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REVIEW

Update on pre-diabetes: Focus on diagnostic criteria and cardiovascular risk

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Abstract

Pre-diabetes, which is typically defined as blood glucose concentrations higher than normal but lower than the diabetes threshold, is a high-risk state for diabetes and cardiovascular disease development. As such, it represents three groups of individuals: Those with impaired fasting glucose (IFG), those with impaired glucose tolerance (IGT) and those with a glycated haemoglobin (HbA_{1c}) between 39-46 mmol/mol. Several clinical trials have shown the important role of IFG, IGT and HbA_{1c}-pre-diabetes as predictive tools for the risk of developing type 2 diabetes. Moreover, with regard to cardiovascular disease, pre-diabetes is associated with more advanced vascular damage compared with normoglycaemia, independently of confounding factors. In view of these observations, diagnosis of pre-diabetes is mandatory to prevent or delay the development of the disease and its complications; however, a number of previous studies reported that the concordance between pre-diabetes diagnoses made by IFG, IGT or HbA_{1c} is scarce and there are conflicting data as to which of these methods best predicts cardiovascular disease. This review highlights recent studies and current controversies in the field. In consideration of the expected increased use of HbA_{1c} as a screening tool to identify individuals with alteration of glycaemic homeostasis, we focused on the evidence regarding the ability of HbA_{1c} as a diagnostic tool for pre-diabetes and as a useful marker in identifying patients who have an increased risk for cardiovascular disease. Finally, we reviewed the current evidence regarding non-traditional glycaemic biomarkers and their use as alternatives to or additions to traditional ones.

Key words: Glycated haemoglobin; Cardiovascular risk; Diagnostic criteria; Non-traditional glycaemic markers; Pre-diabetes

Core tip: Pre-diabetes is a high-risk state for diabetes and cardiovascular disease. There are three diagnostic criteria for pre-diabetes: Impaired fasting glucose (IFG),

impaired glucose tolerance (IGT) and glycated haemoglobin (HbA_{1c}) between 39-46 mmol/mol. The concordance between a pre-diabetes diagnosis made by IFG, IGT or HbA_{1c} is scarce and there are conflicting data as to which of these methods best predicts cardiovascular disease. This review focuses on the evidence regarding the ability of HbA_{1c} for pre-diabetes diagnosis and as a marker for cardiovascular risk. Finally, the evidence regarding non-traditional glycaemic biomarkers as alternatives to the traditional ones is reviewed.

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INTRODUCTION

Pre-diabetes is a general term that refers to an intermediate stage between normal glucose homeostasis and overt type 2 diabetes mellitus. As such, it includes three groups of individuals: Those with impaired fasting glucose (IFG), those with impaired glucose tolerance (IGT) and those with a glycated hemoglobin (HbA_{1c}) between 39-46 mmol/mol (Table 1). As underlined by the American Diabetes Association (ADA), a number of previous studies reported that the concordance between pre-diabetes diagnoses made by IFG, IGT or HbA_{1c} is scarce[1]; according with this consideration, in a study conducted on a large population of Caucasian adults the agreement between the three diagnostic criteria was only 10.4% (Figure 1)[2].

The discordance in the identification of individuals with pre-diabetes using three different diagnostic tests is not entirely unexpected given that fasting plasma glucose, 2 h post oral glucose tolerance test (OGTT), and HbA_{1c} probably reflect different aspects of glucose metabolism, and a diagnosis of pre-diabetes based on IFG, IGT, or HbA_{1c} may represent aetiological factors leading to the development of the different prediabetic states[2]. Indeed, subjects with isolated IFG seem to have a reduced hepatic insulin sensitivity, impaired first-phase insulin secretion, and normal/near-normal muscle insulin sensitivity, while subjects with IGT should be characterized by nearly normal hepatic insulin sensitivity and marked reduced peripheral insulin sensitivity combined with defective late insulin secretion[3,4]. In contrast to IFG and IGT, HbA_{1c} is a marker representing blood glucose concentrations over the preceding 2-3 mo and it is affected by both basal and postprandial hyperglycaemia. To date, it is still not clear if these aspects that are strictly bound to the physiopathology of pre-diabetes may have a clinical relevance in view of a possible therapeutic intervention.

Cardiovascular disease (CVD) is the leading cause of death among individuals with type 2 diabetes, accounting for 40% to 50% of all deaths[5]. Although type 2 diabetes is frequently associated with other cardiovascular risk factors, such as dyslipidemia and hypertension, it is believed that chronic hyperglycaemia *per se* is an independent risk for macrovascular complications. Currently, it is well established that macrovascular disease starts before the development of diabetes, and the slight increase in plasma glucose levels that characterize pre-diabetes have been shown to be an independent predictor for CVD. Much clinical research has focused on lifestyle or pharmacological intervention to prevent diabetes in these high risk subjects[6]; however, few studies have been conducted with specific focus on CVD prevention in this population. Since many clinical trials have failed to demonstrated a reduction in cardiovascular risk from glucose-lowering interventions in patients with overt type 2 diabetes[7,8], it is noteworthy that several studies have reported benefits in improving cardiovascular risk factors, as well as absolute CVD event rates, in people with pre-diabetes treated with glucose lowering drugs[9-11].

Since the utility of a test for pre-diabetes diagnosis is also defined by its capacity to identify the macrovascular complication risk, an important question is whether subjects with pre-diabetes according to IFG, IGT, or HbA_{1c} have an equivalent cardiovascular risk. To date, cardiovascular risk studies comparing IFG, IGT, and HbA_{1c}-pre-diabetic patients are sparse and the results are still controversial[12-14].

This review highlights recent studies and current controversies in the field. In consideration of the increased use of HbA_{1c} as a marker to detect patients with alterations of glycaemic homeostasis, we thought that it could be interesting, and relevant from the clinical point of view, to evaluate the evidence regarding the ability of HbA_{1c} to identify patients who have increased cardiovascular risk. With this specific aim we focused our attention on HbA_{1c} as a diagnostic tool for pre-diabetes. Finally, we reviewed the current evidence regarding non-traditional glycemic biomarkers and their use as alternatives to or additions to the traditional ones.

COMPARISON OF IFG, IGT AND HBA_{1C}, CRITERIA IN PREDICTING TYPE 2 DIABETES

Subjects with pre-diabetes have shown a high conversion rate to overt diabetes and much clinical research has focused on lifestyle or pharmacological intervention to prevent diabetes in these high risk subjects[6]. Subjects with an isolated alteration of glucose homeostasis (IFG, IGT or HbA_{1c} 39-46 mmol/mol) have an incidence of diabetes of 6% per year, a value that is significantly higher compared with subjects with normoglycemia (0.5% per year)[15]. Progression to overt type 2 diabetes is 30%-40% in the next 3-8 years, with an increase of 10% when two alterations of glucose homeostasis are present[6].

According with these considerations, diagnostic and

Table 1 Diagnostic criteria for categories at increased risk of diabetes

Category	Marker	Diagnostic range
IFG	Fasting plasma glycemia	⩾ 5.6 mmol/L (100 mg/dL) < 6.9 mmol/L (126 mg/dL)
IGT	2-h post-load glycemia	⩾ 7.8 mmol/L (140 mg/dL) < 11 mmol/L (200 mg/dL)
HbA_{1c}-prediabetes	HbA_{1c}	⩾ 39 mmol/mol (5.7%) < 47 mmol/mol (6.5%)

IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; HbA_{1c}: Glycated haemoglobin.

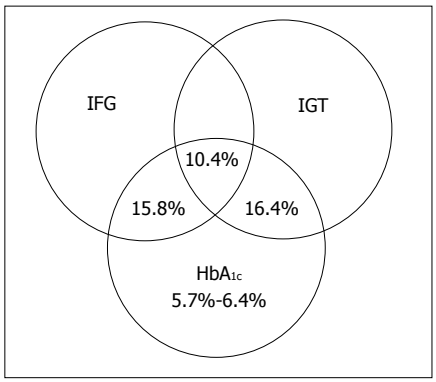

Figure 1 Agreement between glycated haemoglobin pre-diabetes, impaired fasting glucose and impaired glucose tolerance[2]. IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; HbA1c: Glycated haemoglobin.

screening criteria for pre-diabetes have a relevant clinical impact; indeed, it is important to identify individuals at high risk for type 2 diabetes to prevent or delay the development of the disease and its complications.

In 2011, the ADA revised the criteria for the diagnosis of type 2 diabetes and the categories at increased risk for diabetes and the use of HbA_{1c} measurement was recommended as another diagnostic test option already including IFG and IGT[1]. Specifically for the categories of increased risk for type 2 diabetes, the new ADA recommendations state that an HbA_{1c} from 39-46 mmol/mol identifies individuals at high risk for diabetes to whom the term pre-diabetes may be applied.

Indeed, both IFG and IGT present some limitations: They require fasting status and are affected by acute perturbations. Furthermore, the OGTT presents some practical difficulties: It is costly, it needs time, and has lower reproducibility compared with the fasting plasma glucose measurement (FPG)[16]. HbA_{1c} is a "picture" of the average blood glucose level over the period of 2-3 mo[17]. HbA_{1c} has higher reproducibility than FPG; indeed, within subject coefficients of variation are 1.7% for HbA_{1c}, and 5.7% for FPG[17,18]. Furthermore, HbA_{1c} does not need fasting status and could better integrate chronic hyperglycaemia than FPG (Table 2). The predictive value of HbA_{1c} for type 2 diabetes has been reported in several studies. Morris *et al*[19] has shown in a metanalysis conducted on 70 studies that the progression rate to type 2 diabetes of patients with HbA_{1c} pre-diabetes was similar to that for ADA-defined IFG and IFG plus IGT. Moreover, the value of HbA_{1c} in predicting type 2 diabetes has been reported four prospective studies[20-23]; of these, one assessed the use of two glycemic parameters (in particular IFG and HbA_{1c}) for predicting the incidence of type 2 diabetes; the authors supported the combined measurement of FPG and HbA_{1c} for predicting diabetes incidence in a 4 year follow-up using receiver operating characteristic curve (ROC) analysis. When the whole population was analysed, the ROC curve of the model including both FPG and HbA_{1c} was greater those including FPG alone or HbA_{1c} alone. Furthermore, the authors reported a weak correlation between HbA_{1c} and FPG at baseline suggesting that HbA_{1c} is not a surrogate marker of FPG[23].

It is necessary to remember that HbA_{1c} between 39-46 mmol/mol seems to have a lower sensitivity in identify population with pre-diabetes compared with IFG and IGT[24,25]. Conversely, the use of HbA_{1c} may also lead to the reclassification of subjects without IFG or IGT as having pre-diabetes[26]. On the other hand, according to the ADA statement, the lower sensitivity of HbA_{1c} for diagnosing pre-diabetes may be offset by its ability to facilitate establishing a diagnosis[27]. Contrary to these considerations, Rosella *et al*[28] recently reported that the prevalence of undiagnosed pre-diabetes in a representative sample of Canadians was significantly higher using HbA_{1c} measures as screening tool compared with plasma glucose diagnostic criteria. The authors hypothesized that this "reverse association" may be due to a number of factors, such as ethnic differences and the increased prevalence of pre-diabetes from 11.6% in 2003 to 35.3% in 2011[29]. Accordingly, in a study conducted in the Mexican population, Kumar *et al*[30] found a higher prevalence of adults with HbA_{1c} pre-diabetes compared with previous studies conducted in the same population[31]. We reported similar findings in a recent study conducted on 380 subjects attending our out-patients clinic for diabetes and cardiovascular risk evaluation; although we did not perform an opportunistic procedure during recruitment, the group with high HbA_{1c} and normal fasting glucose and normal glucose tolerance (NFG/NGT) represented, in this study, approximately 30% of the entire population and is, therefore, not a rare subset[32]. These observations may not be surprising; in fact, although subjects with NFG and NGT have a lower risk of developing diabetes than patients with either IFG or IGT, in several studies a significant percentage (30%-40%) of all individuals who developed type 2 diabetes had NFG and NGT at baseline[33,34]. This indicates that subjects with NFG and NGT experience a lower risk of developing diabetes compared with IFG and IGT in absolute terms; however, among these subjects there is also a subgroup at increased risk of developing diabetes and, consequently, cardiovascular diseases. From these considerations stems the need to add HbA_{1c}, as a diagnostic tool to identify a new category of high-risk individuals[35]. Further epidemiological data are needed to characterize the real percentage of this group

Table 2 Main points supporting/not supporting the use of glycated haemoglobin as diagnostic tool for diagnosis of pre-diabetes

Supporting	Not supporting
HbA_{1c} may better integrate chronic hyperglycaemia than fasting and 2-h post-load glycaemia	HbA_{1c} seems to have a lower sensitivity in pre-diabetes diagnosis
HbA_{1c} predicts microvascular complications (rethinopathy and nephropathy) similarly to fasting and 2-h post-load glycaemia	Standardization of HbA_{1c} assay needs to be improved
HbA_{1c} has a higher predictive value than fasting plasma glucose in predicting cardiovascular disease	Common, and not always known, clinical conditions (haemoglobinophaties, malaria, anaemia, blood loss) may significantly interfere with HbA_{1c} assay
HbA_{1c} has a greater pre-analytical stability than blood glucose	Ethnic differences in HbA_{1c} assay are not well characterized
HbA_{1c} assay does not need fasting status	The low biological variability of HbA_{1c} provides little information on pathophysiological processes involved in pre-diabetes
HbA_{1c} is not affected by acute perturbations (exercize, stress, diet)	Glucose assessment is cheaper thant HbA_{1c} assay
HbA_{1c} biological variability is lower than fasting and 2-h post-load glycemia	
HbA_{1c} may be an attractive option in settings in which OGTT is not used and rarely repeated	

HbA_{1c}: Glycated haemoglobin; OGTT: Oral glucose tolerance test.

in the overall pre-diabetic population.

To date, it is unclear why the prevalence of pre-diabetes diagnosed by OGTT and HbA_{1c} criteria is substantially discordant. The concentration of HbA_{1c} depends on glucose concentrations and on factors affecting the glycation rate such as systemic oxidative stress. Previous studies reported that some characteristics, such as obesity, are associated with increased oxidative stress[36]; thus, HbA_{1c} may not reflect the real concentration of glucose and be disproportionately high in obese subjects. Several studies investigated the effects of phenotypic characteristics such as obesity on the agreement between OGTT and HbA_{1c}. Li *et al*[37] in a recent study conducted on a large cohort of Chinese subjects without a previous diagnosis of diabetes reported a poor agreement between HbA_{1c} criteria and OGTT in patients independently from body mass index. Moreover, different optimal HbA_{1c} cut-off points for pre-diabetes were reported: 38 mmol/mol for normal weight, 39 mmol/mol for overweight, and 42 mmol/mol for obese subjects.

Also other studies recommend a different cut-off point of HbA_{1c} for diagnosis of pre-diabetes. In particular, longitudinal epidemiological studies have reported that demographic and ethnic factors may contribute to complications in using HbA_{1c} for the diagnosis of diabetes, and the optimal diagnostic HbA_{1c} value is debated and varies because of genetic and biological differences. Yan *et al*[38] identified optimal HbA_{1c} cut-off points for pre-diabetes in two diverse population-based cohorts with different ages. The optimal HbA_{1c} cut-off point for pre-diabetes diagnosis was 38 mmol/mol in the young and middle-aged population, whereas, the optimal cut-off for diagnosing pre-diabetes increased to 39 mmol/mol, in the elderly population. Furthermore, many studies have shown that racial disparities affect the performance of HbA_{1c} for diagnosing pre-diabetes[39]. In summary, it is possible that diagnostic tests for glycemic homeostasis should be used and interpreted considering the individual phenotypic characteristics of the patients; further studies are needed to investigate the clinical usefulness of personalized cutoff values.

COMPARISON OF IFG, IGT AND HBA1C, CRITERIA IN PREDICTING CARDIOVASCULAR RISK

The utility of a test for pre-diabetes diagnosis is also defined by its capacity to identify the risk of micro- and macrovascular complications and from this point of view, the high reproducibility and simplicity may make HbA_{1c} dosage an attractive option. Previous observational studies documented that determination of HbA_{1c}, fasting glucose and OGTT significantly predicted the development of retinopathy and nephropathy but no variables had a significant advantage for detecting the incidence or prevalence of either complication[40,41]. However, fasting glycaemia has a low predictive value in terms of cardiovascular disease, while 2-h post-load glycaemia and HbA_{1c} have a higher predictive value for this chronic complication of diabetes[42].

In a recent work, we showed that arterial stiffness and carotid intima-media thickness were altered in subjects with higher HbA_{1c} levels and similar as that observed in subjects with new onset type 2 diabetes[43]. Furthermore, when we analyzed our population including only subjects with NFG/NGT we found that the NFG/NGT subjects with HbA_{1c} 39-46 mmol/mol showed an alteration of subclinical markers of cardiovascular risk compared with NFG/NGT with lower HbA_{1c} and no significant differences were found compared with IGT and type 2 diabetic patients (Figure 2). According to these data, a reproducible and simple marker such as HbA_{1c} seems to identify subjects at high cardiovascular risk that would be considered normal according to fasting glycaemia and glucose tolerance. Other studies have shown similar data reporting a positive association between the pre-diabetic stage, echogenic plague and progression of coronary artery calcification[44,45]. A recent study has analysed the routine use of HbA_{1c} for diagnosis of pre-diabetes in patients with ST-segment elevation myocardial infarction. The study showed a similar in-hospital and long-term mortality in these patients with pre-diabetes as those with known diabetes. The authors discussed that the

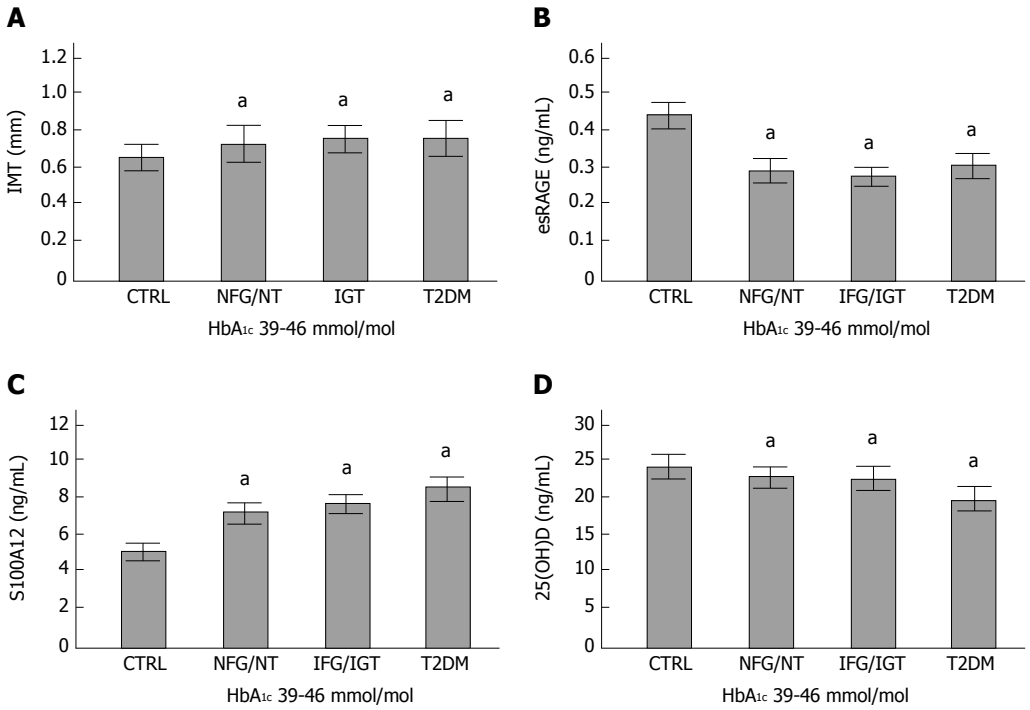

Figure 2 Intima media thickness, endogenous receptor for advanced glycation end-products, S100A12 and 5-hydroxyvitamin D according to glucose tolerance and glycated haemoglobin levels. A: IMT, $^{a}P < 0.05$ *vs* CTRL; B: esRAGE, $^{a}P < 0.05$ *vs* CTRL; C: S100A12, $^{a}P < 0.05$ *vs* CTRL; D: 25(OH)D, $^{a}P < 0.05$ *vs* CTRL. IMT: Intima-media thickness; esRAGE: Endogenous receptor for advanced glycation end-products; 25(OH)D: 25-hydroxyvitamin D; NFG: Normal fasting glucose; NGT: Normal glucose tolerance; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; HbA1c: Glycated haemoglobin.

difficulty in performance and the presence of stress hyperglycaemia in an acutely ill patient with myocardial infarction make OGTT a rarely used diagnostic test in this setting. The use of a simple, one-time HbA1c test allowed them to identify a substantial proportion of patients with previously undiagnosed diabetes or pre-diabetes who could be targeted for risk factor modification with lifestyle interventions and tailored medical therapy[46].

The links between alteration of glucose homeostasis and vascular damage in this population is still unclear, however, several studies have emphasized that the interaction of advanced glycation end products (AGE) with their cell-surface receptor (RAGE) is implicated in triggering inflammatory processes strictly connected with cardiovascular disease[47]. A RAGE soluble form termed endogenous secretory RAGE (esRAGE) may contribute to the removal of circulating ligands, thus competing with cell-surface RAGE for ligand binding[48]. Low levels of esRAGE have been associated with cardiovascular disease and, in a recent study, we found that subjects with pre-diabetes showed low esRAGE plasma levels suggesting a decreased scavenger capacity of these subjects (Figure 2). Further analysis conducted on mononuclear cells isolated from peripheral blood samples of these patients revealed a decreased esRAGE mRNA expression[32]. The regulatory mechanism for alternative splicing to generate esRAGE remains unclear, and environmental or genetic factors may be involved. Further examinations of the molecular mechanism underlying esRAGE regulation will provide potential targets for the prevention and/or treatment of cardiovascular disease.

Our research team has further investigated the characterization of the population with HbA1c pre-diabetes (39-46 mmol/mol) also investigating other markers closely associated with metabolic abnormalities and cardiovascular risk; in a previous study we highlighted a reduced insulin response in combination with impaired suppression of glucagon secretion in subjects with pre-diabetes according to HbA1c undergoing isoglycaemic intravenous glucose infusion[49]. Other data published in 2014 indicated that the presence of pre-diabetes according to HbA1c is associated with hepatic steatosis and with an alteration in the lipid profile known to be predisposing to cardiovascular and liver diseases[50]. Moreover, we showed that the levels of 25 hydroxyvitamin D are reduced and associated with vascular damage in subjects with pre-diabetes by HbA1c with NFG/NGT (Figure 2)[51]. Based on these data, we suggest that among subjects with NFG and NGT, HbA1c may identify subjects with different cardiovascular and glycometabolic risks.

These considerations are, furthermore, supported by previous studies. Indeed, it is important to remember that many authors have documented a significant increase in the incidence of cardiovascular events with HbA1c values substantially lower than those used for diagnosis of diabetes[12]. A recent meta-analysis of six prospective cohort studies in subjects without diabetes mellitus showed a linear association of HbA1c levels with primary cardiovascular events. The observed effect estimates for increased HbA1c levels and was strongly attenuated by adjustment for cardiovascular risk factors but remained statistically significant for primary car-

diovascular events, cardiovascular mortality and all-cause mortality[52].

The majority of randomized controlled trials in non-diabetic subjects with increased HbA_{1c} failed to observe significant effects when aiming to reduce the cardiovascular risk and mortality of these individuals. In the recent IRIS trial, which involved patients without diabetes but with a recent history of ischemic stroke or transitory ischemic attack and who had insulin resistance, the rate of the primary outcome (fatal or non-fatal stroke or fatal or non-fatal myocardial infarction) was lower in the pioglitazone group compared with placebo[11]. These results, although in contrast, at least in part, with other trials conducted on patients with type 2 diabetes (BARI-2D and Pro-active), are of great interest suggesting a favourable effect of pioglitazone on the progression of subclinical atherosclerosis[53,54]. The mechanism that was responsible for the lower rates of stroke and myocardial infarction in the pioglitazone group remains unclear. A recent meta-analysis of prospective, randomized clinical trials has shown a non-significant trend towards reduced risk of fatal and non-fatal myocardial infarction, and fatal and non-fatal stroke were only reduced to borderline. However, the short average follow-up time of 3.75 years was a limitation of previous trials and further RCTs, with a larger sample size and longer follow-up, are required to explore the efficacy of non-drug and drug based approaches to reduce the cardiovascular risk of non-diabetic subjects with increased HbA_{1c}[55].

Other studies have reported similar findings suggesting the role of HbA_{1c} as an early marker of cardiovascular risk; however, it is pertinent to recognize that the determinants of cardiovascular risk in subjects with metabolic alterations are complex and multiple, and individual's cardiovascular risk can't be identified by a single laboratory test[56].

BEYOND TRADITIONAL DIAGNOSTIC CRITERIA: THE ROLE OF NON-TRADITIONAL GLYCAEMIC MARKERS IN PREDICTING DIABETES AND CARDIOVASCULAR RISK

As previously explained, the traditional markers of glucose homeostasis are not definitive, and their use in clinical practice may be biased by a number of clinical and analytical factors. For these reasons, there is growing interest in new serum biomarkers of hyperglycaemia to be used as alternatives or in conjunction with traditional measures. In this review, we will provide a brief overview of the properties and of the existing literature linking these emerging biomarkers with micro- and macro-vascular complications.

One-hour post-load plasma glucose

Recently, an increasing body of evidence has focused on subjects with a plasma glucose concentration of at least 8.6 mmol/L at 1-h during OGTT. In 2008, Abdul-Ghani *et al*[57] demonstrated for the first time that the 1-h post-load plasma glucose concentration may be a clinical indicator that can be used to identify subjects with high risk for type 2 diabetes. These observations were confirmed in other recent studies showing that the incidence rate to type 2 diabetes over a period of 5 years in subjects with NGT and 1-h post-load glycaemia > 8.6 mmol/L was 16.7%[58]. Furthermore, a 1-h post-load glycaemia value > 8.6 mmol/L was strongly associated with different predictors for future cardiovascular events[59,60]. In conclusion, it seems that this glucose value may identify subjects with an intermediate cardiometabolic risk profile between NGT and IGT[57,61]. This has been observed and confirmed in populations of different ethnicities such as Mexican-American, Scandinavian Caucasian, and Asian Indian[59,61,62]. Why 1-h post-load glucose is a good indicator of cardiometabolic risk is still an open question; to date it is known that chronic hyperglycaemia promotes the formation of advanced glycation end products and reactive oxygen species.

One hour post-load glycaemia provides physiopathological information since it is dependent on insulin sensitivity in skeletal muscles and beta-cell function[63].

These data might underline the importance of obtaining intermediate plasma glucose levels during oral glucose tolerance test[59,64]. However, from the clinical point of view, 1-h post-load glycaemia requires, in any case, an OGTT, and, to date, strict lifestyle modification is the only therapy recommended from guidelines for subjects with pre-diabetes, independently from their physiopathologic profile. Furthermore, a study conducted on subjects with HbA_{1c} pre-diabetes reported that most patients with HbA_{1c} in the 39-46 mmol/mol range have a 1-h glucose $\geqslant$ 8.6 mmol/L; these data lead to the consideration that HbA_{1c} may be the most practical tool to identify subjects with impaired glucose homeostasis[43].

Fructosamine and glycated albumin

Fructosamine and glycated albumin are both ketoamines formed from the binding of fructose to total serum protein, mostly albumin, through glycosylation. The fructosamine assay is cheaper and easier to perform than the HbA_{1c} assay and it measures total glycated serum protein, whereas glycated albumin is reported as the proportion of total albumin[65]. Fructosamine and glycated albumin are short-term markers of glucose homeostasis; indeed, they provide information on blood glucose levels over the previous 2-3 wk[66]. This depends on the rapid turnover of glycated proteins, that in contrast to HbA_{1c}, is independent from the turnover of red blood cells or hemoglobin characteristics. Similar to HbA_{1c}, blood for fructosamine dosage can be obtained in any moment of the day, without regard to recent food intake. Both fructosamine and glycated albumin are associated with future risk of diabetes, independently from fasting glucose and HbA_{1c}[67,68]. Another recent study explored the ability of HbA_{1c}, fructosamine and glycated albumin to detect pre-

diabetes and whether there would be added diagnostic value in combining HbA_{1c} with fructosamine or glycated albumin. The study, conducted on United States Africans, showed that HbA_{1c}, fructosamine and glycated albumin detected almost 50% of Africans with pre-diabetes; however, combining HbA_{1c} with glycated albumin (but not with fructosamine) made it possible to identify nearly 80% of Africans with pre-diabetes, as reported in previous studies[69]. Furthermore, the authors reported that pre-diabetic patients identified by glycated protein were younger and with a lower BMI, as previously reported. It is still not clear why glycated plasma proteins are inversely related to body size, however, this observation could be of clinical relevance and it may support the use of glycated albumin to enhance the detection of pre-diabetes in specific populations, such as the non-obese.

Evidence derived from prospective studies regarding the link between non-traditional markers and micro and macrovascular complications are limited. Data from the Atherosclerosis Risk in Communities (ARIC) Study have shown that glycated albumin predicted chronic kidney disease over two decades of follow-up with a similar magnitude to those observed for HbA_{1c}[69]. Other evidence has come from cross-sectional studies. A recent analysis from the ARIC Study has shown an association between glycated albumin and retinopathy, with a pattern of association very similar to that observed for HbA_{1c}[69]. Furthermore, in other studies conducted on adults without diagnosed diabetes, glycated albumin was associated with subclinical atherosclerosis, kidney and cardiovascular disease[70].

A potential limitation to the clinical use of these markers may be that, to date, there is no established clinical cut-off points and the assays are not standardized across instruments. Particular caution should be used in pathological conditions that can impact albumin metabolism including anaemia, malnutrition, nephrotic syndrome and liver cirrhosis.

To date, fructosamine and glycated albumin are not incorporated in clinical guidelines, however, they may be useful complements to HbA_{1c} in clinical practice, mainly when HbA_{1c} testing is inaccessible or when the result might not be reliable.

1,5-anhydroglucitol

1,5-anhydroglucitol (1,5-AG) is a monosaccharide primarily derived from dietary sources and is a non-traditional biomarker of hyperglycaemia. During euglycaemia, serum 1,5-AG is typically maintained at a constant concentration (12-40 μg/mL). It is freely filtered from the glomeruli and a small amount, dependent on dietary intake, is excreted with the urine. The remaining amount is reabsorbed in the renal tubule. In conditions of hyperglycaemia (> 8.9-10 mmol/L) glucose blocks renal tubular reabsorption of 1,5-AG resulting in a drop in 1,5-AG serum levels; therefore, an inverse association exists between hyperglycaemia and 1,5-AG. Clinically, 1,5-AG may be used as a marker of short-term glycaemic variability, reflecting hyperglycaemic episodes over 1-2 wk. 1,5-AG is a non-fasting test and it may include information about glycaemic excursion that is not included in HbA1c dosage.

Previous studies found a significant association between 1,5-AG and the subsequent development of diabetes with a magnitude that was significant but weaker compared with fructosamine and glycated albumin[68]. However, consistent with its pathophysiology, 1,5-AG was no longer associated with incident diabetes among people with a normal fasting glucose < 5.6 mmol/L or HbA_{1c} < 39 mmol/mol, suggesting a limited usefulness for 1,5-AG in the setting of normal glucose and HbA_{1c} levels. According to this data 1,5-AG seems to be a biomarker suitable for detecting glycaemic variations in patients with HbA_{1c} between 53-64 mmol/mol (for example, to monitor a patient's response to changes in medication) rather than in subjects with pre-diabetes.

Few studies have assessed the relationship of 1,5-AG with micro and macrovascular complications. Cross-sectional studies have reported associations between 1,5-AG serum levels, subclinical atherosclerosis, prevalent retinopathy and coronary heart disease in subjects with and without diabetes[71,72]. A recent study observed a threshold effect, with little evidence of risk for cardiovascular events at the "non-diabetic" 1,5-AG concentration of 10-15 μg/mL. However, most of the study group were diabetic subjects, and in the categorical analysis the association with the clinical outcomes was largely confined to the subjects with diabetes[73].

CONCLUSION

The measurement of HbA_{1c} appears to be a reliable diagnostic approach to identify patients at high risk for diabetes and cardiovascular disease; it seems to provide several advantages, especially in settings where OGTT is rarely used and never repeated as a confirmatory test, and eliminates a long series of biological and analytical limits. In most conditions HbA_{1c} could became the reference method, provided that its assay is aligned with international standards. The budget/cost benefit of replacing glucose with HbA_{1c} remains unclear and it is necessary to acquire additional information.

Finally, alternative biomarkers of glucose homeostasis may have a clinical use in identifying subjects at risk for diabetes and cardiovascular disease (mostly 1-h post-load glycaemia) and for short-term evaluation of glucose homeostasis in settings in which HbA_{1c} may present some bias (fructosamine, glycated albumin and 1,5-AG). It is possible that one or more of these biomarkers may be of clinical usefulness, however, long-term prospective studies are needed to demonstrate whether their clinical use may be useful to improve outcomes and patient care.

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MINIREVIEWS

Nitrate-nitrite-nitrosamines exposure and the risk of type 1 diabetes: A review of current data

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Abstract

The potential toxic effects of nitrate-nitrite-nitrosamine on pancreatic β cell have remained a controversial issue over the past two decades. In this study, we reviewed epidemiological studies investigated the associations between nitrate-nitrite-nitrosamines exposure, from both diet and drinking water to ascertain whether these compounds may contribute to development of type 1 diabetes. To identify relevant studies, a systematic search strategy of PubMed, Scopus, and Science Direct was conducted using queries including the key words "nitrate", "nitrite", "nitrosamine" with "type 1 diabetes" or "insulin dependent diabetes mellitus". All searches were limited to studies published in English. Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of type 1 diabetes. A null, sometimes even negative association has been mainly reported in regions with a mean nitrate levels < 25 mg/L in drinking water, while increased risk of type 1 diabetes was observed in those with a maximum nitrate levels > 40-80 mg/L. Limited data are available regarding the potential diabetogenic effect of nitrite from drinking water, although there is evidence indicating dietary nitrite could be a risk factor for development of type 1 diabetes, an effect however that seems to be significant in a higher range of acceptable limit for nitrate/nitrite. Current data regarding dietary exposure of nitrosamine and development of type 1 diabetes is also inconsistent. Considering to an increasing trend of type 1 diabetes mellitus (T1DM) along with an elevated nitrate-nitrite exposure, additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on β-cell autoimmunity and the risk of T1DM.

Key words: Nitrate; Nitrite; Nitrosamine; Type 1 diabetes

Core tip: The potential toxic effects of nitrate-nitrite-nitrosamine on pancreatic β cell have remained a controversial issue over the past two decades. Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of type 1 diabetes. An increased risk of type 1 diabetes was observed in regions with a maximum nitrate levels > 40-80 mg/L. Dietary nitrite could be a risk for development of type 1 diabetes in a higher range of acceptable limit. Additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on β-cell autoimmunity and the risk of type 1 diabetes mellitus.

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INTRODUCTION

An overview of type 1 diabetes

Type 1 diabetes mellitus (T1DM), one of the main autoimmune disorders caused by immune-mediated destruction of pancreatic β-cells, eventually develops to an absolute insulin deficiency, impaired glucose homeostasis, and physiological dependence on exogenous insulin[1]. An overall approximately 3% increased per year in the incidence of T1DM along with a different geographical incidence has been observed worldwide[2,3]. A higher incidence rate of T1DM has been reported in European countries, especially Finland and Sardinia, however recently the incidence of T1DM has risen rapidly in low-incident populations including parts of India, the Middle East, and Sub-Saharan Africa[4]. The incidence of T1DM had an increasing trend in both developed and developing countries during a recent decade[5-8]. According to current trends, it is predicted that new cases of T1DM in European children < 5 year will be doubled and prevalent cases < 15 years will be raised by 70%, between 2005 and 2020[7].

Risk factors of T1DM

T1DM has a multifactorial nature; besides genetic factors and family history which account for about 30%-50% onset of T1DM, several factors such as environmental contaminants, infection agents, drugs, and dietary factors have been known as important etiologically relevant to β-cell autoimmunity and T1DM development[9-11]. Dietary factors implicated in the pathogenesis of T1DM are cow milk proteins (casein, bovine serum albumin, β-lactoglobulin, and bovine insulin), gluten, zinc and vitamin D deficiency, as well as nitrate and nitrite; some ecologic, animal, and human studies have confirmed that *N*-nitroso compounds, nitrate and nitrite play a role in development of T1DM[12-14]. Diabetogenic agents from diet may induce their effects by several suggested pathways, including apoptosis of β-cell, increased oxidative stress, impaired insulin response and immune function, and some postprandial modifications[15].

Diabetogenic hypothesis of nitrate-nitrite exposure

Inorganic nitrate (NO_3) and nitrite (NO_2) are naturally occurring compounds in foods and are also used as food additives; major sources of exogenous nitrate exposure are vegetables and drinking water, whereas processed meat and animal food products are the main sources of nitrite[16]. Considering both acute and chronic potential toxicities, some limitations have been legislated for dietary intakes of nitrate and nitrite; the acceptable daily intakes (ADI) of nitrate and nitrite from food sources as designated by the Scientific Committee on Foods and the Joint Food and Agriculture Organization/World Health Organization (WHO) Expert Committee on Food Additives defined as 3.7 and 0.06 mg/kg body weight, respectively[17]. Moreover, due to substantial concentrations of nitrate-nitrite in drinking water, WHO restricted the acceptable concentrations of drinking water to < 50 mg/L and 3 mg/L for nitrate and nitrite, respectively[18,19].

Recent investigations have however highlighted the beneficial therapeutic effects of nitrate-nitrite against metabolic disorders such as type 2 diabetes[20-22], possible adverse complications such as thyroid disorders and T1DM[23,24] are still remaining due to indiscriminate increased use of fertilizers and nitrite-containing food additives and increased exposure of nitrate-nitrite from both diet and drinking water. It has been proposed that nitrate-nitrite may have toxic effects on pancreatic β-cells due to generation of peroxynitrite, reactive nitrogen intermediates, and nitrosamines[25].

Although data have shown elevated risk of β-cell autoimmunity and T1DM due to high intakes of nitrate-nitrite over the past two decades[26], this data has however not yet led to a consistent confirmed conclusion[27-30].

Aim of this study

This review will focus on the potential effect of nitrate-nitrite-nitrosamines exposure on development of T1DM. We reviewed epidemiological studies investigating the associations between nitrate-nitrite-nitrosamines exposure, from both diet and drinking water to ascertain whether higher nitrate-nitrite may contribute to the development of T1DM.

To identify relevant studies, a systematic search strategy of PubMed, Scopus, and Science Direct was conducted using queries including the key words "nitrate", "nitrite", "nitrosamine" with "T1DM" or "insulin dependent diabetes mellitus". All searches were limited to studies published in English.

EXPOSURE OF NITRATE-NITRITE FROM DRINKING WATER AND THE RISK OF T1DM

Possible relation between quality of drinking water and T1DM, particularly concentrations of nitrate or nitrite has been investigated in several studies. An overview of current data indicates that potential diabetogenic effects of nitrate-nitrite have mainly been investigated by estimation of nitrate-nitrite exposure from drinking water, a relationship also evaluated in the framework of ecological studies[28-32].

An ecological analysis of insulin dependent diabetes mellitus registry data on children aged < 18 year during 1978-1988 in relation to public water supplies and well water systems in Colorado between 1984 and 1988, showed a significant correlation between T1DM incidence and water nitrate level (r = 0.27, P = 0.03)[29]. This correlation was higher in countries where nitrate levels in the public water system were in the highest tertile (r = 0.29, P = 0.02), and the rate of T1DM was higher in the highest compared to the lowest tertile of nitrate exposure (PI = 15/100000 *vs* PI = 7/100000, in 0.77-8.2 mg/L *vs* 0-0.08 mg/L nitrate levels, model R^2 = 0.14); the authors pointed out that findings of the study should be interpreted considering to some limitations such as lack of data on individual's nitrate exposure and inappropriate timing of the exposure measurement[29].

In another ecological analysis, conducted on a population-based study in the framework of Yorkshire Regional Health Authority during 1978-1994, Parslow *et al*[28] reported that the incidence of T1DM was positively associated with mean nitrate levels in drinking water; an increasing trend in standardized incidence ratio (SIR) of T1DM was observed across increasing levels of nitrate in drinking water (SIR = 85, 95%CI: 78-93; SIR = 99, 95%CI: 91-107; SIR = 115, 95%CI: 107-124; in levels of 1.5-3.2, 3.2-14.5 and 14.9-40.0 mg/L, respectively, χ^2 = 26.8, P < 0.001)[28]. Moreover, a 30% higher incidence rate of diabetes was observed among doses in water supply zones with mean nitrate levels 14.9-40.0 mg/L, compared with those in zones with a mean nitrate levels < 3.2 mg/L (IRR = 1.27, 95%CI: 1.09-1.48). In this study, over 30% of drinking water samples contained > 25 mg/L nitrate levels.

Analysis of drinking water in Finland for nitrate, nitrite, nitrate-nitrogen and nitrite-nitrogen, among families with a child, diagnosed as type 1 diabetic compared to controls, showed that higher levels of nitrate in drinking water was related to increased risk of T1DM (OR = 1.32, 95%CI: 1.06-1.64; P = 0.013); nitrite concentrations had no significant association with the risk of T1DM (OR = 0.36, 95%CI: 0.06-2.03; P = 0.25)[33]. Mean nitrate and nitrite levels of drinking water in this population were 4.43 (0-80 mg/L) and 0.02 (0.02-0.16 mg/L), respectively; mean nitrate and nitrite levels in municipalities with high compared to low incidence of T1DM, was lower (1.27 mg/L *vs* 3.25 mg/L, 0.02 mg/L *vs* 0.03 mg/L, for nitrate and nitrite, respectively)[33].

In contrast, some studies report findings to reject diabetogenic hypothesis of the nitrate-nitrite exposure. The incidence of T1DM was not related to nitrate exposure, in an ecological study of children, aged < 15 year, in the Netherlands, conducted using the Dutch Pediatric Surveillance Unit (1993-1995) and nitrate drinking water data from the National Institute of Public Health and Environmental Protection (1991-1995)[31]; standardized incidence rate of T1DM was 1.45 for nitrate levels ranging > 25 mg/L (95%CI: 0.85-2.07). Lack of information on the individual's quantity of water consumption, length of exposure and data on potential risk factors of such as family history of T1DM, were important limitations of this study. Moreover, non-significant findings have been attributed to small number of cases in the > 25 mg/L category; in this study, only 1% of the children were exposed to nitrate levels between 25-41 mg/L[31].

In a Finnish nation-wide case-control study, exposure of nitrate and nitrite in children and their parents from drinking water were assessed in relation to risk of T1DM; no differences were observed in intakes of nitrate or nitrite from drinking water between cases and controls[26].

Analysis of data on nitrate concentration of both tap and bottled water in Italy during 1993-1994, showed no significant association between nitrate exposure and incidence of T1DM during 1989-1998, in the subjects, aged 0-14 y (r = -0.06) or in the group, aged 0-29 year (r = -0.17)[32]. There was no effect from sex in the same age-groups; in contrast with previous reports, a negative trend between nitrate levels and T1DM was also noted[32]; in this study, both tap and bottled water were within the acceptable maximal concentration of 50 mg/L legislated by the European Community and also under the recommended levels of 25 mg/L.

In a retrospective study of 153 Sardinian communes, among 0-14 year Italian children, a significantly inverse trend between childhood diabetes and mean nitrate exposure was observed; higher nitrate of drinking water was reported in districts with low compared to high incidence of T1DM (8.9-14.5 mg/L *vs* 4.3-7.8 mg/L)[34]. The risk of T1DM in subjects exposed to highest compared to the lowest nitrate levels in drinking water (6.5-28.9 mg/L *vs* ≤ 2.5 mg/L) decreased 40% (RR = 0.6, 95%CI: 0.4-1.0, P = 0.027).

An initial assessment of nitrate exposure from domestic water during 1993-1997, in relation to T1DM diagnosed in children aged 0-15 year in England between 1975-1996, suggested that nitrate may had a protective effects against development of T1DM[35]; standardized incidence ratio in the highest compared to the lowest tertile of nitrate levels (7.48-16.58 mg/L *vs* 1-3.65 mg/L) was lower (SIR = 90.2, 95%CI: 77-105 *vs* SIR = 111.8, 95%CI: 96-129; χ^2 = 3.89, P = 0.048), however, Poisson regression analysis failed to support this relationship; mean nitrate levels of drinking water was 6.02 mg/L (min = 0.48 and max = 31.9 mg/L).

Moltchanova *et al*[36] in a study of children aged < 15 in Finland between 1987-1996, showed an increasing

risk of T1DM along with increasing nitrate concentration of drinking water. The posterior mean unit effect of nitrate on diabetes risk was 0.003 (-0.009, 0.0138), *i.e.,* 1 mg/L increased nitrate concentration in the ground water resulted in 0.3% increased risk of T1DM; mean nitrate level of groundwater was 6.22 mg/L (0.20 and 6.64 in the 1st and 4th quartiles, respectively)[36].

In a retrospective study from Saudi Arab, type 1 diabetic patients, diagnosed between 1980 and 2009, no etiological effects for nitrate levels in drinking water of the study areas were observed; mean nitrate level in drinking water showed levels between 0.6 and 4 mg/L, during 30 years which were much lower than the toxic levels[37].

A nested case-control analysis on 95 islet auto-antibody-positive (Islet Ab$^+$) and 139 Islet Ab$^-$ children, conducted in the framework of German BABYDIAB study, indicated no association between nitrate content of drinking water and the risk of islet autoimmunity, whereas higher levels of nitrite ($\geqslant$ 0.009 mg/L *vs* < 0.009 mg/L) had a borderline protective effect (OR = 0.6, 95%CI: 0.4-1.0)[30]; mean nitrate levels of water were 9.5 mg/L (4.8-16.6) and 9.2 mg/L (3.8-21.2) in Islet Ab$^+$ and Islet Ab$^-$ children, respectively; upper nitrite level of drinking water was marginally higher in Islet Ab$^-$ compared to Islet Ab$^+$ children (0.01 mg/L *vs* 0.009 mg/L, P = 0.06). The odds of the progression of islet autoimmunity to T1DM in higher levels of nitrate and nitrite in drinking water ($\geqslant$ 9.58 and $\geqslant$ 0.009 mg/L) was 0.9 (95%CI: 0.4-2.0) and 1.5 (95%CI: 0.6-3.5), respectively[30]. Another important finding was an inverse relation between nitrate concentrations and pH levels of drinking water (r = -0.28, P = 0.001), along with a positive relation between pH of water and progression of T1DM (OR = 2.5, 95%CI: 1.1-5.7)[30]; it may be indirectly provide evidence for hazardous effects of nitrate on T1DM. This study was the first try to investigate the association of nitrate-nitrite exposure during the first year of life in children and the risk of islet autoimmunity; due to importance of this period in developing of islet autoimmunity, this study provided an opportunity to evaluate a potential causal relationship between nitrate-nitrite of drinking water and T1DM progression. Matching for date of birth, duration of follow-up, human leukocyte antigen (HLA), gender and geographical region and also adjustment of main potential risk factors of T1DM including genetic factors (HLA DR 3/4, 4/4) and maternal T1DM were other strengths of the study.

Tables 1 and 2 provide a summary of results from ecological, case-control and cohort studies of mean nitrate-nitrite levels from drinking water in relation to incidence of T1DM.

DIETARY EXPOSURE OF NITRATE-NITRITE AND THE RISK OF T1DM

The risk of T1DM in response to nitrate-nitrite exposure from diet has been evaluated in a limited number of studies. In a prospective case-control study of Swedish children, aged 0-14 years, matched for age, sex, and country of residence, a significant increasing trend of T1DM was noted for higher intakes of foods containing nitrate and nitrite[27]. In this study, fresh green vegetables, boiled vegetables, root vegetables, cheese, sausage and bacon have been defined as high nitrate-nitrite containing foods; mean frequency of nitrate-nitrite rich foods was higher in diabetics compared to controls; highest compared to the lowest (> 75th centile *vs* < 25th centile) frequency of consumption of nitrate-nitrite rich foods was related to an elevated risk of T1DM (OR = 2.41, 95%CI: 1.64-3.54, P = 0.001)[27]. After adjustment of some potential confounding variables including age, sex, maternal age, maternal education, and family history insulin dependent diabetes, the chance of having T1DM was 0.89 and 2.68 in individuals with medium and high nitrate-nitrite exposure from diet[27]. In further analysis, stratified for vitamin C rich foods, risk estimate for medium and highest nitrate-nitrite intakes along with low vitamin C intakes was 0.94 and 2.44 (P < 0.001), respectively; in contrast, higher intakes of nitrate-nitrite were not associated with T1DM in the presence of higher intakes of vitamin C. An indirect estimation of nitrate-nitrite based on food frequency intakes, was an important limitation of this study; lack of data on nitrate-nitrite exposure from drinking water was also another source of bias in estimation of nitrate and nitrite exposure.

In a Finnish nation-wide case-control study, intakes of nitrate and nitrite of children and their parents from food and drinking water were assessed in relation to risk of T1DM[26]. Compared to controls, dietary intakes of nitrite were higher in diabetic children and their mothers (0.9 mg/d *vs* 0.8 mg/d). Higher intakes of nitrate were also observed in cases mother's compared to controls (P < 0.05). The risk of T1DM increased across increasing intakes of dietary nitrite among children (OR = 1.16, 95%CI: 0.82-1.65; OR = 1.49, 95%CI: 1.06-2.10; OR = 2.32, 95%CI: 1.67-3.24 in the second, third, and fourth quartiles, respectively), and their mothers (OR = 1.15, 95%CI: 0.76-1.74; OR = 1.29, 95%CI: 0.87-1.91; OR = 1.98, 95%CI: 1.35-2.90, in the second, third, and fourth quartiles, respectively), a relationship independent of age, mother's education, place of residence or smoking status of mothers[26].

A case-control study on dietary intakes of nitrate and nitrite during the year prior to diagnosis of diabetes, after adjustment of age, sex, and total energy intake, showed a non-significant positive dose-response relationship between risk of T1DM and nitrate intakes from foods (OR = 1; OR = 1.01, 95%CI: 0.0.28-3.61; OR = 1.19, 95%CI: 0.31-4.52, OR = 2.25, 95%CI: 0.45-11.14 in the first to fourth quartiles; P = 0.29); dietary intakes of nitrate were < 5.66, 5.66-7.27, 7.27-9.01, and $\geqslant$ 9.01 mg/d in the first to fourth quartiles, respectively. The risk of T1DM increased 30% (OR = 1.30, 95%CI: 0.30-5.59) in the highest, compared to the lowest quartiles of nitrite intakes ($\geqslant$ 4.82 mg/d *vs* < 1.83 mg/d)[38]. Neither were total intakes of nitrate + nitrite (from both diet and

Table 1 Summary of results from ecological, case-control and cohort studies of mean nitrate levels from drinking water in relation to incidence of type 1 diabetes

Ref.	Country	Exposure levels (mg/L)	Findings
Muntoni *et al*[34]	Italy	≤ 2.5	OR = 1.0
		2.5-4.0	OR = 0.6 (95%CI: 0.4-1.0)
		4.0-6.5	OR = 0.5 (95%CI: 0.3-0.7)
		6.5-28.9	OR = 0.6 (95%CI: 0.4-1.0)
			P = 0.027
Parslow *et al*[28]	United Kingdom	1.5-3.2	OR = 1.0
		3.2-14.9	OR = 1.11 (95%CI: 0.98-1.26)
		14.9-40.0	OR = 1.27 (95%CI: 1.09-1.48)
Winkler *et al*[30]	Germany	< 9.58	OR = 1.0
		≥ 9.58	OR = 0.9 (95%CI: 0.6-1.3)
Zhao *et al*[35]	England	1-3.6	SIR = 1.11 (95%CI: 0.96-1.29)
		3.6-7.8	SIR = 0.99 (95%CI: 0.85-1.15)
		7.8-16.6	SIR = 0.90 (95%CI: 0.77-1.05)
			χ^2 = 3.8, P = 0.048
van Maanen *et al*[31]	The Netherland	< 10	SIR = 0.99 (95%CI: 0.93-1.06)
		10-25	SIR = 0.99 (95%CI: 0.84-1.14)
		≥ 25	SIR = 1.45 (95%CI: 0.85-2.07)
		0.2-2.1	SIR = 1.02 (95%CI: 0.92-1.13)
		2.1-6.4	SIR = 0.95 (95%CI: 0.85-1.06)
		6.4-41.2	SIR = 1.02 (95%CI: 0.92-1.12)
Casu *et al*[32]	Italy	Approximately 10	Simple correlation = -0.17, P = NS
Samuelsson *et al*[33]	Sweden	0-80	OR = 1.32 (95%CI: 1.06-1.64), P = 0.013
Moltchanova *et al*[36]	Finland	0.2-6.64	Posterior mean unit effect = 0.0026 (95%CI: -0.0093-0.0138)
Kostraba *et al*[29]	United States	0-8.2	Correlation = 0.23, P = 0.07

OR: Odds ratio; SIR: Standardized incidence ratio; NS: No significance.

Table 2 Summary of results from ecological, case-control and cohort studies of mean nitrite levels from drinking water in relation to incidence of type 1 diabetes

Ref.	Country	Exposure levels (mg/L)	Findings
Winkler *et al*[30]	Germany	< 0.009	OR for β-cell autoimmunity = 1.0
		≥ 0.009	OR for β-cell autoimmunity = 0.6 (95%CI: 0.4-1.0), P = 0.07
		< 0.009	OR for type 1 diabetes = 1.0
		≥ 0.009	OR for type 1 diabetes = 1.5 (95%CI: 0.6-3.5), P = 0.074
Samuelsson *et al*[33]	Sweden	0.02-0.16	OR = 0.36 (95%CI: 0.06-2.03), P = 0.25

OR: Odds ratio.

drinking water) related to risk of T1DM. It should be noted that the highest intakes of dietary nitrate in this population were much lower than the ADI limit value (9 mg/d *vs* 259 mg/d for an adult subject) whereas dietary nitrite intakes in the highest quartile were higher than the recommended values (4.82 mg/d *vs* 4.2 mg/d for adults). An accurate estimation of nitrate intakes from diet and assessment of the individual's drinking water intakes may be considered as important strengths of this study.

NITROSAMINE EXPOSURE AND THE RISK OF T1DM

N-nitrosodiethylamine and nitrosodimethylamine are two main nitrosamine compounds that contaminate food and water sources; the major known sources of dietary volatile nitrosamines are nitrite-cured meats, especially sausage and fried bacon[39,40]. Nitrosamines mediate their adverse effects due to induction of DNA damage, oxidative stress, lipid peroxidation, and activation of inflammatory signalling pathways, which lead to increased cellular degeneration and death[41].

For the first time in 1981, in a study of children aged 0-14 year in Island, Helgason *et al*[42] provided some primary evidence for the potential role of dietary intakes of nitrosamines in the development of T1DM. Subsequent studies have reported conflicting results. Findings of a case-control study of Australian, children aged 0-15 year, rejected this hypothesis and showed that those children who consumed higher amounts of foods containing nitrosamines did not have an increased risk of diabetes; the odds (95%CI) of T1DM were 0.71 (0.44-1.14) and 1.07 (0.66-1.74), in the middle and highest tertile compared to the lowest tertile of nitrosamine-containing foods, respectively[14].

A prospective case-control study of Swedish children, aged 0-14 year, dietary frequency of nitrosamines rich foods including smoked fish, bacon and sausage, increased risk of T1DM in a dose-response manner[27]. Dietary exposure of nitrosamines was also positively

related to increased risk of T1DM (OR = 1.73, 95%CI: 1.23-2.44 and OR = 2.56, 95%CI: 1.83-3.59 in the medium and high categories, respectively)[27]. Further analysis stratified for different levels of dietary protein intakes, showed that higher nitrosamine intake was risk factor for diabetes, only in the presence of higher levels of protein (OR = 2.08, 95%CI: 0.94-4.60; OR = 2.12, 95%CI: 1.11-4.04, $P = 0.03$)[27].

Another case-control study of Canadian children indicated no significant association between nitrosamines intakes and risk of T1DM (OR = 0.57, 95%CI: 0.21-1.57; OR = 0.66, 95%CI: 0.18-2.45; OR = 0.62, 95%CI: 0.19-2.00; in the second, third and fourth quartiles, $P = 0.51$); daily intakes of nitrosamines were estimated < 0.01, 0.01-0.03, 0.03-0.04, and ⩾ 0.4 mg/d across the quartile categories[38].

CONCLUSION

Ecologic surveys, case-control and cohort studies have indicated conflicting results in relation to nitrate-nitrite exposure from drinking water and the risk of T1DM. A null, sometimes even a negative association has been mainly reported in populations with a mean nitrate levels < 25 mg/L in drinking water, whereas increased risk of T1DM was reported in regions with maximum nitrate levels > 40-80 mg/L. Limited data are available regarding potential diabetogenic effects of nitrite from drinking water, a hypothesis not yet confirmed. Inconsistent findings of the studies may be attributed to a wide variation in nitrate-nitrite exposure, different cut off points used for definition of nitrate-nitrite exposure, differences in the duration of exposure and variation in potential confounding variables, adjusted in the statistical models. Lack of significant association between dietary nitrate intakes with the risk of T1DM, observed in previous studies, may be attributed to mean nitrate intakes lower than ADI. There is evidence which indicates dietary exposure of nitrite may be risk factor for development of T1DM, an effect however seems to be significant in a higher range of acceptable limits. Current data regarding dietary exposure of nitrosamine and development of T1DM is also inconsistent. To conclude findings of previous studies on nitrate-nitrite exposure and risk of T1DM, it should be noted that most studies reviewed had an ecological nature; they provided only an indirect crude estimation of exposure and described only the association between the incidence of T1DM and average level of exposure in a set of data. Considering the fact that nitrate exposure should be assessed based on individual's intake, overall estimation according to nitrate levels of water supplies, lack of data on amount of drinking water and dietary intakes of nitrate-containing foods, were main limitations of previous studies which could lead to potential misclassification of exposure; findings therefore should be considered conservatively. Relevant timing of exposure is also an important issue in assessment of the possibly diabetogenic effect of nitrate, somewhat neglected in the previous studies.

In future studies, a more accurate estimation of nitrate-nitrite exposure at an individual level is recommended to examine the potential effects on β-cell destruction and development of T1DM. Taking into account islet autoimmunity status and assessment of islet autoantibody levels such as insulin autoantibodies, glutamic acid decarboxylase, and IA-2, should also be considered in future investigations of the association between nitrate exposure and the risk of T1DM development, determine the role of nitrate-nitrite at different stages of the disease as initiators, promoters or trigger of the T1DM.

It should be noted the studies investigated possible association of nitrate-nitrite exposure and the risk of T1DM, are mainly limited to European countries, especially high-incidence rate populations including Sweden, Finland, England, Germany and Italy. It is also noteworthy that epidemiological investigations on diabetogenic effects of nitrate-nitrite exposure was of interest during two past decades, and scientific communities have been silent on this issue in recent years; low nitrate-nitrite exposure levels in the mentioned countries may be a reason for this trend.

Considering to an increasing trend of T1DM along with an elevated nitrate-nitrite exposure due to increased use of fertilizers and nitrite-containing food additives, additional research is critical to clarify potential harmful effects of nitrate-nitrite-nitrosamine exposure on β-cell autoimmunity and the risk of T1DM. Given that the incidence of T1DM is alarming among previously secured populations including Middle East, Asian and African countries, and nitrate-contaminated drinking water is currently a public health problem among these populations[43,44], clarifying of the issue should be considered as a public health priority in developing countries.

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Glucagon-like peptide-1 receptor agonists favorably address all components of metabolic syndrome

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Abstract

Cardiovascular death is the leading cause of mortality for patients with type 2 diabetes mellitus. The etiology of cardiovascular disease in diabetes may be divided into hyperglycemia *per se* and factors operating through components of metabolic syndrome (MetS). Hyperglycemia causes direct injury to vascular endothelium and possibly on cardiac myocytes. MetS is a cluster of risk factors like obesity, hyperglycemia, hypertension and dyslipidemia. The incidence of this syndrome is rising globally. Glucagon-like peptide-1 receptor agonists (GLP-1RA) are a group of drugs, which address all components of this syndrome favorably. Experimental evidence suggests that they have favorable actions on myocardium as well. Several compounds belonging to GLP-1RA class are in market now and a large number awaiting their entry. Although, originally this class of drugs emerged as a treatment for type 2 diabetes mellitus, more recent data generated revealed beneficial effects on multiple metabolic parameters. We have studied literature published between 2000 and 2016 to look into effects of GLP-1RA on components of MetS. Results from recently concluded clinical trials suggest that some of the molecules in this class may have favorable effects on cardiovascular outcome.

Key words: Metabolic syndrome; Diabetes; Glucagon-like peptide-1 receptor agonists; Lipids; Body weight; Microalbuminuria

Core tip: The incidence of metabolic syndrome (MetS) is on the rise globally. This will have a negative impact on cardiovascular outcome. Whereas most of the anti-hyperglycemic agents have neutral or negative effects on components of MetS, glucagon-like peptide-1 receptor agonists drugs favorably address all components of MetS. By doing so, they may have a cardio protective role. We have reviewed recent literature to give an updated account on the topic. Results from recently concluded clinical trials suggest that some of the molecules in this class may have favorable effects on cardiovascular

outcome.

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INTRODUCTION

In 1977, Haller[1] used the term "metabolic syndrome (MetS)" for association of obesity, diabetes mellitus, lipid disorder, hyperuricemia, and hepatic steatosis which increase the risk of atherosclerosis.

In 1988, Reaven[2] observed clustering of risk factors for coronary heart disease and stroke - like central obesity, hypertension, hyperglycemia and dyslipidemia, which may have a direct relationship with insulin resistance and termed the cluster, MetS X. This is also known as insulin resistance syndrome or simply, MetS.

The prevalence of diabetes and obesity is on the rise globally. In 2002, the prevalence of MetS in the United States was 34% in the adult population[3]. This will have an impact on cardiovascular mortality and morbidity.

Some authorities have suggested other components like non-alcoholic fatty liver disease (NAFLD), Microalbuminuria, high levels of C-reactive protein (CRP) and polycystic ovary syndrome (PCOS), as parts of MetS[4].

Recent figures in the United States estimate that there has been a reduction in the prevalence of hypertension and dyslipidemia, but an increase is noted in central obesity and hyperglycemia in the year 2010, when compared with figures of the year 1999-2000[5].

As defined by the 2009 Joint Scientific statement, the qualifying criteria for MetS demands the presence of any three or more of the following biological thresholds: (1) waist circumference $\geqslant$ 102 cm (male adults) and $\geqslant$ 88 cm (female adults); (2) fasting plasma glucose $\geqslant$ 5.55 mmol/L (100 mg/dL); (3) blood pressure of 130/85 mmHg; (4) triglycerides 1.69 mmol/L ($\geqslant$ 150 mg/dL); and (5) high-density lipoprotein-cholesterol (HDL-C) 1.03 mmol/L (< 40 mg/dL) (male adults) and 1.29 mmol/L (< 50 mg/dL) (female adults). Prescription drug use was estimated for lipid-modifying agents, anti-hypertensive, and anti-hyperglycemic medications[5,6].

The National Health and Nutrition Examination Survey (NHANES) in 1999 and 2010 (in 2-year survey waves) estimated the prevalence of MetS in adult population ($\geqslant$ 20 years of age). As per the results from 1999-2000 and 2009-2010: There was a reduction in the age-adjusted prevalence of MetS (based on biologic thresholds) by 2.6% (from 25.5% to 22.9%). Further perusal of the different components of MetS during this period revealed the prevalence of hypertriglyceridemia to be decreased by 9.2% (33.5% to 24.3%), as did the hypertension by 8.3% (32.3% to 24.0%). Nevertheless the prevalence of hyperglycemia increased by 7% (12.9% to 19.9%), as did elevated waist circumference by 10.7% (45.4% to 56.1%). These trends varied considerably by sex and race/ethnicity. Changes in the prevalence of hypertension, suboptimal triglycerides, and high-density lipoprotein-cholesterol have corresponded with increases in anti-hypertensives and drugs for dyslipidemia, respectively[6].

As regards to obesity, the prevalence is on the rise. Results from the 2011-2012 NHANES indicate that among United States adults aged 20 and over, 33.9% are overweight (BMI 25.0-29.9), 35.1% are obese (BMI 30.0-34.9), and 6.4% are extremely obese (BMI $\geqslant$ 35.0), The survey indicated wide variation of obesity in terms of age, sex and ethnicity[7].

Native glucagon-like peptide-1 (GLP-1) is a gut hormone, produced by L-cells of distal ileum and colon in response to entry of nutrients, and has a very short half-life of about 2 min. GLP-1 is rapidly destroyed by the circulating enzyme dipeptidyl peptidase-Ⅳ (DPP-Ⅳ). GLP-1 receptors have been found in various tissues like pancreatic islet cells, the gastrointestinal tract, nervous system, cardiovascular system, kidneys and lungs.

Glucagon-like peptide-1 receptor agonists (GLP-1RA) are structurally similar to native GLP-1, but resist degradation by the enzyme DPP-Ⅳ. GLP-1RA is a new class of injectable drugs, emerged for treatment of type 2 diabetes, but has also shown beneficial effects on weight, blood pressure and lipid parameters.

Traditionally GLP-1RAs were developed as an injectable formulation, as they were rapidly degraded by the gastrointestinal enzymes when administered orally[8]. However this scenario is expected to change in the near future, with oral semaglutide preparing to hit the market. It was strongly debated, how a complex protein structure could escape the onslaught from GI juices. In the oral preparation of semagutide, a new carrier termed Sodium N-[8-(2-hydroxybenzoyl) Amino] Caprylate (SNAC) is co-packaged to facilitate its absorption from the gut. SNAC helps in increasing the solubility of semaglutide, as well as increases its permeability across cell membrane by increasing the local (gastric mucosa) pH[9].

We have searched PubMed, Cochrane Library, EMBASE and Google for articles on GLP-1RA, published between 2000 and 2016, and we have found uniformly beneficial effects of GLP-1RA on cardiovascular system, obesity, hyperglycemia, hypertension and lipids. These effects of GLP-1RA have been discussed in the following paragraphs. So, this class of drug may have a favorable effect on cardiovascular mortality and morbidity.

Several GLP-1RA are already in the market and some are in the process of development. Below is a list of GLP-1RA: (1) Exenatide (Byetta) FDA approval in 2006; (2) Liraglutide (Victoza) FDA approval in 2010; (3) Exenatide Long Acting (Bydureon) FDA approval in 2012; (4) Lixisenatide (Lyxumia) EU approval in 2013; (5) Albiglutide (Tanzeum - United States; Eperzan - EU) FDA approval in 2014; (6) Dulaglutide (Trulicity) FDA approval in 2014; (7) Taspoglutide - development has been halted due to injection site skin reaction and gastrointestinal

side effects; and (8) Semaglutide - injectable and oral - undergoing clinical trials.

Fixed ratio combination of GLP-1RA and basal insulin are also available treatment option for patients with inadequate glycemic control: (1) Insulin Degludec/Liraglutide (Xultophy) EU approval in 2014; and (2) Insulin Glargine/Lixisenatide (Lixilan) Regulatory submission to USFDA in 2015.

Insulin Glargine/Lixisenatide combination has been studied in two pivotal Phase Ⅲ trials; LixiLan-L and LixiLan-O, which included 1906 patients with type 2 diabetes. The results of these trials show that LixiLan significantly lowers HbA1c compared to both insulin glargine and lixisenatide. LixiLan-L included 736 patients, whose type 2 diabetes was not adequately controlled on basal insulin alone or combined to 1-2 oral antidiabetic agents. At the end of 30 wk, mean HbA1c declined from 8.1% to 6.3% with LixiLan and from 8.0% to 6.5% with glargine. With LixiLan, 84.4% achieved an HbA1c of < 7.0% compared to 78.3% with glargine. There was also 1.2 kg of weight loss with LixiLan compared to a gain of 0.4 kg with glargine. In addition, there was no difference in rates of hypoglycemia with LixiLan compared to glargine[10,11].

GLP-1RA - ANTIHYPERGLYCEMIC EFFECT

The antihyperglycemic effect of GLP-1RA is substantial. The action is mediated by: (1) glucose dependent insulin secretion by the pancreatic beta cells; (2) suppression of glucagon by the alpha cells; and (3) slowing of gastric emptying[12].

In several clinical trials, with or without metformin, the GLP-RA(s) achieved HbA1c reduction between 0.9%-1.6%. There have been significant reductions of, both fasting and post-prandial plasma glucose levels (vide infra).

Treatment with exenatide 10 mg, twice daily over 30 wk to patients with type 2 diabetes, produced mean reductions in HbA1c of 0.9%-1.0%, compared to placebo, when added to metformin, a sulfonylurea or combination of both[13].

In another 26 wk controlled trial, extended-release exenatide injection once weekly, produced a mean HbA1c reduction of 1.6%, as opposed to reduction of 0.9% by exenatide twice daily ($P < 0.0001$)[14].

In a trial comparing exenatide twice daily *vs* liraglutide once daily, greater post-breakfast plasma glucose lowering was seen with the former while greater fasting plasma glucose was seen with the latter. There was equivalent impact on post-lunch plasma glucose excursion[15].

In another study, adding liraglutide to failing metformin and sulfonylurea therapy, resulted in superior reduction in HbA1c (-1.33%) *vs* basal insulin glargine (-1.09%), and this difference was statistically significant ($P = 0.0015$)[16].

In a head-to-head trial comparing glycemic efficacy of albiglutide once weekly *vs* liraglutide once daily, the latter was found to be more powerful. Patients with type 2 diabetes, inadequately controlled with oral antihyperglycemic agents, were randomized to receive either albiglutide 30 mg once-weekly ($n = 422$) or liraglutide uptitrated from 0.6 mg daily to 1.8 mg once daily ($n = 419$). At the end of 32 wk, there was HbA1c reduction of 0.78% in the albiglutide group and 0.99% in the liraglutide group; treatment difference was 0.21%. However, gastrointestinal side effects were less in the albiglutide group and injection-site reaction was less in liraglutide group[17].

Another head-to-head trial (AWARD-1) compared the efficacy and safety of dulaglutide against exenatide. Patients with type 2 diabetes, receiving metformin (1.5 to 3.0 g) and pioglitazone (30-45 mg) were randomized to four groups of treatment: Dulaglutide 1.5 mg weekly, dulaglutide 0.75 mg weekly, exenatide 10 μg daily, or placebo (placebo-controlled period: 26 wk). Mean baseline HbA1c was 8.1%. Change of HbA1c from baseline to the end of study was -1.51% ± 0.06% for dulaglutide 1.5 mg, -1.30% ± 0.06% for dulaglutide 0.75 mg, -0.99% ± 0.06% for exenatide, and -0.46% ± 0.08% for placebo. Dulaglutide, at both doses, was superior to placebo at 26 wk ($P < 0.001$) and exenatide at 26 and 52 wk ($P < 0.001$). More number of patients reached HbA1c targets with dulaglutide 1.5 mg and 0.75 mg than with placebo and exenatide (all $P < 0.001$). Incidence of hypoglycemia, at 26 and 52 wk, was lower in patients receiving dulaglutide 1.5 mg than in the exenatide group; no dulaglutide-treated patients reported severe hypoglycemia. The common gastrointestinal adverse events for dulaglutide were transient, mild to moderate nausea, vomiting, and diarrhea[18].

The first phase 3a trial results of semaglutide, a once-weekly administered GLP-1RA were announced recently in July, 2015. In this placebo controlled trial, semaglutide was administered in once-weekly doses of 0.5 mg and 1.0 mg, as monotherapy for 30 wk in 388 type 2 diabetes patients, previously on exercise and diet. The trial results showed that from a mean baseline HbA1c of 8.1%, with doses of 0.5 and 1.0 mg of semaglutide, achieved reduction in HbA1c of 1.5% and 1.6%, respectively, compared to no change in the placebo group. Seventy four percent and 73% of the people treated with 0.5 mg and 1.0 mg semaglutide, respectively, achieved the HbA1c target below 7%, compared with 25% of the people treated with placebo[19].

The large amount of data accumulated with the use of different GLP-1RA shows a significant reduction in blood glucose values, with a greater drop seen with higher baseline values of HbA1c, in a dose-dependent manner[12].

GLP-1RA: EFFECTS ON SYSTOLIC AND DIASTOLIC BLOOD PRESSURE

In short term clinical trials (approximately 26 wk), on

patients with type 2 diabetes and hypertension, GLP-1RA(s) have produced a reduction of 1-7 mmHg of systolic blood pressure (SBP), whereas reduction in diastolic blood pressure (DBP) was variable.

A meta-analysis of 16 randomized controlled trials, with 3443 subjects in the GLP-1RA therapeutic arm and 2417 subjects in the control arm, studied effects on blood pressure. The GLP-1RA exenatide, reduced SBP significantly in comparison to both placebo and insulin glargine, with mean differences of -5.24 and -3.46 mmHg, respectively ($P < 0.00001$ for both). In the exenatide-treated group, mean DBP reduction was 5.91 mmHg, compared with the placebo group, -0.99 mmHg ($P < 0.00001$). The meta-analysis studied changes in systolic blood pressure. Results showed a mean reduction of 5.60 mmHg and 2.38 mmHg in the 1.2 and 1.8 mg treatment arms with liraglutide respectively, as compared to placebo and glimepiride arms ($P < 0.00001$; $P = 0.05$ respectively).

In the 1.8-mg-treated group, liraglutide significantly reduced SBP, compared to placebo and glimepiride treatment, with mean differences of -4.49 and -2.62 mmHg, respectively ($P < 0.00001$, and $P < 0.00001$, respectively)[20].

In a study duration of 26 wk, the SBP reduction achieved with exenatide, 10 μg twice daily and liraglutide, 1.8 mg daily were found to be similar (-2.0 mmHg *vs* -2.5 mmHg, respectively; $P = 0.6409$). An additional 14 wk follow-up of the subjects in a partial cross-over design revealed no significant difference in the SBP reduction (ΔSBP of 3.8 mmHg, when patients were switched from exenatide to liraglutide and (ΔSBP of 2.2 mmHg for patients, who continued on liraglutide).

It is interesting to note that blood pressure changes took place before loss of weight was observed-so the effect on blood pressure was independent of weight loss[15].

GLP-1RA: EFFECTS ON LIPIDS

GLP-1RAs have variable effects on different components of lipid profile, the highest being on the triglycerides. Nevertheless, beneficial effects in the LDL cholesterol and HDL cholesterol may also be noted.

Both exenatide and liraglutide resulted in a significantly greater reduction in triglycerides. After 26 wk, there was a significantly greater reduction of triglyceride with liraglutide, 1.8 mg daily as compared to exenatide (-0.22 to -0.40 mmol/L; $P = 0.0485$) [15].

In a study from Greece, 20 obese type 2 diabetes patients were randomized to receive, either liraglutide or exenatide treatment and underwent a standardized meal tolerance test, early in the morning, after 10 h fast at baseline and after a two-week treatment period. Both exenatide and liraglutide, were equally effective in lowering postprandial lipemia, after the first administration and after 2 wk of treatment[21].

In a recent prospective study, the impact of GLP-1 analogs on carotid intima media thickness (CIMT) was assessed using lipid sub-fractions as surrogates. As MetS predisposes an individual to high cardiovascular risk, a reference to this study, may be relevant, to the topic under discussion. Adding liraglutide to type 2 diabetes patients, already on metformin and low CV risk, resulted in statistically significant ($P < 0.001$) improvement in total cholesterol and triglyceride (10% drop from baseline), LDL-cholesterol (19% reduction from baseline) and increase in HDL-C (18% increment from baseline). There was a significant decrease in CIMT from baseline, however, this effect was found to be independent of changes in plasma glucose or lipids[22].

What remains to be determined from long-term prospective trials is, whether these modest improvements in lipids will translate into cardiovascular benefit or not.

GLP-1RA: EFFECTS ON MICROALBUMINURIA

In a study of 16 wk duration on patients with type 2 diabetes, comparing GLP-1RA exenatide with glimepiride, improvement of glucose control was similar, but a 24-h urinary albumin was reduced by 40% in exenatide group, compared to 5% reduction in glimepiride group. Apart from that, urinary transforming growth factor-beta and type Ⅳ collagen in the exenatide group were also significantly reduced, compared to, no change in glimepiride-treated group[23].

Both exendin-4 (exenatide) and liraglutide ameliorated albuminuria, decreased oxidative stress and inflammatory cytokines, in a rat model of diabetic nephropathy. In the exendin-4 study, glomerular macrophage infiltration was prevented by suppression of ICAM-1 production on glomerular endothelial cells and by inhibition of pro-inflammatory cytokine release from macrophages[24].

Clinical experiences with liraglutide from real-life scenario demonstrated significant improvements in urinary albumin excretion rates, as well as decline in eGFR. In an Indian data on type 2 diabetic patients, with mean duration of diabetes of approximately 12 years and baseline clinical albuminuria, there was statistically significant reduction in urinary albumin excretion rate ($P < 0.05$), after 12 wk of treatment with liraglutide[25].

GLP-1RA: EFFECT ON BODY WEIGHT

The mechanisms linking appetite to weight gain has both peripheral sensory inputs and central response. GLP-1RA(s) have consistently demonstrated weight loss in all the clinical trials. Nausea and gastrointestinal slowing were initially postulated as the major mechanisms. However, weight loss was documented independent of gastrointestinal effects. In addition, weight loss was seen with liraglutide despite tachyphylaxis at gastric level. Hence, GLP-1RA(s) are responsible for weight loss by mechanisms, interfering both at central and peripheral sites. Recent studies using structural and functional

Table 1 Body weight parameters: Baseline[24]

	BMI (kg/m^2)	Body weight (kg)	WC (cm)
Metformin	36.6 ± 3.5	103.2 ± 6.3	122.3 ± 7.0
Liraglutide	39.3 ± 4.2	108.9 ± 15.1	124.9 ± 9.9
COMBI	37.6 ± 5.1	105.5 ± 20.6	121.9 ± 17.7

COMBI: Combination of liraglutide and metformin; WC: Waist circumference; BMI: Body mass index.

Table 2 Post-treatment (3-mo) body weight parameters[24]

	BMI (kg/m^2)	Body weight (kg)	WC (cm)
Metformin	36.1 ± 3.8	102 ± 6.8	120.7 ± 7.8
Liraglutide	37.6 ± 5.1	105.1 ± 13.8	121.7 ± 9.6
COMBI	35.5 ± 5.5	99.0 ± 21.2	116.4 ± 18.4

COMBI: Combination of liraglutide and metformin; WC: Waist circumference; BMI: Body mass index.

imaging techniques have demonstrated, reduced activity in the limbic system of the brain, as well as improved hypothalamic connectivity, leading to early satiety and modification of feeding behavior[26-28].

A recent study, comparing the effects of metformin monotherapy *vs* liraglutide monotherapy *vs* combination (COMBI) of both, in patients with polycystic ovary syndrome, documented impressive results as far as weight loss and reduction in waist circumference were concerned. Mean weight loss with COMBI was greatest, 6.5 ± 2.8 kg followed by liraglutide 3.8 ± 3.7 kg and only about 1.2 ± 1.4 kg with metformin. It is interesting to note that there was a significant reduction in waist circumference in the liraglutide arm (3.2 ± 2.9 cm) and in the COBMI arm (5.5 ± 3.8 cm) (Tables 1 and 2). Seventeen patients with PCOS recruited in this study had MetS (6 in metformin group, 4 in COMBI and 7 in liraglutide group). MetS persisted in all the 6 women in metformin arm at the end of the trial whereas it resolved in 3 women in both liraglutide and COMBI groups[29].

Two different doses of liraglutide, 1.8 mg or 3 mg daily, were tried in the SCALE Diabetes trial in patients with type 2 diabetes and obesity or overweight. A total of 846 patients were randomized to receive liraglutide 3 mg daily or liraglutide 1.8 mg daily or placebo in addition to lifestyle intervention. Mean baseline weight was 105.7 kg with liraglutide (3.0-mg dose arm), 105.8 kg with liraglutide (1.8-mg dose arm), and 106.5 kg with placebo. Mean weight loss was 6.4 kg with liraglutide (3.0-mg dose), 5.0 kg with liraglutide (1.8-mg dose), and 2.2 kg with placebo[30].

The results of SUSTAIN 1 trail are highly encouraging in terms of body weight reduction. The absolute weight reduction with 0.5 mg and 1.0 mg semaglutide are 3.8 kg and 4.6 kg as compared to 1 kg weight loss in the placebo arm respectively[19].

GLP-1RA AND HEPATIC AND MUSCLE INSULIN RESISTANCE

In a study, the effect of exenatide on muscle glucose uptake and hepatic glucose production (HGP) was studied in non-diabetic (control) and streptozotocin plus high fat diet induced diabetic rats. With hyperinsulinemic-euglycemic clamp, glucose uptake into gastrocnemius muscles was measured. In the diabetic rats, exenatide reduced the basal production of glucose (94.70 ± 13.46 μmol/kg per minute *vs* 121.07 ± 16.55 μmol/kg per minute, $P < 0.01$). This was effect of exenatide on HGP. Also, there was increased glucose uptake into muscle (0.24 ± 0.02 μmol/g per minute *vs* 0.17 ± 0.02 μmol/g per minute, $P < 0.01$) - an effect on increased muscle insulin sensitivity. These effects of exenatide were absent in the non-diabetic rats[31].

GLP-1RA AND NON-ALCOHOLIC FATTY LIVER DISEASE

As mentioned earlier in the Introduction, some authorities have suggested NAFLD to be a component of MetS. In an interesting review article from Italy, the authors have shown strong correlation of hepatic fat deposition and MetS. They have also commented that, NAFLD is the hepatic component of MetS[32].

In another review article, recently published, the authors concluded NAFLD as a risk factor for type 2 diabetes (28 longitudinal studies) and also for MetS (19 longitudinal studies). As regards to being a part of MetS, the issue has been complicated by documentation of high grade steatosis not associated with insulin resistance. On the contrary, a low-grade fatty liver was found to be genetic angle to this story and a direct cause and effect relationship, is not yet evident. The authors concluded that, NAFLD could be considered as a precursor to MetS, instead of a component of the same[32,33].

Liraglutide was found to be effective in improving NAFLD and non-alcoholic steato-hepatitis (NASH). In a study conducted in Japan, the effect of liraglutide on NAFLD was compared to sitagliptin and pioglitazone. Treatment with liraglutide, significantly reduced liver enzymes, HbA1c and body weight[34].

The effect of liraglutide on NASH, was studied in the recently published LEAN (Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis) trial. Patients were assessed with histology prior to, and at end of study (48 wk). More patients in the 1.8 mg liraglutide arm achieved histological resolution, compared to the placebo arm. However, this was a study in a very small group of patients (26 patients in each arm). Hence a study on a larger population of patients with longer duration needs to be done to come to a definitive conclusion[35].

GLP-1RA AND CORONARY HEART DISEASE

We have seen that, GLP-1RA have favorable actions on components of MetS. Some studies have looked into

their direct effects on coronary artery disease and left ventricular function.

In a study from South Korea, 58 patients with ST-segment elevation myocardial infarction and thrombolysis were put on either exenatide twice daily or placebo. After six months, there was significant reduction of infarct size in the exenatide group, compared to the placebo group. There was also improvement of left ventricular function in the exenatide group, in comparison to placebo[36].

In a recently published meta-analysis of 37 clinical trials with different GLP-1RA, of duration from 24 wk to 208 wk, compared with placebo, or pioglitazone or dipeptidyl peptidase-4 inhibitors, a favorable effect on major adverse cardiovascular event (MACE) was observed with GLP-1RA(s). In placebo-controlled trials, Mantel-Haenzel odds ratio for MACE for exenatide, liraglutide and taspoglutide was 0.45 (0.20-1.02), 0.60 (0.22-1.62) and 0.50 (0.03-8.06), respectively; number of trials 6, 5 and 1 respectively; $P = 0.055$, 0.31 and 0.62 respectively for the three GLP-1RA(s)[37].

The result of "Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA)" study was slightly disappointing. The study recruited patients with type 2 diabetes who had a myocardial infarction or who had been hospitalized for unstable angina within the previous 180 d, to receive either lixisenatide or placebo (1:1 randomization), in addition to locally determined standards of care. The primary composite end points were cardiovascular death, myocardial infarction, stroke, or hospitalization for unstable angina. A total number of 6068 patients were randomized and were followed for a median of 25 mo. A primary end-point event occurred in 406 patients (13.4%) in the lixisenatide group and in 399 (13.2%) in the placebo group [hazard ratio (HR) for lixisenatide, 1.02; 95%CI: 0.89-1.17], which showed the non-inferiority of lixisenatide to placebo ($P < 0.001$) but did not show superiority. There was no significant between-group difference in the rate of hospitalization for heart failure (HR in the lixisenatide group, 0.96; 95%CI: 0.75-1.23) or the rate of death (HR = 0.94; 95%CI: 0.78-1.13). Lixisenatide treatment was not associated with a higher rate of severe hypoglycemia, pancreatitis, pancreatic neoplasms, or allergic reactions, compared to placebo. The ELIXA study also showed that addition of a GLP-1RA, lixisenatide, did not increase risk of myocardial infarction or hospitalization due to heart failure, in such high-risk patients with type 2 diabetes[38].

Another trial "Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER)" was started in September, 2010. The trial enrolled 9340 type 2 diabetes subjects with high risk of cardiovascular disease till April 2012. LEADER is a multicenter, international, randomized, double-blind, placebo-controlled clinical trial. The primary end point is the composite outcome of the first occurrence of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. The result of LEADER trial will throw light regarding the cardiovascular safety of liraglutide relative to the current standard of usual care[39].

On March 04, 2016, in a press release, Novo Nordisk, manufacturer of liraglutide, informed the top-line result of LEADER trial. Treatment with liraglutide has demonstrated, significant reduction of cardiovascular risk in all three components of primary endpoint. The details of the trial results will be presented in the 76th Scientific Session of the American Diabetes Association in June 2016[40].

Once weekly injectable GLP-1RA semaglutide, that has recently completed its Phase 3a clinical trials, has also shown significant reduction in the risk of major adverse cardiovascular events, in its long-term cardiovascular safety SUSTAIN-6 trial. This was announced very recently by its manufacturer on 28 April 2016. SUSTAIN 6 is a 2-year trial to evaluate cardiovascular and other long-term outcomes with semaglutide in approximately 3300 people with type 2 diabetes[41].

A recently published meta-analysis looked into the cardiometabolic efficacy and adverse effects of once-weekly GLP-1RAs, in adults with type 2 diabetes. The authors studied results of clinical trials with albiglutide, dulaglutide, once-weekly exenatide, and taspoglutide and looked into cardiometabolic (primary outcome, fasting plasma glucose and HbA1c) or safety outcome. Results of a total number of 34 trials were studied. All once-weekly GLP-1RAs reduced HbA1c and fasting plasma glucose. Taspoglutide 20 mg, once-weekly exenatide, and dulaglutide 1.5 mg, have also shown a reduction in body weight. The greatest difference in HbA1c reduction was found between dulaglutide 1.5 mg, and taspoglutide 10 mg (-0.4%); for fasting plasma glucose, once-weekly exenatide and albiglutide (-12.6 mg/dL), and for weight reduction, taspoglutide 20 mg, and dulaglutide 0.75 mg (-1.5 kg). Once-weekly exenatide increased heart rate compared with albiglutide and dulaglutide (1.4 to 3.2 beats/min). The risk for hypoglycemia was similar for all; use of taspoglutide 20 mg weekly was associated with the highest risk for nausea (odds ratio, 1.9 to 5.9)[42].

POSSIBLE MECHANISMS OF ACTION: GLP-1 RECEPTOR DEPENDENT OR INDEPENDENT

Endogenous GLP-1 can act through the GLP-1 receptors present on the endothelium, endocardium, cardiomyocytes and vascular smooth muscle cells. Once degraded by DPP-4 the intact GLP-1 (7-36) gets degraded to metabolites some of which like GLP-1 (9-36) can act independent of the GLP-1 receptor and induce vasodilation *via* the cGMP pathway[43].

However exogenous GLP-1RA being resistant to the action of DPP-4 enzyme acts exclusively through the GLP-1 receptor and induce the metabolic and vascular effects. Amongst the various injectable GLP-1RAs, liraglutide is the only one, which is partially resistant to the degrading effect of DPP-4, due to the fatty acid side chain of the molecule, which attaches to plasma albumin

and protects the cleavage site[44]. As a result we can expect both GLP-1R dependent and independent effects on cardio-metabolic parameters from this molecule. GLP-1 (9-36) has been documented to have GLP-1R independent effects in reducing blood pressure as well as improving cardiac function post ischemia[45]. It is worth speculating whether, this additional mechanistic property was responsible for the differential CV results between ELIXA and LEADER trials.

CONCLUSION

MetS has a strong connection with cardiovascular morbidity and mortality. Most of the conventional antihyperglycemic agents address plasma glucose excursions without having any additional impact on the other components of MetS. Some, like insulin, sulphonylureas and thiazolidinediones actually worsen certain components of MetS. The introduction of GLP-1 receptor analogs changed the picture. In addition to reducing plasma glucose, we came across, a group of drugs, which could also reduce body weight, blood pressure, lipids and improve urinary albumin excretion. The drugs have shown a trend toward favorable effects on coronary artery disease and left ventricular function. The entire composite included under the umbrella of MetS can now be tackled more effectively with one single antihyperglycemic agent. Results from recently concluded clinical trials indicate that some the drugs in this class may reduce cardiovascular risk in patients with type 2 diabetes.

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ORIGINAL ARTICLE

Observational Study

Impact of diabetes mellitus on risk of cardiovascular disease and all-cause mortality: Evidence on health outcomes and antidiabetic treatment in United States adults

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Informed consent statement: The current report used data from the National Center for Health Statistics (NCHS) of the United States Centers for Disease Control and Prevention CDC). All informed consents were conducted by the NCHS while the study was carried. No further informed consent forms were requested by using the dataset (https://www.cdc.gov/nchs/nhis/).

Conflict-of-interest statement: We have no conflict of interest to report.

Data sharing statement: Data used in the present study were public use data files provided by the United States CDC NCHS. Those who are interested may apply for and download from NCHS website.

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Abstract

AIM

To examine the epidemic of diabetes mellitus (DM) and its impact on mortality from all-cause and cardiovascular disease (CVD), and to test the effect of antidiabetic therapy on the mortality in United States adults.

METHODS

The analysis included a randomized population sample of 272149 subjects ages ⩾ 18 years who participated in the National Health Interview Surveys (NHIS) in 2000-2009. Chronic conditions (hypertension, DM and CVD) were classified by participants' self-reports of physician diagnosis. NHIS-Mortality Linked Files, and

NHIS-Medical Expenditure Panel Survey Linkage Files on prescribed medicines for patients with DM were used to test the research questions. χ^2, Poisson and Cox's regression models were applied in data analysis.

RESULTS

Of all participants, 22305 (8.2%) had DM. The prevalence of DM significantly increased from 2000 to 2009 in all age groups ($P < 0.001$). Within an average 7.39 (SD = 3) years of follow-up, male DM patients had 1.56 times higher risk of death from all-cause (HR = 1.56, 95%CI: 1.49-1.64), 1.72 times higher from heart disease [1.72 (1.53-1.93)], 1.48 times higher from cerebrovascular disease [1.48 (1.18-1.85)], and 1.67 times higher from CVD [1.67 (1.51-1.86)] than subjects without DM, respectively. Similar results were observed in females. In males, 10% of DM patients did not use any antidiabetic medications, 38.1% used antidiabetic monotherapy, and 51.9% used $\geqslant$ 2 antidiabetic medications. These corresponding values were 10.3%, 40.4% and 49.4% in females. A significant protective effect of metformin monotherapy or combination therapy (except for insulin) on all-cause mortality and a protective but non-significant effect on CVD mortality were observed.

CONCLUSION

This is the first study using data from multiple linkage files to confirm a significant increased prevalence of DM in the last decade in the United States. Patients with DM have significantly higher risk of death from all-cause and CVD than those without DM. Antidiabetic mediations, specifically for metformin use, show a protective effect against all-cause and CVD mortalities.

Key words: Epidemic of diabetes mellitus; Cardiovascular disease; Pharmacoepidemiologic profiles; United States

Core tip: The study is one of the first projects to use a 10-years nationally linked dataset. The results highlight a new epidemic of diabetes in the United States. It addresses the impact of diabetes on cardiovascular disease and all-cause mortality. The study is also one of the first studies to explore the association between glucose lowering drug use and health outcomes using health survey data from the real-world.

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INTRODUCTION

Diabetes mellitus (DM) is the seventh leading cause of death in the United States. Of 2543279 death certificates from all-causes in 2010, 2.9% of deaths (n = 73932) clinically listed DM as the main cause of death, and more than 9% of deaths (n = 234051) were attributable to DM as a comorbid cause of death in the United States. It is estimated that more than 1.4 million Americans are diagnosed with DM every year. In 2012, 29.1 million Americans, or 9.3% of the population, had DM. Of the 29.1 million, 21 million were diagnosed, and 8.1 million were undiagnosed[1]. A similar increased prevalence of DM has been estimated worldwide[2]. It is clear that DM has posed a serious public health problem in the United States and in the world[1-4], not only because DM is a leading cause of death, but also DM is a significant risk factor for cardiovascular disease (CVD). CVD is the number one killer of the Americans[5-10]. Although the overall trend in the prevalence of DM and its impact on risk of CVD have been examined by several studies, some were limited to their small sample sizes[11], some were limited to their study designs [such as findings from the Behavior Risk Factor Surveillance Systems that are conducted using a telephone survey with a very low response rate (< 40%)][12], and some were limited to a cross-sectional analysis design[7,13]. Furthermore, patterns of antidiabetic treatment and its impact on long-term health outcomes are less known. In the present study, we aimed to examine the trend of DM, and the impact of DM on CVD (hypertension, coronary heart disease and stroke), and risk of mortality from CVD and all-cause using a nationally representative sample in the United States. Findings from the study may add new evidence of the burdens of DM to the body of the literatures, the patterns of antidiabetic medications usage, and the magnitudes of DM and drug use on all-cause and CVD mortalities.

MATERIALS AND METHODS

Participants ages 18 years and older in the 2000-2009 National Health Interview Surveys (NHIS) were included in the study. The NHIS has been conducted annually since 1960 by the United States National Center for Health Statistics (NCHS), which is a part of the Centers for Disease Control and Prevention (CDC)[14]. NHIS is a cross-sectional household interview survey that serves as the principal source of information on the health of the noninstitutionalized civilian population of the United States[15]. Uniform sampling and interviewing processes for core variables are continuous throughout each year's survey. The sampling plan follows a multistage area probability design that permits the representative sampling of households and non-institutionalized groups. In NHIS, one adult per household is randomly chosen to participate in a completed interview from approximately 30000 households containing about 85500 persons, of them about 30000 adults ages 18 and older. Participants' vital status (alive or deceased) are followed yearly and linked to death certificates in the National Death Index system (NHIS-Mortality

Linked File). This Linked File provides an important opportunity for health professionals to estimate the risk of mortality prospectively on the basis of the NHIS participants' baseline characteristics[14]. In the present study, we applied the most recently released NHIS-Mortality Linked File, which had follow-up information for subjects who participated in NHIS in and before 2009, and followed up through the end of 2011 (December 31, 2011). We examined the past one decade trend of DM between 2000 and 2009, and risk of mortality in patients with DM. Of total 287530 participants ages 18 and older, we excluded 15381 who had missing information on prevalent DM status at baseline (n = 237), and those who were lost to follow-up (n = 15144) during the course of follow-up between 2000 and 2011, yielding a final analytic sample of 272149 adults (94.7% = 272149/287530). To examine the patterns of medication use in patients with DM, we further linked the study sample at individual participant's level with the Medical Expenditure Panel Surveys (MEPS)[16]. The NHIS, NHIS-Mortality Linked File and MEPS have been approved by the Institutional Review Board of the United States CDC NCHS and are available through the NCHS[14]. The present analysis has been approved by Drexel University Institutional Review Board (# 1605004544).

Two groups of health outcomes in patients with and without DM were examined: (1) all-cause mortality; and (2) CVD mortality. Mortality data were defined using ICD-10: Heart disease (ICD10: I00-I09, I11, I13, I20-I51), cerebrovascular disease (ICD10: I60-I69). CVD includes the two major forms of heart disease and cerebrovascular disease (ICD10: I00-I09, I11, I13, I20-I51 and ICD10: I60-I69). Predictors and covariates included: (1) demographic factors: Age, gender, race/ethnicity and education attainments (< high-school graduate, high-school graduate, and ⩾ college); (2) lifestyle related factors: Body mass index [BMI, calculated by weight (kg)/height (m^2)], cigarette smoking (never smoked, formerly smoked, or currently smoker), alcohol consumption (not a drinker: < 12 drinks in entire life, former drinker: No drinks in previous year, and current drinker), and physical activity. BMI was classified into four groups on the basis of the World Health Organization (WHO) definition (underweight: < 18.5, normal weight: 18.5-24.9, overweight: 25-29.9, and obese: ⩾ 30 kg/m^2). Physical activity status was grouped on the basis of current guidelines (active: ⩾ 150 min per week of moderate-intensity equivalent leisure-time aerobic activity; insufficiently active: 10-149 min per week of moderate-intensity equivalent leisure-time aerobic activity); (3) CVD related chronic conditions: Hypertension, coronary heart disease (CHD) and stroke. Baseline CVD includes patients who had CHD and/or stroke. The baseline chronic conditions were classified by participants' self-reports of diagnoses made by a doctor or health professional; and (4) DM, oral glucose-lowering medication and insulin use were classified according to DM patients' prescription records.

Statistical analysis

A serial analysis was conducted to test the study hypotheses and fit the time-events prediction models. The first group analysis included the basic characteristics description of the study participants and tested gender differences using univariate analysis, including t test for continuous variables, and χ^2 tests for categorical variables. Changes in the prevalence of DM from 2000 to 2009 by sex and ages (18-54, 55-64, 65-74 and ⩾ 75) were tested using simple linear regression models. The second group analysis involved estimates of mortality rates (per 1000 person-year) from all-cause, heart disease, cerebrovascular disease, and total CVD. We used Poisson regression to calculate mortality per person years. The third group analysis estimated the hazard ratios of DM (yes/no) for the risk of mortality from all-cause, heart disease, cerebrovascular disease, and total CVD using Cox's proportional hazard regression models. In the analysis, five multivariate adjusted Cox's models were performed by gender. Model 1 adjusted for age (years) and race/ethnicity (NH-White, NH-Black, Hispanics, and other groups). Model 2 adjusted for age, race/ethnicity and education level (< higher school, high school, and ⩾ college). Model 3 adjusted for the covariates used in Model 2 plus three behavioral factors (smoking, alcohol consumption and physical activity). Model 4 adjusted for the covariates used in Model 3 plus hypertension. Because patients with CVD at baseline may have an increased risk of mortality, we excluded those patients in Model 5 and adjusted the same covariates as used in Model 4. Interactions of gender and DM on risk of mortality were tested using SAS Proc GENMOD. The fourth group analysis involved in estimates of the prevalence of glucose lowering medication and insulin use. We examined hazard ratios of monotherapy and combinations of glucose lowering medication and insulin use for the risk of mortalities compared to those without antidiabetic medication use. In the last group analysis, we compared baseline differences in 5 preventable factors' age-race-adjusted standardized rates (education level, as a marker for economic status, smoking, physical activity, BMI and hypertension) between males and females using logistic regression in order to explain a potential gender difference in the relative risk of all-cause and CVD mortality in patients with DM.

All data analyses were performed using SAS version 9.3, with complex sample modules that take the sample design of NHIS, including stratification, clustering and weight into consideration (SAS Institute, Cary, NC). Statistical significance was determined for a two-sided test at a P value < 0.05.

RESULTS

Baseline characteristics of the study participants

Of 272149 subjects participated in 2000-2009 NHIS, 22305 (8.2%) had diabetes (male: 9892, and female: 12413). The prevalence of DM significantly increased

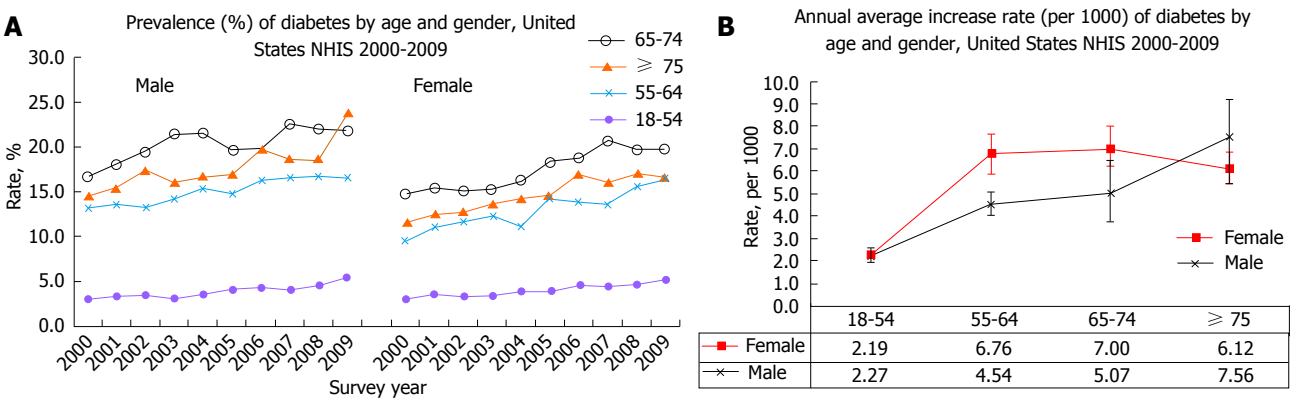

Figure 1 The Burden of diabetes in the United States. A: Prevalence (%) of diabetes by age and gender, United States NHIS 2000-2009; B: Annual average increase rate (per 1000) of diabetes by age and gender, United States NHIS 2000-2009. NHIS: National Health Interview Surveys.

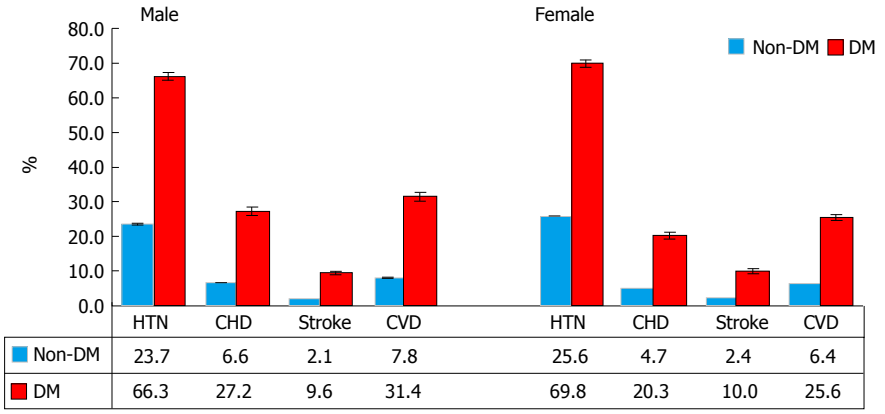

Figure 2 Prevalence (%) of comobid hypertnesion, coronary heart disease, strroke and cardiovascular disease in patients with and without diabetes mellitus, United States National Health Interview Surveys 2000-2009. HTN: Hypertnesion; CHD: Coronary heart disease; CVD: Cardiovascular disease; DM: Diabetes mellitus; NHIS: National Health Interview Surveys.

from 2000 to 2009 in all age groups for males and females (P < 0.001) (Figure 1A). The annual increase rates (per 1000) were 2.27, 4.54, 5.07, and 7.56 for male aged 18-54, 55-64, 65-74 and ⩾ 75, respectively (test for linear trend, all P < 0.01). The corresponding values in females were 2.19, 6.76, 7.00 and 6.12, respectively (P < 0.01). Females had 1.49 times higher annual increase in those aged 55-64, and 1.38 higher in those aged 65-74 compared to males (Figure 1B). There were significant differences in demographic, behavior factors and medical conditions between those with and without DM in males and females (Table 1 and Figure 2).

Mortality from all-cause and CVD in patients with DM vs those without DM

Table 2 shows that mortality from all-cause, heart disease, cerebrovascular disease (CBVD) and CVD increased with age in subjects with or without DM. However, patients with DM had significantly higher mortality than those without DM in both males and females, except for CBVD in males aged 18-54.9 (P = 0.063), and aged ⩾ 75 (P = 0.694), and in females aged ⩾ 75 (P = 0.371). Figure 3A depicts an overall increase in all-cause mortality with increased age in males and females, but a greater increased trend for those aged ⩾ 65. Similar trend for CVD mortality is shown in Figure 3B. Subjects without DM had a much lower mortality rate from all-cause and CVD before the age of 65 as compared to those with DM and age of 65 and older.

Multivariate adjusted hazard ratios of DM for risk of mortality

Of the total study sample, within an average 7.39 (SD = 3) years of follow-up, the results show that after adjustment for age and race/ethnicity, male patients with DM *vs* non-DM had 1.56 times higher risk of death from all-cause (HR = 1.56, 95%CI: 1.49-1.64), 1.72 times higher from heart disease (HR = 1.72, 95%CI: 1.53-1.93), 1.48 times higher from CBVD (HR = 1.48, 95%CI: 1.18-1.85), and 1.67 times higher from CVD (HR = 1.67, 95%CI: 1.51-1.86), respectively (Model 1, Table 3). Similar results were observed in females. After further adjustment for the inclusion of education (Model 2), and behavior risk factors (cigarette smoking, alcohol consumption, and physical inactivity, Model 3), the corresponding HRs of DM for the risk of the mortalities

Table 1 Baseline characteristics of participants, United States National Health Interview Surveys 2000-2009

	Male							Female						
	Non-DM (n = 109507)			DM (n = 9892)				Non-DM (n = 140337)			DM (n = 12413)			
	No.[a]	Rate[b]	(SEP)	No.[a]	Rate[b]	(SEP)	*P* value	No.[a]	Rate[b]	(SEP)	No.[a]	Rate[b]	(SEP)	*P* value[c]
Age, mean, yr	44.9		(0.10)	60.3		(0.14)	< 0.0001	46.6		(0.1)	60.7		(0.16)	< 0.0001
Race/ethnicity														
NH-White	71683	74.5	(0.25)	6062	70.9	(0.55)	< 0.0001	89088	73.8	(0.25)	6782	66.6	(0.55)	< 0.0001
NH-Black	13345	10.4	(0.18)	1712	14.2	(0.40)		21517	12.5	(0.20)	2923	19.2	(0.46)	
Hispanics	19251	10.7	(0.17)	1646	10.4	(0.36)		23779	9.8	(0.15)	2260	10.7	(0.36)	
Others	5228	4.4	(0.10)	472	4.5	(0.25)		5953	3.9	(0.08)	448	3.4	(0.21)	
Education														
Less than HS	19941	15.3	(0.18)	2682	24.0	(0.52)	< 0.0001	25377	15.0	(0.16)	4066	28.8	(0.50)	< 0.0001
HS Graduated	30474	28.0	(0.21)	2887	30.6	(0.58)		39612	28.4	(0.19)	3873	33.1	(0.54)	
⩾ College	58314	56.6	(0.30)	4231	45.3	(0.58)		74451	56.5	(0.27)	4370	38.1	(0.53)	
Smoking status														
No smoker	54430	49.6	(0.24)	3667	36.7	(0.58)	< 0.0001	87384	60.8	(0.21)	7344	58.2	(0.51)	< 0.0001
Former smoker	26644	24.9	(0.19)	4293	44.6	(0.60)		24800	19.1	(0.14)	3075	26.4	(0.47)	
Current smoker	27507	25.5	(0.19)	1834	18.7	(0.46)		27211	20.1	(0.16)	1893	15.4	(0.35)	
Alcohol consumption														
Never	16415	14.3	(0.19)	1660	16.5	(0.43)	< 0.0001	41116	27.0	(0.23)	5159	39.7	(0.55)	< 0.0001
Former	15028	13.8	(0.15)	3348	34.0	(0.61)		19335	14.1	(0.13)	3389	28.2	(0.51)	
Current	75204	71.8	(0.22)	4668	49.5	(0.62)		77255	58.9	(0.25)	3670	32.1	(0.54)	
Exercise														
Inactive	38394	34.5	(0.32)	4485	47.0	(0.62)	< 0.0001	55965	38.9	(0.29)	6416	55.2	(0.62)	< 0.0001
Insufficiently active	27605	27.2	(0.18)	2178	24.6	(0.50)		40073	31.0	(0.18)	2724	25.1	(0.49)	
Sufficiently active	38609	38.3	(0.26)	2473	28.3	(0.59)		38637	30.0	(0.22)	2158	19.8	(0.45)	
BMI, kg/m^2														
Overweight	46884	43.5	(0.18)	3595	36.2	(0.55)	< 0.0001	38254	28.1	(0.15)	3250	28.0	(0.45)	< 0.0001
Obesity	24128	22.4	(0.17)	4372	46.1	(0.59)		31193	22.4	(0.15)	6168	52.8	(0.53)	
Medical condition														
Hypertension	25582	23.7	(0.19)	6501	66.3	(0.56)	< 0.0001	35594	25.6	(0.17)	8662	69.8	(0.48)	< 0.0001
Coronary heart Dis	7020	6.6	(0.09)	2598	27.2	(0.48)	< 0.0001	6435	4.7	(0.07)	2450	20.3	(0.41)	< 0.0001
Stroke	2243	2.1	(0.05)	956	9.6	(0.30)	< 0.0001	3244	2.4	(0.05)	1233	10.0	(0.32)	< 0.0001
CVD	8434	7.8	(0.10)	3019	31.4	(0.51)	< 0.0001	8699	6.4	(0.09)	3109	25.6	(0.43)	< 0.0001

[a]No. = Observed number; [b]Rate for weighted rate using SAS for complex survey; [c]T test for continuous variable, and χ^2 test for categorical variables. Education ⩾ College including those with associate degrees. Overweight: Body mass index (BMI) 25 to 29.9 kg/m^2. Obesity: BMI ⩾ 30 kg/m^2. CVD: Cardiovascular disease; DM: Diabetes mellitus; SEP: Standard error of proportion.

remained statistically significant in patients with DM *vs* those without DM in males and in females. Model 4 shows that after a further control of the effect of hypertension, the HRs were attenuated compared to Model 3, specifically the impact of DM on the risk of death from CBVD became a borderline significance in males (P = 0.06). Finally, we excluded those who had heart disease and stroke at baseline (Model 5), the results show that HRs were further attenuated, except for a slight but non-significant increase in HR for death from CBVD in females.

Females appeared to have a higher HRs of DM for mortality from all-cause, heart disease and CVD than males. However, the increased HRs in females became non-significant after adjusting for age, race/ethnicity and education (Model 2), adjusting for behavior factors (Model 3), adjusting hypertension (Model 4), and excluding those who had heart disease and stroke at baseline (Model 5).

Proportions of antidiabetic medication usage in patients with DM

Table 4 shows that in males, 10% of patients with DM did not use any antidiabetic medications, and 38.1% of DM patients used antidiabetic monotherapy, and 51.9% used ⩾ 2 antidiabetic medications. The corresponding values of the prevalence of those who did not use any antidiabetic medication, those who used 1 only (*i.e.*, monotherapy), and those who used ⩾ 2 were 10.3%, 40.4% and 49.4% in females respectively. Of those with monotherapy in males, 37.2% patients used insulin, followed by metformin (27.3%), sulfonylureas (25.9%) and others (9.6%). The most common three monotherapies in females were metformin (33.2%), insulin (33.0%), and sulfonylureas (21.9%), (gender differences: P = 0.008). Among patients with combined antidiabetic medication therapies, the most frequent combination was metformin and sulfonylureas (20.1% in males, and 21.9% in females). No significant

Table 2 Mortality (per 1000 person year) from all-cause, heart disease, cerebrovascular disease and cardiovascular disease by December 31, 2011 for baseline participants with or without diabetes, United States National Health Interview Surveys 2000-2009

		Male					Female				
		Non-DM		DM			Non-DM		DM		
		Event	Rate	Event	Rate	*P* value	Event	Rate	Event	Rate	*P* value
All-cause											
No. of death		8363		2278			9485		2450		
Age	18-54.9	1765	2.9	317	14.1	< 0.0001	1384	1.9	249	8.6	< 0.0001
	55-64.9	1271	12.5	473	27.8	< 0.0001	989	7.6	403	21.6	< 0.0001
	65-74.9	1874	30.7	677	46.0	< 0.0001	1681	18.3	693	37.6	< 0.0001
	⩾ 75	3453	86.5	811	103.1	< 0.0001	5431	63.2	1105	79.3	< 0.0001
Heart disease											
No. of death		1707		543			1603		506		
Age	18-54.9	288	0.5	62	2.8	< 0.0001	115	0.2	41	1.4	< 0.0001
	55-64.9	244	2.4	116	6.8	< 0.0001	108	0.8	72	3.9	< 0.0001
	65-74.9	350	5.7	164	11.1	< 0.0001	241	2.6	132	7.2	< 0.0001
	⩾ 75	825	20.7	201	25.6	0.032	1139	13.2	261	18.7	< 0.0001
CBVD											
No. of death		376		112			621		134		
Age	18-54.9	50	0.1	4	0.2	0.063	58	0.1	8	0.3	0.002
	55-64.9	38	0.4	19	1.1	< 0.001	33	0.3	19	1.0	< 0.0001
	65-74.9	67	1.1	35	2.4	< 0.001	96	1.0	34	1.8	0.003
	⩾ 75	221	5.5	54	6.9	0.694	434	5.0	73	5.2	0.371
CVD											
No. of death		2083		655			2224		640		
Age	18-54.9	338	0.6	66	2.9	< 0.0001	173	0.2	49	1.7	< 0.0001
	55-64.9	282	2.8	135	7.9	< 0.0001	141	1.1	91	4.9	< 0.0001
	65-74.9	417	6.8	199	13.5	< 0.0001	337	3.7	166	9.0	< 0.0001
	⩾ 75	1046	26.2	255	32.4	0.038	1573	18.3	334	24.0	< 0.0001

P values were given by χ^2 test. Mortality rates (per 1000) were estimated using Poisson regression. CBVD: Cerebrovascular disease; CVD: Cardiovascular disease; DM: Diabetes mellitus.

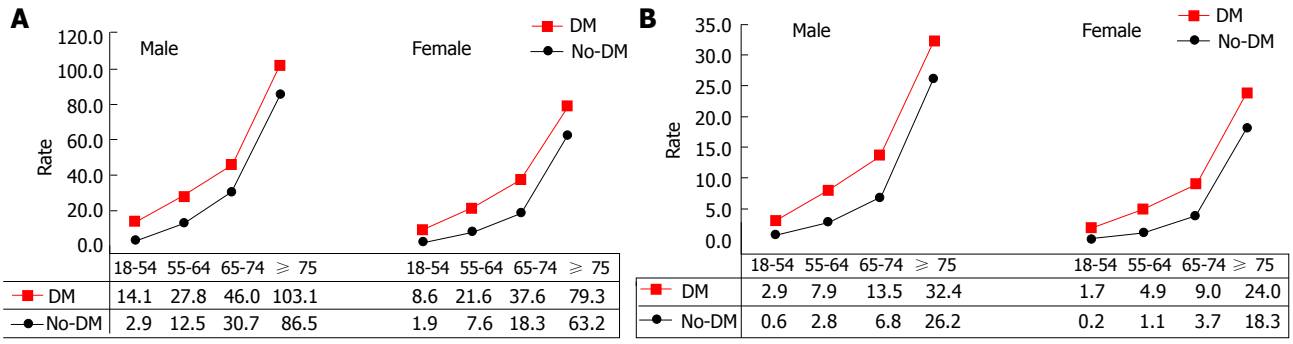

Figure 3 Mortality from all-cause and cardiovascular disease by diabetes status. A: Mortality (per 1000 person year) from all-cause in patients without and with diabetes, United States NHIS 2000-2009; B: Mortality (per 1000 person year) from CVD in patients with diabetes by age and gender, United States NHIS 2000-2009. CVD: Cardiovascular disease; NHIS: National Health Interview Surveys; DM: Diabetes mellitus.

differences in the proportions of combined therapies between males and females were observed (*P* = 0.42).

Hazard ratios of antidiabetic medication use for risk of mortality

Table 5 shows that after adjustment for key covariates, patients with treatment of antidiabetic medication *vs* those without had 7% lower risk of mortality from all-cause (HR = 0.93, 95%CI: 0.73-1.18, *P* = 0.56, Model 2) and 4% lower risk from CVD (HR = 0.96, 95%CI: 0.60-1.54, *P* = 0.87, Model 2), although these associations did not reach statistical significance. However, DM patients with metformin monotherapy had a significantly decreased risk of all-cause mortality (HR = 0.55, 95%CI: 0.38-0.80, *P* = 0.002, Model 2), but those with insulin monotherapy showed an increased risk of all-cause mortality (HR = 1.71, 95%CI: 1.31-2.24, *P* < 0.0001, Model 2). A protective but non-significant effect of the treatment of antidiabetic medications (except for sulfonylureas and insulin use) on CVD mortality was observed.

In patients with DM, a combination of metformin, sulfonylureas and thiazolidinedione showed a significantly reduced risk of all-cause mortality compared to those who did not use a combination therapy (HR = 0.43, 95%CI: 0.27-0.70, *P* = 0.001, Model 2 in Table 5).

Table 3 Multivariate adjusted hazard ratios (HR, 95%CI) of diabetes mellitus for mortality from all-cause, heart disease, cerebrovascular disease and cardiovascular disease by gender

		Male (n = 119399)			Female (n = 152750)			Excess HR[1]	
	Mortality	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value	Rate, %	*P* value
DM *vs* non-DM									
Model 1									
	All-cause	1.56	(1.49-1.64)	< 0.0001	1.69	(1.61-1.78)	< 0.0001	8.3	0.02
	Heart Dis	1.72	(1.53-1.93)	< 0.0001	2.02	(1.81-2.25)	< 0.0001	17.4	0.05
	CBVD	1.48	(1.18-1.85)	0.001	1.43	(1.15-1.77)	0.001	-3.5	0.35
	CVD	1.67	(1.51-1.86)	< 0.0001	1.85	(1.69-2.03)	< 0.0001	10.5	0.16
Model 2									
	All-cause	1.54	(1.47-1.62)	< 0.0001	1.62	(1.55-1.71)	< 0.0001	5.5	0.13
	Heart Dis	1.70	(1.51-1.91)	< 0.0001	1.95	(1.74-2.17)	< 0.0001	14.5	0.09
	CBVD	1.48	(1.18-1.86)	0.001	1.39	(1.12-1.72)	0.003	-6.2	0.70
	CVD	1.66	(1.49-1.84)	< 0.0001	1.79	(1.63-1.96)	< 0.0001	7.8	0.29
Model 3									
	All-cause	1.47	(1.39-1.55)	< 0.0001	1.55	(1.47-1.63)	< 0.0001	5.5	0.15
	Heart Dis	1.62	(1.44-1.82)	< 0.0001	1.80	(1.60-2.03)	< 0.0001	11.2	0.21
	CBVD	1.35	(1.04-1.75)	0.023	1.40	(1.12-1.75)	0.003	3.4	0.85
	CVD	1.57	(1.40-1.75)	< 0.0001	1.68	(1.52-1.86)	< 0.0001	7.4	0.35
Model 4									
	All-cause	1.42	(1.35-1.49)	< 0.0001	1.50	(1.42-1.58)	< 0.0001	5.6	0.15
	Heart Dis	1.58	(1.33-1.69)	< 0.0001	1.65	(1.48-1.89)	< 0.0001	4.4	0.19
	CBVD	1.28	(0.99-1.66)	0.06	1.33	(1.06-1.66)	0.013	3.9	0.83
	CVD	1.46	(1.31-1.63)	< 0.0001	1.58	(1.42-1.75)	< 0.0001	8.2	0.32
Model 5 - in those without baseline CVD									
	All-cause	1.32	(1.23-1.41)	< 0.0001	1.40	(1.32-1.50)	< 0.0001	6.1	0.19
	Heart Dis	1.22	(1.03-1.45)	0.019	1.43	(1.21-1.69)	< 0.0001	17.2	0.19
	CBVD	1.24	(0.93-1.67)	0.137	1.41	(1.10-1.82)	0.008	13.7	0.53
	CVD	1.22	(1.04-1.43)	0.017	1.45	(1.26-1.67)	< 0.0001	18.9	0.08

[1]Excess HR = [(HR in female/HR in male) - 1] × 100. Significant test was estimated based on sex × DM interaction. *P*-values were given by Cox regression models. Model 1: Adjusted for age and race/ethnicity (NH-White, NH-Black, Hispanics, and all other race group); Model 2: Adjusted for Model 1 plus education; Model 3: Adjusted for Model 2 plus smoking, alcohol, and physical activity status; Model 4: Adjusted for Model 2 plus smoking, alcohol, physical activity status, hypertension. DM: Diabetes mellitus; CBVD: Cerebrovascular disease; CVD: Cardiovascular disease.

A significantly reduced risk of all-cause mortality was observed as well in patients with any other combined drug therapies (0.68, 0.50-0.93, P = 0.016, Model 2). No significant association between combination medication use and risk of CVD mortality was observed for DM patients with or without combination therapies, except for those with thiazolidinedione plus any other antidiabetic medications (excluding insulin) (0.52, 0.28-0.98, P = 0.042).

Table 6 shows that DM patients with insulin monotherapy showed an increased risk of heart disease mortality than those without insulin monotherapy (1.91, 1.12-3.26, P = 0.018, Model 2). A combination of metformin, sulfonylureas and insulin was significantly and negatively associated with heart disease mortality (0.35, 0.20-0.62, P < 0.0001, Model 2). No statistical significance in mortality from CBVD was observed in DM patients with or without medication (neither monotherapy nor combinations).

DISCUSSION

The present study, using data from one of the largest national health survey systems and multiple linkage files, examined the burden of DM and its impact on CVD and all-cause mortality among adults in the United States. The study adds new evidence to the body of scientific literatures regarding antidiabetic medication profiles and health outcomes in patients with DM. The main findings show that: (1) the prevalence of DM significantly increased in all age groups for males and females in the last decade; (2) patients with DM had 1.47 to 1.62 times higher risk of death from all-cause and CVD in males, and 1.55 to 1.68 times higher in females compared to those without DM; (3) about 40% of patients with DM used antidiabetic monotherapy, and about 50% used combined antidiabetic therapy, however 10% of patient with DM did not use any medication in both males and females; and (4) in patients with DM, using metformin monotherapy or a combined therapy of metformin with other antidiabetic medications showed a significantly reduced risk of all-cause mortality. This protective association remained significant after adjustment for age, sex, race/ethnicity, survey year, antihypertensive drug, and anti-dyslipidemia medication use.

The present study confirmed an increased prevalence of DM in the last decade in the United States This finding is consistent with previous reports[1-4], and provides new evidence at the national level. Several factors may contribute to the increased rates. Of them, an increased prevalence of obesity across the

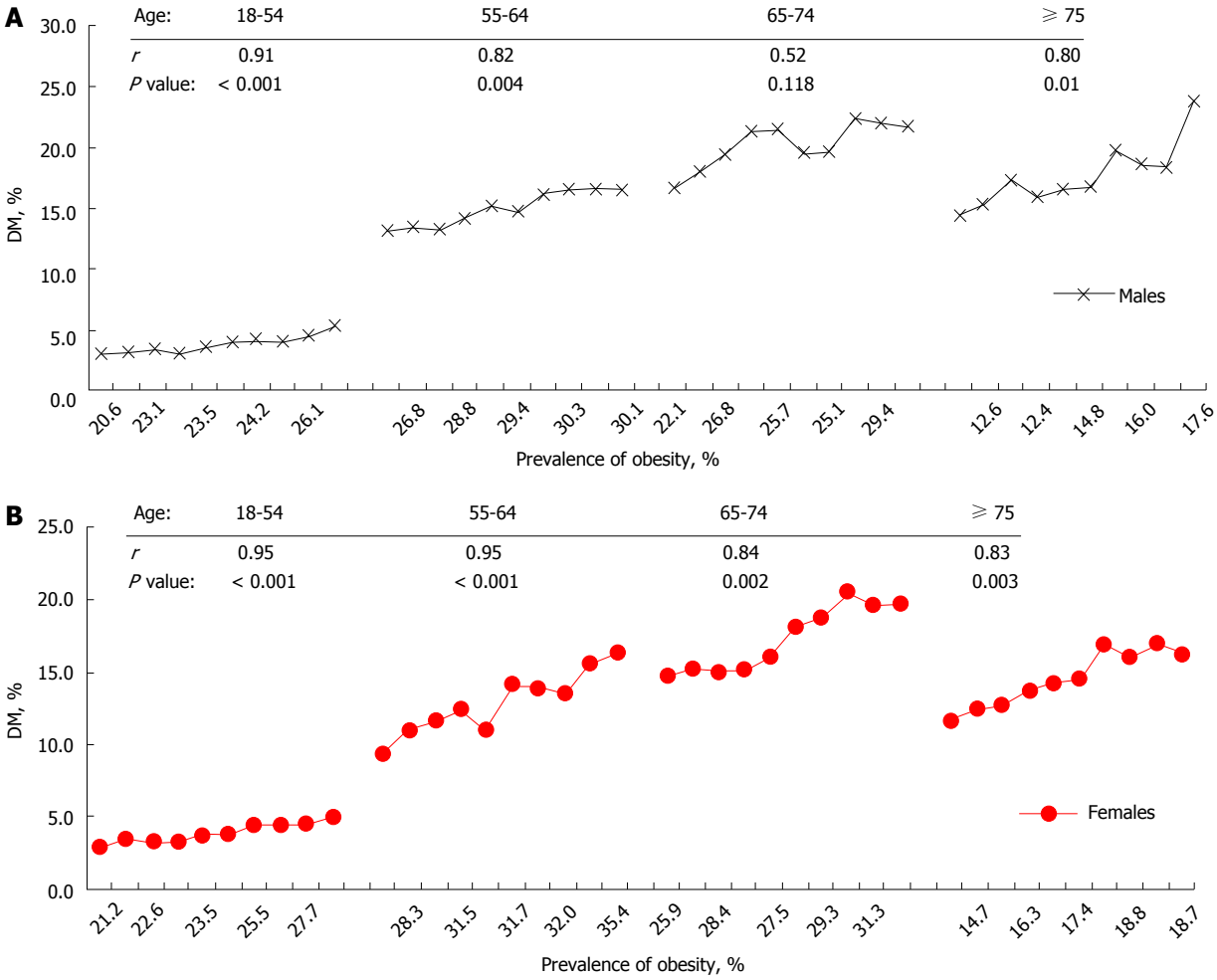

Figure 4 Chnages in obesity rates and its corelation with diabetes rates. A: Correlation between prevalence of obesity and diabetes by age in males, United States NHIS 2000-2009; B: Correlation between prevalence of obesity and diabetes by age in females, United States NHIS 2000-2009. NHIS: National Health Interview Surveys; DM: Diabetes mellitus.

nation may contribute to the increased trend of DM. We analyzed obesity rate using the same NHIS data. Figure 4 depicts a positive correlation trend between the prevalence of obesity and DM between 2000 and 2009 by four age groups in males (Figure 4A) and in females (Figure 4B) between 2000 and 2009. The highest correlation coefficient (r) was shown in ages 18-54 (r = 0.91, P < 0.001) in males, and ages 18-54 (r = 0.95, P < 0.001), and 55-64 (r = 0.95, P < 0.001) in females. Given the well-known pathophysiological mechanisms of obesity and risk of DM, this finding suggests that control of obesity would play a pivotal role in stopping the unwelcome trends of DM. In addition, females aged 55-74 have a greater increased trend of DM than males (Figure 1B). Although it is unclear why there is a notable increase in this age group for females, changes in female hormone at pre- and post-menopausal ages may partly explain this gender difference in the risk of DM and other chronic diseases[5,7,17-21]. Data from the Women's Health Initiative Hormone Trial suggest that combined therapy with estrogen and progestin reduces the incidence of DM[21].

As demonstrated in several studies, we observed an excess relative risk (*i.e.*, hazard ratio) of DM for all-cause and heart disease in females *vs* males. However, this excess risk became non-significant after adjustment for key covariates. Findings using data from the earlier Framingham Heart Study (FHS) surveys (1970s and 1980s) demonstrated a significant excess risk of recurrent myocardial infarction and fatal coronary heart disease for women with DM *vs* men with DM[22,23]. Our non-significant results are not consistent with the previous report. It may be attributable to the different datasets we used from the FHS. For example, the majority of participants in FHS were white middle class individuals who may have different risk profiles from minorities and people with lower social status. Furthermore, a decreased relative risk of DM for CVD in recent generations has been observed because of early diagnosis and disease prevention as well as more advanced treatment than two or three decades ago. Nevertheless, this relatively higher risk of DM for coronary heart disease in women *vs* men should be still taken into consideration in CVD risk assessment and disease prevention. In the study, among 5 preventable CVD risk factors that we examined, 4 (percent of

Table 4 Proportion of antidiabetic medication use in patients with diabetes by gender

		Male		Female		
		%	(SEP)	%	(SEP)	*P* value
By groups						
	Monotherapy	38.11	(1.19)	40.36	(1.06)	0.291
	Combination	51.89	(1.27)	49.38	(1.10)	
	No drug	10.00	(0.83)	10.26	(0.66)	
Monotherapy						
	Biguanides (Metformin)	27.32	(1.89)	33.24	(1.57)	0.008
	SU	25.86	(1.72)	21.88	(1.34)	
	Insulin	37.20	(1.85)	33.01	(1.42)	
	Others	9.63	(1.16)	11.87	(0.95)	
Combination						
	Metformin + SU	20.05	(1.34)	21.94	(1.10)	0.422
	TZD + Any (insulin excluded)	13.56	(1.25)	13.11	(0.87)	
	Insulin + Any (TZD excluded)	15.25	(1.38)	16.91	(1.07)	
	Metformin + SU + TZD	7.72	(0.86)	6.75	(0.69)	
	Metformin + SU + Insulin	6.89	(0.87)	4.97	(0.61)	
	Any other combinations	36.52	(1.85)	36.32	(1.44)	

P values were given by χ^2 test. Others include alpha glucosidase inhibitors, meglinitides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. TZD: Thiazolidinedione; SU: Sulfonylureas; SEP: Standard error of proportion.

Table 5 Multivariate adjusted hazard ratios (HR, 95%CI) of antidiabetic medication use for mortality from all-cause and cardiovascular disease in patients with diabetes

	All-cause mortality						Mortality from CVD					
	Model 1			Model 2			Model 1			Model 2		
	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Medication use *vs* no-use	0.87	(0.68-1.11)	0.251	0.93	(0.73-1.18)	0.556	0.88	(0.54-1.44)	0.613	0.96	(0.60-1.54)	0.873
Monotherapy (ref: Non-drug use)												
Biguanides (Metformin)	0.53	(0.36-0.77)	0.001	0.55	(0.38-0.80)	0.002	0.82	(0.42-1.61)	0.564	0.87	(0.45-1.68)	0.681
SU	0.89	(0.66-1.21)	0.456	0.91	(0.67-1.23)	0.529	1.10	(0.66-1.83)	0.716	1.11	(0.67-1.84)	0.696
Insulin	1.65	(1.26-2.16)	< 0.001	1.71	(1.31-2.24)	< 0.0001	1.51	(0.86-2.66)	0.153	1.58	(0.92-2.70)	0.094
Combination												
Metformin + SU	0.75	(0.55-1.01)	0.059	0.81	(0.60-1.10)	0.168	0.77	(0.41-1.43)	0.403	0.87	(0.48-1.57)	0.632
TZD + Any (insulin excluded)	0.87	(0.60-1.26)	0.468	0.98	(0.67-1.42)	0.905	0.43	(0.23-0.82)	0.011	0.52	(0.28-0.98)	0.042
Insulin + Any (TZD excluded)	1.27	(0.90-1.78)	0.171	1.33	(0.94-1.86)	0.103	1.37	(0.69-2.72)	0.365	1.45	(0.75-2.81)	0.264
Metformin + SU + TZD	0.40	(0.25-0.65)	< 0.001	0.43	(0.27-0.70)	0.001	0.54	(0.24-1.18)	0.120	0.58	(0.27-1.25)	0.164
Metformin + SU + Insulin	0.64	(0.41-1.01)	0.053	0.67	(0.43-1.05)	0.080	0.75	(0.44-1.29)	0.293	0.80	(0.47-1.37)	0.418
Other combination	0.63	(0.46-0.86)	0.004	0.68	(0.50-0.93)	0.016	0.59	(0.31-1.09)	0.091	0.64	(0.35-1.19)	0.157

Others: Include alpha glucosidase inhibitors, meglinitides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. *P* values were given by Cox regression models. Model 1: Adjusted for age, sex, race/ethnicity, and survey year; Model 2: Adjusted for Model 1 plus anti-hypertension and anti-hyperlipidemia drugs. TZD: Thiazolidinedione; SU: Sulfonylureas.

individuals with lower socioeconomic status, assessed by education level, the proportion of individuals who were physically inactive, the proportion of individuals with obesity, and the proportion of individuals who had hypertension) were significantly higher in females than males, although males had a higher smoking rate than females (Table 7). These risk factors differences may partly explain the relative risk difference between genders. It is clear further studies are needed to assess the gender differences, including studies of the established and emerging risk predictors[15,24-28].

The present study provides new evidence of the patterns of antidiabetic medicine usage and their impact on all-cause and CVD mortalities in patients with DM. Treatments with metformin, insulin, and sulfonylureas were the top three medications in the study population. More than one third of patients took insulin, which is commonly given to patients either for a short-term use because of significantly out of control serum glucose, or for long-term glucose control because

Table 6 Multivariate adjusted hazard ratios (HR, 95%CI) of antidiabetic medication use for mortality from heart disease and cerebrovascular disease in patients with diabetes

	Heart disease mortality						Cerebrovascular disease (CBVD) mortality					
	Model 1			Model 2			Model 1			Model 2		
	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Medication use *vs* no-use	1.01	(0.62-1.65)	0.982	1.08	(0.67-1.74)	0.741	0.58	(0.19-1.78)	0.339	0.67	(0.23-1.95)	0.457
Monotherapy												
Biguanides (Metformin)	0.80	(0.38-1.67)	0.550	0.83	(0.40-1.70)	0.608	0.94	(0.22-4.01)	0.938	1.20	(0.30-4.82)	0.792
SU	1.26	(0.76-2.11)	0.373	1.27	(0.76-2.13)	0.364	0.71	(0.23-2.24)	0.560	0.76	(0.25-2.34)	0.629
Insulin	1.85	(1.06-3.24)	0.031	1.91	(1.12-3.26)	0.018	0.60	(0.16-2.23)	0.445	0.71	(0.23-2.21)	0.547
Combined												
Metformin + SU	0.82	(0.41-1.67)	0.586	0.91	(0.47-1.80)	0.794	0.59	(0.14-2.47)	0.472	0.71	(0.17-2.94)	0.632
TZD + Any (insulin excluded)	0.55	(0.28-1.08)	0.082	0.68	(0.35-1.30)	0.242	0.14	(0.02-1.18)	0.070	0.15	(0.02-1.32)	0.088
Insulin + Any (TZD excluded)	1.79	(0.89-3.61)	0.103	1.85	(0.94-3.64)	0.074	0.44	(0.13-1.53)	0.196	0.50	(0.15-1.66)	0.257
Metformin + SU + TZD	0.61	(0.27-1.40)	0.245	0.65	(0.30-1.42)	0.280	0.34	(0.04-2.83)	0.316	0.41	(0.04-3.95)	0.438
Metformin + SU + Insulin	0.33	(0.19-0.59)	0.000	0.35	(0.20-0.62)	< 0.0001	1.66	(0.55-5.02)	0.368	1.91	(0.64-5.73)	0.245
Other combinations	0.71	(0.37-1.37)	0.307	0.78	(0.42-1.47)	0.444	0.28	(0.06-1.31)	0.106	0.31	(0.07-1.41)	0.129

Others: Include alpha glucosidase inhibitors, meglinitides, dipeptidyl peptidase 4 inhibitors, amylin analogs, and incretin mimetics. *P* values were given by Cox regression models. Model 1: Adjusted for age, sex, race/ethnicity, and survey year; Model 2: Adjusted for Model 1 plus anti-hypertension and anti-hyperlipidemia drugs. TZD: Thiazolidinedione; SU: Sulfonylureas.

their DM has progressed over many years (commonly between 10 and 20 years) and their pancreas can no longer make enough insulin to respond to other glucose-lowering medications[5,29]. Similar to previous studies, findings from the present study suggest a significant protective effect on all-cause mortality, and a protective effect on CVD mortality for those using metformin or metformin combined with other glucose-lowering medications. Metformin, a class of medications known as "biguanides" and a first-line agent for type 2 DM (T2DM) pharmacotherapy, is one of the most prescribed drugs worldwide[30]. It has been suggested that the potential mechanisms by which metformin reduces the risk of mortality is lowering blood glucose by reducing hepatic glucose output, decreasing intestinal glucose absorption, and controlling body weight by decreasing food intake[30-32], The mechanism of the cardiovascular effect of metformin was reported to improve lipoprotein profiles in diabetic patients by decreasing plasma concentrations of free fatty acid, triglycerides, total cholesterol and LDL cholesterol and increased HDL cholesterol[30]. Meanwhile, all-cause mortality includes deaths from cancer as well. Several studies have shown a significant risk reduction in cancer incidence and mortality among diabetic patients on metformin use relative to other antidiabetic drugs use[33]. Furthermore, in considering that biguanides demonstrate a better safety profile than most oncology drugs in current anticancer drug use, nonconventional routes for administering diabetobiguanides for cancer treatment has been suggested[34]. Findings from the present study support the current knowledge of metformin therapy in the reduction of CVD and total mortality, although further studies are needed in detail on its specific association with CVD and cancers.

In addition to the strength of using a large-scale sample size, the present study has several other advantages. First, using the NHIS-Mortality Linked Files, we were able to test the association between DM and risk of outcomes prospectively. Second, by using NHIS-MEPS linkage Files, we were able to test the patterns of medications which paves the way for us to further test more details on the association between pharmacotherapy and disease outcomes using a nationally representative dataset.

Similar to any study, however, the present study has several limitations. First, we were unable to classify whether a patient with DM was type 1 DM (T1DM) or T2DM because the NHIS data did not collect the information. Therefore, findings from the study cannot be applied to interpret risk differences between T1DM and T2DM. However, although T1DM can occur at any age, it is most often diagnosed in children, adolescents, or young adults. The NHIS's participants were aged 18 and older. Furthermore, it is well-known that the majority of total DM are T2DM in general population, we may be able to assume the majority DM cases in the NHIS data were T2DM. Second, baseline predictors were measured once only, that any changes in the study variables after baseline may affect the prospective estimates of the associations between baseline predictors and health outcomes. Findings of the study should be on the basis of the hypothesis that these changes, if any, were randomized across all participants, so that a potential time-varying bias would be small when a study uses a large-scale sample size[35]. Third, participants' medical conditions at baseline were self-reported physician-diagnosis of disease (hypertension, CHD, stroke and DM), therefore possible recall bias may occur. However, the recall bias might have a relatively small effect, because the use of self-reports of physician-diagnosis of disease have been confirmed as a valid approach in large-scale population health surveys in the United States[36,37]. Fourth, the NHIS did not have data

Table 7 Age-race-adjusted rates of five selected predicators for cardiovascular disease in patients with diabetes at baseline, 2000-2009 National Health Interview Surveys

	Patients with DM at baseline				
	Male (n = 9892)		Female (n = 12413)		
	Rate	(SEP)	Rate	(SEP)	P value
Education					
Less than HS	21.53	(0.50)	24.82	(0.47)	< 0.0001
HS Graduated	28.44	(0.59)	30.61	(0.55)	0.0100
⩾ College	50.03	(0.60)	44.57	(0.56)	< 0.0001
Smoking status					
No smoker	40.92	(0.58)	61.60	(0.53)	< 0.0001
Former smoker	36.03	(0.58)	17.81	(0.49)	< 0.0001
Current smoker	23.05	(0.46)	20.59	(0.35)	< 0.0001
Exercise					
Inactive	42.80	(0.63)	49.34	(0.62)	< 0.0001
Insufficiently active	26.88	(0.52)	27.98	(0.49)	0.1000
Sufficiently active	30.32	(0.61)	22.68	(0.47)	< 0.0001
BMI, kg/m^2					
Overweight	34.21	(0.55)	25.82	(0.46)	< 0.0001
Obesity	47.13	(0.60)	53.88	(0.51)	< 0.0001
Medical condition					
Hypertension	52.54	(0.58)	54.55	(0.46)	0.0100

Education ⩾ College including those with associate degrees. Overweight: Body mass index (BMI) 25 to 29.9 kg/m^2. Obesity: BMI ⩾ 30 kg/m^2. P values were given by χ^2 test. HS: High school.

on participants' physical exams and laboratory tests (*i.e.*, without exact blood pressure measures, and measures from serum lipids and metabolic biomarkers), which may not only lead to underestimate the prevalence of hypertension and DM, but also limit us to quantitatively estimate the association between antidiabetic drug use and changes in serum HbA1c (a biomarker of glycaemia control status in diabetic patients) and lipid profiles, and their impacts on the study outcomes. Therefore, the findings of the study provide a relatively conservative estimate of the burdens of disease. Fifth, we were unable to test subgroups of antidiabetic drugs' effects on the study outcomes, such as the subgroups of sulfonylureas, because the detail data was not available from NHIS-MESP Linkage File. Sixth, in multivariate analysis, we cannot always be able to control adequately for confounding factors. We may not even know about them and chance cannot be discarded although it is highly unlikely.

Despite the limitations discussed the above, three clear and important conclusions follow the present study. First, the prevalence of DM significantly increased in all age groups in the past decade, with specific increase in females aged 55-74 compared to males. Second, DM is a significant predictor for mortality from all-cause and CVD in both genders, with a slightly higher excess relative risk in females *vs* males. Third, about 10% of patients with DM do not receive antidiabetic therapy. DM patients who received metformin monotherapy or combination of metformin with other antidiabetic medications (except insulin) showed a significant protective effect on all-cause mortality.

ACKNOWLEDGMENTS

The study used data from the National Health Interview Surveys (NHIS), NHIS Mortality Linked Files and Medical Expenditure Panel Surveys (MEPS) from the United States Centers for Disease Control and Prevention - National Center for Health Statistics (CDC-NCHS). Points of view however, are those of the authors and do not necessarily represent the position of the CDC-NCHS. The authors gratefully acknowledge the assistance of Miss Gabrielle Pyronneau for her careful proofreading and editing of the manuscript.

COMMENTS

Background

Diabetes mellitus (DM) is a leading cause of death in the United States. The present study examined the trend of DM using data from nationally representative surveys from 2000 to 2009. It is one of the first studies that address the epidemic of DM in the nation, and its serious impact on population health. Furthermore, findings from the study add new evidence of glucose-lowering treatment and health outcomes in patients with DM using data from the real world, instead of using a sample from very selective participants that are commonly applied in clinical trials.

Research frontiers

The amount of data in the real world has been exploding, and analyzing large-scale data sets, so called Big data is becoming a key basis of competition and productivity in epidemiological studies of DM. The present study using a large-scale dataset from multiple sources not only addresses the epidemic of DM in the United States, but also advances the research methods to build a national cohort sample by taking the advantages of national health survey data at baseline linking health medication prescription and vital statistics for a decade time period. Findings from this approach provide a unique opportunity to address drug effects on health outcomes using data from real world.

Innovations and breakthroughs

The innovations of the study are characterized by its research design linking data from multiple sources, and building up a representative sample of national cohort study.

Applications

The study design adds new research approach to the body of study designs using data from population based studies. Findings from the study are very informative for counties that are experiencing an increasing trend of obesity and diabetes in the world.

Terminology

Cross-sectional study design; Prospective study design; Linked Files.

Peer-review

This is a timely, interesting and informative report.

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ORIGINAL ARTICLE

Observational Study

Cost of illness among patients with diabetic foot ulcer in Turkey

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Abstract

AIM

To evaluate the annual cost of patients with Wagner grade 3-4-5 diabetic foot ulcer (DFU) from the public payer's perspective in Turkey.

METHODS

This study was conducted focused on a time frame of one year from the public payer's perspective. Cost-of-illness (COI) methodology, which was developed by the World Health Organization, was used in the generation of cost data. By following a clinical path with the COI method, the main total expenses were reached by multiplying the number of uses of each expense item, the percentage of cases that used them and unit costs. Clinical guidelines and real data specific to Turkey were used in the calculation of the direct costs. Monte Carlo Simulation was used in the study as a sensitivity analysis.

RESULTS

The following were calculated in DFU treatment from the public payer's perspective: The annual average per patient outpatient costs $579.5 (4.1%), imaging test costs $283.2 (2.0%), laboratory test costs $284.8 (2.0%), annual average per patient cost of intervention, rehabilitation and trainings $2291.7 (16.0%), annual average per patient cost of drugs used $2545.8 (17.8%)

and annual average per patient cost of medical materials used in DFU treatment $735.0 (5.1%). The average annual per patient cost for hospital admission is $7357.4 (51.5%). The average per patient complication cost for DFU is $210.3 (1.5%). The average annual per patient cost of DFU treatment in Turkey is $14287.70. As a result of the sensitivity analysis, the standard deviation of the analysis was $5706.60 ($n$ = 5000, mean = $14146.8, 95%CI: $13988.6-$14304.9).

CONCLUSION

The health expenses per person are $-PPP 1045 in 2014 in Turkey and the average annual per patient cost for DFU is 14-fold of said amount. The total health expense in 2014 in Turkey is $-PPP 80.3 billion and the total DFU cost has a 3% share in the total annual health expenses for Turkey. Hospital costs are the highest component in DFU disease costs. In order to prevent DFU, training of the patients at risk and raising consciousness in patients with diabetes mellitus (DM) will provide benefits in terms of economy. Appropriate and efficient treatment of DM is a health intervention that can prevent complications.

Key words: Diabetic foot; Diabetes complications; Cost of illness; Burden of illness; Amputation

Core tip: The purpose of this study is to evaluate the annual cost of patients with Wagner grade 3-4-5 diabetic foot ulcer (DFU) in Turkey. Cost-of-illness methodology was used in the generation of cost data. Monte Carlo Simulation was used in the study as a sensitivity analysis. The average annual per patient cost of DFU treatment in Turkey is $14287.70. As a result of the sensitivity analysis, the standard deviation of the analysis was $5706.60 ($n$ = 5000, mean = $14146.8, 95%CI: $13988.6-$14304.9). Hospital costs are the highest component in DFU disease costs.

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INTRODUCTION

Diabetes mellitus (DM) is a health problem, the severity of which is gradually increasing all over the world. DM exists in approximately 8.3% of the world's population. In 2013, 10.8% ($548 billion) of global health expenses were for DM and its complications[1]. DM amounts to 23% (approximately 10 billion Turkish liras - TL) of the total health expenses of Turkey in 2012[2].

Diabetic foot ulcer (DFU) is a frequently observed, serious, and chronic complication of DM. The risk of occurrence during diagnosis can be up to 25%, and 2% of cases require amputation[3]. Half of the cases of non-traumatic foot amputation are due to DM[4]. It is estimated that a patient's foot is amputated due to DFU once every 30 s worldwide. The rate of recurring amputation is between 30% and 50% in the following three years in the patients who are amputated once. The rate of death within five years following amputation is 50%[5].

Approximately 400000 DFU cases are observed in Turkey, and 7700 amputation procedures are performed annually due to DFU[6]. As the prognosis of cases after amputation is considered, the importance of rational treatment in DFU becomes significant. Surgical and non-surgical basic wound care principles are essential in the efficient recovery of the wounds. Prevention of ulcers and fighting against wound site infections that are difficult to heal are as important as its treatment. DFU treatment requires multidisciplinary treatment procedures. DFU has direct costs as well as indirect costs and it is very important to try to increase quality of life of the patients during treatment, minimize the disease costs, and administer correct treatment that enables the person to remain as a productive and value-adding individual as well as to prevent occurrence of the disease.

The purpose of this study is to evaluate the annual cost of patients with Wagner grade 3-4-5 DFU from the public payer's perspective in Turkey.

MATERIALS AND METHODS

This study was conducted focused on a time frame of 1 year from the perspective of the Turkish reimbursement institution. Cost-of-illness (COI) methodology, which was developed by World Health Organization, was used in the generation of cost data[7].

COI methodology

Cost is a monetary measure for the sacrifices made for achieving a certain goal. Cost is the value of a source. Economists use the concepts of "opportunity cost" or "monetary cost" in COI studies. Even though no money is spent, it is always considered that scarce resources that can be used in other areas are used. The basic idea behind cost estimation is that once a health service is provided to a person, the resources that are used will not be available anymore for other people or alternative social uses.

COI studies are used by policymakers for budget justification, determining the priorities in financing biomedical research, and development of intervention programs for preventing and treating diseases[8,9].

Cost studies can be based on either prevalence or incidence, depending on the purpose of the analysis. The approach based on prevalence is more frequently used. In the approach based on prevalence, the total costs are calculated for a patient population in a certain area in a certain period of time[8,9]. The period of time is usually 1 year. Said studies are required for health policymakers for budget planning and decisions[8]. Studies based on

incidence calculate the lifetime cost of a patient who has a disease, starting from diagnosis to treatment, or if it is a chronic disease, until death[8,9]. The analysis perspective determines which resources will be used in the calculation[8]. Perspective shows who is affected as the resource allocation preference is made and in whose name the decisions are made[10,11]. COI analyses can be performed with different perspectives, such as societal perspective, patient perspective, or perspective of the third person/public payer[8].

Health economy research defines the costs in two main categories. The first one is the medical costs that occur due to disease, and the second one is the other disease-associated costs including non-medical costs that occur due to disease[9-12]. There are direct and indirect costs in each category. Direct costs refer to which payments are made and indirect costs refer to which resources are lost[9]. The direct medical costs include all types of exclusive and non-exclusive uses of resources (not only monetary expenditures) such as costs related to hospital services, outpatient services, laboratory tests, supplies, prescriptions, physical therapy, care services at home and care centers, caregiver costs, and services such as ambulance, etcetera, and the use of health personnel and departments of hospitals. In addition, they include the future costs or savings such as costs of other tests with false positive or true positive results during monitoring associated with said disease and hospital admissions and treatment costs. Direct medical costs are calculated by classification according to the types of payments and expenses[10]. Indirect costs are the costs of morbidity and mortality[9].

Assessment and evaluation

By following a clinical path with the COI methodology, the main total expenses were reached by multiplying the number of uses of each expense item, the percentage of cases that used them, and unit costs. The direct medical costs, which are the outpatient, laboratory and imaging methods, prescribed drugs, medical supplies that are directly used during the course of treatment of disease, and the amount spent for the hospital admissions and interventions, were calculated, and non-medical direct expenses were ignored, as there were no sufficient data for Turkey. The intangible costs including pain, unhappiness, distress, misery, stress, et cetera, caused by the disease in the individual were also not taken into consideration in this study. The indirect costs including the societal costs caused by the disease, disabilities, or premature deaths were also excluded from the study.

The clinical guidelines were followed in calculating the direct costs and actual data were used for some cases.

The cost of disease was calculated by rating the Wagner classification that shows the grade of foot ulcer[13]. The Wagner classification rates of patients with DFU in Turkey were as follows: Grade 1: 7.7%, grade 2: 27.2%, grade 3: 35.2%, grade 4: 25.4%, and grade 5: 4.5%[14-16]. The costs of patients of grades 3-4-5 according to the Wagner classification were calculated.

The Medical Enforcement Declaration (MED), which is officially declared by the institution, is used for the payment of health services by the reimbursement institution in Turkey[17]. The costs for all medical services used in the calculations were obtained from MED. The drug expenses were based on the 2015 list of the Republic of Turkey Ministry of Health Turkish Medicines and Medical Devices Agency. The drugs were classified according to the active ingredients and all forms of all products included in the reimbursement list related to the active ingredients and were included in the analysis, and their average values were reflected to the calculations. Public discounts, current public paid costs, and costs related to medical materials such as all orthosis and prosthesis devices were obtained from MED. The costs were calculated according to United States dollars by using the foreign exchange rate in 2014 ($1.00 = 1.179 TL).

The average institution cost was calculated as the admission fee for treatments administered in an outpatient clinic. The health organizations, clinical branches, and surgical branches that can administer DFU treatment were chosen, the prices of related outpatient were obtained from MED, and the average values were calculated. The average costs of pricing per admission of the patients with DFU to the outpatient clinic were included in the analysis according to said average value calculated for each branch. The cost for outpatient to which the patients were transferred for consultation was 10.12 procedure points in accordance with MED. Some of the medical materials used in payment per admission to the outpatient clinic were included in the admission fee. The examinations that were not included in the outpatient clinic admission fee were included in the costs according to MED.

Monte Carlo Simulation (MCS) was used in the study as the sensitivity analysis. MCS is a technique that uses random numbers and a trial-and-error method without using any formula. MCS provides an estimate for the statistical distribution of the possible costs. At the same time, the distribution of variables that constitute the costs is obtained. Simulation technique is a methodology employed to solve problems, not a theory. Approach of this technique to the problems varies depending on system structure and the model to be constructed based on this structure. During the simulation process, a sample is generated by the distribution of the variance observed in the proper distribution forms. Random values are used for uncertain variables. MCS can assign random values to all variables and parameters in accordance with the probabilities. The simulation is based on the random number generation. For example, for a possibility of 66 that requires osteomyelitis treatment, probability distribution according to the random numbers drawn from a normal distribution is as follows: The patient will receive the osteomyelitis treatment if the random number drawn is between 00 and 66, and for the numbers drawn between 67 and 99, the patient will be treated without a need for osteomyelitis treatment. In this study, distribution parameters were calculated at the

Table 1 The average unit cost of outpatient to which the admissions are made in the treatment of diabetic foot ulcer

	Average unit cost ($-PPP 2014)
Outpatient clinics	
Endocrinology and metabolic diseases	27.1
Orthopedics and traumatology	25.4
Plastic and reconstructive surgery	26.8
Dermatology	22.4
Infectious diseases	27.3
Neurology	27.5
Nephrology	27.4
Cardiovascular surgery	27.8
Physical therapy and rehabilitation	26.4
Algology	31.0
Medical ecology and hydroclimatology	25.6
Consultations	
Infectious diseases - consultation	5.2
Orthopedics and traumatology - consultation	
Cardiovascular surgery - consultation	
Plastic and reconstructive surgery - consultation	
Dermatology - consultation	

Table 2 Unit costs of imaging - laboratory tests used in the treatment of diabetic foot ulcer

Name of test	Average unit cost ($-PPP 2014)
Imaging tests	
Direct foot X-ray	Included in outpatient clinic admission fee
Electrocardiogram	
Unilateral chest X-ray	
Doppler ultrasonography	32.4
Magnetic resonance angiography	55.1
Angiography	392.3
Scintigraphy	131.7
Laboratory tests	
Bone biopsy	97.6
Tissue culture	97.6
Aspiration/swab culture	33.8
Bone curettage culture	97.6
HbA1c	3.4
Bleeding profile (Pre-op)	12.2
Glucose	Included in outpatient clinic admission fee
Hemogram	
C-reactive protein	
Red blood cell sedimentation rate	
Albumin	
Kidney function tests	
Liver function tests	
Hepatitis markers	

HbA1c: Hemoglobin A1c.

rates of diagnosis-treatment and follow-up steps for DFU. In this analysis, the distribution values were provided with the results obtained by performing 5000 simulations for each possible situation. The time horizon is 1 calendar year.

RESULTS

The outpatient clinics visited and complications experienced by the patients according to proportional distribution vary during the treatment for DFU, and this creates different cost items in admissions made to the outpatient clinic[14,18-27]. According to the public payer's perspective, the average annual per patient outpatient cost was $579.5 in DFU treatment (Table 1).

The distribution of imaging tests and laboratory tests that are required to be performed during the treatment of DFU was obtained from the literature. While bone curettage culture and bone biopsy were required in 66% of the patients[14,18,25-27] scintigraphy is performed in Wagner grade 4 and 5 gangrene patients (20.6%)[15,16]. Some of the laboratory tests and imaging procedures performed for the patients with DFU are included in the payment per admission made to the outpatient clinic. The items that are not included in the payment per admission were added to the calculations according to the MED list. Culture, gram staining, and antibiogram analyses must be performed in patients with DFU. In DFU treatment, the average annual per patient cost for imaging tests was $283.2, and laboratory test cost was $284.8 (Table 2).

Wagner 3-4-5 DFU patient groups (44.9%) are admitted to inpatient for an average of 23 d a year[18,19,21,23,26,27]. The average rate of amputation in said patients is 53.9%[14,18,19,22-24,26,27]. The average hospitalization period for the patients who are amputated is 42 d[23]. Six percent of patients are hospitalized for five days due to revascularization surgery, and the patients who have graft/flap (24%) are hospitalized for an average of 12 d (Table 3)[28,29].

The average annual per patient cost of inpatient care due to DFU was $7357.4.

All of the patients received training on a diabetic foot. The average rate of patients receiving treatment for osteomyelitis is 66%[14,18,25-27]. Wound debridement is performed in patients at an average of 10.1%. The average rate of patients who had graft/flap was 24%. Revascularization surgery is performed in two ways: Percutaneous transluminal angioplasty (6%) or bypass (6%)[18-20,26]. An average of 8.0% of the patients receives hyperbaric oxygen treatment[18] over an average of 40 sessions[20,24]. The rate of patients receiving physical therapy and rehabilitation is 16.6% (30.8% of the amputated patients) (Table 4)[18,19,22,25,27].

The average annual per patient cost of intervention, rehabilitation, and training for DFU was $2291.7.

Antibiotic treatment of DFU can be grouped into three categories: Low risk, high risk, and serious risk. In wounds with low risk (24%), clindamycin (4 × 300 mg) or cephalexin (4 × 500 mg) is used for 14 d. In wounds with high risk (60.3%), the patients are admitted to the hospital and one of the following parenteral treatments is administered for 14 d: Piperacillin/tazobactam, ampicillin sulbactam, cephalexin, third generation cephalosporin + clindamycin, or ciprofloxacin + clindamycin. The patients with wounds with serious risk (15.3%) must be admitted to the hospital and one of the following treatments is administered for 14-21 d (for 6 wk if osteomyelitis exists): Ampicillin + gentamicin + clindamycin, imipenem/meropenem, vancomycin, piperacillin/clavulanate, or ticarcillin/clavulanate[30,31].

Table 3 Hospitalizations in departments for treatment of diabetic foot ulcer

Admission to department	Rate of patients (%)	Hospitalization period
Wound follow-up	44.9	23
Amputation surgery	53.9	42
Revascularization surgery	12.0	5
Plastic and reconstructive surgery - graft/flap	24.0	12

Table 4 Medical and surgical interventions performed in the treatment of diabetic foot ulcer and their costs

Interventions	Rate of patients (%)	Average cost ($-PPP 2014)
Osteomyelitis treatment	66.0	605.0
Wound debridement	10.1	813.7
Graft/flap	24.0	602.1
Percutaneous transluminal angioplasty	6.0	6250.9
Bypass	6.0	6512.4
Amputation	53.9	961.7
Hyperbaric oxygen treatment	8.0	70.0[1]
Physical therapy and rehabilitation	16.6	31.3[1]
Diabetic foot patient training	100.0	1.5

[1]Cost per session.

Table 5 Distributions of annual drug use of patients regarding drugs and other medical materials

Drugs and medical materials	Average cost ($-PPP 2014)
Insulin	1118.9
Antibiotics - in the group of wounds with low risk	78.1
Antibiotics - in the group of wounds with moderate risk	240.0
Antibiotics - in the group of wounds with serious risk	764.5
Anti-thrombotic	1348.4
Orthosis and prosthesis devices	961.5
Wound sheath	101.8

Table 6 The average annual cost per patient in diabetic foot treatment ($-PPP 2014)

Cost components	Average per patient annual cost ($-PPP)
Outpatient costs	579.5
Laboratory costs	284.8
Imaging test costs	283.2
Inpatient costs	7357.4
Intervention costs	2291.7
Drug costs	2545.8
Medical material costs	735.0
Complication costs	210.3
Total cost per patient	14287.7

Insulin is used in all of the patients. Furthermore, the cost of anti-thrombotic treatment was added to the calculation for 85% of the patients.

The average annual per patient cost of medication used in the treatment of DFU was $2545.8.

A total of 42.6% of the patients (non-ischemic wounds) use wound sheath as a medical supply[18,20,26,27]. For 53.9% of the patients, the costs of orthosis-prosthesis devices were reflected in the calculation by considering the average values for the supplies and their weighted use (Table 5).

The average annual per patient cost of medical supplies used in the treatment of DFU was $735.0.

Some complications of methods applied in the treatment of DFU can be observed as well. During treatment of DFU, infection can be observed after amputation in 12.8% of the patients and re-amputation can be observed in 11.5% of the patients[16]. Complications such as barotraumatic otitis (10.26%) and hypoglycemia (0.85%) can be observed in patients treated with hyperbaric oxygen treatment[20]. The average cost of complications per patient with DFU was $210.3.

The average annual per patient cost of DFU treatment in our country was $14287.7 (Table 6).

Sensitivity analysis

DFU includes use of some interventional procedures and pharmacological agents as well as various services provided by outpatient, inpatient, and laboratory units during diagnosis and treatment stages and also includes the cost of side effects of said procedures. Separate calculations were made for each variable for the distribution and accuracy of the results. Thus, the results of each variable are represented by the probabilities calculated within. As a result of the sensitivity analysis (Table 7), the standard deviation of the analysis was $5706.6 ($n$ = 5000; mean = $14146.8, 95%CI: $13988.6-$14304.9).

The health expenses per person are $-PPP 1045 in 2014 in Turkey and the average annual per patient cost for DFU is 14-fold of said amount. The total health expense in 2014 in Turkey is $-PPP 80.3 billion and the total DFU cost has a 3% share in the total annual health expenses for Turkey.

DISCUSSION

In this study, the direct medical costs of DFU were investigated from the public payer's perspective in Turkey. In similar studies conducted on a limited number of patients and in a single center, the estimated treatment costs of DFU patients were investigated in Turkey. In a retrospective study conducted by Keskek *et al*[21] in 2010 on patients with type 2 diabetes mellitus (T2DM) in Turkey, it was demonstrated that the costs of treatment in the hospital per patient in patients with DFU in a tertiary hospital were higher than those of the patients with T2DM without any chronic complications. The cost of one hospitalization for each patient was calculated in the study conducted by Keskek *et al*[21]. The cost of the hospital per admission in patients with DFU was $976.10. The cost of supplies was calculated at 42.6%, and 57.4% was calculated as cost of service. In the cost study related to DM and chronic complications conducted with 7095 patients in 2009 in Turkey, the direct costs of DFU

Table 7 Result of sensitivity analysis for the total costs of diabetic foot ulcer

Cost components	$-PPP ($n$ = 5000)			
	Average	SD	95%CI	Median
Outpatient costs	576.2	196.1	568.0-584.4	565.5
Cost of imaging tests	279.4	205.6	267.9-291.0	219.2
Laboratory costs	283.3	64.7	278.7-287.7	254.8
Inpatient costs	7290.3	5047.9	6864.8-7715.8	8969.5
Intervention costs	2212.3	2347.7	1980.3-2444.4	1568.2
Drug costs	2554.4	566.3	2490.7-2618.2	2707.4
Cost of medical supplies	742.0	538.0	673.9-810.2	961.5
Cost of complications	208.7	452.7	145.0-272.5	0.0
Total cost	14146.8	5706.6	13988.6-14304.9	14615.4

were TL 1545, and in cases of amputation, the annual cost was TL 2386. In said study, the prevalence of DFU was 9.0% and its incidence was 2.0% in patients with DM, and the incidence of amputation was 0.2% and its prevalence was 1.0% in patients with DM[32].

The costs of treatment vary according to the distribution of outpatient clinics visited by DFU patients, medication and medical materials used in treatment, laboratory and imaging tests performed, and the need for admission to a hospital and surgical intervention. The period of hospitalization is an important factor that causes high costs. The period of hospitalization is prolonged due to uncontrolled hyperglycemia, long-term wound care, infections, debridement, amputation, and newly occurring complications; therefore, the cost of treatment increases. In our study, the average direct total cost of DFU treatment per patient in our country is $14287.7. Hospital admissions are $7357.4 (51.5%) of said cost.

In the studies conducted based on prevalence from the perspective of the health care payer, the cost of DFU in the United States was between $1892 and $48354[33-36]. In the study conducted by Harrington *et al*[33], calculations were made using the insurance database of 1995 in the United States. The cost of DFU was $15309. Inpatient costs are 74% of the total cost[33]. The study conducted by Stockl *et al*[34] was performed by using the insurance database of 2000 and 2001. The cost per episode increases according to the severity of DFU. While the cost of grade 1 was $1892 per episode, the cost of grade 4/5 was $27721 per episode. Inpatient costs amount to 77% of the total cost[34]. In the study conducted by Sargen *et al*[35], the cost of DFU was studied using the insurance database of calendar year 2007. In said study, the cost of DFU was $31363, and if amputation was performed, said cost was $48354[35]. In the study conducted by Margolis *et al*[36] based on the Medicare database of the United States, the amounts of reimbursement payments made for DFU and lower extremity amputations between 2006 and 2008 were calculated. The cost per patient in patients with DM with DFU was $31600 for 2006, $33100 for 2007, and $35100 for 2008. The cost per patient in patients with DM who had a lower extremity amputation was $49300, $51200, and $54100, respectively[36].

Kerr *et al*[37] calculated the cost of DFU for the National Health Service (NHS) in England in 2010-2011. Outpatient care, inpatient care, and post-amputation care were calculated in the study conducted by Kerr *et al*[37]. Moreover, calculations for materials such as wheelchairs, et cetera, were performed as well. In the study, it was found that 0.6% of the expenditures of NHS consisted of DFU for 2010-2011. Half of the total cost consisted of primary and community care of DFU. Some 8.8% of the total hospital costs associated with diabetes were spent for DFU. The existence of DFU increases the period of hospitalization of the patients by 2.51-fold. The outpatient cost was £4994. The inpatient cost was £3620 per admission. The post-amputation care cost was £2879 per patient.

In the study conducted by Girod *et al*[38] in 2003 in France, the monthly cost of DFU was €697 for outpatient care and €1556 for hospital care. While 70% of the total cost consisted of hospital costs in the patients admitted to the hospital, the percentage of drug costs was 10%[38].

Prompers *et al*[39] prospectively calculated the societal disease cost for DFU in Europe in 2003-2004 with the approach based on incidence. In the study, in which 14 sites from 10 European countries were included, the direct cost of DFU per patient was €9446 and the cost per patient in amputated patients was €24540. The indirect cost of DFU was €645 per patient and said cost was €681 in the amputated patients. Hospital costs were 39% of the total cost of DFU[39].

In the study conducted by Rezende *et al*[40] in 2008 in Brazil with a simulated hypothetical cohort, approximately 30% of patients with DFU were admitted to the hospital. It was stated that extremity amputation was performed in 14% of patients with DFU. The total annual cost of patients admitted to the hospital due to DFU was approximately $264 million ($51 million-461 million) and said cost was $128 million ($24.5 million-$222.3 million) for the amputated patients[40].

In a study conducted in Pakistan in 2005 for investigating the direct cost of DFU treatment in a tertiary hospital, it was demonstrated that the cost of treatment increases as DFU progresses. The cost for University of Texas Classification grade 1 phase B was £21 and the same cost was £288 for grade 2 phase D and £378 for grade 3 phase D. In the study, in which 62% of the patients had a grade 2 ulcer, the average cost was £376 for major amputations and £389 for minor amputations. The average annual health expense per patient was £1.7

in Pakistan for the period the study was conducted[41].

In a study comparing the costs of United States Medicare and private insurance patients in 2013, it was calculated that the annual treatment cost of DFU was $11296 for Medicare ($27040 *vs* $15743) and $15329 for privately insured ($25931 *vs* $10602) patients[42].

In conclusion, despite the fact that it is difficult to compare the costs between countries due to the social and economic differences in terms of methods used in the treatment of DFU, said disease is a complication that decreases the quality of life of the patient, is life threatening, and significantly increases the socioeconomic costs of DM.

The annual cost of DFU in Turkey was found to be similar to the results of cost studies conducted based on prevalence for the other countries.

DM-related complications are severe and will often require hospitalization for long periods. In some cases, it exposes a necessity for major surgery. The highest cost component was the hospital cost in the COI for DFU. Improvements in inpatient durations and health interventions will reduce the costs of related disease. The second leading cost component was found to be the pharmacy costs. Among these costs, antithrombotic drugs have the largest share. Increased use of generic anti-thrombotic drugs may be a powerful factor for reducing this cost.

The most effective way of reducing the costs related to DFU is the prevention of the complication itself. Another alternative is delaying the complication as long as possible. In order to prevent DFU, it will be helpful to provide training to the patients at risk and to raise awareness in patients with DM in terms of economy. Appropriate and efficient treatment of DM is a health intervention that can prevent complications. Further studies may help in discovering more effective healthcare strategies and improving the healthcare quality.

COMMENTS

Background

Diabetes mellitus (DM) has the highest proportion in health expenses globally. A major part of these expenses are caused by DM complications. Diabetic foot ulcer (DFU) is a frequent and severe DM complication. DFU might cause disability by going all the way to amputation. Studies have shown that DFU substantially increases mortality rates. The study has been done from Turkey Healthcare Payer's perspective. In Turkey, there are no previous studies of DFU costs done according to cost-of-illness (COI) methodology.

Research frontiers

The current research hotspot is to identify how much DFU's cost is among all DM complications that are high in cost and which resources cost the highest among all the components of DFU costs. This way, the areas that should be intervened to lower the DFU costs will be easier to determine.

Innovations and breakthroughs

DFU's annual mean per patient cost is $14287.7. Hospitalization costs constitute 51.5% of these expenses. Studies done in United States and Europe report that hospitalization costs for DFU are approximately 70%. This rate is lower in Turkey. Also, hospitalization costs are a major part of DFU costs. Pharmacy costs, which are mostly anti-thrombolytic drugs and insulin treatment, constitute 18% of all costs. In Turkey, DM constitutes 23% of all healthcare costs, and 1/6 of this is DFU expenses, which are approximately 3% of all health care expenses in Turkey.

Applications

With this study, it has been shown that DFU constitutes 3% of all health care costs in Turkey. Shortening the time spent hospitalized and improving the interventions done in hospitals should lower the costs substantially. Using generic anti-thrombolytic agents and manufacturing insulin locally in Turkey are powerful moves that might decrease these costs. The study, which is done by COI methodology, will supply the convenient data needed to compare the costs between Turkey and other countries.

Terminology

Cost in health economics refers to the resources consumed during the provision of health care. COI study aims to determine the total economic impact of a disease or health condition on society through the identification, measurement, and valuation of all direct and indirect costs. Sensitivity analysis is a way to analyze the impact of uncertainty on an economic analysis or a decision. Simulation is a modeling technique that makes it possible to observe the causation in the system and the actions of the real system under different circumstances. Monte Carlo Simulation (MCS) is a technique that uses random numbers and a trial-and-error method without using any formula. MCS provides an estimate for the statistical distribution of the possible costs.

Peer-review

In the present study, the authors evaluate the annual cost of patients with diabetic foot ulcers in Turkey. In general, the manuscript is well written, straightforward and very descriptive.

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ORIGINAL ARTICLE

Observational Study

Predictors of hypoglycemia in insulin-treated patients with type 2 diabetes mellitus in Basrah

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Abstract

AIM

To measure the incidence and determinants (predictors) of hypoglycemia among patients with type 2 diabetes mellitus (T2DM) who were on insulin treatment for at least one year.

METHODS

The present study is an out-patients based inquiry about the risk and predictors of hypoglycemia among patients with T2DM seeking care at the Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center, in Basrah over a period of 7 mo (from 15th of April, 2013 to 15th of October, 2013). The data used in the study were based on all detailed interview and selected laboratory investigations. A total of 336 patients could be included in the study.

RESULTS

The incidence of overall hypoglycemia among the studied patients was 75.3% within the last 3 mo preceding the interview. The incidence of hypoglycemia subtypes were 10.2% for severe hypoglycemia requiring medical assistance in the hospital, 44.36% for severe hypoglycemia treated at home by family; this includes both confirmed severe hypoglycemia with an incidence rate of 14.6% and unconfirmed severe hypoglycemia for which incidence rate was 29.76%. Regarding mild self-treated hypoglycemia, the incidence of confirmed mild hypoglycemia was 21.42%, for unconfirmed mild

hypoglycemia the incidence rate was 50.0% and for total mild hypoglycemia, the incidence rate was 71.42%. The most important predictors of hypoglycemia were a peripheral residence, increasing knowledge of hypoglycemia symptoms, in availability and increasing frequency of self-monitoring blood glucose, the presence of peripheral neuropathy, higher diastolic blood pressure, and lower Hemoglobin A1c.

CONCLUSION

Hypoglycemia is very common among insulin-treated patients with T2DM in Basrah. It was possible to identify some important predictors of hypoglycemia.

Key words: Diabetes mellitus; Insulin; Hypoglycemia; Out-patient; Type 2

Core tip: Outpatients study aimed to assess the frequency of hypoglycemia and their predictors among patients with type 2 diabetes mellitus on insulin for at least one year. The majority of patients (75.3%) had hypoglycemia in the preceding 3 mo. We identify some important predictors of hypoglycemia.

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INTRODUCTION

Hypoglycemia is very frequent and serious complication of insulin therapy, especially in those with intensive treatment and unawareness of hypoglycemia is a very dangerous situation that complicated the problem more[1].

Severe hypoglycemia is defined to be an episode of hypoglycemia in which a patient requires help from another people. Thus, patients who are more compliant or precise in using their medication to lower their glucose levels are at greatest risk of hypoglycemia and its sequels[2,3].

Confirmed symptomatic hypoglycemia is an event during which classic symptoms of hypoglycemia was confirmed simultaneously by measured plasma glucose concentration < 70 mg/dL (3.9 mmol/L)[3]. Asymptomatic hypoglycemia is an attack not accompanied by classic symptoms of hypoglycemia but with a measured plasma glucose concentration < 70 mg/dL (3.9 mmol/L). Furthermore, probable symptomatic hypoglycemia is defined as symptoms of hypoglycemia that not proven by measuring simultaneous plasma glucose and assumed to be due to a plasma glucose concentration < 70 mg/dL (3.9 mmol/L).

It's well known that people with diabetes most of the times treat symptoms of hypoglycemia with the diet without measuring their plasma glucose at the same time. That why these episodes can be considered as probable hypoglycemia. These unconfirmed hypoglycemic episodes reported by the patients may affect the results of studies intended to evaluate the drugs that affect plasma glucose, but they should be declared by any mean as self-reported hypoglycemic episodes that are not confirmed.

Finlay, we have to define relative hypoglycemia. These symptoms of hypoglycemia reported by patients with diabetes but associated with simultaneously measured plasma glucose concentration > 70 mg/dL (3.9 mmol/L).

This last group of hypoglycemic episodes is seen more in those with long-standing diabetes with poor control. They per say may not be harmful, and they are no suitable outcome measures in clinical studies needed to evaluate drug therapy in diabetes, but again have to be reported though the symptoms happen with plasma glucose levels > 70 mg/dL (3.9 mmol/L).

In this study, we assess the frequency of hypoglycemia among insulin-treated patients with type 2 diabetes mellitus (T2DM) who were on insulin for at least one year.

MATERIALS AND METHODS

Study design

The study is a cross-sectional study investigating retrospectively the experience of hypoglycemia among patients with type T2DM receiving insulin for at least one year preceding the time of study who attended Al-Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC). The study extended in the data collection phase over five month period from 15th of April to 15th of October 2013.

Sampling methods and sample size

A total of 336 patients were enrolled in the study. Data were collected through direct interview with the patients after ensuring their verbal agreement to take part in the study. On average 5-6 patients could be fully interviewed each working day.

Inclusion criteria

All patients with T2DM (no age limit) including men and non-pregnant women receiving insulin for at least one year proceeding the time of study who attended FDEMC. Informed verbal consent was taken from all patients, and the ethical committee of Basrah College of Medicine approved the study.

Questionnaire and data collection

A special questionnaire form was prepared for the purpose of data collection for this study. It covered the following

aspects. Personal characteristics including information on name, age, sex, job, address, level of education, marital status. Medical characteristics including family history of diabetes mellitus, duration of diabetes, duration of insulin use, type of insulin use, frequency of insulin used per day, dose of insulin per time of administration per day, total dose of insulin per day (for the preceding 3 mo), whether the patient is on oral hypoglycemic drug, its type dose, and frequency. Other questions include who inject insulin to the patient, state of patient's vision, patient's mobility, the source of medication, knowledge of the patient about symptoms of hypoglycemia.

Information on hypoglycemic attacks, including whether the patient had hypoglycemic attacks during the preceding three before the interview, type of hypoglycemic attack and timing during the day, events precipitating hypoglycemia, whether self-monitoring blood glucose (SMBG) device was available and the frequency of its use, did hypoglycemic attack was confirmed by SMBG or by venous blood and what was the blood glucose level, awareness of the patient for hypoglycemia.

History of other co-morbidities such as hypertension (HTN), ischemic heart disease (IHD), cerebrovascular accidents (CVA), amputation, chronic kidney disease (CKD), diabetic foot, and peripheral neuropathy (PNP).

The use of insulin by another family member at home or outside the home and whether the patient takes other concomitant medications with the insulin. Measurement of height and weight to obtain a body mass index (BMI) (done by a nurse on the day of the visit). Investigations were done in the laboratory of FDEMC on the day of the visit, and these include measurement of glycated hemoglobin (HbA1C), serum creatinine, and urine for albumin.

Definition of variables

Details related to hypoglycemia: Respondent's knowledge of hypoglycemia symptoms was grouped into yes or no. An incident of hypoglycemia, the respondent was asked if he or she developed, at least, one episode of hypoglycemia during the last 3 mo; this includes asking about the symptoms of hypoglycemia, and the answer was grouped into yes or no.

Type of hypoglycemia: By adopting the ADA definition of hypoglycemia[3,4], it was classified into: (1) severe need third party help in the hospital by a doctor; severe need second party help at home by family. Severe hypoglycemia also subdivided into confirmed severe hypoglycemia, and unconfirmed severe hypoglycemia; and (2) mild self-treated hypoglycemia was also subdivided into confirmed mild hypoglycemia and unconfirmed mild hypoglycemia. Confirmation of hypoglycemia (what was blood glucose level at the time of the attack?) was grouped into: By SMBG, by venous blood or not (hypoglycemia not confirmed). Awareness of hypoglycemia was grouped into yes or no[1].

Statistical analysis

Data were coded according to the variable definition and entered into a computer program: Statistical Package for Social Science (SPSS - version 20). Data were analyzed and presented in suitable tables. Three layers of tables are presented: Descriptive tables describing patients socio-demographic and medical characteristics, Cross-tabulations of the history of hypoglycemia with probable risk factors. χ^2 or Fisher's Exact test was used to find out the statistical association, P value < 0.05 was considered significant. Logistic regression analysis was done to identify significant predictors of hypoglycemia.

RESULTS

Socio-demographic characteristics of the studied patients included age range was 29-88 years with mean age of 54.47 years; 38.1% were in the age group 50-59 year; 28.0% were in the age group 60-69 year. Regarding gender, female cases showed predominance forming 61.9% compared to males who accounted for 38.1% of cases. More than one-quarter of patients (29.8%) had completed primary schooling. The majority were married accounting for 80.1%. Regarding residence, most of the respondents lived in Basrah city (67.3%).

Some medical aspects of the studied patients, where 40.2% have more than one 1st and 2nd degree relative with DM, regarding the frequency of insulin administration/day; 44.6% of patients received insulin three times daily, 42.9% received insulin twice daily.

On co-morbidities, 74.4% of them had HTN, 17.3%, and CVA reported IHD was reported by 6%. Amputation was evident in 3%, CKD in 26.8% and diabetic foot in 27.1%, and PNP in 90.2%.

Most of the patients (75.6%) injected themselves insulin and needed no external support, about vision; 81% of patients reported good vision, 87.8% were mobile alone without assistance. The majority of patients (66.1%) received insulin from more than one source. Regarding knowledge of hypoglycemia symptoms; 95.2% reported that they knew hypoglycemia symptoms.

Table 1 shows the incidence (%) of hypoglycemia (total and subtypes) in the last 3 mo as reported by the patients. The majority of patients (75.3%) had hypoglycemia in the preceding 3 mo. The incidence of hypoglycemia subtypes was 10.2% for severe hypoglycemia requiring medical assistance in the hospital, 44.36% for severe hypoglycemia treated at home by family; this includes both confirmed severe hypoglycemia with an incidence rate of 14.6% and unconfirmed severe hypoglycemia for which incidence rate was 29.76%.

Regarding mild self-treated hypoglycemia, the incidence of confirmed mild hypoglycemia was 21.42%, for unconfirmed mild hypoglycemia the incidence rate was 50.0% and for total mild hypoglycemia, the incidence rate was 71.42%.

More than half of the patients who had experienced hypoglycemia during the preceding 3 mo (57.6%) had

Table 1 Incidence, types, timing, and causes of hypoglycemia in 336 patients

Variable	*n* (%)
Hypoglycemia in the last 3 mo	253 (75.3)
Type of hypoglycemia	
Severe treated in hospital	34 (10.2)
Sever confirmed hypoglycemia treated at home by family (≤ 70 mg/dL)	49 (14.6)
Severe unconfirmed hypoglycemia treated at home by family or blood glucose > 70 mg/dL	100 (29.7)
Mild confirmed hypoglycemia (≤ 70 mg/dL)	72 (21.4)
Mild unconfirmed hypoglycemia or blood glucose > 70 mg/dL	168 (50.0)
Total severe hypoglycemia treated at home by family	149 (44.3)
Total mild hypoglycemia	240 (71.4)
Timing of hypoglycemia in the last 3 mo	
Nocturnal	22 (8.7)
Day time	83 (32.8)
Nocturnal and day time	148 (58.5)
Precipitating factors hypoglycemia	
Missed meal, delayed meal, eating a less amount of food	214 (84.6)
Performing an exercise	42 (16.6)
Doctor change the dose of insulin recently	12 (4.7)
Insulin dose adjusted by the patient, errors in the dose of insulin	7 (2.8)
No obvious cause	22 (8.7)
Awareness of hypoglycemia in the last 3 mo	
No	19 (7.5)

Table 2 Relation of hypoglycemia in the last 3 mo with age, gender, education, marital status and residence among 336 patients

	Hypoglycemia in the last 3 mo		Total (*n*)	*P* value
	Yes	No		
Age (yr)	*n* (%)	*n* (%)	27	0.944
29-39	22 (81.5)	5 (18.5)		
40-49	48 (73.8)	17 (26.2)	65	
50-59	97 (75.8)	31 (24.2)	128	
60-69	70 (74.5)	24 (25.5)	94	
⩾ 70	16 (72.7)	6 (27.3)	22	
Gender				
Male	93 (72.7)	35 (27.3)	128	0.776
Female	160 (76.9)	48 (23.1)	208	
Education				
Illiterate	61 (76.2)	19 (23.8)	80	0.016
Just literate	37 (75.5)	12 (24.5)	49	
Primary school	83 (83.0)	17 (17.0)	100	
Intermediate school	44 (77.2)	13 (22.8)	57	
Secondary school	8 (50.0)	8 (50.0)	16	
College and more	20 (58.8)	14 (41.2)	34	
Marital status				
Single	5 (83.3)	1 (16.7)	6	0.604
Married	200 (74.3)	69 (25.7)	269	
Divorced	3 (60.0)	2 (40.0)	5	
Widowed	45 (80.4)	11 (19.6)	56	
Residence				
Basrah city	164 (72.6)	62 (27.4)	226	0.215
Northern Basrah	40 (72.4)	13 (27.65	53	
Southern Basrah	5 (83.3)	1 (16.7)	6	
Eastern Basrah	12 (100.0)	0 (0.0)	12	
Western Basrah	32 (82.1)	7 (17.9)	39	

developed both nocturnal and daytime hypoglycemia.

The most common causes of hypoglycemia are factors related to a meal including missed meal, delayed meal or eating a less amount of food, and the majority of the patients are aware of hypoglycemia symptoms in the preceding 3 mo.

Determinants of hypoglycemia during the preceding 3 mo

In Table 2, although a higher percentage of hypoglycemia was reported in the younger age group 29-39 year and among females; there is no significant association between age and gender with experience of hypoglycemia during the preceding 3 mo; $P > 0.05$. There is a highly significant association with the education of respondents; $P = 0.016$ with the highest percentage in those who had completed primary schooling. There is no significant association between marital status and residence with experience of hypoglycemia during the preceding 3 mo ($P > 0.05$).

Table 3 shows there is no significant association of DM family history, duration of DM and duration of insulin treatment with experience of hypoglycemia during last 3 mo; $P > 0.05$. No significant association between type of insulin and experience of hypoglycemia during the preceding 3 mo ($P > 0.05$); while there is a highly significant association between the frequency of insulin administration per day and total dose of insulin per day with hypoglycemia in the last 3 mo ($P < 0.05$).

In Table 4, there is no significant association between dose of regular, premix and Neutral Protamine Hagedorn (NPH) insulin and experience of hypoglycemia during last 3 mo ($P > 0.05$). There is no significant association between family support, vision, mobility and source of medications with experience of hypoglycemia during the preceding 3 mo; $P > 0.05$. While there was a significant association between knowledge of hypoglycemia symptoms and experience of hypoglycemia ($P < 0.05$).

Table 5 shows there is no significant association regarding availability and frequency of SMBG with experience of hypoglycemia during last 3 mo ($P > 0.05$).

Also, there is no significant association between HTN, CVA, CKD, amputation and diabetic foot with experience of hypoglycemia during the preceding 3 mo ($P > 0.05$), but a significant association does exist between IHD and PNP with experience of hypoglycemia during the preceding 3 mo ($P < 0.05$).

There is no significant association between insulin use by other family members, concomitant use of other medication and type of medication with experience of hypoglycemia during the preceding 3 mo ($P > 0.05$).

Table 6 shows that there is no significant association between BMI; systolic blood pressure; diastolic blood pressure; HbA1c; serum creatinine; urine for albumin with experience of hypoglycemia during last 3 mo ($P > 0.05$).

Logistic regression analysis

To overcome some of the interaction and confounding effects of the various predictors used in this study; a logistic regression analysis was done. Experience of hypoglycemia in the last 3 mo was used as the dependent

Table 3 Relation of hypoglycemia in the last 3 mo with diabetes mellitus family history, duration of diabetes mellitus, duration of insulin treatment, type of insulin, frequency and total dose

	Hypoglycemia in the last 3 mo		Total (*n*)	*P* value
	Yes *n* (%)	No *n* (%)		
Family history of DM				
None	85 (78.7)	23 (21.3)	108	0.601
One	68 (73.1)	25 (26.9)	93	
More than one	100 (74.1)	35 (25.9)	135	
Duration of DM (yr)				
1-10	140 (75.7)	45 (24.3)	185	0.877
11-20	92 (76.0)	29 (24.0)	121	
21-30	18 (69.2)	8 (30.8)	26	
⩾ 31	3 (75.0)	1 (25.0)	4	
Duration of insulin treatment (yr)				
1-10	238 (74.8)	80 (25.2)	318	0.578
> 10	15 (83.3)	3 (16.7)	18	
Type of insulin				
Premix	75 (72.1)	29 (27.9)	104	0.239
Regular	6 (75.0)	2 (25.0)	8	
NPH	24 (64.9)	13 (35.1)	37	
Combination of 2 or 3 insulin types	148 (79.1)	39 (20.9)	187	
Frequency of insulin administration/d				
Once	3 (30.0)	7 (70.0)	10	0.001
Twice	107 (74.3)	37 (25.7)	144	
Thrice	126 (84.0)	24 (16.0)	150	
⩾ Four times	17 (53.1)	15 (46.9)	32	
Total dose of insulin (unit/d)				
< 20	3 (37.5)	5 (62.5)	8	0.007
21-40	46 (75.4)	15 (24.6)	61	
41-60	121 (79.1)	32 (20.9)	153	
61-80	59 (81.9)	13 (18.1)	72	
81-100	17 (56.7)	13 (43.3)	30	
> 100	7 (58.3)	5 (41.7)	12	

DM: Diabetes mellitus; NPH: Neutral protamine hagedorn.

outcome variable, only peripheral residence, knowledge of hypoglycemia symptoms, availability and increasing frequency of SMBG, presence of PNP, higher diastolic blood pressure, and lower HbA1c were significant and independent predictors. All other studied variables were not predictors (Table 7).

DISCUSSION

The results of this study showed that most of the studied patients had experienced at least one episode of hypoglycemia during the last 3 mo (75.3%). The reported risk of hypoglycemia in this study is higher than the 43.3% that was reported by Fritsche *et al*[5], 45% by Donnelly *et al*[6] and the 64% by Henderson *et al*[7].

Although it is agreed that patients remember major events such as major hypoglycemia requiring second party help by medical personnel or by family easier than minor self-treated events; in the present study patients seemed to recall both minor and major hypoglycemic episodes including those hypoglycemic episodes which were treated in hospital or at home by family; this can be explained by the fact that hypoglycemic events including minor ones cause stress, anxiety and other sympathoadrenal symptoms that can be remembered even if it happened several mo ago especially if they are frequent[8,9].

Incidence rates of hypoglycemia subtypes (severe and mild) in the present study were generally higher than that reported in other studies. By Donnelly *et al*[6] the incidence of severe hypoglycemia requiring assistance was 3%, by Henderson *et al*[7] it was 15% and by United Kingdom Hypoglycemia Study Group it was 7% (incidence of mild hypoglycemia 51%)[10]. This excess in incidence may be due to poor adherence to the prescribed treatment regimens, fluctuation in the timing of meals and insulin doses, low education, presence of other diabetes complications especially diabetic nephropathy and autonomic neuropathy. Some patients who experienced minor hypoglycemia may receive unnecessary help from their relatives or unnecessary treatment in the emergency room; this could have lead to overestimation of severe hypoglycemia.

The incidence of severe hypoglycemia treated at home by the family and was confirmed by blood glucose measurement was lower than the incidence of severe unconfirmed hypoglycemia (14.6% *vs* 29.76%) and the same thing for mild self-treated hypoglycemia (incidence of confirmed hypoglycemia was 21.42% *vs* 50.0% for mild unconfirmed ones), this might be due to many patients choose to treat hypoglycemia without measuring blood glucose by SMBG or it is unavailable

Table 4 Relation of hypoglycemia during the last 3 mo with dose of insulin, family/social support, mobility, source of medications and knowledge of hypoglycemia symptoms

	Hypoglycemia in the last 3 mo		Total (*n*)	*P* value
	Yes *n* (%)	No *n* (%)		
Regular dose (unit)				
1-10	7 (100.0)	0 (0.0)	7	0.347
11-20	84 (80.2)	21 (19.8)	105	
21-30	57 (77.0)	17 (23.0)	74	
> 30	5 (62.5)	3 (37.5)	8	
Total	153 (79.0)	41 (21.0)	194	
Premix dose (unit)				
1-10	2 (100.0)	0 (0.0)	2	0.45
11-20	78 (78.8)	21 (21.2)	99	
21-30	86 (77.3)	25 (22.7)	111	
> 30	10 (62.5)	6 (37.5)	16	
Total	176 (77.1)	52 (22.9)	228	
NPH dose (unit)				
1-10	4 (100.0)	0 (0.0)	4	0.528
11-20	37 (73.1)	14 (26.9)	51	
21-30	27 (66.7)	14 (33.3)	41	
> 30	6 (85.7)	1 (14.3)	7	
Total	74 (72.4)	29 (27.6)	103	
Family/social support				
Self	190 (74.8)	64 (25.2)	254	0.914
Others	51 (76.1)	16 (23.9)	67	
Self and others	12 (80.0)	3 (20.0)	15	
Vision				
Good	203 (74.6)	69 (25.4)	272	0.560
Poor	50 (78.1)	14 (21.9)	64	
Mobility				
Mobile alone	223 (75.6)	72 (24.4)	295	0.698
Mobile with assistance or use wheel chair	9 (81.8)	2 (18.2)	11	
Walk on stick	21 (70.0)	9 (30.0)	30	
Source of medications				
FDEMC[1]	63 (75.0)	21 (25.0)	84	0.507
Public clinic	7 (58.3)	5 (41.7)	12	
Private sector	13 (72.2)	5 (27.8)	18	
More than one source	170 (76.6)	52 (23.4)	222	
Knowledge of hypoglycemia symptoms				
Yes	246 (76.9)	74 (23.1)	320	0.003

[1]Al-Faiha Specialized Diabetes, Endocrine and Metabolism Center. NPH: Neutral protamine hagedorn.

or not functioning; this is called (probable symptomatic hypoglycemia). Besides, patients with poor glycemic control and persistently high blood sugar levels could experience hypoglycemia at blood glucose level > 70 mg/dL (3.9 mmol/L), this is called (relative hypoglycemia)[5].

By the present study it was found that factors related to meal (missed meal, delayed meal and eating less amount of food in meals) were the most common precipitating factors of hypoglycemic events, this is agreed with what is known by most literatures[2,8,9,11].

Nocturnal hypoglycemia is a dangerous problem in patients with T2DM on insulin, if it is severe enough; it may lead to death or serious neurological impairment, it occurs in about two thirds of the studied patients. Eating less amount of food in dinner and use of bed time intermediate acting NPH human insulin may contribute to nocturnal hypoglycemia[2,8,12].

Hypoglycemia unawareness occurs in a minority of the studied patients who report episodes of severe hypoglycemia that necessitate medical management in hospitals. Long standing T2DM and recurrent hypoglycemic episodes are possible risk factors[1]. These results agreed with those study of Akram *et al*[13].

No relation was found in the present study of hypoglycemia to age. The same findings were obtained by Davis *et al*[14], while contradictory results were reported in other studies that concluded aging as an important risk factor of hypoglycemia[15-18].

This may be due to that elderly people constitute a small proportion of the studied patients (only 6.5%).

Although in our study females predominates males; no association was found between gender and hypoglycemia, several recent studies support our findings[14,19,20].

There is a significant association between hypoglycemia and level of education at the level of univariate analysis ($P < 0.05$) but this association has disappeared at the level of logistic regression. Hypoglycemia is more prevalent among illiterate patients or those with lower than secondary school qualification. Low educational attainment may mean less understanding and carelessness regarding the dangerous complications of hypoglycemia and the importance of adherence to

Table 5 Relation of hypoglycemia during the last 3 mo with availability, frequency of self-monitoring of blood glucose, common co-morbidities, and concomitant medication use (other than OHD)

	Hypoglycemia in the last 3 mo		Total (*n*)	*P* value
	Yes *n* (%)	No *n* (%)		
Availability of SMBG				
Available and used	124 (75.2)	41 (24.8)	165	0.996
Not available	102 (75.6)	33 (24.4)	135	
Available and not used	27 (75.0)	9 (25.0)	36	
Frequency of SMBG use				
Once/mo	19 (79.2)	5 (20.8)	24	0.164
1-2 times/wk	48 (67.6)	23 (32.4)	71	
Once daily	23 (74.2)	8 (25.8)	31	
Twice daily	8 (88.9)	1 (11.1)	9	
Thrice daily	2 (50.0)	2 (50.0)	4	
According to patients condition	25 (89.3)	3 (10.7)	28	
Common co-morbidities				
HTN	106 (72.1)	41 (27.9)	147	0.232
IHD	50 (86.2)	8 (13.8)	58	0.034
CVA	15 (75.0)	5 (25.0)	20	0.975
Amputation	7 (70.0)	3 (30.0)	10	0.693
Diabetic foot	72 (79.1)	19 (20.9)	91	0.322
CKD	66 (73.3)	24 (26.7)	90	0.614
PNP	235 (77.6)	68 (22.4)	303	0.004
Insulin use by other family members				
At home	21 (80.8)	5 (19.2)	26	0.764
Outside home	42 (76.4)	13 (23.6)	55	
Non	190 (74.5)	65 (25.5)	255	
Concomitant medication use (other than OHD)				
Yes	215 (74.1)	75 (25.9)	290	0.216

SMBG: Self-monitoring of blood glucose; HTN: Hypertension; IHD: Ischemic heart diseases; CVA: Cerebrovascular accidents; CKD: Chronic kidney disease; PNP: Peripheral neuropathy.

the treatment plan and those patients may be unable to adjust insulin doses according to their daily activities or meals. These results did agree with results found by ACCORD[21].

There was no significant association between marital status and hypoglycemia in our study, a result that agrees with what was found by Bruce *et al*[22] but contradicts the results of Akram *et al*[13] in that being married is a risk factor for hypoglycemia.

Although there is no significant association between residence and hypoglycemia at univariate analysis; a strong negative association does exist at the level of multivariate analysis ($P < 0.05$) which implies that patient from periphery of Basrah (outside the major city of Basrah) experienced hypoglycemia more than patients from Basrah city, this could be explained partially by difficult access to these patients to FDEMC according to their appointments to adjust their insulin regimens and partly because patients living in rural areas may have relatively low education than those living in Basrah city which is found to be significantly associated with hypoglycemia in our study and others[21,23].

No relationship was found in our study between duration of DM and hypoglycemia, the same is found by some studies[14,19,22]. But not in ACCORD[21] which is a large randomized controlled trial that follow-up large number of patients for several years most of them were elderly and have longer duration of diabetes and Akram *et al*[13] who found that the risk of hypoglycemia increased progressively when the duration of diabetes was more than 16 years and United Kingdom Hypoglycemia Study Group[10] who found that risk of hypoglycemia in insulin treated patients increased after 5 years of therapy. The present study is a cross sectional one that investigated retrospectively the experience of hypoglycemia among diabetic patients in the last 3 and 12 mo and more than half of them were diagnosed with diabetes for less than 10 years. Thus the duration of diabetes in the studied patients is relatively short and could not allow the effect of duration to be identified.

In addition, no significant association between types of insulins studied [regular human, premix human (70:30) and NPH] with hypoglycemia. The risk of hypoglycemia is seems to be similar with these types. Akram *et al*[13] and Miller *et al*[24] found that the relationship between type of insulin and risk of severe hypoglycemia is inconsistent.

There is a significant association between the frequency of insulin administration per a day with the experience of hypoglycemia in the preceding 3 mo, which is an established fact in insulin therapy[25].

There is a significant association between presence of IHD and hypoglycemia at the level of univariate analysis ($P < 0.05$) but this association has disappeared at the level of logistic regression. IHD as a part of macrovascular complications of DM is found to be a significant predictor of hypoglycemia[26].

Table 6 Relation of hypoglycemia during the last 3 mo with body mass index, systolic blood pressure; diastolic blood pressure; hemoglobin A1c; serum creatinine; urine for albumin

Variables	Hypoglycemia in the last 3 mo		Total (*n*)	*P* value
	Yes *n* (%)	No *n* (%)		
BMI (kg/m^2)				
Thin or normal (< 25.00)	46 (83.6)	9 (16.4)	55	0.123
Overweight (25.0-29.9)	95 (77.2)	28 (22.8)	123	
Obese (30.00-39.99)	93 (68.9)	42 (31.1)	135	
Morbid obesity (≥ 40)	18 (81.8)	4 (18.2)	22	
Total	252 (75.2)	83 (24.8)	335[1]	
Systolic blood pressure (mmHg)				
Normal (< 130)	91 (79.8)	23 (20.2)	114	0.157
Prehypertension (130-139)	62 (79.5)	16 (20.5)	78	
Stage 1 hypertension (140-159)	76 (71.0)	31 (29.0)	107	
Stage 2 hypertension (≥ 160)	24 (64.9)	13 (35.1)	37	
Total	25 (375.3)	83 (24.7)	336	
Diastolic blood pressure (mmHg)				
Normal (< 80)	63 (78.8)	17 (21.2)	80	0.792
Pre-hypertension (80-89)	148 (74.4)	51 (25.6)	199	
Stage 1 hypertension (90-99)	38 (74.5)	13 (25.5)	51	
Stage 2 hypertension (≥ 100)	4 (66.7)	2 (33.3)	6	
Total	253 (75.3)	83 (24.7)	336	
HbA1c (%)				
< 7.0	10 (83.3)	2 (16.7)	12	0.117
7.0-10.0	136 (79.1)	36 (20.9)	172	
10.1-13.0	85 (73.3)	31 (26.7)	116	
> 13.0	22 (61.1)	14 (38.9)	36	
Total	253 (75.3)	83 (24.7)	336	
Serum creatinine (mg/dL)				
< 0.7	66 (72.5)	25 (27.5)	91	0.632
0.7-1.4	167 (75.9)	53 (24.1)	220	
> 1.4	3 (100.0)	0 (0.0)	3	
Total	236 (75.2)	78 (24.8)	314[2]	
Urine for albumin (positive)	65 (75.6)	21 (24.4)	86	0.947
Total	235 (75.3)	77 (24.7)	312[3]	

[1]BMI had not been measured for one patient due to bilateral lower limb amputation; [2,3]Unequal numbers because some of the patients did not complete their investigations. BMI: Body mass index; HbA1c: Hemoglobin A1c.

Furthermore a significant association between the presence of PNP and risk of hypoglycemia ($P < 0.05$) both at the level of univariate and logistic regression analyses was found. PNP may reflect advanced diabetes and its associated microvascular complications, *e.g.*, autonomic neuropathy. This result agrees with what was found by Miller *et al*[27].

No relation was found between family/social support, vision and mobility with risk of hypoglycemia. These factors were not applied as risk factors in the previously mentioned large randomized controlled trials UKPDS[28], ACCORD[21], VADT[29], United Kingdom Hypoglycemia Study Group[10]. We explored their effect as indicators of severity of diabetes and thus we assumed a patient who needed support and restricted vision and mobility was likely to develop hypoglycemia.

At the level of univariate analysis there is a significant relationship between knowledge of hypoglycemia symptoms and hypoglycemia ($P < 0.05$), while at the level of logistic regression also there is a strong positive association with knowledge of hypoglycemia symptoms ($P < 0.05$), *i.e.,* the more knowledge of hypoglycemic symptoms the more hypoglycemia was reported. Although most patients who experience hypoglycemia have prior knowledge of hypoglycemia symptoms; this knowledge did not protect them from hypoglycemia and this may be due to low education, poor understanding of the importance of adjusting insulin dose and time of injection according to daily activities or the amount and time of meals. Also it may indicate that health education is inadequate, medical practitioners should spent more effort to teach their patients about signs, symptoms, and proper treatment of hypoglycemia, as well has how to prevent it[30].

There is no significant relationship between availability and frequency of SMBG with risk of hypoglycemia; while at the level of logistic regression analysis we found that the availability of SMBG *per se* decreases the risk of hypoglycemia ($P < 0.05$) and frequent use of SMBG associated with more hypoglycemia. Frequent use of SMBG does not protect patients from hypoglycemia nor predict it but probably remind the patient with signals of hypoglycemia, or this may be due to bad storage conditions of the device and strips, high temperature and humidity, absence of hand washing prior to testing. Anyhow, our result agrees with a number of other studies[31-33].

No significant association was found between CVA and amputation with risk of hypoglycemia, same findings

Table 7 Results of logistic regression showing significant predictors of hypoglycemia in the last 3 mo

	B	Sig.	Exp (B)
Significant predictors			
Residence	-0.247	0.030	0.782
Knowledge of hypoglycemia symptoms	1.133	0.044	3.104
Availability of SMBG	-0.599	0.030	0.550
Frequency of SMBG	-0.228	0.031	0.796
PNP	-1.391	0.002	0.249
Diastolic blood pressure	-0.046	0.013	0.955
Systolic blood pressure	0.020	0.053	1.020
HbA1c	0.153	0.021	1.165
Non-significant predictors			
Age	0.002	0.960	1.002
Gender	-0.425	0.200	0.654
Education	0.067	0.505	1.069
Duration of DM	-0.019	0.42	0.981
Frequency of insulin administration/d	-0.381	0.259	0.683
Dose of regular insulin	-0.021	0.215	0.979
Dose of premix insulin	-0.027	0.305	0.974
Dose of NPH	-0.022	0.429	0.979
A total dose of insulin	0.018	0.148	1.018
Mobility	0.117	0.440	1.124
HTN	0.594	0.117	1.811
IHD	-0.758	0.081	0.469
CKD	0.307	0.614	1.359
BMI	0.033	0.208	1.033

SMBG: Self-monitoring of blood glucose; HbA1c: Hemoglobin A1c; HTN: Hypertension; IHD: Ischemic heart diseases; CKD: Chronic kidney disease; PNP: Peripheral neuropathy; BMI: Body mass index; DM: Diabetes mellitus; NPH: Neutral protamine hagedorn.

obtained by other studies in that there is no significant association between macrovascular complications of diabetes including CVA and amputation with risk of hypoglycemia[13,19].

Also no significant association was found between diabetic foot and risk of hypoglycemia, this agree with what is found by other studies which suppose that no significant association between microvascular complications of diabetes and risk of hypoglycemia[14].

Although it is agreed that in advanced kidney diseases, insulin excretion from kidneys will decrease and thus the risk of hypoglycemia will increase[14,27]. No significant association was found between, CKD and risk of hypoglycemia, this looks similar to what is found by other studies[19,24]. There was no significant association between BMI and hypoglycemia, similar results were found by other studies[14,19,24].

No significant association was found between systolic and diastolic blood pressure and risk of hypoglycemia at level of univariate analysis but there is a significant positive association between diastolic blood pressure and risk of hypoglycemia at the level of logistic regression ($P < 0.05$), *i.e.,* as the diastolic blood pressure increase; the risk of hypoglycemia will increase too. Similar results were found by other studies[34]. This association may be related to antihypertensive drugs those patients use, namely the ACE inhibitors, which are suggested to be a risk factor for hypoglycemia[35].

Although no significant association was found between HbA1c and hypoglycemia at the level of univariate analysis; there was a strong negative association, *i.e.,* the lower the HbA1c; the more the risk of hypoglycemia, this is consistent with what is found by several large studies[21,28]. In that intensive glycemic control and HbA1c goal < 7 is associated with increased risk of hypoglycemia (both major and minor).

Taking the results as a whole, particularly the logistic regression analysis, the only residence (rural), knowledge of hypoglycemia symptoms, availability and increasing frequency of SMBG, the presence of the PNP, high diastolic blood pressure and low HbA1c were significant and independent predictors of hypoglycemia. All other studied variables were not predictors.

Limitations of the study

Although every patient entering this center (FDEMC) on the day of the interview was checked to see if he or she met the inclusion criteria; selection bias cannot be excluded. Another limitation is that a small proportion of patients did not complete their investigations regarding fasting glucose (12.5%), random glucose (12.3%), serum creatinine (6.5%), urine for albumin (7.1%) measurement.

In conclusion, hypoglycemia is very common among insulin treated patients with T2DM Basrah. It was possible to identify a number of important predictors of hypoglycemia.

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COMMENTS

Background

Hypoglycemia is one the important barrier for initiating and continuing insulin therapy in type 2 diabetes (T2DM) for patients and doctors. Overcoming this barrier will be fundamental to start insulin at earlier stage.

Research frontiers

Basrah is one the largest city in Iraq. Data on the hypoglycemia frequency is lacking in Iraq and this city. This study will start to give baseline hypoglycemia frequency in insulin treated patients with T2DM.

Innovations and breakthroughs

This study showed that some form of hypoglycemia accord in more than three quarter of patients with T2DM treated with insulin. The important predictors of hypoglycemia were residence (rural), knowledge of hypoglycemia symptoms, availability and increasing frequency of self-monitoring blood glucose, the presence of the peripheral neuropathy, high diastolic blood pressure and low hemoglobin A1c (HbA1c).

Applications

This study provided for the first time data on the frequency of hypoglycemia for the first time in Basrah (Southern Iraq), which seems to be very common.

Terminology

Hypoglycemia is state of low blood glucose that ranges from mild that can be

self-treated to severe which the need help by the others including the hospital. It can be symptomatic or a symptomatic, documented by blood glucose estimation or not and nocturnal or daytime.

Peer-review

This paper is well written and the information that contains is a useful tool for physiology and the correlation between miRNAs and impaired fracture healing.

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Response to comment on: Statin use and risk of diabetes mellitus

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Abstract

In letter to the editor "Comment on: Statin use and risk of diabetes mellitus" authors found the statement "pravastatin 40 mg/d reduced the risk of diabetes by 30% in West of Scotland Coronary Prevention study" erroneous. As per our opinion the statement is right but had been referenced incorrectly.

Key words: Pravastatin; Diabetes mellitus

Core tip: The statement "Pravastatin has shown to decrease the risk of developing diabetes by 30%" is correct and had been wrongly referenced in "Statin use and risk of diabetes mellitus" by Chogtu *et al*.

Chogtu B, Magazine R, Bairy KL. Response to comment on: Statin use and risk of diabetes mellitus. *World J Diabetes* 2016; 7(18): 481-482 Available from: URL: http://www.wjgnet.com/1948-9358/full/v7/i18/481.htm DOI: http://dx.doi.org/10.4239/wjd.v7.i18.481

TO THE EDITOR

We thank Eren *et al* for showing interest in the review article "statin use and risk of diabetes mellitus"[1]. Eren *et al*[2] have pointed to an apparent factual error in the following statement: "pravastatin 40 mg/d reduced the risk of diabetes by 30% in West of Scotland Coronary Prevention study (WOSCOPS)". However, in our opinion, this statement is correct if we refer to WOSCOPS 2001, in which authors have put forth that pravastatin in a dose of 40 mg/d resulted in a 30% reduction (P = 0.042) in the risk of diabetes[3]. Haffner[4] in an editorial in the same issue of Circulation has also alluded to the fact that Pravastatin reduced incidence of diabetes by 30% - though with a caveat that these results should be cautiously interpreted as the statistical significance in WOSCOPS 2001 was modest.

Regarding the review article "Statin use and risk of diabetes mellitus" the error is not in the statement but in quoting the reference. We should have referenced it as Freeman *et al*[3] rather than Kotseva *et al*[5]. We again thank Eren *et al*[2] to bring this error to our notice.

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