scales measured, a change attributed to IR attenuation and other metabolic parameter modification[60]. Post-treatment behavioral normalization was also noted in a study by Koshal *et al*[61] including mice manifesting with depression secondary to seizure activity. Some of the other changes in the treatment group were the reduction of oxidative stress and seizure activity[61]. Cognitive-deficient rodents with T2DM treated with liraglutide presented with ameliorated functional potential as a result of activation and modification of downstream signaling pathways of AMPK, mTOR and phosphoinositide 3-kinase (PI3K)[62]. The involvement of the mTOR pathway in the neuroprotective action of liraglutide was further confirmed in a study of streptozotocin-induced T2DM[63].

In a manner similar to other GLP-1 RAs, a pattern of reduced proinflammatory mediators and increased amyloid plaque clearance in APP/PS1/tau mice models of AD is observed with the administration of both lixisenatide and dulaglutide, resulting in improved neurocognitive potential. The pathways involved include those of p38-MAPK, protein kinase A and Akt/PI3K[64-66]. Both the neuroprotective attributes of semaglutide and its superiority to liraglutide in improving cognition have been observed in mice models of Parkinson's disease caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine[67,68].

SGLT-2 inhibitors

Sodium-glucose cotransporter 2 (SGLT2) inhibitors exert their actions on several tissue types, with their potency as antidiabetic substances stemming from their ability to hinder renal glucose reabsorption in the proximal tubule of the nephron. Members of this class currently in use are canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, ipragliflozin, luseogliflozin, tofogliflozin with sotagliflozin, a dual SGLT1/SGLT2 inhibitor, in phase III clinical trials. The relationship between neural functional capacity and memory integrity and SGLT2 inhibition has been explored in studies utilizing canagliflozin, dapagliflozin and empaglilozin.

Arafa *et al*[69] studied the effects of canagliflozin treatment on memory dysfunction secondary to scopolamine administration. As an end-result of SGLT2 inhibitor treatment, neural tissue monoamine and acetylcholine levels were increased with M1 receptor activity, a biochemical shift culminating into improved cognitive function on MWM and Y maze trials[69]. Similar patterns of altered acetylcholine signaling post-canagliflozin treatment were described on a similar study with diabetic rodents that included a metformin treatment group as well[70].

Dapagliflozin both as monotherapy and in combination with liraglutide has shown beneficial effects on memory and cognition, following remodeling of neural tissue with increased expression of doublecortin and synaptophysin (biomarkers of neural proliferation and synaptic formation respectively), as well as reduced IR[71].

The effect of empagliflozin on cognitive function was documented in the study by Lin *et al*[72], after a 9-wk regimen on db/db mice. Assessment with the MWM test and immunohistological examination of cortical tissue was subsequently performed. The cognitive function of the treatment group was superior to that of age-matched controls, with concurrent attenuation of oxidative stress and increased BDNF levels[72].

Given the relative lack of data for this antidiabetic class, combined with the fact that the possible mediating mechanisms, either direct molecular or indirect *via* modification of hemodynamic parameters, for their action on neural tissue have not been elucidated as of yet, there is definite need for further research on the subject.

Insulin

Neural tissue IR is an important substrate for the cognitive decline observed on diabetic subjects, especially in the hippocampal region. Numerous architectural and molecular changes fuel the pathologic process, including increased amounts of oxidative stress, activation of inflammatory cascades, peptide formation and aberrant deposition, commonly in the form of amyloid, as well as dysregulation of the hypothalamic-pituitary-adrenal axis[73]. As would be expected, since the basis of diminished functional capacity in T2DM is formed on the existence of IR, treatment with insulin, in many forms, has proven to be beneficial in ameliorating the relevant pathophysiological alterations.

Several studies have emerged, exploring the use of insulin *via* intranasal delivery, so as to bypass the blood brain barrier. This route allows for rapid achievement of therapeutic concentrations in the target tissue and treatment effectiveness, with accompanying cognitive improvement post-therapy[74-76]. Some of the proposed mechanisms for explaining the attenuation of neurofunctional deterioration caused by T2DM include altered activation of electrolyte channels (mostly calcium-related),

neuropeptide expression pattern differentiation, increased clearance of peptides (hyperphosphorylated tau and Aβ) that deposit as neurofilaments, synaptic remodeling and activation, upregulation of N-methyl-D-aspartate receptors turnover and improvement of hemodynamic parameters such as neural tissue perfusion[75].

On a study performed by Maimaiti *et al*[75], short-acting insulin lispro (Humalog) and long-acting insulin detemir (Levemir) were administered intranasally on a rat model of age-related mental impairment. Both the long- and short-acting compounds were equally effective in improving memory recall, matching the performance of aged members in the treatment group, to that of young rodents in the control group[75]. Slightly different results came from the study of Benedict *et al*[77] where despite both regular and fast-acting insulin improving cognition when compared to the control group, the short-acting insulin aspart was more efficient than regular insulin in memory recall testing.

In a different research project, long-acting insulin analogs (glargine, detemir, degludec) were compared to regular insulin by use on cultured cortical neurons of rodents. Glargine, detemir and regular upregulated cortical BDNF, and activation of the Akt signaling cascade, with degludec having marginally inferior efficacy. Furthermore, regular and glargine ameliorated memory and cognition (as estimated by performance on the Y maze), showing superiority over detemir[78].

Finally, many of the physiological actions of insulin in the neural system are mediated by insulin-like growth factor-1 (IGF-1) receptors. Due to the afore-mentioned, similarly to insulin, use of neurostimulating factors with analogous activity on target tissues, such as IGF-1 has yielded promising results in the setting of neural proliferation and damage recovery post-trauma[79,80], neurodevelopmental disorders[81], neurovascular dysfunction[82] and IR[83].

Research data pertaining the use of insulin in the setting of cognitive decline, confirm the relationship between IR and mental deterioration, a state reversible by treatment with insulin or insulin-sensitizers. Further research could provide insight on the appropriate insulin delivery methods for achieving maximum therapeutic concentrations and treatment efficacy while minimizing risk, so as to fully utilize the potential of this therapeutic approach for diabetic and non-DE. A brief table containing all the aforementioned cognitive capacity experimental tests used on rodents is provided below (Table 1).

CONCLUSION

DE is term describing a multifactorial state of neural dysfunction resulting from T2DM and its hallmark, IR. Current antidiabetic regimens appear to have a beneficial effect on cognitive decline and memory impairment secondary to diabetes and other causes. Most of the research data on the subject derives from studies on metformin, TZDs and incretins, with further elucidation being required for the role and mechanisms of sodium-glucose cotransporter inhibition on neural functionality. As has been shown by the intranasal delivery of insulin, the development of vectors allowing for direct access to the CNS without inhibition from the blood-brain-barrier could open up some very interesting perspectives for repurposing the antidiabetic therapy as means to effectively treat mental dysregulation states. Moreover, the extensive elucidation of the underlying pathophysiology allowing for oral antidiabetic medication to affect neural functionality could provide insight on the reasons behind cognitive impairment in T2DM, while also allowing for formulation of proper guidelines for hinderance of its development and ultimately, treatment.

Table 1 Experimental trials for the evaluation of cognitive capacity and memory impairment on rodent study populations

Type of test	Method of operation
Y-Maze Spontaneous Alternation	Evaluates willingness of subjects to explore new paths on a 3-arm structure with each pathway angled at 120°
Morris Water Maze	Evaluates spatial and long-term memory by testing the escape capacity and velocity of subjects on a water tank
Passive avoidance	Evaluates learning and memory integrity by introducing aversive stimuli
Rotarod	Evaluates balance and motor coordination by assessing the ability of the subject to remain standing on a rotating cylinder
Open field	Evaluates willingness of subjects to explore new paths, anxiety and motor coordination though observing the subject's movement patterns on a walled-off area
Novel object recognition	Evaluates recognition memory though habituation of test subjects with novel objects and subsequent evaluation of their capacity to discriminate between familiar and unfamiliar.

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Case Control Study

Screening the RFX6-DNA binding domain for potential genetic variants in patients with type 2 diabetes

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Institutional review board statement: The study was ethically approved by the IRB board of Jordan University Hospital (JUH) No. 10-2017-1737, Decision No. 2017-134. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent statement: Informed consents were obtained from human participants in this

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Abstract

BACKGROUND
The regulatory factor X6 (RFX6), a member of regulatory factor X family, is known to play a key role in the development and differentiation of pancreatic beta cells as well as insulin production and secretion. However, the potential role of RFX6 in type 2 diabetes (T2D) is still unclear.

AIM
Recent studies have indicated that RFX6 binding to DNA could be disrupted in diabetes. Therefore, in this study we investigated whether genetic mutations are present in the DNA binding domain of *RFX6* gene that could abrogate its function in T2D.

METHODS
A cohort of T2D patients was enrolled in this study, and the gene encoding the DNA binding domain of RFX6 was amplified by polymerase chain reaction and then analysed by direct DNA sequencing.

RESULTS
The DNA sequence analysis revealed the absence of any exonic mutation. However, we have identified a new heterozygous single nucleotide polymorphism (IVS6+31 C>T) in the intronic region of DNA binding domain gene that is present in 9.2% and 8.5% of diabetic and control people, respectively ($P = 0.97$).

CONCLUSION
We report the absence of any significant genetic variant that could affect the function of RFX6-DNA binding domain in T2D.

Key words: Regulatory factor X6; Genetic variant; Diabetes; DNA binding domain

Core tip: Regulatory factor X6 (RFX6) protein plays a key role in the differentiation of pancreatic beta cells as well as insulin production and secretion. Several lines of evidence have indicated that RFX6 binding to DNA could be disrupted in diabetes; however, the mechanism underlying this process is still unknown. In this case-control study, we analysed the genotype of RFX6-DNA binding domain in diabetes patients in comparison to healthy controls. Our results indicate the absence of any significant genetic variant in the DNA binding domain that could affect the function of RFX6 in type 2 diabetes.

INTRODUCTION

Diabetes mellitus is a group of glucose metabolism disorders characterized by high levels of blood glucose. The disease affects millions of people worldwide, which is usually associated with serious complications that affect various systems of human body[1]. Type 2 diabetes (T2D) is mainly manifested by low insulin production by pancreatic cells and/or the produced insulin does not function effectively[2]. Alterations in both beta cells average mass and function have been observed and reported in people with diabetes[3-5].

Regulatory factor X (RFX) proteins constitute a family of DNA binding proteins that is conserved in the eukaryotic kingdom[6]. In humans, RFXs act as regulatory transcription factors that bind to a conserved *cis*-regulatory element called the X-box motif, which is typically 14-mer DNA sequences located in specific promoter regions of the genome[7]. The function of mammalian RFX proteins has only recently started to emerge, and has shown to play an important role in regulating growth and development, immune response and endocrine secretions[7]. The RFX family has seven members of RFX1-7 in mammals, which have wide expression in various tissues and organs. RFX6 is a main member of RFX family that is predominantly expressed in pancreatic islets and encoded by a gene on chromosome 6[7]. RFX6 possesses a highly conserved DNA binding domain which is critical for binding of RFX6 to X-box promoter motifs and thus regulating their function. Recent studies conducted in mice demonstrated that RFX6 is specifically required for pancreatic beta cells differentiation during embryonic development[8]. Moreover, RFX6 was shown to be an important factor to maintain key features of functionality of mature beta cells, and *RFX6* gene deletion in adult mice beta cells was shown to disrupt glucose homeostasis and caused glucose intolerance, impaired beta cell glucose sensing and defective insulin secretion[9].

In 2017, Varshney *et al*[10] published an interesting study in the Proceedings of the National Academy of Sciences, where they performed an integrated analysis of molecular profiling data of the genomic DNA, epigenome and transcriptome in diabetic pancreatic beta islets, to understand the potential connections between genetic variants, chromatin landscape, and gene expression in T2D. The study showed that most of the reported genetic variants in T2D are enriched in regions of the DNA where RFX transcription factors are predicted to bind. The study also concluded that these genetic variants that increased the risk of T2D are predicted to disrupt mainly the binding of RFX6 to genomic DNA[10], indicating that RFX6 binding to X-box promoter motifs could be disrupted in T2D.

In this study, we sought to investigate if any structural genetic defects could be

present in the RFX6-DNA binding domain in T2D patients that could potentially inhibit its function in diabetes.

MATERIALS AND METHODS

Patient and control samples

Initially, a total of 98 blood samples (49 samples from T2D patients, 49 from healthy volunteers (control group)) were collected from Jordanian population (Table 1). The study was then extended to investigate the association between the identified intronic variant (IVS6+31 C>T) and diabetes. A total of 283 blood samples (141 from T2D patients, 142 from healthy volunteers) were included in the extended study (Table 1). Diabetic participants who enrolled in this study were Jordanian adults (age ≥ 20 years), including both females and males, with known history of diabetes and recruited from Jordanian medical centres during the time period between Dec 2015 and July 2017. Controls were unrelated to diabetic patients and had no history of diabetes, as determined by history and lab examination. All blood samples were collected according to protocols approved by the Institutional Review Board, and informed consents were obtained from participants included in the study.

Biochemical examination

The levels of blood glucose and glycosylated Hb (HbA1c) were evaluated in the participants of this study. Blood glucose level was measured by the glucose oxidase method using Cobas c111 analyzer (Switzerland). The percentage of HbA1c in blood was determined using ion-exchange high-performance liquid chromatography (D-10™ Bio Rad, United States).

Molecular techniques

For molecular assays, DNA was extracted from venous whole-blood samples, and then collected in EDTA containing tubes, using Wizard genomic DNA purification kit (Promega, United States). Extracted DNA was stored at −20°C. Polymerase chain reaction (PCR) was used to amplify genomic DNA encompassing the coding sequences and intronic borders of exons 3, 4, 5 and 6 of the *RFX6* gene (NCBI: NG_027699.1). The primers used for PCR assay were as described in (Table 2). The PCR amplification reactions were performed in a total volume of 25 µL in 0.2 mL PCR tubes containing 200 ng of genomic DNA, 5 µL of 5× FIREPol® Master Mix (Solis BioDyne) with 7.5 mmol $MgCl_2$ and 10 pmol of each primer (Gene Link, United States). All PCR reactions were performed using C1000 Touch™ Thermal Cycler (Bio-Rad; United Kingdom) and the reaction conditions were as follows: initial denaturation of 5 min at 95°C, followed by 29 cycles of 30 s at 95°C, 30 s at specific annealing temperature (Table 2), 1 min at 72°C and a final extension of 6 min at 72°C. All PCR products were checked by gel electrophoresis to verify correct product size, then purified and sequenced using the same forward primer used for the gene amplification. DNA sequencing reactions were performed at Macrogen Inc., South Korea, using BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, United States) and the ABI PRISM 3730XL analyzer (Applied Biosystem, United States).

Statistics

Data analyses were performed using IBM Statistical Package for the Social Sciences software (SPSS, version 19). Pearson's chi-squared or Fisher's exact tests were used to test an association between categorical variables (*i.e.*, gender and genetic variant) and case-control status. Student t-test was used to assess the significance of difference of means of age, blood glucose and glycosylated Hb between case and control groups. A 95% confidence interval for the odds ratio was calculated and used to describe the results. For sample size calculation, the Cochran's formula was used[11].

RESULTS

In this study, the screening for structural genetic variants in specific regions of RFX6 gene was carried out in T2D patients in attempt to discover new potential mutations that could mediate the pathogenesis of T2D. Evaluation of levels of blood glucose and HbA1c of enrolled subjects was performed to discriminate between diabetic and non-diabetic (Table 1). The DNA sequence analysis of exons 3, 4, 5 and 6 of *RFX6* gene in 49 diabetic patients revealed the absence of any genetic mutation. However, the DNA sequencing of introns borders revealed the presence of a heterozygous genetic variant

Table 1 Demographic and clinical characteristics of diabetic patients and healthy controls			
	Control group	**Diabetic patients**	***P*-value**
Primary screening, (*n* = 98)			
Male, *n* (%)	32 (65%)	24 (49%)	*0.285*
Age (mean ± SD)	50.1 ± 12.3	54.7 ± 10.7	0.149
Female, *n* (%)	17 (35%)	25 (51%)	0.217
Age (mean ± SD)	47.7 ± 15.5	56.2 ± 13.2	0.065
FBG (mg/dL)	97± 10	192± 94	< 0.001
HbA1c (%)	5.4± 0.4	8.2± 2.2	< 0.001
Screening the IVS6+31 C>T, (*n* = 283)			
Male, *n* (%)	78 (55%)	59 (42%)	0.105
Age (mean ± SD)	48.9 ± 15.2	57.4 ± 11.3	0.001
Female, *n* (%)	64 (45%)	82 (58%)	0.136
Age (mean ± SD)	51.8 ± 15.2	57.1 ± 11.5	0.024
FBG (mg/dL)	98± 10.4	177± 80	< 0.001
HbA1c (%)	5.4± 0.5	7.8± 1.9	< 0.001

P-value is significant at ≤ 0.05. FBG: Fasting blood glucose; HbA1c: Glycosylated haemoglobin.

(IVS6+31 C>T) within the intron 6 of *RFX6* gene, where C is substituted by T, 31 nucleotides downstream of the end of exon 6 (Figure 1). To investigate the significance of the identified intronic variant in T2D, the study was extended and the IVS6+31 C>T was screened in 283 samples (141 diabetic and 142 healthy controls). DNA sequence analysis of the samples revealed the identification of the heterozygous IVS6+31 C>T in 9.2% and 8.4% of diabetic and control groups respectively, with no significant association in genotype or allele frequency between diabetic and control groups (Table 3).

The demographic characteristics of the study population (age and gender) are shown in Table 1. In primary screening study, there was no statistically significant difference in male/female proportion (*P* = 0.285 and *P* = 0.217, respectively) or age of gender (*P* = 0.149 and *P* = 0.065, respectively) between case and control groups. As for the extended screening study of IVS6+31 C>T variant, no statistically significant difference in male/female proportion was found between case and control groups (*P* = 0.105 and *P* = 0.136, respectively). However, there was a significant difference in age of male/female of case–control status (*P* = 0.001 and *P* = 0.024, respectively).

DISCUSSION

Recently, it has been proposed that a loss of pancreatic beta-cells mass, differentiation and function is a hallmark of T2D[1,2]. Concurrently, the regulation of beta cells growth and differentiation has been under intensive investigation, and several lines of evidences have indicated the role of key transcription factors in controlling the function state of beta cells. For example, evidences coming from loss-of-function studies in adult mice beta cells have revealed that transcription factors such as NeuroD1[12], Nkx6.1[13] and Pdx1[14] are important in maintaining the differentiation and function state of pancreatic beta cells. Thus, it appears that loss of function of key beta cell transcription factors results in the loss of both beta cell identity and function. More recently, RFX6 transcription factor has been shown to play a key role in regulating the state of pancreatic beta cells differentiation and function[9].

RFX6 contains a highly conserved DNA binding domain that facilitates their binding to X-box promoter motif of certain genes, which is essential to regulate the transcription of *RFX6*-target genes[7]. It has been shown that genetic alterations in the RFX6-DNA binding domain could be associated with neonatal diabetes. In fact, mutations in the RFX6-DNA binding domain are assumed to be the cause of neonatal diabetes in Mitchell-Riley syndrome, through the production of a defective RFX6 protein[15]. In this project, we sought to detect if any genetic mutation could be present in the RFX6-DNA binding domain in T2D. Based on our findings we conclude that structural mutations in the DNA binding domain of RFX6 are unlikely to exist in T2D. However, another large-scale study could increase the statistical power of our results. In addition, it is noteworthy to mention that RFX6 proteins contain other conserved

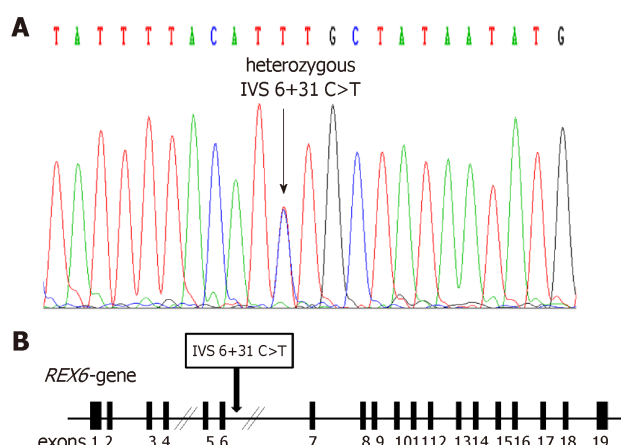| Table 2 Primer sequences used in DNA amplification of DNA binding domain sequence of *RFX6* gene			
Primer	**Sequence (5'-3')**	**Ta (°C)**	**Size (bp)**
RFX6-3	F: 5- CTT ATG TCT ACT CAT TAC CTC -3	50	306
	R: 5- TCA TGC TAT CTG CCT GAC -3		
RFX6-4	F: 5- CAG TTC ATT CAG AGT TCA AC -3	56	216
	R: 5- CTT CAT GCA CAA GAG CAG -3		
RFX6-5	F: 5- GTC ATC AGG GTT TGC AGT TC -3	50	258
	R: 5- ATT CAA TAG GTA TCA TGC -3		
RFX6-6	F: 5- GTA AGT TGA GAA AGA TGC -3	56	258
	R: 5- CAT GTA TTG CTC AGC TTG -3		

Ta indicates annealing temperature.

regions including B, C, and D domains[6]. These domains are thought to be involved in RFX6 oligomerization which are required for DNA binding and activation[7]. Therefore, screening the other functional domains of RFX6 may provide more insights into the potential mechanism by which RFX6 binding to DNA is abrogated in diabetes.

Table 3 Association between the variant IVS6+31 C>T investigated in *RFX6* gene and type 2 diabetes risk					
	Control, *n* (%)	T2D, *n* (%)	*P*-value	OR	95%CI
genotype					
C \ C	130 (91.5)	128 (90.8)	0.97	0.9	0.39-2.06
C \ T	12 (8.5)	13 (9.2)		1.1	0.48-2.50
T \ T	0	0		1	0.02-51.1
Allele					
C	272 (95.7)	269 (95.3)	0.82	0.9	0.41-2.03
T	12 (4.3)	13 (4.7)		1.1	0.49-2.44

P-value is significant at ≤ 0.05. OR: ODDs Ratio; CI: Confidence Interval; T2D: Type 2 diabetes.

Figure 1 Regulatory factor X6 intronic genetic variant. A: The heterozygous IVS6+31 C>T as determined by automated DNA sequencing analysis; B: *RFX6* gene structure and the location of the IVS6+31 C>T genetic variant (as indicated by the black arrow).

ARTICLE HIGHLIGHTS

Research background

Diabetes mellitus is a global health challenge, which is usually associated with the loss/dysfunction of insulin-producing pancreatic beta cells. Hence, understanding the molecular mechanisms that control beta cells differentiation and function represents a major interest in the medical field. Regulatory factor X6 (RFX6) is DNA binding protein that is predominantly expressed in pancreatic islets of human and plays a key role in regulating pancreatic beta cells differentiation and insulin production, and it has been recently. RFX6 contains a highly conserved DNA binding domain which is critical for binding of RFX6 to DNA and consequently regulates the amount of messenger RNA produced by the gene. Several lines of evidence have indicated that RFX6 binding to DNA could be disrupted in diabetes. However, the mechanism by which this could happen is still unknown.

Research motivation

The presence of genetic mutations in the gene coding for the RFX6-DNA binding domain could result in inhibition of binding of RFX6 to DNA and consequently loss of function. Defining such genetic mutations will provide valuable information to diagnose, treat, prevent and cure type 2 diabetes (T2D).

Research objectives

In this study, we sought to investigate if any structural genetic mutations could be present in the RFX6-DNA binding domain in T2D patients and whether they are associated with diabetes.

Research methods

A case-control study was conducted in T2D patients and healthy volunteers. The DNA was extracted from all subjects and polymerase chain reaction (PCR) was used to amplify genomic DNA encompassing the coding sequences and intronic borders of exons 3, 4, 5 and 6 of the *RFX6* gene, then PCR samples were analysed by DNA sequencing.

Research results

Our data showed the absence of any mutation in the exons coding for the RFX6-DNA binding

domain. However, we have identified a new heterozygous single nucleotide polymorphism (IVS6+31 C>T) in the intronic region of DNA binding domain gene that is present in 9.2% and 8.5% of diabetic and control people, respectively ($P = 0.97$).

Research conclusions

We conclude that genetic mutations in the DNA binding domain of RFX6 are unlikely to exist in T2D.

Research perspectives

RFX6 binding to DNA is mediated by multiple of domains. Indeed, RFX6 proteins contain other conserved regions, including B, C, and D domains, which play a critical role in oligomerization of the protein and are required for DNA binding and activation. Thus, testing the other functional domains of RFX6 in future will provide more insights into the role of RFX6 in diabetes.

ACKNOWLEDGEMENTS

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Retrospective Cohort Study

Targeted genotyping for the prediction of celiac disease autoimmunity development in patients with type 1 diabetes and their family members

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Abstract

BACKGROUND
Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) have an increased risk of developing celiac disease (CD) compared to the general population. This is largely explained by the shared association with major histocompatibility class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states.

AIM
To describe the frequency of CD autoimmunity (CDA) and the distribution of HLA and haptoglobin genotypes in patients with T1D and their FDRs. Additionally, we aimed at identifying predictors associated with an increased risk of developing CDA in patients with T1D and their family members.

METHODS
We obtained clinical information and blood samples from 1027 participants (302 with T1D and 725 FDRs) over a five-year period. Samples were tested for autoantibodies associated with CD, HLA-DQ alleles, and haptoglobin genotype.

We fit univariate and multiple logistic regression models for CDA separately for subjects with T1D and for FDRs of subjects with T1D.

RESULTS
Implementation of a screening program increased the frequency of CDA by 2-fold in participants with T1D and 2.8-fold in their FDRs. Multivariate analysis found that, in participants with T1D, having both DR7-DQ2 and DR4-DQ8 was associated with an increased frequency of CDA. In FDRs of T1D patients, reported CD in the family was associated with an increased frequency of CDA during screening. Haptoglobin 2 genotype was not associated with developing CDA in the multivariate analysis.

CONCLUSION
Patients with T1D and their FDRs have a high frequency of CDA. Carrying both DR7-DQ2 and DR4-DQ8 was associated with development of CDA in patients with T1D.

Key words: Screening; Gluten; Diabetic; Coeliac; Haptoglobin; Human leukocyte antigen

Core tip: Serological screening for celiac disease (CD) autoimmunity in subjects with type 1 diabetes (T1D) and their first-degree relatives (FDRs) found an underestimation of CD by 2 fold in T1D patients and 2.8 fold in their FDRs. Participants with T1D who carry DR7-DQ2/DR4-DQ8 were more likely to screen positive for CD autoimmunity. There was no association between carrying zonulin genetics and an increased risk of developing CD in our cohort. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population and, given the often-asymptomatic nature of disease, physicians should have a low threshold for screening.

INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy that occurs in genetically susceptible individuals in response to ingestion of gluten. While the worldwide prevalence of this condition is estimated at 1%, prevalence is known to vary among geographical locations and ethnic groups[1]. The prevalence of CD and other autoimmune conditions appears to be on the rise, and yet most patients with CD remain undiagnosed[2,3]. Many of these individuals may be asymptomatic and identified through the screening of high-risk groups. Patients with type 1 diabetes (T1D) constitute a high-risk group given their risk of CD is reported at 3-8 times higher than that of the general population[4-7]. This increased risk is likely due to a shared genetic predisposition with the major histocompatibility (MHC) class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states[8,9]. Recent evidence suggests that, like T1D patients, relatives of those with T1D have an increased risk of autoimmune disease. Screening studies detecting celiac-associated antibodies have found a prevalence of CD in relatives of those with T1D ranging between 2.5% and 6%[7,10].

Both T1D and CD are diseases for which increased permeability is crucial to the pathogenesis[11,12]. Zonulin, a family of proteins belonging to the serine proteases group, is a master regulator of paracellular permeability and works through reversibly altering intercellular tight junctions[13-15]. Serum zonulin levels correlate with increased intestinal permeability and have been associated with many chronic inflammatory disorders, including CD and T1D[14-16]. One of the zonulin isoforms is the precursor of haptoglobin-2 (*HP2*)[17]. In humans, haptoglobin (*HP*) exists as two

common alleles, *HP1* and *HP2*, giving rise to three different *HP* genotypes (*HP1-1*, *HP2-1*, *HP2-2*). The *HP*2 allele is found only in humans and only individuals who possess an *HP2* can produce zonulin. Worldwide, the frequency of *HP1-1* varies from 0.07-0.70[18]. In the United States, the frequency ranges from 0.31-0.55, with a frequency of 0.41 reported in Caucasians[19]. Presence of the *HP2* allele has been shown to influence the course of inflammatory disease due to differences in antioxidants, scavenging, and immunomodulatory properties[18]. Previous work has shown that the zonulin gene (*HP2*) is more frequent in chronic inflammatory diseases such as inflammatory bowel disease[20], CD[21], and lupus[22], and that homozygosity for *HP2-2* is associated with more severe clinical manifestations of inflammatory conditions[21]. In patients with T1D, the frequency of *HP2-2* and *HP2-1* genotypes is increased compared to that reported in the general population[23]. Further, the *HP2-2* genotype has been associated with increased risk of coronary artery disease in patients with type-2[24] but not type-1[25] diabetes. In patients with T1D, *HP2-2* has not been significantly associated with diabetic nephropathy[23], but has been associated with an increased risk of cardio-renal mortality[26], including a decline in kidney function and progression to end-stage renal disease[27,28]. Overall, the contribution of *HP* genotype to development of other autoimmune diseases, specifically CD, has not been evaluated. However, given the role of zonulin in the pathogenesis of both T1D and CD, we postulated that patients with T1D or their first-degree relatives (FDRs) would be more likely to develop CD if they carried the *HP2-2* genotype.

Since approximately half of T1D patients who are diagnosed with CD present asymptomatically, and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs, we employed a prospective program to serologically screen patients with T1D and their FDRs for CD[29,30]. We aimed at identifying predictors that may increase the risk of developing CD in patients with T1D, and to understand which individuals from these high-risk families were more likely to develop CD to identify which subjects may benefit most from screening. We hypothesized that patients with T1D who develop CD are younger at the age of T1D diagnosis, more likely to carry DR3-DQ2/DR4-DQ8 and more likely to have zonulin genetics, *HP2*, than patients with T1D without CD. We also hypothesized that FDRs of subjects with T1D will be more likely to develop CD if they are female, complain of GI symptoms, carry DR3-DQ2, and carry the zonulin gene *HP2*.

MATERIALS AND METHODS

Setting
This study was performed during the Children with Diabetes (CWD) annual conference. CWD is a United States based organization that provides educational and social support for families of children with T1D. We conducted serological screening for CD at CWD's annual conference over five consecutive years (2013-2017).

Subjects
Children and adults attending the CWD conference diagnosed with T1D or with a FDR (parent, child, or sibling) with T1D were eligible for participation. Participants self-selected to participate by visiting our "booth" to conduct study procedures. Written informed consent was obtained from all participants. All study procedures were reviewed and approved by the Partners Human Research Committee Institutional Review Board.

Clinical information
Participants and, when necessary, their caregivers (on behalf of a child), completed a brief self-report clinical questionnaire targeted to assess the family history of T1D and CD, presence or absence of CD-associated symptoms in the individual, current diet, and other pertinent medical information.

Serology
All subjects underwent venipuncture with an on-site phlebotomist. A minimum of 8 cc of blood was collected from each participant. Serum was evaluated for antibodies to IgA tissue transglutaminase (tTG) and IgG deamidated gliadin peptide (dGP) using QUANTA Lite Rh-tTG IgA ELISA (INOVA Diagnostics, San Diego, CA, United States) on the BioFlash platform. Individuals found to have IgA tTG levels above the kit reference value (> 20 CU) were subjected to confirmatory testing for IgA endomysial antibodies (EMA) using the NOVA Lite Monkey Oesophagus IFA Kit (Inova Diagnostics, San Diego, CA, United States). Subjects found to have elevated IgG dGP in the absence of elevated IgA tTG were further evaluated for potential IgA

deficiency. Serum samples for these individuals were sent to an outside lab (LabCorp, Burlington, NC, United States) and a total IgA level was performed using immuno-turbidimetric methods.

HLA determination
HLA was determined from whole blood samples using the DQ-CD Typing Plus (BioDiagne, Palermo, Italy) according to the manufacturer's instructions.

HP genotyping
HP genotype was determined by either PCR or immunoblot depending on availability of biological samples. For determination by PCR, genomic DNA was extracted from venous blood using QIAamp DNA kit (Qiagen, Hilden, Germany). The genotypes were determined by a novel one step PCR method using primers designed with Primer3 in exon 2 and exon 5 of *HP1* corresponding to exons 2 and 7 of *HP2*. The primers were designed as follows: forward: TTTCTGGCTGCTAAGTTG and reverse: AATGTCTTTCGCTGTTGC. The PCR was performed in 10 uL reactions containing 100 ng purified DNA, 5 uL of 2× MyTaq Red Mix (Bioline, Taunton, MA, United States), and 300 nM of each primer. After PCR, the amplicons were electrophoresed on a 1% agarose gel and read under a UV bulb. The duplication in *HP2* results in a size difference of the PCR products (2.5 kb *HP1* and 4.3 kb *HP2*) allowing for differentiation of the two genotypes.

   Following screening, all participants were informed of their serological status and genetic compatibility (in regard to HLA only). In the event of abnormal serological findings, patients were instructed regarding necessary follow-up procedures with a local physician or specialist.

Definition of CD, Celiac disease autoimmunity (CDA) and IgA deficiency
Participants who self-reported a diagnosis of CD prior to screening were classified as "previous CD" if their diagnosis was based on biopsy or "history of CDA" if their diagnosis was based on bloodwork alone. Patients with positivity for both IgA tTG and IgA EMA at screening were considered, for this study, positive for CD. In the absence of IgA EMA (IgA tTG elevated alone), subjects were classified as demonstrating CDA. Patients with elevated IgG dGP in the absence of elevated IgA tTG were evaluated for potential IgA deficiency. Serum IgA levels less than 7 mg/dL were regarded as IgA deficient. Individuals found to have elevated IgG dGP and IgA deficiency were classified as CDA. For the purposes of the univariate and multivariate analyses, all patients with CD and CDA were combined and are referred to having CDA.

Statistical analysis
Categorical data are presented as frequency (percentage). Continuous data are described as mean ± SD if normally distributed and median (interquartile range; IQR) otherwise. All tests of significance were two-sided with $\alpha = 0.05$, and all analysis was performed with SAS 9.4 (Cary, NC). We fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D. We included all covariates that had *P*-values less than 0.10 in univariate analyses and in the multiple regression models. We used generalized estimating equations to account for the correlation within families in all analyses; we used an exchangeable working correlation matrix, except for the multiple regression models for individuals with T1D for which we used an independence working correlation matrix due to convergence issues.

RESULTS

Demographics
Demographic data for participants with T1D, T1D and CDA (T1D+CDA), and FDRs of T1D patients with (FDR + CDA) and without CDA are shown in Table 1. The majority of patients in the study were female, White and not Hispanic. As expected, since screening took place at a conference for children with T1D and their family members, participants with T1D were younger than their FDRs at the time of screening. More than 50% of participants reported being asymptomatic at screening. Participants with CDA prior to or at the time of screening had a higher frequency of reporting a relative with CD and a higher frequency of reporting a relative diagnosed with any autoimmune disease.

HLA and haptoglobin genetics
Table 1 demonstrates the frequency of the HLA and haptoglobin genetics for

Table 1 Demographic data: Participants with type 1 diabetes and their first-degree relatives *n* (%)				
Demographics	T1D only (*n* = 280)	T1D + CD (*n* = 22)	FDR only (*n* = 689)	FDR +CDA (*n* = 36)
White	249 (88.9)	22 (100)	629 (91.3)	35 (97.2)
Not hispanic	188 (67.1)	15 (68.2)	451 (65.5)	27 (75.0)
Female	178 (63.6)	17 (77.3)	409 (59.4)	26 (72.2)
Age at screening (yr), median (range)	19 (2-72)	14.5 (7-43)	40 (1-74)	39.5 (3-55)
Age at diagnosis of T1D (yr), median (range)	10 (0.1-64)	6.5 (1-21)		
Presence of gastrointestinal symptoms (GI sx)	113 (40.5)	6 (27.3)	216 (31.4)	13 (36.1)
Presence of extraintestinal symptoms (Ex sx)	78 (27.9)	3 (13.6)	176 (25.5)	7 (19.4)
Both GI and Ex symptoms	56 (20)	3 (13.6)	98 (14.2)	4 (11.1)
Asymptomatic	144 (51.6)	16 (72.7)	394 (57.3)	20 (55.6)
Human leukocyte antigen (HLA) genotype				
DR3-DQ2	49 (18)	2 (9.1)	148 (21.8)	10 (28.6)
DR3-DQ2 homozygote	18 (6.6)	3 (13.6)	25 (3.7)	3 (8.6)
DR7-DQ2	12 (4.4)	1 (4.6)	41 (6.1)	2 (5.7)
DR7-DQ2 homozygote	2 (0.7)	0 (0)	13 (1.9)	0 (0)
DR3/DR7-DQ2 homozygote	1 (0.37)	0 (0)	20 3.0)	3 (8.6)
DR4-DQ8	83 (30.5)	5 (22.7)	205 (30.2)	10 (28.6)
DR3-DQ2/DR4-DQ8	70 (25.7)	7 (31.8)	50 (7.4)	6 (17.1)
DR7-DQ2/DR4-DQ8	6 (2.2)	4 (18.2)	33 (4.9)	0 (0)
DQ2/DQ8 negative	31 (11.4)	0 (0)	142 (20.9)	1 (2.9)
Haptoglobin genotype (HP) (Zonulin)				
HP 1-1	46 (16.4)	2 (9.1)	120 (17.4)	8 (22.2)
HP 2-1	118 (42.1)	10 (45.5)	278 (40.4)	21 (58.3)
HP 2-2	116 (41.4)	10 (45.5)	290 (42.2)	7 (19.4)
Any HP2	234 (83.6)	20 (90.9)	568 (82.6)	28 (77.7)

T1D: Type 1 diabetes; CDA: Celiac disease autoimmunity; FDRs: First-degree relatives; GI sx: Gastrointestinal Symptoms; Ex sx: Extraintestinal Symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

participants who underwent screening. All individuals with known or newly diagnosed CDA, except for one, carried HLA-DQ2 or 8. The T1D + CDA and FDR + CDA groups demonstrated a higher frequency of HLA DQ2 compared to T1D and FDRs without CDA. Overall, participants in this cohort had a higher frequency of carrying *HP2* (in heterozygosity or homozygosity) than previously published work reporting the frequency of *HP* genotypes in the general population[18,19].

Prevalence of CD and CDA

Table 2 reports the prevalence of CD and CDA in the screened participant cohort. Prior to our screening program, 3.7% of participants with T1D and 1.8% of FDRs reported a diagnosis of CD or history of CDA. After screening, the estimated prevalence of CDA in our cohort increased by two-fold in patients with T1D and by 2.8-fold in FDRs. One participant in the cohort was found to have elevated IgG dGP and IgA deficiency and was classified as CDA.

Univariate and Multivariate Analysis

Participants with T1D: The univariate analyses of participants with T1D (Table 3) showed that an older age at study entry and older age of onset of T1D are associated with lower risk of screening positive for CDA. In addition, the following characteristics (in T1D patients) were associated with higher risk of CDA in our cohort: absence of symptoms, carrying DR7-DQ2/DR4-DQ8, first-degree relation to an individual with CD and first-degree relation to an individual with thyroid disease and/or any autoimmune disease. In multiple regression analysis of subjects with T1D (Table 3), carrying DR7-DQ2/DR4-DQ8 remained highly significantly associated with screening positive for CDA.

FDRs of Participants with T1D: The univariate analyses of FDRs of participants with T1D (Table 4) showed that absence of DQ2/DQ8 and presence of *HP2-2* are both associated with lower risk of CDA. Carrying DR3-DQ2 in homozygosity, DR3-DQ2/DR4-DQ8, and *HP2-1* are all associated with a higher risk of screening positive

Table 2 Cohort prevalence of celiac disease and celiac disease autoimmunity

	n	CD	CDA	CD at screening	CDA at screening	CDA prevalence estimation
Type 1 diabetes	302	8 (2.7)	3 (1.0)	8 (2.5)	3 (0.7)	22 (7.3)
First-degree relative	725	9 (1.2)	4 (0.6)	18 (2.7)	5 (1.0)	36 (5.0)

T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity.

for CDA. Risk of CDA was increased in those who reported a diagnosis of CD in a FDR and a history of any other autoimmune disease in a FDR. In multiple regression analyses of FDRs of subjects with T1D (Table 4), including the significant predictors from the univariate analyses, only reporting CD in a FDR is highly significantly associated with screening positive for CDA.

DISCUSSION

Though patients with T1D have been identified as a population at risk for CD who would benefit from routine screening, the optimal timing, frequency, and provider to spearhead this effort remain the object of debate. Implementation of our screening program in this known high-risk population and their FDRs revealed that a large proportion of individuals with undiagnosed CDA. Indeed, our active screening uncovered a two-fold increase in CDA in participants with T1D and a 2.8-fold increase in CDA in their FDRs. Furthermore, participants with T1D who screened positive for CDA were more frequently asymptomatic than participants with T1D only suggesting that routine screening is necessary to identify these patients.

Our study aimed at identifying clinical and laboratory characteristics to predict which individuals among this high-risk subgroup (T1D patients and their FDRs) may benefit most from screening for CD. While we did not find any significant clinical predictors of developing CD in our cohort, patients with T1D who screened positive for CDA had a trend towards a younger age of T1D onset and were less likely to report extra-intestinal or gastrointestinal symptoms than participants with T1D alone. Previous work has shown that up to 85% of patients with T1D who screen positive for CD are asymptomatic[4]. While data is mixed, some studies suggest gastrointestinal symptoms are more frequent in patients with long-term T1D compared to control patients[31]. Given that patients with T1D and CDA had a lower median age at the time of screening and had a narrower age range than patients with T1D alone, it is possible that patients with both T1D and CDA were diagnosed with T1D for a shorter period of time. Additionally, studies suggest that patients with T1D and poor glycemic control have more frequent GI symptoms[31,32]. Since we did not perform additional testing to assess glycemic control it is possible that more patients with T1D alone had poor glycemic control and a greater frequency of GI symptoms. Finally, our analysis of patients with T1D and CDA included eight patients who were diagnosed with CD prior to our screening program and thus were already on treatment for CD. Therefore, the low frequency of symptoms in patients with T1D and CDA may be explained by the inclusion of these patients already on a gluten free diet.

We also sought to describe the distribution of HLA and haptoglobin genotypes in our cohort and for the first time utilize the haptoglobin genotypes in a translational approach to identify predictors that may help to establish which patients among this unique cohort are more likely to develop CD. Our findings that participants with T1D are more likely to carry HLA DQ8 and participants with CD are more likely to carry HLA DQ2 compared to those without these conditions are in agreement with the published literature[33-35]. Moreover, our findings that *HP2-1* and *HP2-2* are more frequent in this cohort compared to the general population is expected and in agreement with previous work due to the association of *HP2* with autoimmune conditions[23]. These findings, along with those from our univariate analysis showing that lack of HLA DQ2/8 is associated with a lower risk of CDA, further establish that our cohort is well defined and that HLA DQ typing and analysis is robust.

The HLA genetics DR7-DQ2/DR4-DQ8 was significantly associated with screening positive for CDA in participants already diagnosed with T1D in our cohort. This is particularly interesting given that, while DR3-DQ2 is known to have a strong association with CD, DR7-DQ2 for some time had been overlooked as a risk allele for CD, with commercial clinical labs often not evaluating for this allele or mistakenly interpreting it as not increasing the risk of CD. While DR3-DQ2 is more frequent in patients with CD, 4.4% of patients carry DR7-DQ2[36]. Furthermore, studies suggest

Table 3 Univariate and multiple regression models: Factors related to celiac disease autoimmunity in participants with type 1 diabetes

	Estimate	Se	Lower limit	Upper limit	Z stat	*P*-value
Univariate Model (exchangeable correlation)						
Female	0.6562	0.5151	-0.3534	1.6659	1.27	0.2027
Age	-0.0314	0.0152	-0.0611	-0.0017	-2.07	0.0383[A]
Onset of T1D	-0.0658	0.0342	-0.1329	0.0013	-1.92	0.0546
Gastrointestinal (GI) symptoms	-0.5812	0.491	-1.5435	0.3811	-1.18	0.2365
Extraintestinal (EX) symptoms	-0.9204	0.6391	-2.1731	0.3323	-1.44	0.1498
Both GI and EX symptoms	-0.4724	0.6385	-1.7239	0.7791	-0.74	0.4594
No GI or EX symptoms	0.9074	0.4931	-0.059	1.8737	1.84	0.0657
Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous	-0.7402	0.7571	-2.2241	0.7437	-0.98	0.3282
HLA DQ2-DR3 Homozygous	0.7843	0.67	-0.5332	2.1019	1.17	0.2433
HLA DQ2-DR7 Heterozygous	0.028	1.0649	-2.0592	2.1152	0.03	0.979
HLA DQ8	-0.3975	0.5234	-1.4233	0.6283	-0.76	0.4475
HLA DQ2-DR3/DQ8	0.2767	0.4814	-0.6668	1.2203	0.57	0.5654
HLA DQ2-DR7/DQ8	2.4851	0.6507	1.2098	3.7604	3.82	0.0001[B]
HLA DQ2 Heterozygous	-0.575	0.6367	-1.823	0.6729	-0.9	0.3665
HLA DQ2 Homozygous	0.6192	0.666	-0.6862	1.9246	0.93	0.3525
Haptoglobin genotype (HP) 1-1	-0.6932	0.7671	-2.1967	0.8104	-0.9	0.3662
HP 2-1	0.1539	0.4414	-0.7112	1.0191	0.35	0.7273
HP 2-2	0.1525	0.4422	-0.7141	1.0192	0.34	0.7301
Any HP2	0.693	0.767	-0.81	2.197	0.9	0.366
First degree relative (FDR) with celiac disease (CD)	1.4091	0.6188	0.1962	2.622	2.28	0.0228[C]
FDR with Type 1 diabetes (T1D)	0.0724	0.557	-1.0193	1.1641	0.13	0.8966
FDR with thyroid disease	0.7839	0.4619	-0.1215	1.6892	1.7	0.0897
FDR with other autoimmune disease	1.1634	0.4432	0.2947	2.0321	2.62	0.0087[D]
CD in another relative	0.4136	0.6598	-0.8796	1.7068	0.63	0.5307
Multiple Regression Model (independence working correlation)						
Intercept	-3.3213	0.927	-5.1383	-1.5043	-3.58	0.0003
Age	-0.0121	0.019	-0.0494	0.0252	-0.64	0.5246
Onset of T1D	-0.0396	0.0425	-0.1229	0.0436	-0.93	0.3509
DQ2-DR7/DQ8	2.4131	0.8401	0.7666	4.0596	2.87	0.0041[E]
No GI or EX symptoms	1.1905	0.6566	-0.0963	2.4774	1.81	0.0698
FDR with CD	0.6927	1.3443	-1.9421	3.3274	0.52	0.6064
FDR with thyroid disease	-0.8976	1.6214	-4.0756	2.2804	-0.55	0.5799
FDR with other autoimmune disease	1.9765	1.7861	-1.5241	5.4771	1.11	0.2685

Significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

that, in patients at-risk for CD, the presence of DR7-DQ2 with DR3-DQ2 is associated with an increased frequency of developing CD[37]. Our findings are similar given that, despite a low frequency of participants with T1D carrying DR7-DQ2, those that do in combination with DR4-DQ8 have a high frequency of screening positive for CDA.

Our analysis did not demonstrate an association between carrying *HP2* and an increased risk of developing CD in participants with T1D or their family members. While we did not identify significant differences in *HP* genotype in this cohort, *HP2* was highly represented in our cohort. Additionally, there was a trend towards an underrepresentation of *HP1* in patients with both T1D and CD. Interestingly in FDRs there was a trend towards increased representation of *HP2-1*. These trends require further investigation with larger cohorts and should be compared to a group of individuals without a risk of autoimmune disease. Considering this, true trends may be somewhat masked by the nature of our study population; our cohort is made up of patients with a personal or family history of autoimmune disease, and thus a higher overall frequency of *HP2*. However, the purpose of our study was to identify predictors from a high-risk group. In our study, HLA type and having a family history of CD were the strongest predictors of developing CD. While we found that

Table 4 Univariate and multiple regression models: Factors related to celiac disease autoimmunity in first degree relatives of participants with type 1 diabetes

	Estimate	Se	Lower limit	Upper limit	Z stat	*P*-value
Univariate model (exchangeable correlation)						
White	1.26	0.88	-0.47	2.98	1.43	0.154
Female	0.5433	0.3674	-0.1768	1.2635	1.48	0.1392
Age	-0.0046	0.0097	-0.0236	0.0144	-0.47	0.6355
Gastrointestinal (GI) symptoms	0.2551	0.3395	-0.4103	0.9206	0.75	0.4524
Extraintestinal (EX) symptoms	-0.3748	0.4596	-1.2756	0.526	-0.82	0.4148
Both GI and EX symptoms	-0.2715	0.5305	-1.3113	0.7682	-0.51	0.6087
No GI or EX symptoms	-0.085	0.3419	-0.7551	0.5851	-0.25	0.8036
Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous	0.4255	0.383	-0.3251	1.1761	1.11	0.2666
HLA DQ2-DR3 Homozygous	0.8042	0.7654	-0.696	2.304	1.05	0.2934
HLA DQ2-DR7 Heterozygous	-0.0376	0.6636	-1.3382	1.263	-0.06	0.9548
HLA DQ2-DR3/DR7 Homozygous	1.02	0.715	-0.381	2.42	1.43	0.153
HLA DQ8	-0.0981	0.4058	-0.8934	0.6972	-0.24	0.809
HLA DQ2-DR3/DQ8	0.9272	0.472	0.0021	1.8523	1.96	0.0495[A]
HLA DQ2/DQ8 Negative	-2.1836	1.0232	-4.1891	-0.1781	-2.13	0.0328[B]
HLA DQ2 Heterozygous	0.3561	0.388	-0.4043	1.1166	0.92	0.3586
HLA DQ2 Homozygous	0.6842	0.5435	-0.3811	1.7495	1.26	0.2081
Haptoglobin genotype (HP) 1-1	0.2901	0.4341	-0.5607	1.1409	0.67	0.5039
HP 2-1	0.6823	0.3429	0.0103	1.3544	1.99	0.0466[C]
HP 2-2	-1.0763	0.4252	-1.9096	-0.2429	-2.53	0.0114[D]
Any HP2	-0.29	0.434	-1.141	0.561	-0.67	0.503
First degree relative (FDR) with CDceliac disease (CD)	1.6768	0.3809	0.9303	2.4233	4.4	<.0001[E]
FDR with thyroid disease	-0.503	0.4849	-1.4533	0.4473	-1.04	0.2995
FDR with other autoimmune disease	0.6817	0.3606	-0.025	1.3884	1.89	0.0587
CD in other relative	0.5217	0.7115	-0.8728	1.9161	0.73	0.4634
Multiple regression model (independence working correlation)						
Intercept	-2.8829	0.404	-3.6746	-2.0912	-7.14	< 0.0001
HLA DQ2-DR3/DQ8	0.9407	0.5381	-0.1139	1.9953	1.75	0.0804
HLA DQ2/DQ8 Negative	-1.9348	1.0275	-3.9487	0.0791	-1.88	0.0597
HP 2-1	0.0804	0.4492	-0.8001	0.9609	0.18	0.858
HP 2-2	-1.0149	0.563	-2.1185	0.0886	-1.8	0.0715
FDR with CD	2.3635	0.7576	0.8787	3.8483	3.12	0.0018[F]
FDR with other autoimmune disease	-0.8269	0.7245	-2.2468	0.5932	-1.14	0.2538

Significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

carrying *HP2* is not a predictor of developing autoimmune disease in this already high-risk population there was a trend towards an increased representation of *HP2* in patients with T1D and CDA. Further, it is unclear why FDR have a lower risk of developing CDA if they carry *HP2* in homozygosity and future work evaluating this finding in a larger cohort is needed.

Limitations of our study include utilization of self-reported family history of CD, T1D, and other autoimmune diseases, and lack of endoscopy to confirm CD in patients found to have CDA at screening. While this was not feasible in this screening study, all patients that had a positive serologic test for CD were advised to undergo further confirmatory testing with repeat blood work and an endoscopy. All patients with a positive IgA tTG had a second confirmatory test with IgA EMA. The majority of patients in our cohort with elevated IgA tTG additionally tested positive for IgA EMA making a diagnosis of CD likely. However, for the purposes of analysis, we combined participants with a positive IgA tTG alone and those with a positive IgA tTG and EMA in our CDA estimate. The possibility for falsely elevated or transiently elevated IgA tTG in patients with T1D and other autoimmune disorders is well known, thus our CDA estimate may be an overestimation. However, our prevalence estimates are in line with previously published work[4,5,7]. Finally, to assess follow-up

care in our cohort, a questionnaire was sent to participants during three years of the five-year study. Approximately 40% (*n* = 24) of participants with CDA responded to the questionnaire. Of those 38% (*n* = 9) of participants sought follow-up of their positive serology with a physician and the majority underwent endoscopy (*n* = 7). This highlights an additional limitation of screening studies in that appropriate follow-up is not ensured despite our efforts to provide educational materials and guidance.

In conclusion, implementation of a screening program increased identification of CDA in participants with T1D and their FDRs by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. Haptoglobin genotype did not predict the development of CDA in this high-risk population. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population, and given the often asymptomatic nature of disease; physicians should have a low threshold for screening.

ARTICLE HIGHLIGHTS

Research background
Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) are at increased risk of developing celiac disease (CD). The majority of patients with T1D and CD are asymptomatic at diagnosis and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs. We employed a prospective program to serologically screen patients with T1D and their FDRs for CD. We then retrospectively aimed to identify clinical and genetic predictors that may increase the risk of developing CD in this cohort of individuals at high-risk of developing CD.

Research motivation
Patients with T1D are up to eight times more likely to develop CD, and their FDR's are up to six times more likely to develop CD. Given that many may be asymptomatic, there is a need to identify predictors of CDA development in this high-risk cohort. The main topics, the key problems to be solved, and the significance of solving these problems for future research in this field should be described in detail.

Research objectives
Our objective was to identify clinical and genetic predictors that may increase the risk of developing CD in patients with T1D. In addition, we aimed to understand which FDRs of the patients with T1D, who are already at an increased risk of developing autoimmune disease, were more likely to develop CD. Our ultimate goal was to identify which subjects may benefit most from screening to help guide future screening recommendations.

Research methods
Participants included patients diagnosed with T1D or FDR of a patient with T1D attending the annual Children with Diabetes (CWD) conference over a 5 year time period. Participants answered clinical questionnaires and had blood drawn for CD serological testing and genotyping. Prevalence of celiac disease autoimmunity (CDA) was described. We then retrospectively fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D accounting for the correlation within families when indicated in order to identify predictors of developing CDA.

Research results
Implementation of a prospective screening program in patients with T1D and their FDRs increased identification of CDA by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. In FDRs of patients with T1D, screening positive for CDA was significantly increased in those who reported having a family member diagnosed with CD. Haptoglobin genotype did not predict the development of CDA in this high-risk population.

Research conclusions
CDA is under recognized in patients with T1D and their FDR's and that prospective screening in this high-risk cohort increased the identification of CDA by at least 2 fold. Clinical symptoms were not helpful in distinguishing patients with CDA, as the majority of patients reported no symptoms. Haptoglobin genotype was not found to be a predictor of CDA in this cohort. In our cohort, FDRs of patients with T1D were more likely to screen positive for CDA if they had a family history of CD, while patients with T1D who carried the HLA genotype DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA.

Research perspectives
Given the high frequency of CDA in patients with T1D and their FDRs, physicians should have a low threshold to screen for CDA even in the absence of symptoms.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Observational Study

Burden of diabetic foot ulcer in Nigeria: Current evidence from the multicenter evaluation of diabetic foot ulcer in Nigeria

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Institutional review board statement: Approval for the study was given by the local Research and Ethics committee of each of the participating centers.

Informed consent statement: Participation in this study was voluntary. Verbally granted informed consent was obtained from each patient prior to enrollment into the study. Confidentiality was ensured at all stages by means of unique coding system consisting of patients' initials and assigned numbers.

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Abstract

BACKGROUND
Nigeria bears the greatest burden of diabetes prevalence in Sub-Saharan Africa. Diabetic foot ulcer (DFU) is a serious and potentially life-threatening complication of diabetes. Significant improvements in diabetic foot incidence and outcomes have been recorded in many Western countries in the past decade. However, the current burden of DFU in Nigeria is largely unknown.

AIM
To evaluate the patients' profile, ulcer characteristics, associated co-morbidities and outcome of patients with DFU in Nigeria.

METHODS
Multicenter evaluation of diabetic foot ulcer in Nigeria was a one year multicenter observational study of patients hospitalized for DFU in six tertiary health institutions in Nigeria from March 2016 to March 2017. Demographic and diabetes information, ulcer characteristics and associated co-morbidities were assessed. Relevant laboratory and imaging studies were performed. All patients received appropriate multi-disciplinary care and were followed up until discharge or death. Outcome variables of interest were ulcer healing, lower

extremity amputation (LEA), duration of hospitalization and mortality.

RESULTS
A total of 336 patients (55.1% male) with mean age of 55.9 ± 12.5 years were enrolled into this study. Majority (96.1%) had type 2 diabetes. Only 25.9% of the subjects had prior foot care knowledge. Most of the subjects presented late to the hospital and median (IQR) duration of ulcer at presentation was 39 (28-54) d. Ulcers were already advanced (Wagner grades ≥ 3) in 79.2% of the subjects while 76.8% of the ulcers were infected at the time of admission. The commonest co-morbidities were systemic hypertension, anemia and hyperglycemic emergencies. One hundred and nineteen subjects (35.4%) suffered LEA while 10.4% left against medical advice. The median (IQR) duration of hospitalization was 52.0 (29-66) d with case fatality rate of 20.5%.

CONCLUSION
The burden of DFU in Nigeria is very high. The major gaps include low level of foot care knowledge among diabetic patients, overdependence on self-medication and unorthodox medicine following development of foot ulceration, late hospital presentation, and high amputation and mortality rates. Extensive foot care education within the framework of a multi-disciplinary foot care team is highly desirable.

Key words: Burden; Diabetes; Epidemiology; Foot ulcer; Amputation; Mortality; Multicenter evaluation of diabetic foot ulcer in Nigeria; Nigeria; Africa

Core tip: The multicenter evaluation of diabetic foot ulcer in Nigeria was a one year observational study of 336 adults who were hospitalized for diabetic foot ulcer in six tertiary hospitals in Nigeria. The subjects were managed by multi-disciplinary diabetic foot care teams and were followed up until discharge or death. This study demonstrated a high burden of diabetic foot ulcer in Nigeria which accounted for about a quarter of diabetes related hospital admissions over the study period. The study recorded high amputation and mortality rates of 35.4% and 20.5% respectively. Major challenges in diabetic foot care identified in this study include low level of foot care knowledge among the patients, poor health-seeking behavior and late hospital presentation.

INTRODUCTION

Although diabetes mellitus (DM) prevalence is rising globally, Sub-Saharan Africa appears to be the worst-hit[1]. In the last two decades, Nigeria, for instance has witnessed more than a 100% increase in the prevalence of the disease, from 2.2% in 1997 to nearly 6% in 2015[2]. It is generally reported that Nigeria which is the most populous country in Africa has the greatest burden of diabetes within the Sub-Saharan sub-continent[3]. This disproportionate increase in diabetes prevalence has largely been blamed on changing demographic dynamics including increasing urbanization and adoption of unhealthy lifestyles. Paralleling this increase in disease burden is also an upsurge in the prevalence of diabetes-related complications and death. One of the most devastating of these complications is diabetic foot ulcer (DFU), a costly, disabling but preventable complication of diabetes that is associated with significant morbidity and mortality.

DFU refers to a breech in the continuity of the skin epithelium involving its full thickness or beyond, distal to the ankle joints, in a person living with DM[4]. Foot ulceration is common in patients with DM with current global prevalence of about 6.3%[5]. It is estimated that a person with diabetes has a 25% lifetime risk of developing

DFU[6]. In Africa with constrained resources and fractured health systems, the prevalence of DFU is higher at about 7.2%[5]. The burden of DFU in Africa is substantial, constituting a major source of hospitalization and mortality[7]. With the rapidly rising diabetes prevalence in Africa, the burden of DFU in this region is expected to be on the increase.

Many of the predisposing factors for DFU are well established and include advancing age, long duration of diabetes, poor glycemic control, presence of neuropathy and peripheral vascular disease[8,9]. However, majority of DFUs are usually as a result of interplay among an at-risk foot, repeated micro-trauma and super-imposed infection[4]. Managing DFUs is usually very challenging especially in resource-constrained settings. The cost of managing DFU is substantial, and DFUs account for up to 40% of diabetes-related expenditures, making it one of the most expensive diabetes complications to deal with[10]. DFUs often heal very slowly resulting in prolonged hospitalization, or may fail to heal completely. They are also very prone to infection with resultant tissue necrosis and gangrene. Consequently, foot ulcerations are the commonest cause of lower extremity amputation (LEA) in persons with diabetes, accounting for up to 85% of LEAs in this population[4]. The International Diabetes Federation estimates that at least one limb are lost to DFU somewhere in the world every 30 s[1]! Lower limb amputation is associated with significant disabilities including loss of productivity and reduced quality of life[11]. Furthermore, it has been observed that 5-year survival after an LEA is worse than many cancers[12]. Foot ulceration in diabetic patients is therefore a medical, economic and psychosocial issue requiring serious attention.

The burden of DFU in Nigeria has been reportedly high, with prevalence rates ranging from 11%-32% among hospitalized patients[13,14]. At about half a decade ago, amputation rate from DFU in Nigeria was as high as 52%[15]. Furthermore, DFU is the commonest cause of diabetes-related mortality in Nigeria after hyperglycemic emergencies[2]. Diabetic foot ulceration is therefore a matter of serious public health concern in Nigeria. Contemporary data on the actual burden of DFU in Nigeria are however very scanty, and available studies on this subject were single-centered and mostly retrospective. In order to fill this gap, we sought to evaluate the current burden of DFU in a larger population across multiple centers in Nigeria.

MATERIALS AND METHODS

Study areas and design

The multicenter evaluation of diabetic foot ulcer in Nigeria (MEDFUN) was an observational study conducted in six tertiary healthcare institutions in Nigeria, between March 2016 and April 2017. These centers include Enugu State University Teaching Hospital located in South-Eastern Nigeria, Lagos State University Teaching Hospital in the South-West, Aminu Kano Teaching Hospital in the North-West, Ahmadu Bello University Teaching Hospital Zaria also in the North-West, Federal Medical Center Keffi in the North-Central and Federal Medical Center Umuahia also located in the South-East. The locations and geographic spread of these study sites are indicated in a map of Nigeria hereby presented (Supplementary). All the centers render specialized tertiary health care and serve as referral centers for primary and secondary health facilities within and outside their geopolitical zone. The Research and Ethics committee of each of the participating centers approved the study protocol while verbal informed consent was obtained from each patient prior to recruitment.

Subjects and recruitment

In the present study, subjects with type 1 DM (T1DM) or type 2 DM (T2DM) hospitalized for DFU in any of the participating centers were consecutively enrolled after obtaining verbal consent. Distinction between T1DM and T2DM was made clinically as follows: Subjects who reported dependence on insulin for diabetes control since the time of diagnosis were classified as having T1DM while those who had been controlled on oral anti-diabetic drugs with or without insulin were adjudged to have T2DM. Pregnant women, subjects with diabetes other than types 1 and 2, and those with wounds limited to above the ankle joints were excluded.

Data collection and clinical measurements

Using a specially designed structured proforma, relevant socio-demographic and diabetes-related information such as gender, age, occupation, cigarette smoking status, diabetes type and duration, as well as the type of healthcare facility where the patient was receiving diabetes care prior to development of foot ulcer were obtained and documented. Knowledge of foot care was assessed and patients were interviewed

on whether they had received foot care education prior to foot ulceration. History of development and progression of ulcer including mechanism of ulceration, site of ulcer, duration of ulcer and prior ulcer treatment methods were also assessed. Clinical wound infection was determined according to the International Working Group on Diabetes Foot (IWGDF) guideline by the presence of purulent exudates or any two or more of the following: Periwound edema, periwound redness, local warmth, foul smell, pain or tenderness on palpation and fever[4]. Commonly known risk factors for DFU were also evaluated, including history of previous DFU, barefoot walking, improper foot wear, visual impairment, foot deformity, peripheral neuropathy and peripheral artery disease (PAD). Peripheral neuropathy was diagnosed by loss of pressure perception to Semmes-Weinstein 10 g monofilament test or diminished vibration sense using the 128 Hz tunning fork. PAD was diagnosed based on impalpable dorsalis pedis and/or posterior tibial artery pulsations on manual palpation or significant arterial narrowing (> 50%) on Doppler ultrasonography of the lower limbs. The severity of ulcer was graded using two different ulcer classification systems, namely, the Wagner's grading system and the University of Texas wound classification system[16,17].

Relevant laboratory and imaging studies were performed for each subject including urine protein using dipstick detection, full blood count, erythrocyte sedimentation rate, glycated hemoglobin (HbA1c), blood culture, ulcer specimen culture, lipid profile, plain radiograph of the foot and Doppler ultrasonography of both lower limbs. Co-morbid complications including hypertension, anemia, shock, hyperglycemic emergency, hypoglycemia, stroke, kidney disease and cardiac failure were explored and documented.

Patient management and outcome indicators

Every patient received appropriate multi-disciplinary care including bed rest, wound debridement, daily wound dressing, antibiotic therapy, skin grafting and limited amputation in addition to control of blood glucose and treatment of associated co-morbidities. All the primary investigators who led the multidisciplinary team were endocrinologists. Other relevant specialists including nutritionists, plastic surgeons, orthopedic surgeons and vascular surgeons were co-opted based on need and availability. None of the centers had a podiatrist, an important foot care specialist that is grossly in short supply in Nigeria. The decision to amputate or not was an exclusive prerogative of the multi-disciplinary footrace team at each study center. All the enrollees were followed up until discharge or death. Outcome variables of interest included ulcer healing, amputation, duration of hospitalization and mortality. We defined amputation above the mid-tarsal bone or involving the big toe as major amputation, otherwise it was minor. At the stoppage of data collection, records of medical admissions over the study period were reviewed retrospectively in all the centers to determine the total number of medical admissions and diabetes-related admissions.

Statistical analysis

Data were collated in all the six participating centers and analyzed using the Statistical Package for Social Sciences (IBM version 23.0; SPSS Inc., Chicago, IL, United States). Categorical variables were presented as numbers and percentages while continuous variables were presented as means and standard deviations or medians and interquartile ranges as appropriate. Analysis at this stage was mainly descriptive. Data were presented in frequency tables, bar charts, pie charts and line graphs as deemed appropriate. The Chi-Square test was used to test differences in categorical proportions while continuous variables were compared between two or more groups of interest using the Student's *t*-test. Statistical significance was established at $P < 0.05$.

RESULTS

There were 9778 total and 1350 (13.8%) diabetes related admissions in the medical wards over the study period. Out of this number, 336 patients with a male: female ratio of 1:0.8 had DFU, and this number accounted for 24.9% of DM-related admissions. Majority of the DFU subjects (96.1%) had type 2 diabetes. The mean ± SD age and mean ± SD duration of DM were 55.9 ± 12.5 years and 8.5 ± 5.7 years respectively. Most of the patients (71.7%) were not accessing diabetes care at the study centers but were referred because of the foot ulcer. Glycemic control was generally poor with mean HbA1c of 9.6 ± 1.9%. Only 87 subjects (20.4%) had received foot care education prior to development of ulcer. Neuropathic and neuro-ischemic ulcers predominated in 37.2% and 40.2% of the subjects respectively. Ulcers were adjudged advanced (Wagner grade ≥ 3) in 79.2% of the subjects and majority were already

infected. The commonest co-morbidities were systemic hypertension (56.8%), anemia (53.6%) and hyperglycemic emergencies (36.6%). Table 1 shows the clinical profile of the study participants while the ulcer grades are shown in Figure 1.

Identifiable factors that probably predisposed the patients to developing DFU are presented in Figure 2. Diabetic peripheral neuropathy (DPN) and PAD were present in 78.0% and 52.4% of the participants respectively. About 48.2% of the subjects admitted to barefoot walking while 28.6% have had previous foot ulceration. As shown in Figure 3, majority of the subjects have multiple risk factors such that up to 44.9% have four or more risk factors operating simultaneously.

Only 21.1% of our subjects sought treatment in hospital as their first option following development of foot ulcer. The most preferred initial treatment option was self-medication which was practiced by 42.0% of the patients. 19.6% of the subjects patronized traditional healers/herbalists while 9.2% relied on prayer houses. These are summarized in Figure 4.

Figure 5 shows the admission outcomes of the patients studied. Of the 336 subjects hospitalized for DFU, satisfactory wound healing occurred in 147 subjects (43.8%). One hundred and nineteen subjects (35.4%) underwent LEA of which 75.6% were major amputations. Thirty-five subjects (10.4%) left against medical advice, mainly due to refusal of amputation (48.6%) and financial constraint (42.9%). Sixty-nine deaths (20.5%) were recorded, including 34 deaths post LEA. The median time between admission and death was 16 d (interquartile range 10-33 d). The median (IQR) duration of hospitalization for the study population excluding those who discharged against medical advice was 52.0 (29-66) d. Both amputation and mortality rates significantly increased with higher ulcer grades (Figure 6).

DISCUSSION

With a population estimated at about 200 million people, Nigeria is the most populous black nation. And with diabetes prevalence of nearly 6% in adults, representing about 5-7 million adults, Nigeria currently harbors the largest number of people living with diabetes in the West African sub-region[1]. Understanding the burden of diabetes and its complications in Nigeria is therefore a reliable sneak peek into the rest of Africa. Diabetic foot ulceration is one of the most challenging complications of DM. Due to absence of national data, prevalence rates of DFU in Nigeria from several single center studies vary widely from 11.7%-32%[13,14]. Similar wide variations have also been reported for DFU outcomes with amputation rates ranging from 12.6%-52%[14,15] and mortality rates ranging from as low as 8.7% to above 40%[13,14,18]. The need to have a current and more representative national data on the outlook of DFU in Nigeria therefore became the driving force that birthed the MEDFUN study.

Our data shows that DFU constitutes about a quarter of diabetes related hospital admissions in Nigeria. This represents a much higher burden than what is obtainable in developed nations where DFU generally accounts for less than 10% of medical admissions[19,20]. Our findings closely mirror the scenarios in some other African settings where the burden of DFU is also reportedly high[21,22]. Worrisome too is the fact that the bulk of our patients (73.8%) belonged to the young and middle-age categories and nearly three-quarter have had diabetes for less than 10 years duration. This finding is supported by previous local studies and suggests that in Nigeria, DFU affects predominantly the actively-working segment of the population who are often their family bread winners[14,15,18]. In contrast, majority of patients with DFU in Netherlands and Thailand for instance are above the age of 60 years and have had diabetes for longer duration[20,23]. The socio-economic consequences of this scenario on a people already groaning under poverty and many communicable diseases could be better imagined.

The prevalence of diabetic foot disease largely reflects the quality of diabetes care as this complication of DM is largely preventable through proper diabetes management[24]. Approximately 83% of our study subjects had HbA1c above 7%, a reflection of the poor quality of diabetes care in our locality especially at primary and secondary healthcare levels where the bulk of our patients came from. Poor glucose control has been widely reported in Nigeria even among subjects attending tertiary health institutions[25,26]. Factors that may be responsible for this include poverty, poor drug compliance, poor access to healthcare, shortage of trained diabetes care manpower and diabetes status unawareness. It is noteworthy that 14.6% of the subjects in this study were unaware of their diabetes status until they presented with foot ulcer. Chronic hyperglycemia is generally known to predispose to many diabetes-related complications including peripheral neuropathy, and the latter is a potent risk
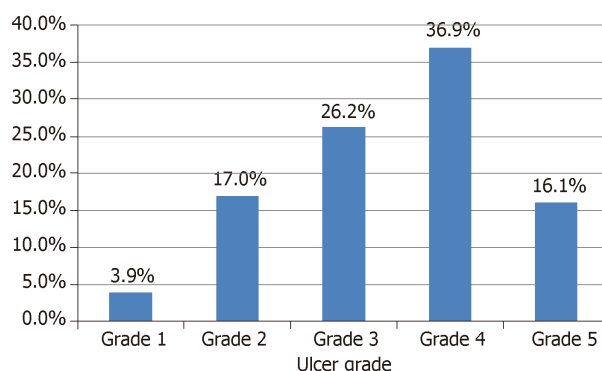
Table 1 Clinical profile of the patients with diabetic foot ulcers

Variable	n (%)	mean ± SD
Age (yr)		55.9 ± 12.5
< 45 yr	48 (14.3)	
45-64 yr	200 (59.5)	
≥ 65 yr	88 (26.2)	
Gender (male)	185 (55.1)	
Occupation		
Civil servants	61(18.2)	
Traders	137 (40.8)	
Artisans	12 (3.6)	
Farmers	40 (11.9)	
Unemployed	86 (25.6)	
Cigarette smoking (current smokers)	17 (5.1)	
Diabetes type (type 2)	323 (96.1)	
Diabetes duration (yr)		8.5 ± 5.7
≤ 10 yr	250 (74.4)	
11-20 yr	79 (23.5)	
> 20 yr	7 (2.1)	
Newly diagnosed diabetes	49 (14.6)	
Glycated hemoglobin (%) (n = 296)		9.6 ± 1.9
HbA1c < 7%	17 (5.7)	
Referred from outside the study centers	241 (71.7)	
Ever had foot care education	87 (25.9)	
Type of Ulcer		
Neuropathic	125 (37.2)	
Ischemic	42 (12.5)	
Neuro-ischemic	135 (40.2)	
Non-neuropathic, non-ischemic	34 (10.1)	
Duration of ulcer before admission (d)		39 (28-54)[1]
Ulcer > 30 d duration	237 (70.5)	
Previous history of ulcer	96 (28.6)	
Advanced ulcer (Wagner grade ≥ 3)	266 (79.2)	
Presence of wound infection	258 (76.8)	
Co-morbid complications		
Hypertension	191 (56.8)	
Shock	40 (11.9)	
Anemia	180 (53.6)	
Hyperglycemic emergency	123 (36.6)	
Hypoglycemia	33 (9.8)	
Cardiac failure	23 (6.8)	
Renal impairment	66 (19.6)	
Stroke	32 (9.5)	

[1]Data presented as median (interquartile range).

factor for development of DFU[8,9,27]. Up to 78% of our study subjects presented with DPN which we also identified as a major risk factor for DFU. DPN predisposes to DFU by causing loss of protective sensation in the feet as well as foot deformities, resulting in abnormal weight bearing, recurrent micro-trauma, callous formation and eventual ulceration[8,9].

Our study uncovered very low levels of foot care knowledge among the participants. We observed that nearly three-quarter of the patients had never received foot care education since diagnosis of diabetes. This finding is of great concern owing to the strategic importance of proper foot care knowledge in the prevention of DFU and amputation. It has been demonstrated that diabetic patients who are
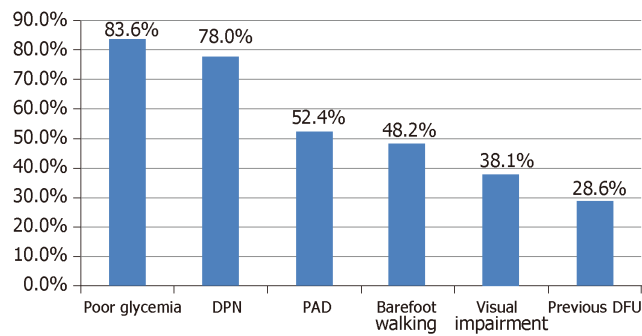
Figure 1 Distribution of diabetic foot ulcer severity by Wagner grading system.

knowledgeable about foot care are 3 times less likely to develop DFU and to suffer LEA[28,29]. Patients who have adequate foot care knowledge are less likely to engage in harmful foot practices that could predispose to ulceration. They are also more likely to present earlier to the hospital following ulceration thereby reducing the likelihood of amputation. Other authors in Nigeria have observed low level of foot care knowledge both among the general diabetic population and those with DFU[14,30]. In a recent multi-center study, 78.4% of the 352 diabetic patients surveyed had poor knowledge of foot care and the authors lamented that high risk behaviors such as bare foot walking and improper foot wear were rampant among the patients[30]. Anumah *et al*[14] recently reported that 84.7% of patients who were hospitalized for DFU at a tertiary hospital had no prior foot care education. Certified diabetes educators are grossly in short supply in Nigeria and almost non-existent in rural and semi-urban areas. This manpower shortage may largely explain this serious gap in diabetes care in our locality.

The poor health-seeking behavior of patients with DFU in Nigeria was also brought to bear in this study. Our data show that the practice of self-medications and patronage of unorthodox treatment outlets including native/herbal homes and prayer houses were common initial treatment options among the patients. Although this attitude may be partly attributable to poverty and poor access to healthcare, it may not be totally unconnected with the negative illness perceptions that are pervasive in Africa. In many traditional African cultures, diseases are often ascribed to diabolism and spiritual etiologies[31-33]. In Lagos, Nigeria, as many as 46% of diabetic patients take alternative herbal medicines[34]. The presence of a non-healing wound may therefore be misinterpreted as the outcome of "stepping on poison" or "spiritual attack" and orthodox care is usually not sought until the disease is advanced. This may partly account for the late hospital presentation which was observed among our study subjects. The ulcer had lasted more than 1 mo in over 70% of our subjects prior to hospitalization, with 79.3% of patients presenting with at least Wagner grade 3 ulcers. Delayed hospital presentation is also a common denominator in many previous studies of DFU in Nigeria[13-15].

Amputation and mortality rates of 35.4% and 20.5% respectively that were observed in this study are unacceptably high and not in tandem with the trends in the civilized world. In Australia for instance, LEA rate from DFU is less than 2% with over 70% being minor amputation[19]. Very low amputation and mortality rates were also reported in Netherlands, Thailand, and Scotland[20,23,35]. Differences in the quality of healthcare systems are likely to be responsible for these discrepancies. We hypothesize that the poor glucose control and delay in hospital presentation following development of DFU contributed significantly to these unpleasant outcomes. Lavery *et al*[36] demonstrated that duration of ulcer more than 30 d increased the probability of wound infection by nearly 5 times and that amputation was 154 times more likely in infected wounds. This is probably due to the higher propensity of accelerated tissue necrosis and gangrene in such wounds especially in a limb with compromised vascular supply. Wound infection was present in 76.8% of our patients while over half had developed some form of gangrene. Such patients are expected to suffer more amputation and death from overwhelming sepsis. Not surprisingly therefore, we observed significant associations between ulcer severity as measured by Wagner grading, and amputation as well as mortality ($P < 0.001$ respectively). Our data agree with many other previous studies in Nigeria that also reported high amputation and mortality rates among patients with DFU[13,18,37]. Nigeria is therefore in dire need of total overhaul of diabetes care to stem this ugly tide. Importantly, appropriate multi-

Figure 2 Prevalence of risk factors for diabetic foot ulcers in the study population. DFU: Diabetic foot ulcer; DPN: Diabetic peripheral neuropathy; PAD: Peripheral artery disease.

disciplinary care team approach led by an endocrinologist has been found to drastically improve diabetic foot outcomes and is hereby advocated[38].

Conclusion
The results from this study revealed that the burden of DFU in Nigeria is still alarming even in this 21st century. This study has exposed several treatment gaps including poor knowledge of foot care among patients, high patronage of self-medications and unorthodox treatment, and delayed hospital presentation with advanced foot ulcers, resulting in prolonged hospitalization, high LEA rate and high mortality. Bridging these gaps through intensive public enlightenment programmes, foot care education of diabetic patients and establishment of well-trained diabetic foot care team may go a long way in reversing this ugly trend.

Strenghts and limitations
To our knowledge, MEDFUN is the largest, most extensive and the only multi-center study on DFU both in Nigeria and the West-African sub-region. The limitations of this study however need to be highlighted. Firstly, the study centers covered only 4 out of the six geo-political zones of Nigeria. However, the 2 geo-political zones that were not included in this study share common characteristics with one or more of the other 4 zones. It is therefore arguable that our results are largely generalizable as a true reflection of the burden of DFU in Nigeria. Secondly, each of the participating centers adopted its own DFU management protocol based on availability of manpower. Clinical decisions were therefore dependent on the clinicians at each center. It is not unlikely that this lack of uniformity might have affected the outcome of this study. This is also applicable to the clinical measurements which are prone to inter-observer bias and laboratory tests which might have been influenced by performance variations of diagnostic equipments at the different study centers. However, this lack of uniformity is common in studies of this nature, including the widely cited Eurodiale study which was the largest multi-center DFU study in Europe[30].
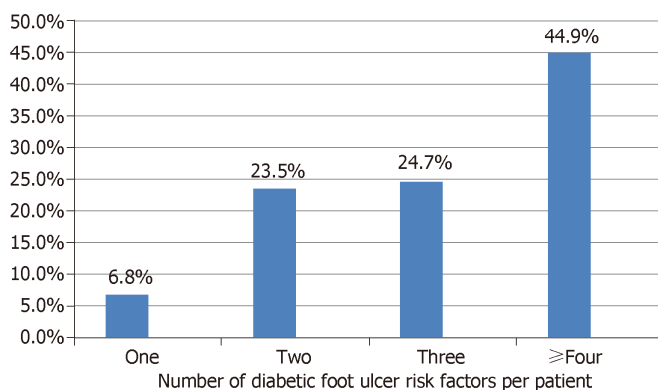
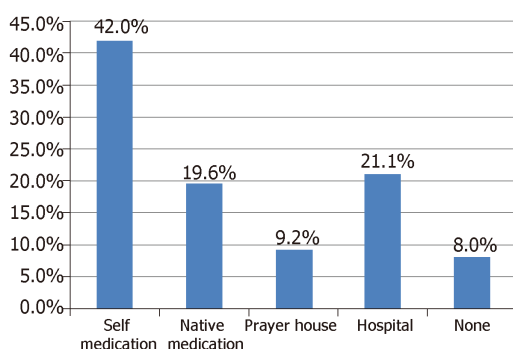**Figure 3 Per patient burden of diabetic foot ulcer risk factors.**

Figure 4 Preferred initial treatment options for patients with diabetic foot ulcers in Nigeria.
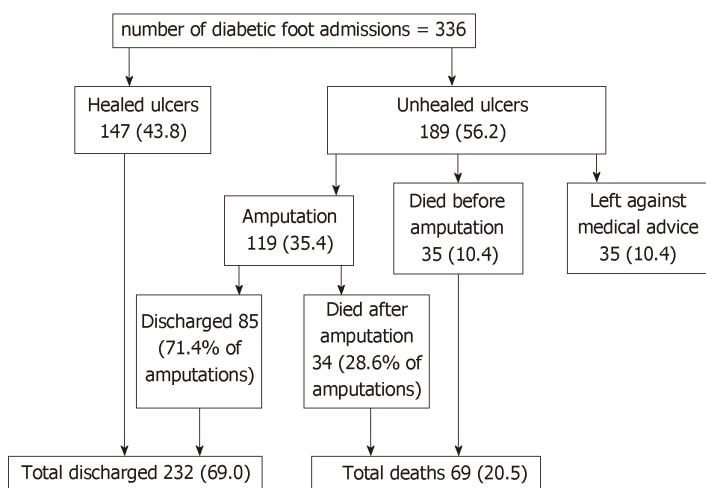

Figure 5 Outcomes of diabetic foot ulcer admissions in Nigeria.
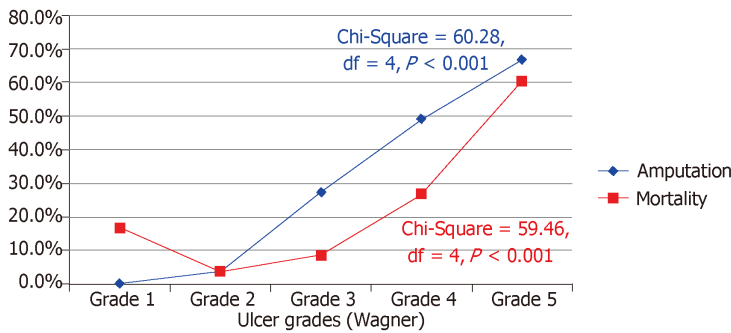
Figure 6 Amputation and mortality rates by ulcer grades.

ARTICLE HIGHLIGHTS

Research background

Diabetic foot ulcer (DFU) is a serious and costly complication of diabetes that is associated with high morbidity and mortality. However, DFU-related lower extremity amputation (LEA) and death are both preventable through appropriate healthcare measures.

Research motivation

The prevalence of diabetes in Nigeria is steadily rising with the country currently harboring the largest burden of diabetes in Sub-Saharan Africa. Evaluation of a disease burden helps in identifying healthcare gaps that need to be addressed. However, the current burden of DFU in Nigeria is largely unknown.

Research objectives

We evaluated the patient and ulcer characteristics as well as the outcomes of patients hospitalized for DFU in six tertiary healthcare centers in Nigeria over a one year period.

Research methods

In an observational study design, we followed up a total of 336 type 1 and type 2 diabetic patients who were hospitalized for DFU until they exited the hospital. Then we documented their baseline profile, clinical progress, disease outcomes and mode of exit.

Research results

The study revealed that DFU accounted for about a quarter of diabetes related hospitalization in Nigeria. It further showed that most of the affected patients lacked knowledge of foot care and resorted to self-medications or alternative medicine approaches following development of foot ulcer. Consequently, over three-quarter of the patients presented late to the hospital with advanced ulcer. The study revealed a high LEA and mortality rates of 35.4% and 20.5% respectively.

Research conclusions

We concluded that the burden of DFU in Nigeria is still substantial and decried the high degree of foot care ignorance and poor health-seeking behavior among patients with DFU in our country.

Research perspectives

We advocate for massive public enlightenment programmes about diabetic foot with emphasis on its prevention and timely treatment. Massive training of diabetes educators and podiatrists in Nigeria to improve foot care knowledge and foot care practice is strongly recommended.

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Observational Study

Prevalence and associated factors of hospitalization for dysglycemia among elderly type 2 diabetes patients: A nationwide study

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Abstract

BACKGROUND
The prevalence of older individuals with type 2 diabetes mellitus (T2DM) is increasing due to the aging population and improved medical care. These patients are very susceptible to disease and treatment-related hospitalizations, resulting in higher health care costs, morbidity, and decreased quality of life. However, data of treatment-related complications, especially dysglycemia-related hospitalizations, are lacking.

AIM
To assess the prevalence and associated factors for dysglycemia-related hospitalizations among elderly diabetic patients in Thailand using nationwide patient sample.

METHODS

T2DM patients aged ≥ 65 years who received medical care at public hospitals in Thailand in the year 2014 were included. The prevalence of hospitalization due to dysglycemia within one year was examined. Multivariable logistic regression was performed to assess the independent factors associated with hospitalization due to hypoglycemia and hyperglycemia

RESULTS
A total of 11404 elderly T2DM patients were enrolled in this study. The mean age was 72.9 ± 5.5 years. The prevalence of hospital admissions due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, hyperglycemic dehydration syndrome, and hypoglycemia among elderly T2DM patients in the year 2014 was 0.1%, 0.1%, 1.7% and 3.1%, respectively. Increased hospitalization due to hypoglycemia was associated with older age, female sex, had hypertension, dementia, lower body mass index, elevated hemoglobin A1C (HbA1C), decreased kidney function, insulin use. Increased hospitalization due to hyperglycemia was associated with dementia, depression, lower body mass index, elevated HbA1C, and insulin use.

CONCLUSION
The prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was 4.9%. Close monitoring of blood glucose should be provided in high-risk patients for prevention and early detection for these complications.

Key words: Type 2 diabetes mellitus; Hospitalization; Diabetes in elderly; Dysglycemia; Hypoglycemia; Hyperglycemia

Core tip: Currently, the numbers of older individuals over 65 years of age with type 2 diabetes mellitus (T2DM) are rising. However, data of treatment-related complication especially, dysglycemia-related hospitalization is lack. In this study, we conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. We demonstrated that the prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was 4.9%. The close monitoring of blood glucose should be provided in high-risk patients for prevention and early detection for these complications.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common chronic disease worldwide that poses a significant crisis in the global health system[1,2]. The associated morbidity and mortality can be caused by the disease itself or its associated multisystem complications that can increase in incidence and severity with prolonged disease duration. This disease, which has a higher prevalence in the older population, is also increasing in the elderly population likely due to the aging population[3,4]. The prevalence of individuals over 65 years of age with T2DM in Thailand reached 17.2% as reported by the InterASIA study in 2003[5]. Furthermore, increased age unsurprisingly has been found to be a significant predictor of higher health-care costs among diabetic patients[6,7].

Older patients with T2DM are more susceptible to dysglycemia-related complications requiring hospitalizations and associated morbidity and mortality[8]. Several recent studies showed that intensive glucose control strategies may derive less benefit and have demonstrated increased harms[3,9]. Increasingly, the importance of specialized care and management for the geriatric population on clinical outcomes has

been recognized, and as such the treatment approach used T2DM in the elderly population should differ from those in the younger patients[8]. However, DM care quality metrics established more than a decade ago have primarily focused on prevention of hyperglycemia and its complications[9]. The current state of clinical practice in relation to established quality metrics and its impact on dysglycemic-related hospitalizations in elderly T2DM in Thailand is unknown. Thus, the aim of this study sought to determine whether prevalence of dysglycemia-related hospitalization in elderly T2DM in Thailand and the associated factors.

MATERIALS AND METHODS

Study design and population

This was an analysis on the DM/HT dataset in 2014[10]. This was a nationwide survey conducted annually in Thailand to evaluate the status of medical care in T2DM patients who visited the public hospitals of the Thai Ministry of Public Health and the clinics in the Thailand National Health Security Office's program. The Inclusion criteria of this DM/HT survey consisted of T2DM patients aged ≥ 35 years who received regular medical care in the targeted hospital for at least 12 mo. Patients who received care at primary care units outside Bangkok and University hospitals were excluded from the study. A two-stage stratified cluster sampling method was used to select a nationally and provincially representative sample of T2DM patients in Thailand. The first stage of sample collection consisted of the provinces that constituted 77 strata. The second stage of sample collection was the hospitals' levels in each province, which were stratified into five strata according to the size of the hospital. These five strata were regional (> 500 beds), provincial (200-500 beds), large community (80-120 beds), medium community (60 beds), and small community (10-30 beds) hospitals. All regional (*n* = 25) and provincial (*n* = 70) hospitals were enrolled, but only 456 (62% out of 736) community hospitals were included. Of 456 community hospitals, 10%, 20%, and 70% were large, medium and small community hospitals, respectively (Figure 1).

All patients were recruited from the outpatient clinic. Written informed consent was obtained from patients before enrollment. This study was approved by both the Institutional Review Board of the Royal Thai Army Medical Department and the Ethical Review Committee for Research in Human Subjects, the Ministry of Public Health of Thailand due to the regulations of bureaucratic systems in Thailand. Well-trained research nurses reviewed medical records and collected data into a case record form. Data entry into the case record form was then transferred to the central data management of the Medical Research Network of the Consortium of Thai Medical Schools to adjudicate that the process of data collection was compiled according to study protocol. The data management team was responsible for inquiries to study sites to verify data. Site monitoring was randomly performed in approximately 10% of study sites. To focus on the hospital admission due to dysglycemia in elderly T2DM patients during 2014, we selected only patients aged ≥ 65 years for analysis in this study.

Data collection

Clinical characteristics, demographic information, medication, and laboratory data were collected using manual data retrieval from the medical record as described above. Body mass index (BMI) was stratified by using criteria for an Asian population[11]. GFR was estimated based on age, sex, race and the most recent creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation[12]. We examined the prevalence of hospitalization due to hypoglycemia and hyperglycemia in the year 2014. Hyperglycemia complication included diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome.

Statistical analysis

Continuous variables were presented as mean ± SD. Categorical variables were presented as count with percentage. Backward stepwise multivariable logistic regression analysis was performed to identify factors associated with hospital admission due to hypoglycemic and hyperglycemic complications. Odds ratio (OR) with 95%CI was reported. Possible interactions and collinearities were also tested. A *P*-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, United States).
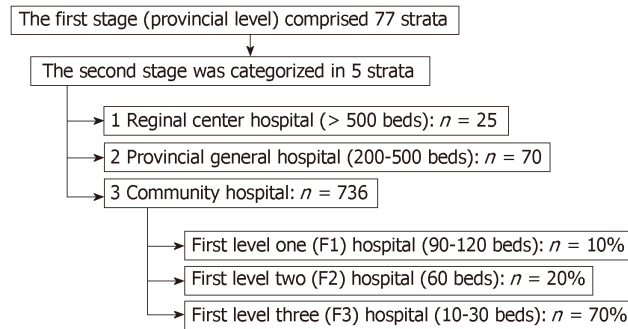
A two-stage stratified cluster sampling method

Figure 1 Flow chart of the participant selection.

RESULTS

Clinical characteristics

A total of 11404 elderly T2DM patients were included in the analysis. The clinical characteristics are summarized in Table 1. The mean age was 72.9 ± 5.5 years. Twenty-nine point four percent of the patients were aged > 75 years. Thirty-one point five percent were male. The mean diabetic duration was 7.5 ± 4.4 years. Twenty point nine percent of the patients used insulin. The mean BMI was 24.3 ± 4.3 kg/m². The mean hemoglobin A1C (HbA1C) was 7.5% ± 1.9%. The mean estimated glomerular filtration rate (eGFR) was 54.7 ± 21.5 mL/min per 1.73 m² (Table 1).

Prevalence of dysglycemia-related hospitalizations among elderly T2DM patients

The prevalence of dysglycemia-related hospitalizations among elderly T2DM patients during the year 2014 was 4.9% (*n* = 558). Among elderly T2DM patients, 11 (0.1%), 16 (0.1%), and 192 (1.7%) were admitted in hospital due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome, respectively, whereas 356 (3.1%) were admitted in hospital due to hypoglycemia.

Associated factors associated with hypoglycemia-related and hyperglycemia-related hospitalizations

In multivariable analysis, older age, female sex, had hypertension, dementia, lower BMI, insulin use, elevated HbA1c, and decreased eGFR were associated with increased risk of hospital admission due to hypoglycemia, whereas overweight and obesity were associated with decreased risk of hospital admission (Table 2).

In multivariable analysis, dementia, depression, insulin use, and HbA1C 8.5% and above were associated with increased risk of hospital admission due to hyperglycemia, whereas overweight was associated with decreased risk of hospital admission (Table 3).

DISCUSSION

This was a large nationwide, multicenter, one-year period cross-sectional study that examined prevalence and associated factors for hospitalization due to dysglycemia among elderly T2DM patients during the year 2014. Despite a prior study that showed an overall decreasing trend in dysglycemia-related hospital admissions among elderly Thai T2DM patients[13], our data describe that the prevalence of hypoglycemia-related hospitalization is higher than previously reported from United States[7,14], England[15], Canada[16], Italy[17], Denmark[18] and South Korea[19] (Table 4). This might be due to several reasons. First, the United States report consisted of combined data from both diabetic and non-diabetic patients. Second, our study included different sized hospitals, and the smaller sized hospital may have had limited availability of specialists. This may have negatively affected the quality of care for these special populations. Moreover, our study found that hypoglycemia-related hospitalization is higher than hyperglycemia in old diabetic patients. It could translate that elderly T2DM patients were likely to treat with rigorous glycemic control.

Our study revealed that age, female sex, hypertension, dementia, insulin use, low BMI, elevated HbA1C and low eGFR are associated with hypoglycemia-related hospitalizations. Older age and its association with severe hypoglycemia is consistent

Table 1 Baseline characteristics

Characteristics	All
N	11404
Age (yr)	72.9 ± 5.5
65-75	8055 (70.6)
> 75	3349 (29.4)
Male	3594 (31.5)
Duration of diabetes (yr)	7.5 ± 4.4
Hypertension	9831 (86.2)
Dyslipidemia	8048 (70.6)
Cancer	96 (0.8)
Dementia	24 (0.2)
Depression	106 (0.9)
Cerebrovascular disease	451 (4.0)
Cardiovascular disease	1129 (9.9)
Peripheral artery disease	82 (0.7)
Peripheral neuropathy	448 (3.9)
Diabetic retinopathy	688 (6.0)
Smoking	326 (2.9)
Insulin	2385 (20.9)
BMI (kg/m^2)	24.3 ± 4.3
< 17.5	391 (3.6)
17.5-22.9	3992 (36.6)
23.0-27.9	4655 (42.6)
≥ 28.0	1882 (17.2)
HbA1C (%)	7.5 ± 1.9
< 7.0	3760 (43.7)
7.0-8.5	2892 (33.6)
> 8.5	1947 (22.6)
eGFR (mL/min per 1.73 m^2)	54.7 ± 21.5
≥ 60	4034 (38.5)
< 60	6448 (61.5)
Prevalence (%)	
Dysglycemia-related hospitalization	558 (4.9)
Hypoglycemia-related hospitalization	356 (3.1)
Diabetic ketoacidosis related hospitalization	11 (0.1)
Hyperosmolar hyperglycemic state-related hospitalization	16 (0.1)
Hyperglycemic dehydration syndrome-related hospitalization	192 (1.7)

BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate.

with several reports[19,20]. The Korean cohort demonstrated that older patients, females, several comorbidities such as chronic kidney disease and dementia, and insulin use were associated with a high risk of hypoglycemia[19]. Another previous study found that in patients who are aged ≥ 80 years, severe hypoglycemia accounted for up to one in six hospital admissions[20]. Older patients may have multiple factors that can predispose them to develop hypoglycemia such as polypharmacy, age-related changes in pharmacokinetics and pharmacodynamics, decreased hormonal regulation and counter-regulation, suboptimal intake of water and/or food, decreased intestinal absorption, and cognitive impairment[21,22].They may also be burdened with diseases that affect their ability to effectively metabolize hypoglycemic agents or respond to hypoglycemia, such as heart failure, liver disease, sarcopenia[23] and kidney dysfunction[24]. Female sex is associated with hypoglycemia related hospitalizations, consistent with a Korean cohort[19]. Females are hypothesized to develop hypoglycemia more readily due to lower muscle mass, less tolerability of hypoglycemic symptoms, stricter diet control, and less access to medications than males[25,26]. Our study found that elderly T2DM patients with hypertension are

Table 2 Crude and adjusted odds ratios of factors that were independently associated with hospital admission due to hypoglycemic complication, using multivariate analysis

Variables	Crude OR (95%CI)	*P*-value	Adjusted OR (95%CI)[1]	*P*-value
Age (yr)				
65-75	Reference		Reference	
> 75	1.87 (1.51-2.32)	< 0.001	1.79 (1.37-2.35)	< 0.001
Gender				
Male	Reference		Reference	
Female	1.64 (1.27-2.11)	< 0.001	1.63 (1.20-2.21)	< 0.01
Duration of diabetes (yr)	1.02 (1.01-1.03)	0.04		
Hypertension	1.48 (1.04-2.11)	0.03	1.63 (1.04-2.56)	0.03
Dyslipidemia	1.10 (0.87-1.39)	0.42		
Cancer	0.66 (0.16-2.68)	0.56		
Dementia	6.27 (2.13-18.43)	0.001	6.98 (1.80-26.98)	< 0.01
Depression	0.29 (0.04-2.11)	0.22		
Cerebrovascular disease	1.31 (0.81-2.12)	0.28		
Cardiovascular disease	1.33 (0.97-1.83)	0.08		
Peripheral artery disease	2.48 (1.07-5.72)	0.03		
Peripheral neuropathy	1.24 (0.75-2.03)	0.40		
Diabetic retinopathy	1.51 (1.04-2.20)	0.03	1.45 (0.93-2.24)	0.10
Smoking	0.58 (0.25-1.30)	0.18		
Insulin	3.36 (2.71-4.15)	< 0.001	3.47 (2.61-4.60)	< 0.001
BMI (kg/m^2)				
< 17.5	1.80 (1.20-2.71)	<0.01	1.80 (1.12-2.90)	0.02
17.5-22.9	Reference		Reference	
23.0-27.9	0.54 (0.43-0.69)	< 0.001	0.44 (0.33-0.60)	< 0.001
> 28.0	0.39 (0.27-0.57)	< 0.001	0.30 (0.19-0.48)	< 0.001
HbA1C (%)				
< 7.0	Reference		Reference	
7.0-8.5	1.42 (1.05-1.92)	0.02	1.41 (1.03-1.93)	0.01
≥ 8.5	1.97 (1.45-2.68)	< 0.001	1.49 (1.06-2.09)	0.02
eGFR (mL/min per 1.73 m^2)				
≥ 60	Reference		Reference	
< 60	2.59 (1.97-3.39)	< 0.001	1.53 (1.12-2.11)	0.01

[1]Adjusted for age, gender, duration of diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, diabetic retinopathy, smoking, insulin, body mass index, hemoglobin A1C and estimated glomerular filtration rate using backward (Wald) method. BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate; OR: Odds ratio; 95%CI: 95% confidence interval.

associated with hypoglycemic admissions. This finding provides novel insights. A possible cause might be due to polypharmacy since these elderly T2DM patients with hypertension had more comorbidities and more diabetic complications that affect hypoglycemic drugs metabolism.

A previous community-based study in Sweden study[27] found that glycemic control is worse in patients with T2DM alone than in those with T2DM combined with hypertension. It is related to different degrees of insulin resistance and insulin secretion. Normotensive T2DM patients had reduced insulin secretion which accounts for the higher HbA1C and lower risk for developing hypoglycemia[27]. Dementia in elderly T2DM patients has previously been described as correlating with a higher risk for hypoglycemia[28,29]. The possible contributing factors in dementia patients included low compliance, medication errors and increased susceptibility to medication overdoses[30]. Furthermore, dementia patients generally have lower dietary intake (*e.g.*, dysphagia), loss of functional capacity[31], increased difficulty preparing food, and higher likelihood of hypoglycemic unawareness[32,33]. Consequently, these patients may not be diagnosed with hypoglycemia until they develop severe life-threatening symptoms or signs that require hospitalization.

Exogenous insulin use as an associated risk factor for hypoglycemia makes inherent

Table 3 Crude and adjusted odds ratios of factors that were independently associated with hospital admission due to hyperglycemic complication, using multivariate analysis

Variables	Crude OR (95%CI)	P value	Adjusted OR (95%CI)[1]	P value
Age (yr)				
65-75	Reference			
> 75	0.81 (0.59-1.10)	0.18		
Gender				
Male	Reference			
Female	0.95 (0.72-1.27)	0.74		
Duration of diabetes (yr)	0.98 (0.97-1.01)	0.09		
Hypertension	0.63 (0.45-0.88)	< 0.01		
Dyslipidemia	0.86 (0.65-1.15)	0.31		
Cancer	1.09 (0.27-4.46)	0.90		
Dementia	10.44 (3.54-30.79)	< 0.001	19.08 (4.42-82.45)	< 0.001
Depression	2.58 (1.04-6.39)	0.04	3.98 (1.48-10.71)	< 0.01
Cerebrovascular disease	1.69 (0.97-2.93)	0.06		
Cardiovascular disease	1.70 (1.18-2.47)	< 0.01		
Peripheral artery disease	0.63 (0.09-4.56)	0.65		
Peripheral neuropathy	1.70 (0.98-2.95)	0.06		
Diabetic retinopathy	1.68 (1.07-2.65)	0.03		
Smoking	0.80 (0.33-1.94)	0.61		
Insulin	7.77 (5.85-10.32)	< 0.001	6.37 (4.30-9.45)	< 0.001
BMI (kg/m^2)				
< 17.5	1.79 (1.03-3.13)	0.04	1.59 (0.75-3.36)	0.23
17.5-22.9	Reference		Reference	
23.0-27.9	0.73 (0.53-0.99)	0.04	0.64 (0.43-0.95)	0.03
> 28.0	0.75 (0.50-1.14)	0.18	0.62 (0.37-1.05)	0.07
HbA1C (%)				
< 7.0	Reference		Reference	
7.0-8.5	1.93 (1.07-3.45)	0.03	1.83 (0.99-3.40)	0.05
≥ 8.5	9.88 (6.01-16.23)	< 0.001	5.97 (3.46-10.28)	< 0.001
eGFR (mL/min per 1.73 m^2)				
≥ 60	Reference			
< 60	2.04 (1.48-2.82)	< 0.001		

[1]Adjusted for age, gender, duration of diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, diabetic retinopathy, smoking, insulin, body mass index, hemoglobin A1C and estimated glomerular filtration rate using backward (Wald) method. BMI: Body mass index; HbA1C: Hemoglobin A1C; eGFR: Estimated glomerular filtration rate; OR: Odds ratio; 95%CI: 95% confidence interval.

sense[34], especially when compared with medications that work primarily via other mechanisms, such as decreasing hepatic gluconeogenesis, decreasing intestinal absorption of glucose, increasing endogenous insulin sensitivity or increasing gluconeogenesis in the muscles. Elderly T2DM patients may be at higher risk for hypoglycemia as they may require insulin due to a longer chronicity of T2DM resulting in subsequent decreased endogenous insulin production, may require multiple hypoglycemic drugs, and often may have hepatic and/or renal impairments[35]. Insulin should be used with caution in older adults. The administration of insulin therapy requires good visual acuity, motor skills and cognitive ability in the patient, especially for regimens that require multiple daily injections. This may be too complex for patients with several comorbidities and limited functional status. Our study also demonstrated that low BMI is associated with hypoglycemia related hospitalizations as well. A low BMI in elderly patients may cause higher risk for hypoglycemia due to lower muscle mass, suboptimal nutrition status and low glycogen storage[36,37]. Our study additionally revealed that an elevated HbA1C is associated with hypoglycemic admissions. An elevated HbA1C may increase hypoglycemic risk due to more aggressive blood glucose control resulting in labile blood sugars, or a higher association with polypharmacy.

Table 4 Rate of dysglycemia related hospitalization stratified by country

Ref.	Outcome	Populations	Country	Rate of admission (per year)
Kaewput *et al*[13], 2019	Dysglycemia	Type 2 diabetes, age ≥ 65 years old	Thailand	3.7%
Lombardo *et al*[17], 2013	Dysglycemia	Any type of diabetes, subgroup; age ≥ 65 years old	Italy	6.7 per 1000 person-years
Lipska *et al*[7], 2014	Hypoglycemia	General population, subgroup; age ≥ 65 years old	United States	612 per 100000 person-years
Fu *et al*[14], 2014	Hypoglycemia	Type 2 diabetes, age ≥ 65 years old	United States	0.59 per 1000 person-years
Zhong *et al*[15], 2017	Hypoglycemia	Any type of diabetes, age ≥ 65 years old	England	3.52 per 1000 person-years
Clemens *et al*[16], 2015	Hypoglycemia	Patients with treated diabetes, age ≥ 65 years old	Canada	0.4%
Lombardo *et al*[17], 2013	Hypoglycemia	Any type of diabetes, all age groups	Italy	0.4 per 1000 person-years
Kim *et al*[19], 2016	Hypoglycemia	Type 2 diabetes, age ≥ 65 years old	Korea	9.3 per 1000 person-years
Lipska *et al*[7], 2014	Hyperglycemia	General population, subgroup; age ≥ 65 years old	United States	367 per 100000 person-years
Lombardo *et al*[17], 2013	Hyperglycemia	Any type of diabetes, all age groups	Italy	6.7 per 1000 person-years
Henriksen *et al*[18], 2007	Diabetic ketoacidosis	General population, all age groups	Denmark	12.9 per 100000 person-years

Our study also supported that a low eGFR is associated with severe hypoglycemia in elderly T2DM patients, consistent with a prior report[19]. Impaired kidney function has been shown to significantly increase the risk of hypoglycemia[24]. The kidney may play an essential role in glucose metabolism and serve as a defense mechanism against hypoglycemia. Furthermore, impaired renal function limits insulin and other antidiabetic drugs clearance. Patients with advanced kidney disease may also have chronic inflammation and anorexia that leads to suboptimal nutrition and a reduction in glycogen stores[24]. Hence, in addition to their risk of polypharmacy, cognitive decline, dementia, sarcopenia, and frailty, older adults with diabetes and impaired renal function are at risk of hypoglycemia[24,38].

There were certain factors that appeared to protect against hypoglycemia-related hospitalizations. An elevated BMI is associated with decreased risk of hypoglycemia-related hospitalization, consistent with a prior Korean report[19]. Overweight and obese patients may have protective factors against hypoglycemia such as a higher dietary intake and more glucose reserves in the form of higher glycogen storage, muscle mass, and fat mass.

Our report describes a higher prevalence of hyperglycemia-related hospitalizations than a previous report from the United States[7] (Table 4). Similar to hypoglycemia-related hospitalizations, the difference in prevalence reported may be due to differences in study design, where other study included both diabetic and nondiabetic patients and our study included patients from varying hospital sizes.

This study showed that dementia, depression, insulin use, and HbA1C were associated with an increased risk of hyperglycemia-related hospitalization. Conversely, overweight patients had a decreased risk of hyperglycemia-related hospitalization. Dementia and depression as risk factors for hyperglycemia are supported by other reports[39]. It may be due to poor compliance or inability to access medications[31]. Insulin use and elevated HbA1C were also risk factors for hyperglycemic hospitalizations. This could be due to an association with more advanced diabetic diseases, higher patient prevalence of comorbidities and their associated complications and poor compliance. Conversely, overweight was found to be inversely associated with hyperglycemia-related hospitalizations. This can probably be explained by the fact that improved glycemic control is associated with weight gain.

The strengths of this study included the large representative patient cohort and the adjustment for multiple clinical variables. This report is a large nationwide cross-sectional study that included varying hospital sizes in Thailand. The multivariable logistical regression statistical analysis performed for assessing associated hypoglycemia and hyperglycemia-related hospitalization factors included several possible confounders such as age, gender, smoking, BMI, duration of diabetes, comorbidities, insulin use and laboratory parameter. The comorbidities included hypertension, dyslipidemia, cancer, dementia, depression, cerebrovascular disease, coronary artery disease, peripheral artery disease, peripheral neuropathy, and diabetic retinopathy. Laboratory parameters included HbA1C and eGFR for its final model adjustment.

There are several limitations of this study. First, data collection was performed using retrospective medical record review; therefore, incomplete data records with missing diagnoses cannot be verified. Second, the study population does not include patients from university hospitals. Consequently, the prevalence of hypoglycemia and

hyperglycemia-related hospitalization may be significantly underestimated. Third, we only measured hospitalization rates for dysglycemia. Dysglycemic events resulting in death prior to hospital admission were not captured. Fourth, we did not adjust the final statistical models for several possible confounders such as alcohol consumption and former tobacco use as this data was unavailable. One previous study had demonstrated an associated risk of hypoglycemia in T1DM patients with alcohol use[40]. Conversely, another study did not show an association between alcohol consumption and risk of hypoglycemia in T2DM patients. Pietraszek *et al*[41] had instead reported that acute intake of alcohol does not increase hypoglycemic risk in diet-controlled T2DM subjects; an alcohol-related hypoglycemic effect only occurred when sulfonylurea was co-administered. Furthermore, long-term alcohol use seems to be associated with improved glycemic control in T2DM probably due to improved insulin sensitivity. Former tobacco use may have also affected our outcomes if patients had decided to quit smoking due to health conditions related to the dysglycemic admission. Last, this was a cross-sectional study which had duration of only one year.

Serial blood glucose monitoring is already recommended for prevention of glycemic complications in elderly T2DM patients. However, the importance of timely and more frequent blood glucose monitoring in elderly T2DM patients with associated risk factors for dysglycemic hospitalization should be emphasized. Increased awareness, dedication of resources, and early intervention to prevent complications related to the management of diabetes is key to improving both the care of elderly patients and healthcare expenditure. The American Diabetes Association guidelines recognizes the need to incorporate geriatrics components into the assessment and management of diabetes[9]. By recognizing the multiple risk factors in elderly T2DM patients identified in our study, a multidisciplinary approach[9,42,43] to the individualization of a patient's optimal glucose level and its medical management can be performed. Future clinical practice improvements can then be compared to the prevalence rates identified in this study.

In conclusion, prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand was higher than developed countries at 4.9%. Elderly T2DM patients should be evaluated for risk factors of dysglycemia and may benefit from more frequent blood glucose monitoring and individualization of care in order to prevent dysglycemia-associated hospitalizations.

ARTICLE HIGHLIGHTS

Research background
The prevalence of older individuals with type 2 diabetes mellitus (T2DM) is increasing due to the aging population and improved medical care. These patients are very susceptible to disease and treatment-related hospitalizations, resulting in higher health care costs, morbidity, and decreased quality of life. However, data of treatment-related complications, especially dysglycemia-related hospitalizations, are lacking

Research motivation
This study would provide further support to the importance of regular monitoring of blood glucose, and glucose control should be individualized for the elderly T2DM patients. To further investigate the association between each variable and dysglycemia were assessed using multivariate logistic regression. Furthermore, it would motivate future research on whether more intensive monitoring of T2DM patients may allow earlier detection and prevention of dysglycemia-related hospitalization. The authors conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools.

Research objectives
We conducted this study to determine the prevalence and associated factors for hospitalizations due to dysglycemia among elderly T2DM patients in Thailand using nationwide patient sample.

Research methods
We conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. This study evaluated adult T2DM patients from 831 public hospitals in Thailand in the year 2014. We examined the prevalence of hospitalization due to hypoglycemia and hyperglycemia. Hyperglycemia complication included diabetic ketoacidosis, hyperosmolar hyperglycemic state, and hyperglycemic dehydration syndrome. The association factors between dysglycemia-related hospitalizations were assessed using multivariate logistic regression.

Research results
In this study, a total of 11404 elderly T2DM patients were enrolled in this study. The mean age

was 72.9 ± 5.5 years. The prevalence of hospital admission due to diabetic ketoacidosis, hyperosmolar hyperglycemic state, hyperglycemic dehydration syndrome, and hypoglycemia among elderly T2DM patients in the year 2014 was 0.1%, 0.1%, 1.7% and 3.1%, respectively. Increased hospitalization due to hypoglycemia was associated with older age, female sex, had hypertension, dementia, lower body mass index, elevated hemoglobin A1C (HbA1C), decreased kidney function, insulin use. Increased hospitalization due to hyperglycemia was associated with dementia, depression, lower body mass index, elevated HbA1C, and insulin use.

Research conclusions
We found that prevalence of dysglycemia-related hospitalization in elderly T2DM patients in Thailand had higher than developed countries. Elderly T2DM patients, especially in patients with associated factors, should be closely monitored blood glucose.

Research perspectives
Serial blood glucose monitoring should be recommended to prevent glycemic complications especially, in the elderly T2DM patients with associated factors cannot be over-emphasized. The early intervention to prevent further complications and adequate control of diabetes is a key to the reduction of complications of diabetes itself and treatment-related complications. Among elderly T2DM patients with multiple morbidities, the glucose control should be individualized.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Prospective Study

Optimized health care for subjects with type 1 diabetes in a resource constraint society: A three-year follow-up study from Pakistan

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Abstract

BACKGROUND
Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with type 1 diabetes (T1D) in Pakistan.

AIM
To observe effectiveness of diabetes care through development of model clinics for subjects with T1D in the province of Sindh Pakistan.

METHODS
A welfare project with name of "Insulin My Life", was started in province of Sindh, Pakistan. This was collaborative work of Baqai Institute of Diabetology and Endocrinology, World Diabetes Foundation and Baqai Medical University between February 2010 to February 2013. Under this project thirty-four T1D clinics were established. Electronic database was designed for demographic, biochemical, anthropometric and medical examination. Monthly consultation was part of the standardized diabetes care. All the recruited subjects with T1D were provided free insulins and related materials.

RESULTS
Out of 1428 subjects, 795 (55.7%) were males and 633 (44.3%) were females. Subjects were categorized into ≤ 5 years of age 103 (7.2%), between 6-12 years 323 (22.6%), between 13–18 years 428 (29.7%) and ≥ 19 years of age 574 (40.2%) groups. Glycemic control as assessed by HbA1c was significantly improved (*P* <

0.0001) at three years follow up as compared to baseline in all age groups. Decreasing trends of mean self-monitoring blood glucose were observed at different meal timings in all age groups. No significant change was found in the frequency of neuropathy, nephropathy and retinopathy during the study period ($P > 0.05$).

CONCLUSION
This study gives us long-term longitudinal data of people with T1D in a resource constraint society. With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of microvascular complications was observed over 3 years.

Key words: Insulin My Life; Type 1 diabetes; Insulin; Care; Pakistan

Core tip: This study adds the three years follow up of subjects with type 1 diabetes (recent) by providing all healthcare related facilities. This study will highlight the impact of integrated and comprehensive care on the glycemic control and complications of diabetes.

INTRODUCTION

Annually, more than 132600 subjects under 19 years of age have been diagnosed with type 1 diabetes (T1D) globally[1]. It is also estimated that currently around 1106500 subjects (0-19 years) are living with T1D worldwide[1]. Although, there are clear geographic differences in trends but the estimated annual increase in T1D is around 3%[2]. In 2015, according to IDF, more than 7 million cases of diabetes are reported in Pakistan out of which 2% are suffering from T1D[3]. The incidence of T1D in Pakistan has been reported as 1.02 per 100000 per year[4].

Uncontrolled T1D can lead to microvascular and macrovascular complications mostly in young age group posing a challenge for health care professionals[2,5]. Majority of subjects with T1D living in developing countries have minimum or no access to optimal care[2,6] As a result, these subjects are prone to acute and chronic complications of T1D affecting their quality of life[7].

Limited studies are available on acute and chronic complications in people with T1D from Pakistan[8]. A study conducted in the province of Sindh, showed higher rate of complication in subjects with T1D. Authors have reported that every fourth person with T1D is suffering from any one of the chronic complication while 2% subjects with T1D had diabetic ketoacidosis (DKA) and 21% had history of DKA[9]. Similar trend was noted in smaller scale studies from this region[8,10].

Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with T1D in Pakistan[11-13]. In Pakistan, 33% people lives with poverty and most of the populations (40%) does not receive basic health services[14]. Health expenses are 0.7%-0.8% of gross domestic product of Pakistan, while 3.5% of total governmental budget. Overall health care system in Pakistan also offers the support for diabetes but subjects with T1D needs specific attention and optimal care[7,14].

The study aims to observe effectiveness of optimal care for subjects with T1D including (free periodic consultations, education, dietary advice, provision of insulin and syringes, glucometers, and assessment of glycemic control through HbA1c 6 monthly) by establishing model clinics throughout the province of Sindh, Pakistan.

MATERIALS AND METHODS

A welfare project with name of "Insulin My Life (IML)", was started in the province of Sindh in between February 2010 to February 2013. This was a collaborative work of Baqai Institute of Diabetology and Endocrinology (BIDE), World Diabetes Foundation and Baqai Medical University. Ethical approval was obtained by the Institutional Review Board (IRB) of BIDE with approval/reference number: BIDE/IRB/Prof.Yakoob-IML/02/11/10/025. Subjects with only T1D were included in this study. Informed consent was obtained from above 19 years of age and below 19 years were enrolled after obtaining informed consent from their parents by diabetes educators and physicians.

Three days' workshop for doctors and educators

A total of 34 physicians with post graduate diploma in diabetes and 30 diabetes educators were identified from each district of Sindh. A three days structured training program as per the standard guidelines[15,16] for the management of T1D and prevention of complications was designed for the physicians and for the educators separately.

Community based awareness and education sessions through camps and media coverage

More than 0.3 million teachers were sensitized about T1D specifically for the identification and management of emergencies in subjects with T1D. A total of 654 community based awareness camps and group sessions were held in the vicinity of identified clinics. In these awareness camps knowledge of self-monitoring blood glucose (SMBG), insulin using techniques, dose regime, optimal targets for glycemic control, adequate diet, physical activity, sick day rule, signs and symptoms of hypoglycemia and hyperglycemia were provided to subjects with T1D and their family members.

Printed educational material in English, Urdu and regional language (Sindhi) was also provided to subjects with T1DM, their parents and community. Eighteen televisions and 30 radio programmes in local and regional languages were also telecasted as a part of awareness campaign. A dedicated website www.insulinmylife.com was also launched to dessiminate relevant information regarding T1D[17].

Establishment of model Type 1 diabetic clinics and 24-h helpline service

Thirty-four model type 1 diabetic clinics were established at least one in each district of Sindh during the initial phase of the project (Figure 1)[17]. A 24 h telephonic helpline service was made available to all project registrants. Through 24 h helpline service trained diabetes educators in consultation with primary consultant gave advises and sort out day to day problems including dose adjustments, hypo and hyperglycemic management. In case of emergency these registered subjects with diabetes were advised to contact emergency services.

Diagnosis of T1D

Biochemical parameters include glucose level in fasting, after 2-h of postprandial glucose, HbA1c, proteinuria and urinary ketones. Polyphagia, polyuria, polydipsia, weight loss history, and DKA history which are confirmed if previous records are present were recorded. In suspected cases of DKA, blood pH, HCO3 was done[9].

Provision of optimal care for T1D

No single subject had free insulin and blood sugar testing equipment at the start of the study. All registered subjects with T1D were asked to have free of cost consultation with physician, diabetes educators, free coverage for insulin and glucose testing equipment after every six months.

Subjects with other than T1D were excluded from the study. HbA1c, microalbuminuria test and consultation with a dietitian were offered after every 6 months. Free medical supplies including insulin, glucometers, glucose strips, lancets and insulin syringes, SMBG recording booklets were provided to the participants and they were asked to monitor their glucose readings with a record of these readings to be maintained in diaries. All children (less than and equal to 12 years of age) with T1D were referred to pediatrician as and when needed. The Growth chart with growth velocity was also followed throughout the study period.

Glycemic control assessment

Glycemic control was assessed by checking FBS and RBS at baseline and end of the study along with fasting HbA1c at baseline and after every 6 mo during 3 years
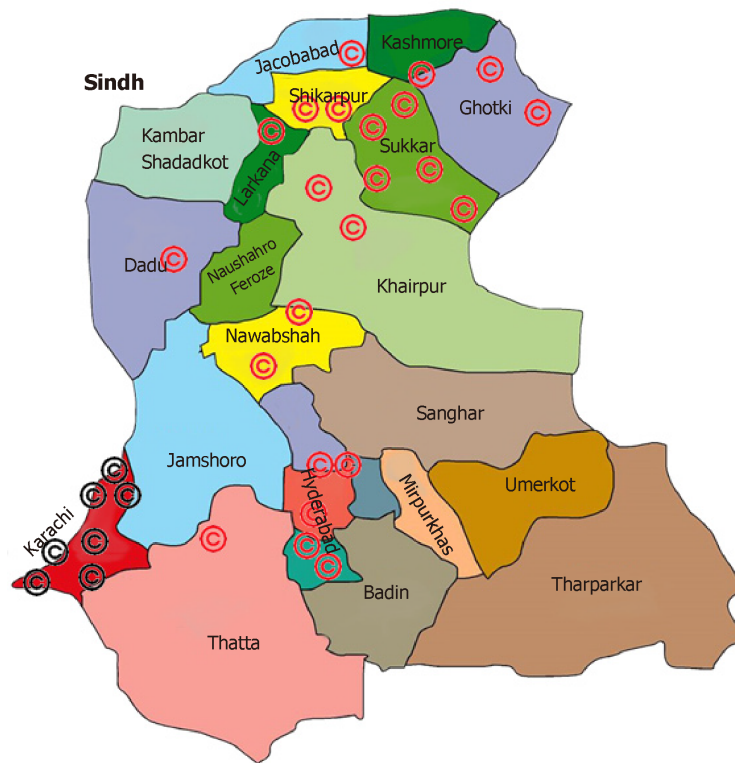
Figure 1 Type 1 diabetic model clinics in the province of Sindh.

follow up. Glycemic control was also assessed by using SMBG level at different meal timings in all age groups. Those who have HbA1c between 6.5%-8%, 9%–10% and ≥ 10% were considered good, fair and poor control, respectively[15,18].

Screening of micro vascular complications
Vista 20 direct ophthalmoscope was used for fundus examine. Retinopathy was confirmed by normal, microdots, hard exudates, pre-proliferative and proliferative or maculopathy. Protein > 1+ on dipstick (Combur 10, Roche Diagnostics) show nephropathy. Twenty-four hours quantitative analyses of urine for protein and creatinine were done. Neuropathy was known as absent touch or vibratory sensations of the feet. The 10-g monofilament and vibration sensation by 128 Hz tuning fork was used for touch sensation.

Data collection
Electronic and centralized database was designed to records demographic, bio-chemical, anthropometric and medical examination.

Statistical analysis
Statistical Package for Social Sciences (SPSS, version 20) was used for demographic and biochemical data. Continuous data was presented as mean, standard deviation and categorical data as numbers and percentages. Chi-square test was used for comparison of percentages and t test was performed for the mean difference comparison. Statistically significant was considered as P-value < 0.05.

RESULTS

T1D model clinics in the province of Sindh-Pakistan are shown in Figure 1. Out of 1428 subjects 790 (55.3%) were males and 638 (44.7%) were females. Subjects were categorized into four groups according to age as ≤ 5 years of age (n = 103, 7.2%), between 6-12 years (n = 323, 22.6%), between 13–18 years (n = 428, 29.7%) and ≥ 19 years of age (n = 574, 40.2%) groups. Mean age (years) at the time of diagnosis in ≤ 5 years of age was 3.2 (± 1.5) and at the time of recruitment 3.5 (± 1.5), between 6-12 years was 8.3 (± 2.5) and 9.5 (± 1.9), between 13–18 years 13.7 (± 3.6) and 15.6 (± 1.7) and in ≥ 19 years of age groups 22 (± 6.3) and 25.7 (± 5.5), respectively. Duration of diabetes, family history of diabetes, weight, systolic and diastolic blood pressure were

noted in all age groups along with serum creatinine at baseline (Table 1).

Mean HbA1c at baseline *vs* end of study in ≤ 5 years of age subjects was (11.5 ± 2.04 *vs* 10.2 ± 2.12, *P* = 0.026), between 6-12 years was (10.7 ± 2.28 *vs* 8.9 ± 2.24, *P* ≤ 0.0001), between 13–18 years was (10.5 ± 2.76 *vs* 8.7 ± 2.49, *P* ≤ 0.0001) and (9.6 ± 2.52 *vs* 8.5 ± 2.17, *P* < 0.0001) in ≥ 19 years of age. A significant decrease in HbA1c was observed in all age categories (*P* < 0.05) (Table 2). The comparison of systolic, diastolic blood pressure along with fasting and random blood glucose were also presented in Table 2. Glycemic control as retrieved by HbA1c was significantly improved at final visit as compared to the baseline in all age groups. At baseline visit good glycemic control was observed in 3.6% subjects which increased to 25.9% at the end of study for ≤ 5 years of age. Similar trend can be seen in age 6-12 years (baseline 13.5% *vs* end line 36.3%, *P* < 0.0001), for age 13–18 years (14.7% *vs* 37.7%, *P* < 000001) and (26.8% *vs* 62.1%, *P* < 0.0001) for ≥ 19 years of age group (Table 3).

During three years follow up decreasing trends of mean SMBG were also observed at different meal timings in all age groups (Table 3). Comparatively lower mean SMBG values were observed compared to first month during the study period (Table 4). Graphical representation of microvascular complications was shown in Figure 2. The frequency of retinopathy shows a slight increasing (non-significant) trend, while the frequency of nephropathy and neuropathy almost remained the same during the study period. Significant improvement in HbA1c levels was observed in all age groups at end of study period (at 3 years) (Figure 3).

DISCUSSION

In this observational study, a three year follow up of people with T1D registered under project of IML in the province of Sindh Pakistan. Significant improvement in the glycemic control was noted with provision of comprehensive care, awareness and treatment free of cost.

Though it is difficult to achieve optimum glycemic control among adolescents, regardless the type of diabetes[19], what we have observed that with proper care fewer people remained in the poor glycemic category and many people achieved fair to good control (Table 2). This has been shown by Diabetes Patient Verlaufs-dokumentation (DPV) registry also that healthy outcomes can be achieved in individuals with T1D when provided with optimized and personalized care[20]. Good glycemic control not only important for decreasing the morbidity, but it can decrease diabetes related mortality rate as well as shown by Nordwall M related DM registry[21,22]. On the other hand, without proper access to standardized care people with T1D suffer from adverse results even at an earlier age[23]. In our study, over 3 years, people with T1D in each age category showed downward trend of HbA1c and this decline was statistically significant.

With provision of free glucostrips and glucometers it was made possible for study registered participants to check blood glucose at least 2 times/d. However, the annual cost per participant which include consultation fee, lab diagnosis, glucometers, insulins, strips, lancets and syringes, *etc*. was 61000pkr (436USD), per month 5083pkr (36USD) and per day 169pkr (1.2USD). SMBG profile of our cohort also showed downward trend at different mealtimes and this proves that by continuous education and pursuing its effectiveness enhances the motivation of subjects and their families to achieve better glycemic control. Study from Bulgarian suggests that due to families' devotion to diabetes control, children under six years achieved good glycemic control[24]. Glycemic control with chronic complications was clearly shown by landmark study that is in Diabetes Control and Complication Trial (DCCT)[25,26]. On the contrary association between poor glycemic control and increase risk of chronic complication was shown by several studies[26].

In study from Southeast Sweden, prolonged uncontrolled HbA1c was closely associated with the development of severe complications in individuals with T1D[22]. Another observational, population based study from DPV registry indicates that poor HbA1c was found to be a powerful biomarker for the development of retinopathy, nephropathy and neuropathy in patients with T1D[27]. Time to onset of complications was also influenced by HbA1c as in the primary prevention cohort of DCCT[22]. However, in our study rate of complication including nephropathy, and neuropathy remained the same throughout the study period through there was non-significant rise in frequency of retinopathy.

This study with best of our knowledge, concludes that it is first of its kind from Pakistan, giving us long-term longitudinal data of patients with T1D in a resource constraint society. With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of

Table 1 Baseline demographic and clinical characteristic of study subjects				
Variables	0-5 yr	6-12 yr	13-18 yr	19 and above
n (%)	103 (7.21)	323 (22.62)	428 (29.97)	574 (40.2)
Male	50 (48.5)	155 (48)	251 (58.6)	334 (58.2)
Female	53 (51.5)	168 (52)	177 (41.4)	240 (41.8)
Age at diagnosis (yr)	3.2 ± 1.5	8.3 ± 2.5	13.7 ± 3.6	22 ± 6.3
Age at recruitment (yr)	3.5 ± 1.5	9.5 ± 1.9	15.6 ± 1.7	25.7 ± 5.5
Duration of diabetes (yr)	0.3 ± 0.7	1.1 ± 2	1.9 ± 3.3	3.7 ± 5.4
Family history of diabetes	37 (35.9)	131 (40.6)	199 (46.5)	276 (48.1)
Weight (kg)	13.90 ± 2.61	28.31 ± 13.01	42.86 ± 10.64	53.11 ± 10.34
Serum Creatinine (mg/dL)	0.64 ± 0.18	0.80 ± 0.19	0.94 ± 0.43	0.92 ± 0.31
Cholesterol (mg/dL)	---	153.67 ± 33.06	155.63 ± 40.01	163.18 ± 28.58
Triglyceride (mg/dL)	---	83.25 ± 42.96	103.47 ± 73.66	94.46 ± 60.24
High density lipoproteins (mg/dL)	---	39.48 ± 12.21	42.46 ± 11.73	41.52 ± 10.45
Low density lipoproteins (mg/dL)	---	79.70 ± 26.07	86.34 ± 30.67	98.94 ± 30.44

Data presented as mean ± SD and *n* (%).

microvascular complications was observed over 3 years.

Limitations
In a resource constraint society like Pakistan, there is lack of an infrastructure for current study to provide health care system in a proper way. But, with available resources such kind of data was considered as the best available option. All the study participants during the study duration were coming to their respective medical centers for the required care. However, in remote areas the follow-up HbA1c was not completely available. This study helps us to know more about T1D in Pakistan than ever before, but much is still to be learned. This study need to be replicated at Nationwide level.

Table 2 Comparison of clinical measures from baseline to last follow up

Variables		Age (yr)			
		0-5 yr	6-12 yr	13-18 yr	19 and above
HbA1c (%)	Baseline (n = 1428)	11.5 ± 2.04	10.7 ± 2.28	10.5 ± 2.76	9.6 ± 2.52
	End line (n = 516)	10.2 ± 2.12	8.9 ± 2.24	8.7 ± 2.49	8.5 ± 2.17
	P-value	0.026	< 0.0001	< 0.0001	< 0.0001
Systolic blood pressure (mmHg)	Baseline (n = 1428)	108.3 ± 20.8	105.4 ± 14.7	106.3 ± 14.7	108.2 ± 14.9
	End line (n = 1428)	92.4 ± 10.3	99.1 ± 13.3	105.3 ± 13.3	112.7 ± 14.2
	P-value	0.001	0.0002	0.500	0.001
Diastolic blood pressure (mmHg)	Baseline (n = 1428)	74.6 ± 11.2	72.9 ± 10.6	73.7 ± 10.5	74.3 ± 10
	End line (n = 1428)	65 ± 6.6	66.2 ± 7.7	71.3 ± 8.3	74.6 ± 8.5
	P-value	0.001	0.0004	0.002	0.747
Fasting blood sugar (mg/dL)	Baseline (n = 1428)	199.2 ± 109.4	266.4 ± 118.9	282.4 ± 107	266.20 ± 112.6
	End line (n = 1428)	77.0 ± 2.64	293.86 ± 105.57	270.96 ± 104.83	252.43 ± 117.13
	P-value	0.059	0.292	0.550	0.415
Random Blood Sugar (mg/dl)	Baseline (n = 1428)	326.2 ± 161.1	342.4 ± 149.7	360.3 ± 135.4	338.9 ± 131
	End line (n = 1428)	522.33 ± 267.54	479.24 ± 189.99	394.63 ± 171.36	354.86 ± 157.43
	P-value	0.002	<0.0001	0.138	0.476

Data presented as mean ± SD.

Table 3 Age distributed glycemic status on first and last visit of the study period

Glycemic category	Age (yr)							
	0-5 yr		6-12 yr		13-18 yr		19 and above	
	Baselinevisit	Lastvisit	Baselinevisit	Last visit	Baselinevisit	Last visit	Baselinevisit	Last visit
Good glycemic control (HbA1c < 6.5%-8%)	3.6	25.9	13.5	36.3	14.7	37.7	26.8	62.1
Fair glycemic control (HbA1c 9%-10%)	14.3	40.7	16.2	19.1	14.7	14	26.8	12.4
Poor glycemic control (HbA1c ≥ 10%)	82.1	33.3	70.3	44.5	70.7	48	46.5	25.5

Data presented as percentages (%).

Table 4 Trends of mean self-monitoring blood glucose readings during the study period

Timing	Month 1	Year 1 (month 2-12)	Year 2(month 13-24)	Year 3(month 25-36)
Before breakfast				
0-5 yr	213.5	202.6	184.1	150.9
6-12 yr	197.3	173.6	193.1	146.2
13-18 yr	180.3	169.9	168.3	137.8
19 and above	163.2	151.5	139.6	138
After 2 h of breakfast				
0-5 yr	328.5	246.9	167.7	194
6-12 yr	215.5	209.5	203.5	190.3
13-18 yr	232.5	203.5	215.5	173.8
19 and above	200.3	178.6	166.2	184.6
Before lunch				
0-5 yr	248.2	265.8	132.3	140.2
6-12 yr	180.1	191.2	194.3	184
13-18 yr	200.3	189.7	192.8	148.9
19 and above	164	160.2	147.4	151.7
After 2 h of lunch				

0-5 yr	281.6	252.2	204.7	257.5
6-12 yr	269.8	226.8	237.3	207.6
13-18 yr	246.6	230.6	237.4	194.8
19 and above	223.1	211.8	202.8	203.8
Before dinner				
0-5 yr	299.2	276	216.2	239.9
6-12 yr	262	261.1	242.6	218.1
13-18 yr	255	228.4	234	205.5
19 and above	223.6	191.8	211.4	182.1
After 2 h of dinner				
0-5 yr	297.3	254.3	196.8	239.8
6-12 yr	239.3	247.8	244.7	192.5
13-18 yr	240.1	219.2	213.7	179.8
19 and above	217.4	195.9	212.1	180.7
Before sleeping				
0-5 yr	273.3	254.9	212.5	214.7
6-12 yr	230.7	230.6	181.1	211.3
13-18 yr	195	223.3	218.8	165.7
19 and above	167.3	167.9	196.4	195

Figure 2 Complications rate of patients with ≥ 10 years diabetes duration.

Figure 3 Trends of glycosylated hemoglobin (HbA1c levels).

ARTICLE HIGHLIGHTS

Research background

Inadequate health infrastructure and poverty especially in rural areas are the main hindrance in the optimal management of subjects with type 1 diabetes (T1D) in Pakistan.

Research motivation

The current study with lack of an infrastructure provides health care system in a proper way with available resources, to evaluate patient centered outcomes in the measurement of progression and treatment. Such kind of data was considered as the best available option.

Research objectives
The objective of this study is to observe the effectiveness of diabetes care through development of model clinics for subjects with T1D in the province of Sindh Pakistan.

Research methods
In this welfare project "Insulin My Life (IML)", subjects with only T1D were included. Thirty-four model T1D clinic were established and total of 654 community based awareness camps and group sessions were held. All registered subjects with T1D were asked to have free of cost consultation with physician, diabetes educators, free coverage for insulin and glucose testing equipment after every six months. Glycemic control was assessed by checking FBS and RBS at baseline and end of the study along with fasting HbA1c at baseline and after every 6 mo during 3 years follow up. Glycemic control was also assessed by using self-monitoring blood glucose level (SMBG) at different meal timings in all age groups.

Research results
Out of 1428 subjects 790 (55.3%) were males and 638 (44.7%) were females. Glycemic control as retrieved by HbA1c was significantly improved at final visit as compared to the baseline in all age groups. At baseline visit good glycemic control was observed in 3.6% subjects which increased to 25.9% at the end of study for ≤ 5 years of age. Similar trend can be seen in age 6-12 years, 13–18 years, and ≥ 19 years of age group. Comparatively lower mean SMBG values were observed compared to first month during the study period.

Research conclusions
With provision of standardized and comprehensive care significant improvement in glycemic control without any change in the frequency of microvascular complications was observed over 3 years.

Research perspectives
This study helps us to know more about T1D in Pakistan than ever before, but much is still to be learned. This study need to be replicated at Nationwide level.

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