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

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EDITORIAL

Long live the Liver!

Nishant Raizada[1] · S. V. Madhu[1]

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As the new-fangled COVID-19 pandemic begins to recede, the spectre of the old and familiar pandemic of obesity looms large once again. Of the many diseases, obesity predisposes to one of the most sinister albeit silent is non-alcoholic fatty liver disease.

The recent worldwide prevalence estimates of NAFLD are alarming to say the least. A meta-analysis published in July 2022 estimates the NAFLD worldwide prevalence to be 32.4% with a suggestion of a rapid increase in the past decade [1]. The numbers in patients with diabetes are even more unsettling. The NAFLD prevalence in patients living with type 2 diabetes mellitus is more than twice that of the general population. A 2019 meta-analysis pegs the prevalence of NAFLD in type 2 diabetes mellitus to be 55.5% [2].

Closer home, while the data may not be that extensive, the numbers here are far from reassuring. Data on overall prevalence of NAFLD in India is scanty—small studies suggest that the prevalence ranges between 8 and 32% [3]. Notably, most of these studies are from the past decade and do not take into account the temporal effects observed in the Western data. Hence, it may be wise to assume a much higher current prevalence of NAFLD.

In a recently published study from India, a review of electronic medical records of more than 1.5 lakh patients with diabetes suggests that 44.48% of them have NAFLD, the prevalence being close to 60% for males [4].

The link between NAFLD and diabetes appears to be sinister. Insulin resistance is proposed to be a major player in the pathogenesis of NAFLD. Increase in lipolysis, de novo triglyceride synthesis, hepatic triglyceride uptake and accumulation are consequences of insulin resistance which promote NAFLD. Features of metabolic syndrome (MS) are present in most patients with NAFLD—some authors refer to NAFLD as the hepatic manifestation of MS [3]. The close association between NAFLD and other metabolic factors explains the high prevalence of NAFLD in diabetes.

It is however interesting to study which type 2 diabetes patients are likely to develop NAFLD. In a study from Brazil, patients with diabetes and NAFLD had more obesity, waist circumference, hypertriglyceridemia and elevated alanine transaminases as compared to those without NAFLD [5]. A large data set from Italy suggests that patients with diabetes and NAFLD are likely to be older and have higher age adjusted prevalence of macrovascular complications when compared with those with diabetes but without NAFLD [6]. Interestingly, a small study from Sri Lanka found that patients of diabetes who had NAFLD were younger than those without NAFLD. Increased BMI, waist circumference and transaminitis were present in those with NAFLD [7]. Among patients with diabetes with normal aminotransferases, those with NAFLD had worse HbA1c and higher insulin resistance than those without NAFLD [8].

In this issue, Ayaz et al. [9] studied diabetes patients with and without hepatic steatosis. Surprisingly, they found that glycemic status was not significantly different between the two groups. Previous studies have reported positive correlation between HbA1c and NAFLD in both diabetes patients and those without diabetes [10, 11]. This discrepancy probably hints at the now well-known concept of type 2 diabetes being a heterogeneous group of disorders rather than a single disease. Novel subtypes of type 2 diabetes have now been identified. Some of these subtypes have less insulin resistance and more decrements in insulin secretion. If insulin resistance is the underlying cause of NAFLD, the insulin deficient subtypes may not share the burden of NAFLD equally. More studies on subtypes of diabetes and NAFLD would be informative.

Keeping the heterogeneity of diabetes in mind, racial factors are important in NAFLD as well. Asian Indians can have nearly two times higher hepatic fat for the same BMI when compared with Caucasians [12]. Petersen et al.similarly reported two times higher hepatic triglyceride content and IL-6 levels in Asian Indians as compared to Caucasians [13]. This suggests that in our country, type 2 diabetes

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mellitus with NAFLD would be a common clinical scenario. Not only do we, as physicians involved in diabetes care, need to understand this in detail to better manage our patients, but there is also a need to further characterize our diabetes clusters to see if those with NAFLD emerge as a unique cluster with distinct pathogenesis, natural history and prognosis.

Other factors in the pathogenesis of NAFLD also need to be explored. Depletion of antioxidants such as vitamin E, vitamin A and glutathione may lead to hepatic inflammation. In this issue, Saber et al. demonstrate that in an animal model of NAFLD, the levels of vitamin A, vitamin E and selenium reduce significantly as compared to controls. Other data has looked at role of hormones, such as leptin and adiponectin, and incretins as well as intestinal microbes and bile acids in pathophysiology of NAFLD. These and other studies on pathophysiology are important as they provide vital clues on developing therapeutic options for NAFLD. Although the mainstay of NAFLD therapy continues to be lifestyle intervention and weight reduction, several pharmacotherapeutic measures are now available. Vitamin E has shown promise in therapy of non-alcoholic steatohepatitis (NASH) in patients without diabetes [14]. Similarly, biopsy-proven NASH appears to respond well to pioglitazones [15]. These pharmacotherapeutic measures have been shown to be beneficial in biopsy-proven NASH, but their utility in other milder forms of NAFLD has yet to be demonstrated. Metformin, omega-e fatty acids, obeticholic acid and ursodeoxycholic acids are other drugs which have shown possible benefit [16]. More recently, data on potential benefits of diabetes medications including SGLT-2 inhibitors and GLP-1 analogues has emerged [16]. As these drugs cause significant weight reduction and improve glycemic control, these findings were not unexpected. Although this data is still evolving, semaglutide has shown improvement in cases of biopsy-proven NASH [17]. While conclusive evidence is awaited, it is prudent to prioritize these drugs in the treatment of patients with co-existent diabetes mellitus and NAFLD.

The silent nature of NAFLD often results in the disease being ignored in its initial stages. However, once inflammation progresses, this disorder can lead to serious morbidity as well as mortality. While on one hand efforts to elucidate the pathophysiology and management aspects of NAFLD need to be expedited, the benefits of the same cannot be harnessed unless the awareness of this disorder grows.

Healthcare providers at all levels need to be sensitized about the looming threat on the liver so that appropriate advice and screening can be provided to patients. Keeping obesity in check, eating a healthy diet and exercising regularly can have myriad benefits including reduced risk of NAFLD. As is true for other lifestyle disorders, for NAFLD, prevention would be much better than cure.

References

1. Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan GG, Shaheen A-A. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7:851–61.
2. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A, Nader F. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol. 2019;71:793–801.
3. Duseja A, Singh SP, Saraswat VA, Acharya SK, Chawla YK, Chowdhury S, Dhiman RK, Jayakumar RV, Madan K, Misra SP, Mishra H, Modi SK, Muruganathan A, Saboo B, Sahay R, Upadhyay R. Non-alcoholic fatty liver disease and metabolic syndrome—position paper of the Indian National Association for the Study of the Liver, Endocrine Society of India, Indian College of Cardiology and Indian Society of Gastroenterology. J Clin Exp Hepatol. 2015;5:51–68.
4. Kalra S, Das AK, Tiwaskar M, Vg MP, Singh M. Assessment of prevalence and associated risk factors of NAFLD in people living with diabetes in India: a retrospective, multicenter, electronic medical records based study. J Assoc Physicians India. 2022;70:11–2.
5. Leite NC, Salles GF, Araujo ALE, Villela-Nogueira CA, Cardoso CRL. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int. 2009;29:113–9.
6. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, Day C, Arcaro G. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30:1212–8.
7. Herath HMM, Kodikara I, Weerarathna TP, Liyanage G. Prevalence and associations of non-alcoholic fatty liver disease (NAFLD) in Sri Lankan patients with type 2 diabetes: a single center study. Diabetes Metab Syndr Clin Res Rev. 2019;13:246–50.
8. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, Subbarayan S, Webb A, Hecht J, Cusi K. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. J Clin Endocrinol Metab. 2015;100:2231–8.
9. Ayaz T, Polat HB, Kilictas B. Evaluation of the reciprocal interaction between hepatic steatosis and type 2 diabetes: a comparative analysis with respect to anti-diabetic treatment, glycemic control, renal and hepatic function. Int J Diabetes in Developing Countries. 2021. https://doi.org/10.1007/s13410-021-01009-4.
10. Masroor M, Haque Z. HbA1C as a biomarker of non-alcoholic fatty liver disease: comparison with anthropometric parameters. J Clin Transl Hepatol. 2021;9:15–21.
11. Kanwal S, Ghaffar T, Aamir AH, Usman K. Frequency of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus and its associated risk factors. Pak J Med Sci. 2021;37:1335–41.
12. Luthra A, Nigam P, Misra A. Metabolic correlation and management strategies of non-alcoholic fatty liver disease: an Asian Indian perspective. Diabetes Metab Syndr Clin Res Rev. 2007;1:279–85.
13. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla Man C, Cobelli C, Shulman GI. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. Proc Natl Acad Sci U S A. 2006;103:18273–7.
14. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia

J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR, NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362:1675–85.
15. Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. Ann Intern Med. 2016;165:305–15.
16. Kanwal F, Shubrook JH, Younossi Z, Natarajan Y, Bugianesi E, Rinella ME, Harrison SA, Mantzoros C, Pfotenhauer K, Klein S, Eckel RH, Kruger D, El-Serag H, Cusi K. Preparing for the NASH epidemic: a call to action. Diabetes Care. 2021;44:2162–72.
17. Newsome PN, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, Sanyal AJ, Sejling A-S, Harrison SA, NN9931-4296 Investigators. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. N Engl J Med. 2021;384:1113–24.

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REVIEW ARTICLE

A systematic review of diabetes risk assessment tools in sub-Saharan Africa

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Abstract

Objectives To systematically review all current studies on diabetes risk assessment tools used in sub-Saharan Africa (SSA) to diagnose diabetes in symptomatic and asymptomatic patients.

Methods Tools were identified through a systematic search of PubMed, Ovid, Google Scholar, and the Cochrane Library for articles published from January 2010 to January 2020. The search included articles reporting the use of diabetes risk assessment tool to detect individuals with type 2 diabetes in SSA. A standardized protocol was used for data extraction (registry #177726).

Results Of the 825 articles identified, 39 articles met the inclusion criteria, and three articles reported tools used in SSA population but developed for the Western population. None was validated in SSA population. All but three articles were observational studies (136 and 58,657 study participants aged between the ages of 15 and 85 years). The Finnish Medical Association risk tool, World Health Organization (WHO) STEPS instrument, General Practice Physical Activity Questionnaire (GPPAQ), Rapid Eating and Activity Assessment for Patients (REAP), and an anthropometric tool were the most frequently used non-invasive tools in SSA. The accuracy of the tools was measured using sensitivity, specificity, or area under the receiver operating curve. The anthropometric predictor variables identified included age, body mass index, waist circumference, positive family of diabetes, and activity levels.

Conclusions This systematic review demonstrated a paucity of validated diabetes risk assessment tools for SSA. There remains a need for the development and validation of a tool for the rapid identification of diabetes for targeted interventions.

Keywords Type 2 diabetes · Validation · Diabetes risk assessment · Africa · Sub-Saharan region · Risk factors

Introduction

Diabetes mellitus (DM) is a major public health problem globally as the prevalence and burden are uncontrolled in urban areas due to significant lifestyle choices [1, 2]. Globally, it was reported in 2016 that more than 422 million adults were living with diabetes and estimated to increase by 55% to over 591.9 million by 2035 [3]. Three-quarters of those with diabetes live

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in low- to middle-income countries (LMICs), and this is projected to increase [4] with a median prevalence estimate at 5% in sub-Saharan Africa [5].

Due to increasing urbanization, demographics, and nutritional changes in the region [6–8], compounded by a lack of awareness of the lifestyle risk factors for diabetes, inadequate healthcare infrastructure, and lack of access to quality healthcare on the sub-continent [2, 9], the prevalence of diabetes is predicted to increase from 4.85% in 2013 to 5.35% in 2035 [10]. In addition, the proportion of adults aged 20–79 years with undiagnosed diabetes was estimated to be 90% of the diabetic population in LMICs compared with 33% in high-incom`e countries [10]. The burden of the disease is evident and keeps increasing [2, 9].

Diabetes has become one of four prioritized non-communicable diseases (NCDs) by the World Health Organization (WHO) [11]. The projected increase in the prevalence of diabetes mellitus (DM) in SSA will exert considerable economic and human resource costs on the healthcare system that is already lacking in funding and trained human resource [12, 13]. To mitigate this effect, there is a need for an effective, non-invasive screening tool for DM in this region such as the diabetes risk assessment tools which are convenient for early screening and detection of diabetes to avoid diabetes-related morbidity, reduce the cost of healthcare, and improve quality of life [11).

Screening for diabetes in general practice by measuring fasting blood glucose levels is feasible, but expensive, invasive, and time-consuming. This could be more efficient if targeted at high-risk patients [14]. Different strategies have been suggested to improve diabetes detection including opportunistic screening and population-based screening [15].

Over the last decade, many diabetes risk assessment tools used for identifying previously undiagnosed diabetes and individuals at high risk of diabetes have been developed and validated in various countries. However, these tools were developed for different population groups using both community- and population-based studies [16–27], predominantly among Caucasian [20, 28, 29]Biostatistics, Asian [18, 21, 22, 30, 31], and Middle Eastern [32, 33] populations. The tools identified age, sex, obesity, family history of diabetes, and hypertension as the most common factors associated with diabetes [18, 20–22, 28–30, 32, 33]. In a population-based systematic review conducted using 5 qualitative analysis tools (two each from Mexico and Peru and another from Brazil), researchers found that the area under the curve (AUC) ranged from 66 to 72% and recommended the use of different diabetes risk scores for the Latin American populations [34].

In Africa, there is paucity of data on the use of non-invasive tools for diabetes risk assessment despite the growing population and prevalence of diabetes. For example, in a population-based study conducted in Egypt on 1032 individuals without a history of diabetes, the authors found that a predictive model could easily detect undiagnosed diabetes [35]. Another study conducted on 3094 Mauritian Indians using risk prediction models found that the AUC was 62% for men and 64% for women [36]. In Ogun State, Nigeria, using a community-based diabetes risk assessment tool on 56,567 individuals, authors found higher risk scores for diabetes and a higher rate of obesity among females than males. A study by Alebiosu et al. suggested that current evidence should be examined in order to implement diabetes preventative strategies [37]. Overall, these studies [35–37] are in agreement that diabetes assessment tools are limited to the population for which they were developed, and when used in different populations including among SSAs, their validity could be affected resulting in an inferior predictive model.

Therefore, this study was designed to provide evidence on the availability and use of diabetes risk assessment tools in SSA by systematically reviewing all current studies on diabetes risk assessment tools within the region. This was supported by reviewing studies conducted outside SSA countries on diabetes risk assessment tools so that the findings are generalizable to a wider population. Identifying the diabetes risk assessment tool models available in the SSA region would be valuable at the primary care level, for clinicians and public health workers to facilitate early detection of DM among those who are unaware of their status. Also, the study will provide evidence of the risk factors and diabetes risk scores that could be further studied in different SSA countries or integrated into the guidelines for policy-makers as a standard of practice for diabetes screening at the population level. Findings can also be used to develop diabetes prevention and education programs across SSA communities.

Research design and methods

This study was conducted in conformance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement on reporting items for systematic reviews and meta-analyses [38] following a protocol for this systematic review which was registered in Prospero (registry #177726). The search was performed in the following databases: PubMed, Ovid, Google Scholar, and the Cochrane Library. The databases were searched and articles published from January 2000 to January 2020 were included and no language limits were applied. A literature search strategy was developed and implemented using the Population, Intervention, Comparison, Outcome, and Study (PICOS) framework, as shown in Table 1. The search goals were first to identify studies on the development of tools for non-invasive diabetes risk assessment in Africa, and then to expand that search to other continents. Search terms included diabetes risk assessment tools, diabetes mellitus risk assessment

Table 1 The search strategy for literature selection

PICOS	Description
Population	Indigenous African population aged 15 years and over
Intervention	Application of diabetes risk assessment tools
Comparison	Comparison of DM risk tools to other previously validated tools
Outcome	Accuracy levels of risk assessment tools
Study	All studies including but not limited to clinical trials, cohort, case-control, cross sectional, and reviews

tools, diabetes risk assessment tools in Africa, "diabetes" AND "risk assessment tools" AND Africa.

Search strategy and selection criteria

Two experienced reviewers, EE and GO, independently carried out the searches on two separate dates. PubMed and Ovid databases were searched on January 13, 2020, while Cochrane Library was searched on January 15, 2020. Both reviewers used the same predefined search terms as detailed in the Supplementary file (S1). The search hits were then manually screened for relevance and collated for more detailed scrutiny using predefined inclusion and exclusion criteria. All articles selected by both reviewers were collated for data extraction. A third experienced reviewer, KO, adjudicated disputed articles. The full electronic search strategy for PubMed database, including the limits used, is presented (S1).

Data extraction and quality assessment

A total of 825 articles were selected for review of the abstracts, and 786 articles were excluded after duplicate removal and after applying the inclusion and exclusion criteria below. Data were extracted after assessing the quality of the studies by using Cochrane collaboration's tool for assessing risk of bias in publications. The following data were extracted from each study where available: authors, year of publication, number of study participants, the location, mean age and/or age range of study participants, sample size, predictor variables (age, body mass index (BMI), waist circumference, waist to height ratio), diabetes risk assessment tools used, accuracy level of the tools and/or risk of developing diabetes, and tool validation.

The article selection process is presented in the PRISMA flow chart (Figure 1). For synthesis of results, qualitative description of data was performed using area under the receiver operating curve (AUC), sensitivity, specificity, positive predictive value, negative predictive value, and odds ratios.

Inclusion and exclusion criteria

Eligible articles for inclusion were those that reported on the following: individuals 15 years and older; quantitative scores of predictive models; diabetes risk assessment tools developed and used in an African population; validated tools for assessing risk factors for diabetes in an African population; and on specific tools developed for assessing diabetes risk factors in an African population.

Exclusion criteria included articles that reported on validated tools for assessing risk factors for diseases other than DM; on health topics other than DM; and on invasive diabetes risk assessment tools. In addition to those included, articles were also selected and grouped if they included information on diabetes risk assessment tools developed for non-African populations. The same exclusion criteria were adopted but these articles were considered separately from the African studies, only if they met the inclusion criteria.

Results

Diabetic risk assessment tools in Africa

Thirty-nine articles met the inclusion criteria for a full review. Only three of those articles were from sub-Saharan Africa [37, 39, 40] and involved the use of non-invasive diabetes risk assessment tools in local populations (Table 2). The quality of the data in each of the studies conducted in SSA countries is presented in Table 2.

In the current review, the age range of participants in the studies that included Africans was 18–62 years and the sample size ranged from 136 to 58,657 persons. All the studies were observational. The most common non-invasive predictor variables used in the three studies on the African population were age, body mass index (BMI), waist circumference, family history of diabetes, and activity levels. Others include vegetable consumption, waist-to-height ratio (WHtR), and history of use of antihypertensive agents.

Type of tools and tool accuracy

Five different tools were used in the selected studies including the Finish Medical Association DM risk tool, WHO STEPS instrument, General Practice Physical Activity Questionnaire (GPPAQ), Rapid Eating and Activity Assessment for Patients (REAP), and an anthropometric tool. The accuracy of disease

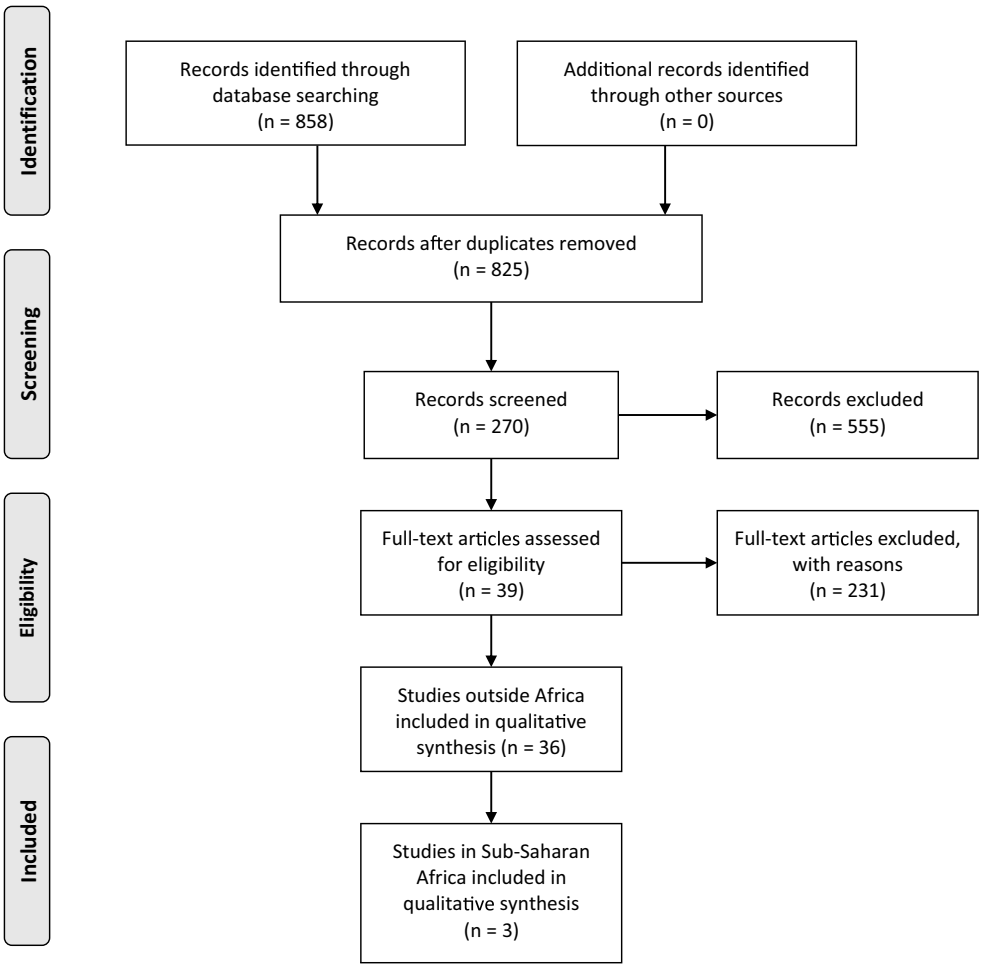

Fig. 1 Flow chart of the article selection process. Of the 42 studies that met the inclusion criteria, only three were from sub-Saharan Africa

assessment tools was measured in terms of sensitivity, specificity, or as the area under the receiver operating curve (AUC). Among residents in Nigeria, one study [40] reported that the predictor WHtR has the highest AUC for predicting two or more cardiometabolic syndrome (CMS) in women (0.70); BMI has the highest AUC for predicting two or more CMS in men, and another [37] measured the risk of developing DM in 10 years using logistic regression and found a mean risk score of 5.60 ± 3.90 for developing DM for their sample population. Among Ghanaians, Gudjinu and Sarfo [39] found total cholesterol greater than 6.3, REAP score greater than 51, BMI greater than 35 kg/m^2, GPPAQ score for inactivity, serving of fruit per meal less than one, and middle socio-economic status (SES) to be predictors with the highest odds ratios for developing DM. None of the studies reported validation of the tools used for the study population.

Diabetic risk assessment tools outside Africa

Given the few studies in Africa, in addition, we reviewed the 36 articles that met our criteria but were outside of SSA. This was done to broaden the understanding and the depth of interpretation of the African tools since the tools used in the African studies have been used previously in non-African populations. The continents covered include North America, South America, Europe, Asia, and Australia and two studies from the Middle East Region [54, 55]. The age range of subjects was between 15 and 85 years with a sample size ranging from 44 to 15,768 participants. The most used predictor variables common to all studies were anthropometric variables including BMI, WHtR, and waist circumference (WC), as well as age and family history. Other less commonly used variables were visceral adiposity index (VAI), body adiposity index (BAI), triglyceride (TG) level, smoking, ethnicity, sedentary lifestyle, and hypertension.

Type of tools and accuracy

Twenty different tools were used in seven studies but the anthropometric and Finnish diabetes risk assessment tools accounted for about 40% of all tools. Others used locally adapted questionnaires and compared accuracy results with published diabetic risk tools [18, 22, 49, 51, 54, 59, 65]. Most (69.2%) of the articles reviewed use AUC to report the

Table 2 Characteristics of studies included in the review

Author	Age (years)	*N*	Predictors	Tools	Tool accuracy	Validation
Studies conducted in sub-Saharan Africa						
Alebiosu et al. 2013 [37]	25–54	58,657	Age, BMI, waist circumference below ribs, exercise, vegetable consumption, use of anti-hypertensives, previous record of high blood sugar level, family history	Finnish Medical Association DM risk tool	5.05 % of study sample had DM. Mean risk score 5.60 ± 3.90. 5.05% had high risk of developing DM in 10 years. 15% of study population had moderate to very high risk of developing DM in 10 years	No
Gudjinu et al. 2017 () 45	35–62	136	Rapid eating and activity level, weight, height, waist circumference, fasting venous blood, fasting lipids	WHO STEPS instrument, GPPAQ and REAP	Risk factors with the highest risk scores of developing DM: Total cholest > 6.3 = 10.67; REAP score > 51 = 7.34; BMI > 35 = 6.06 GPPAQ score for inactivity = 7.3; serving per eating < 1 = 5.76; middle SES = 5.03	No
Oguoma et al. 2016 [40]	18–59	422	Hyperglycemia, HTN, hypertriglyceridemia, LDL-HDL	Anthropometric tools	WHtR highest AUC for predicting ≥ 2 CMS in women (0.701); BMI highest AUC for predicting ≥ 2 CMS in men (value)	No
Studies conducted outside of sub-Saharan Africa						
Skogberg et al. 2017 [41]	30–64	917	BMI, WHtR, WC, and WHR	Anthropometric	0.81 AUC	No
Agarwal et al. 2019 [42]	36–69	200		Finnish Diabetes Risk Score (FINDRISC), Canadian diabetes risk questionnaire (CANRISK), Indian Diabetes Risk Score (IDRS), American Diabetes Association (ADA) risk score, undiagnosed diabetes mellitus, Filipino	Filipino tool: highest specificity (0.73); IDRS and undiagnosed DM highest NPV (0.96); highest AUC (FINDRISK, CANRISK) 0.8; overall FINDRISK more effective with sensitivity of 0.96	Yes
Elizalde-Barrera et al. 2019 [43]	47.14	280	BMI, WHtR, VAI, BAI, and TG	Anthropometric	The measurements with the highest area under the curve were TG (0.631, 95% confidence interval [CI] 0.566–0.697), VAI (0.628, 95% CI 0.563–0.693), and WHtR (0.622, 95% CI 0.557–0.688), and in the adjusted binary logistic regression model, were found to be independently associated with impaired fasting glucose (IFG), odds ratio of 2.665, (95% CI 1.567–4.533) 2.567 (95% CI 1.527–4.317) and 2.171 (95% CI 1.102–4.276)	No
McGrath et al. 2018 [44]	65.3 ± 10.5	278		HARP		No
Rauh et al. 2018 [45]	28–85	3544	Age, BMI, waist circumference, use of anti-hypertensives, smoking, family history of myocardial infarction/ stroke, and family history of diabetes	Anthropometric	AUC of 0.78 (95% CI 0.75–0.81) in men and 0.78 (95% CI 0.74–0.81) in women. Calibration was poor (HL statistic: p <0.001) but improved considerably after intercept recalibration. Examination of individual outcomes showed that in men, AUC was highest for CKD (0.85 [95% CI 0.78–0.91]) and lowest for T2D (0.69	Yes

Table 2 (continued)

Author	Age (years)	*N*	Predictors	Tools	Tool accuracy	Validation
					[95% CI 0.65–0.74]). In women, AUC was highest for CVD (0.88 [95% CI 0.83–0.94]) and lowest for T2D (0.71 [95% CI 0.66–0.75]).	
Zhang et al. 2017 [46]	< 30–60	15768	Drinking tea frequently, body mass index ≥28.0 kg/m^2, waist to height ratio ≥ 0.5, triglycerides level 1.70 to 2.25 and ≥2.26 mmol/L, and fasting plasma glucose 5.6 to 6.0 and ≥6.1 mmol/L	Cox proportional hazard score	The sensitivity, specificity, and AUC (95% confidence interval) for this full model were 69.63%, 75.56%, and 0.791 (0.783–0.799), respectively.	Yes
Bould et al. 2017 [47]	> 45	1035		FINDRISC		No
Liu et al. 2016 (48)	≥ 55	1857	Impaired FPG, poor self-assessment of health, overweight, obesity, and reduced physical activity	Anthropometric;	AUC was 0.76 (95% confidence interval: 0.72–0.80), and the optimism-corrected AUC was 0.78 (95% confidence interval: 0.69–0.87)	Yes
Khunti et al. 2016 [49]	40–75	577		Leicester Practice Computer Risk Score (LPCRS); Leicester Self-Assessment Score (LSAS)		No
Dugee et al. 2015 [31]	15–64	1018		FINDRISK; Rotterdam risk score	AUC for FINDRISK= 61, Rotterdam = 64; adapted risk score = 77 (95% CI: 71–88%).	No
Robinson et al. 2011 [50]	40–74	6223		CANRISK; FINDRISK	AUC CANRISK = 0.75; FINDRISK = 0.69	Yes
Xie et al. 2010 (51)	35–74	15540		Self-administered tool	Sensitivity of 0.61 (95% CI 0.55 to 0.67), a specificity of 0.71 (95% CI 0.70 to 0.73) in women, and a DRL of 3 or greater predicted type 2 diabetes status with a sensitivity of 0.59 (95% CI 0.52 to 0.65) and a specificity of 0.63 (95% CI 0.62 to 0.65) in men.	Yes
Chen et al. 2010 [25]	≥ 25	6,060		AUSDRISK	The AUROC of the diabetes risk tool was 0.78 (95% CI, 0.76–0.81) and HL chi^2 statistic was 4.1 (p = 0.85). Using a score > or = 12 (maximum, 35), the sensitivity, specificity, and positive predictive value for identifying incident diabetes were 74.0%, 67.7%, and 12.7%, respectively. The AROC and HL chi^2 statistic in the two independent validation cohorts were 0.66 (95% CI, 0.60–0.71) and 9.2 (p = 0.32), and 0.79 (95% CI, 0.72–0.86) and 29.4 (p < 0.001), respectively.	Yes
Otero et al. 2011 [52]	30–74	44	BMI, WC, physical exercise, consumption of fruits, and vegetables, HTN, hx of high blood sugar, smoking	CANRISK	CANRISK tool identified 11·4% of the sample to be at high risk, 9·1 at moderate risk, and 43·2% at slightly elevated risk for developing DM.	Yes
Guo et al. 2018 [53]	35–74	194	Height, weight, and Waist circumference	CHINARISK (adapted CANRISK) FINDRISK	AUC 0.705 (95% CI 0.632, 0.778), demonstrating moderate diagnostic value at a cut-off score of 30. The sensitivity was 73%,	Yes

Table 2 (continued)

Author	Age (years)	*N*	Predictors	Tools	Tool accuracy	Validation
					with a positive predictive value of 57% and negative predictive value of 78%.	
Carrillo-Larco 2019 [34]	42–50	711–6995	Age, waist circumference, and family history of diabetes, and only one study used oral glucose tolerance test as the outcome	FINDRISK, simplified FINDRISK, Latin America FINDRISK, Peruvian Risk score	AUC 66–72	9 models were validated
Al-Lawati and Tuomilheto, 2007 [54]	≥ 20	4881 and 1432	Strongest predictors (age > 60years, +ve family history of diabetes); moderate predictors (WC, BMI, current hypertension status)	Developed the model for diabetes risk assessment of Oman using two existing data sets	Sensitivity = 78.6%, Specificity = 73.4%; AUC = 83% (for cohort 1); 62.8%; 78.2%; 76% (for cohort 2)	Yes
Al-Khalaf et al. 2010 [55]	36.2 ± 8.9	562	Age ≥ 35 years (3.72), WC ≥ 100 cm (6.89), BP med (2.66), +ve Fam Hx (2.66)	Blood glucose, anthropometric, self-administered questionnaire	AUC = 0.82; NPV = 99%	No
de Leon et al. 2008 [56}	18–75	6237	Age, waist/height ratio, +ve family history of diabetes, systolic blood pressure	Blood glucose, anthropometric, self-administered questionnaire	AUC = 0.837 (M), 0.874 (F), sensitivity = 84.2%, specificity = 39.8%; PPV = 17.2% (M), 15.3% (F)	Yes
Gao et al. 2010 [18]	20–74	1986/4336	Age, WC, +ve FHx	Lifestyle questionnaire with validation and anthropometric measurement	AUC = 62.4 (M), 63.2 (F); sensitivity = 72.5%; specificity = 60.1%; PPV = 17%; NPV = 95.1%	Yes
Mohan et al. 2005 [22]	>30	2600	Age, abdominal obesity, +ve FHx, physical activity	OGTT, lifestyle questionnaire, anthropometric measurement	AUC = 69.8%, 61.3%; sensitivity = 72%; specificity = 56%; PPV = 6.5%; NPV = 98%	Yes
Bang et al. 2009 (17)	≥ 20	5258	Age, sex, + FHx, HTN, obesity, physical activity	National Health and Nutrition Examination Survey	AUC = 0.83	Yes
Bindraban et al. 2008 [57]	35–60	336, 593, 486 respec-tively	Age, BMI, WC, resting HRt, + FHx, HTN, Hx of CVD, ethnicity	National Health and Nutrition Examination Survey	Sensitivity = 76%; specificity = 72%	No
Glumer et al. 2004 [58]e	30–60	6784	Age, sex, BMI, HTN, physical activity, + FHx	Diabetic symptoms and risk factors questionnaire with validation and OGTT	AUC = 0.804; sensitivity = 81%; specificity = 45%	Yes
Gray et al. 2010 [20]	40–75	6390	Age, sex, ethnicity, + FHx, WC, BMI, HTN, BP drugs	UK screening study, OGTT	AUC = 0.72; sensitivity = 77%; specificity = 72%; PPV = 11.3%; NPV = 98.2%; PLR = 2.76, NLR = 0.32	Yes
Griffin et al. 2000 [59]	40–64	1077	Age, sex, BMI, steroids medication, BP drugs, + FHx, Smoking Hx	Demographic data and medical records form general practices, lifestyle questionnaire, OGTT, anthropometric measures	AUC = 0.80; sensitivity = 88% (DM), 75% (pre-DM); specificity = 75% (DM), 65% (pre-DM); PPV = 14% (M), 49% (pre-DM); NPV = 99.3% (DM), 85% (pre-DM)	Yes
Heikes et al. 2008 [60]	≥ 20		Age, WC, gest DM, ht, ethnicity, HTN, + FHx, exercise	National Health and Nutrition Examination Survey	AUC = 0.85 (DM), 0.75 (pre-DM)	Yes
Li et al. 2009 [61]		771	Age, BMI, Hx Bld glc	FINDRISC	AUC = 0.88 (95% CI = 0.85–0.92) for continuous predictors, 0.86 (95% CI = 0.82–0.90) for categorical predictors; sensitivity = 78.5%; specificity = 62.5%; PPV = 18.3%; NPV = 96.4%	Yes

Table 2 (continued)

Author	Age (years)	*N*	Predictors	Tools	Tool accuracy	Validation
Pires da Sousa et al. 2009 [62]	≥ 35	1224	Age, BMI, abdominal circumf, HTN, Tot Chol, LDL, TG	Anthropometric measures	AUC = 77%; sensitivity = 66%; specificity = 70%; PPV = 31%; NPV = 39%	Yes
Saaristo et al. 2005 [63]	45–74	4622	Undiagnosed DM2, AGT, metabolic syndrome, and CV risk factors	FINDRISC	AUC = 72% (M), 73% (F); sensitivity = 73%; specificity = 56%; PLR = 1.6	No
Chaturvedi et al. 2008 [64]	35–64	4044	Age, WC, BP, +FHx,	Lifestyle questionnaire with validation; anthropometric measures,	Sensitivity = 83.3%; specificity = 65.5%; PPV = 12.83%; NPV = 98.53%	Yes
Dong et al. 2011 [30]	35–74	5348	Age, BMI, WtHR, syst/diast BP, HRt, +Fhx	OGTT and lifestyle questionnaire with validation	Sensitivity = 96.8%; specificity = 24%; PPV = 17.8%; NPV = 97.8%	Yes
Keesukphan et al. 2007 [65]	48.4 ± 10.9	429	Age, BMI, HTN	Lifestyle questionnaire with validation	Sensitivity = 81%; specificity = 54%; PPV = 6%; PLR = 1.8	No
Lee et al. 2012 [66]	≥ 20	9602	Age, +FHx, HTN, WC, smoking, alcohol	Korean National Health and Nutrition Examination Survey, anthropometric measurements	AUC = 73%	Yes
Pongchaiyakul et al. 2011 [67]	15–85	4314	Age, sec, BMI, Sys BP	Anthropometric measurement and lifestyle questionnaire with validation	AUC = 75%; sensitivity = 76.6%; specificity = 59.9%; PPV = 9.4%; NPV = 97.9%	Yes
Ramachandran et al. 2005 [68]	≥ 20	4993	Age, BMI,WC, +FHx, sedentary lifestyle	Lifestyle questionnaire with validation and OGTT	Sensitivity = 59% (M), 61% (F); specificity = 63% (M), 71% (F); PPV = 11 % (M), 14% (F); NPV = 95% (M), 96% (F); PLR = 1.61 (M). 2.16 (F); NLR =	Yes

Included studies that used populations that were not Africans. *BMI*, body mass index; *DM*, diabetes mellitus; *WHO STEPS*, World Health Organization STEPS Instrument for Chronic Disease Risk Factor Surveillance; *GPPAQ*, General Practice Physical Activity Questionnaire; *REAP*, Rapid Eating and Activity Assessment for Patients; *SES*, socio-economic status; *HTN*, hypertension; *L-HDL*, low- and high-density lipoprotein; *WHtR*, waist-to height ratio; *CMS*, cardiometabolic syndrome; *AUC*, area under the curve; *WC*, waist circumference; *NPV*, negative predictive value; *VAI*, visceral adiposity index: *BAI*, blood adiposity index; *TG*, triglycerides; *HARP*, Hospital Admission Risk Program; *NA*, not available. *PLP*, positive likelihood rate; *NLP*, negative likelihood rate; *M*, male; *F*, female

accuracy of the tools with values ranging from 0.60 to 0.88, while others used sensitivity and specificity for performance validation of the risk assessment tools (51.3%). Validation of tools was recorded in 77.1% of the studies using methods such as internal, external, measurement, and split-sample validation.

Discussion

This study found that the diabetes risk factors pertinent to SSA included waist circumference to height ratio, age, BMI, waist circumference below the ribs, exercise, vegetable consumption, use of anti-hypertensives, previous history of hyperglycemia, positive family history of diabetes, rapid eating, fasting venous blood glucose, and fasting lipids. Different diabetes risk assessment tools have been used in different populations in SSA, but none was developed and/or validated for use in the SSA populations. The findings provide insights and guidelines in a first phase for developing a more population-based diabetes risk assessment tool for SSA based on the reported risk factors in the available studies, and in a second phase for devising and building new risk assessment methods based upon the data collected from the tool design project. The study also reviewed evidence from non-African populations to ensure results are applicable to a wider population. By reviewing both African and non-African studies, this study provides the basis for the selection of the most relevant risk score to be implemented within the population.

It is typical to validate a newly developed tool for use in a population, or adapt a tool previously validated for use in another population. Studies [41] have indicated that available diabetes risk assessment tools perform differently for different populations. This underscores the need to validate a tool prior to its use in a population that is different from its original design. Differences in lifestyle, diet, and other sociodemographic characteristics could account for this variability warranting the need for validation of the tool. In this review, we found that the studies used various validation methods including split-sample cross-validation [50, 69], validation with another external population [18, 45, 61, 70], and bootstrapping [31, 48].

Studies conducted in sub-Saharan Africa

This review study did not identify a diabetic risk assessment tool specifically designed and validated for the population of SSA and that reported tool accuracy metrics like AUCs, sensitivity and specificity, and positive and negative predictive values. The implication is that clinicians may have to depend on tools developed for other populations to screen for diabetes in the sub-region. Given the vast variation in social, economic, nutritional, demographic, and genetic factors between different populations, and how these factors interplay in the etiology of diabetes, the accuracies of these external diabetic risk assessment tools may be in question. This claim seems to have empirical support from the study done by Skogberg et al. [41] who demonstrated higher accuracy levels among Finns compared to Russian, Somali, and Kudish immigrants when the variables WC and WHtR were used to predict DM. With the prevalence of DM in SSA expected to grow significantly, the need for accurate early detection is imperative. Our study argues strongly in favor of developing such tools.

We found some issues with three studies [55, 59, 65] identified in this review. Paramount among them was the fact that tools developed for other populations were used in some local SSA populations without validating them for the local populations. The study by Alebiosu et al. [55] provides one such example. They used the Finnish Medical Association diabetes risk assessment (FINDRISK) tool, without validating the tool in the local SSA population. Although they reported a significant correlation between total risk score and fasting blood sugar, they did not report any of the standard measures of tool accuracy like the AUC, sensitivity and specificity, and negative and positive predictive values. Without accurate results, it is not clear if the tool could accurately screen for DM particularly at the early stages in the population they studied.

The WHO STEPS instrument, GPPAQ, and REAP were used in Ghana by another study [51] to assess the risk factors for type 2 DM but did not predict DM directly. Although the tools have utility in associating DM risk factors to scores, robust predictive values were not reported and as with the study by Alebiosu et al. [55], the tools were not validated for the local population. One study [59] reported accuracy levels using the area under the receiver operating characteristics curve (AUC). Anthropometric variables were not used to predict DM directly but of cardiometabolic syndrome (CME), a syndrome which comprises hyperglycemia, hypertension, and hypertriglyceridemia. Although they demonstrated similar AUCs for female and male anthropometric variables like WHtR and BMI, the tool was designed specifically to predict CMS; therefore, its predictive utility for DM remains unclear.

An accurate DM risk assessment tool validated for local SSA populations does not seem to exist yet. However, the few related studies we reviewed showed pertinent anthropometric risk factors for DM that could be used to develop and validate a DM tool in SSA. These anthropometric factors include waist circumference to height ratio, age, BMI, waist circumference below the ribs, exercise, vegetable consumption, use of anti-hypertensives, previous history of hyperglycemia, positive family history of diabetes, and rapid eating. The cut-off values of the anthropometric variables used in the reviewed studies are presupposed normative values for populations outside Africa and could lower the predictive value of the tools in the African population. Perhaps a non-invasive

tool that uses normative values for WC, WHtR, BMI, dietary intake, exercise regimen, etc., which are pertinent to the local population, could give higher overall accuracy including sensitivity and specificity values. These findings therefore provide insights and guidelines in a first phase for developing a more population-based diabetes risk assessment tool for SSA based on the reported risk factors identified for local populations. A second phase will involve devising and building new risk assessment methods based on the data collected from the tool design project.

Studies conducted outside sub-Saharan Africa

Regarding the studies conducted outside of SSA, a total of 36 articles on diabetes risk assessment tools were reviewed. In general, the tools were developed in Europe, Asia, the USA, and the Middle East. However, none of the tools was developed or validated for the African population [57]. A similar lack of validated risk assessment tools found in SSA has been noted among some Latin American populations with the study recommending that the health authorities prioritize the development, validation, and implementation of a risk assessment tool [58]. Considering the high prevalence of diabetes and undiagnosed diabetes in the SSA region [10], the health authorities in the various countries should take similar actions by prioritizing the development and validation of a tailored diabetes risk assessment tool. Such tool should ideally be non-invasive, easy to use, free, and easily available to clinicians, public health workers, researchers, and individuals to assess their level of risk for developing diabetes. As with other Western countries, making the tool available on Ministry of Health websites will enable screening and early diagnosis of diabetes to reduce the rate of complications related to undiagnosed diabetes.

The number of cases of diabetes used in the derivation model for risk factors in the studies conducted outside of Africa varied from 48 [60] to 207 [62], and for validation, it was between 29 and 582 cases. Compared to studies in SSA populations, these studies reported similar risk factors outside of Africa [62–64] including age, waist circumference, and family history of diabetes. These were the most common predictors of diabetes and in one study oral glucose tolerance test was the outcome. Of five studies that assessed the use of diabetes risk assessment tools in South America [58], one review study found a high discrimination performance (AUC 70%, range: 66–72%) across studies, and the highest metric was always the negative predictive value [30, 60, 63, 64]. Although discrimination estimates in those studies were largely acceptable, calibration metrics were not reported. For countries such as Brazil [66], Mexico [67], and Peru [30] where risk assessment tools were developed and validated both cross-sectionally and prospectively, there was enough scientific evidence to implement these tools as part of the standard of care for type 2 DM screening at the population level [30]. Two studies from the Middle East also reported a high discrimination performance (AUC ranged from 76 to 83%) and only one study reported the tool's accuracy in terms of sensitivity and specificity [43] while another study reported the negative predictive value of the tool [41].

Generally, diabetes risk assessment tools cannot be directly transferred from one demographic group to another, due to the variation in factors such as diet and activity levels in different population groups. This is because the accuracy level of the tool varies with racial demographics [61] and since the three studies conducted in the African population used tools that were neither developed nor validated for the SSA population, it may be problematic to assume that their results can be replicated. In addition, only one of the studies reported an accuracy level for the tools used making it impossible to assess the performance of these tools in the SSA population. These findings indicate the need for the development of an accurate and validated non-invasive diabetes risk assessment tool for the SSA population. This tool should be cost-effective and able to identify persons at high risk of developing type 2 diabetes with reasonably high accuracy levels. Such a tool is important because the projected increase in the prevalence of diabetes in Africa will no doubt exert an enormous cost on the healthcare delivery systems in SSA, which are already chronically underfunded and understaffed [17, 18].

Limitations and strengths

One of the limitations of the current review relates to the fact that there was no standardized format for reporting the outcome variables in the different studies. For example, only one of the three studies in sub-Saharan Africa reported the level of accuracy of the screening tool used. The variation in the different tools used in the studies did not allow for comparison of the performance of the screening tools when used for the African population. Another limitation of this study was the small number of published reports on the diabetes risk assessment tools, leading to the inclusion of studies published in non-African regions to enlarge the scope of the discussion as suggested by a reviewer. Despite these limitations, this is the first study to provide evidence on the diabetes risk assessment tools used in SSA. The study reviewed evidence from non-African populations to ensure results are applicable to a wider population. By reviewing both African and non-African studies, this study provides the basis for the selection of the most relevant risk score to be implemented within the population such that the findings can be used as a reference to developing a tool for use among the African population.

Conclusions

This comprehensive review of the available literature found that no available diabetes risk assessment tool was developed and validated for the SSA population, despite the disproportionately high prevalence of diabetes in this region and the projected increase. This review found only three articles for the SSA region over a 20-year period which demonstrates the limited published research on diabetes risk assessment tools in the region. Although the existing European or American diabetes risk tools cannot be adopted in SSA countries without prior validation in the specific population, the findings of this study provided useful evidence of the risk factors and diabetes risk scores that could be further studied in different SSA countries. There is need for practical strategies to address the barriers to the implementation of diabetes risk assessment tools including that a low-cost, reliable screening tool for undiagnosed diabetes be developed and internally validated for the SSA population. The potential for cost and morbidity savings could be significant. Development of such tools should take into account the peculiar demographic characteristics of the sub-region identified in this study. Having a validated diabetes risk assessment tool with sufficiently high sensitivity and specificity will help healthcare policy-makers make informed decisions in the prudent allocation of scarce resources. The tool could then be deployed by trained healthcare workers in the screening of those at risk of diabetes for further clinical examination and possible care and it can be adapted by different SSA countries for validation in different communities. As in many developed countries, such tools can be integrated into the guidelines for policy-makers as a standard of practice for diabetes screening at the population level.

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Data availability The data that support the findings of this study are available on request from the corresponding author, ULO.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
2. Mbanya JCN, Motala AA, Sobngwi E, Assah FK, Enoru ST. Diabetes in sub-Saharan Africa. Lancet. 2010;375(9733):2254–66.
3. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JEJDr, et al. Global estimates of diabetes prevalence for 2013 and projections for 2035. 2014;103(2):137-49.
4. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017
5. Manne-Goehler J, Atun R, Stokes A, Goehler A, Houinato D, Houehanou C, Hambou MMS, Mbenza BL, Sobngwi E, Balde N, Mwangi JK, Gathecha G, Ngugi PW, Wesseh CS, Damasceno A, Lunet N, Bovet P, Labadarios D, Zuma K, et al. Diabetes diagnosis and care in sub-Saharan Africa: pooled analysis of individual data from 12 countries. Lancet Diabetes & Endocrinol. 2016;4(11):903–12.
6. Kengne AP, June-Rose McHiza Z, Amoah AG, Mbanya JC. Cardiovascular diseases and diabetes as economic and developmental challenges in Africa. Prog Cardiovasc Dis. 2013;56(3):302–13.
7. Kengne AP, Amoah AG, Mbanya JC. Cardiovascular complications of diabetes mellitus in sub-Saharan Africa. Circulation. 2005;112(23):3592–601.
8. Jakovljevic MB, Milovanovic O. Growing burden of noncommunicable diseases in the emerging health markets: the case of BRICS. Front Public Health. 2015;3:65.
9. Peer N, Kengne A-P, Motala AA, Mbanya JC. Diabetes in the Africa region: an uDiabetic Res Clin Pract. 2014;103(2):197–205.
10. Diabetes I. Brussels: International Diabetes Federation atlas; 2011. International Diabetes Federation. 2012.
11. World Health Organization. Global report on diabetes. 2016. 2017.
12. Mufunda J, Chatora R, Ndambakuwa Y, Nyarango P, Kosia A, Chifamba J, Filipe A, Usman A, Sparks VH. Emerging noncommunicable disease epidemic in Africa: preventive measures from the WHO Regional Office for Africa. Ethn Dis. 2006;16(2):521–6.
13. de Graft AA, Addo J, Ofei F, Bosu WK, Agyemang C. Ghana's burden of chronic non-communicable diseases: future directions in research, practice and policy. Ghana Med J. 2012;46(2):1–3.
14. Lawrence JM, Bennett P, Young A, Robinson AM. Screening for diabetes in general practice: cross sectional population study. BMJ. 2001;323(7312):548–551.
15. Organization WH, editor Screening for type 2 diabetes. Report of the World Health Organization and International Diabetes Federation meeting, 2003; 2003.

16. Aekplakorn W, Bunnag P, Woodward M, Sritara P, Cheepudomwit S, Yamwong S, Yipintsoi T, Rajatanavin R. A risk score for predicting incident diabetes in the Thai population. Diabetes Care. 2006;29(8):1872–7.
17. Bang H, Edwards AM, Bomback AS, Ballantyne CM, Brillon D, Callahan MA, Teutsch SM, Mushlin AI, Kern LM. Development and validation of a patient self-assessment score for diabetes risk. Ann Intern Med. 2009;151(11):775–83.
18. Gao WG, Dong YH, Pang ZC, Nan HR, Wang SJ, Ren J, et al. A simple Chinese risk score for undiagnosed diabetes. Diabet Med. 2010;27(3):274–281.
19. Glümer C, Carstensen B, Sandbæk A, Lauritzen T, Jørgensen T, Borch-Johnsen K. A Danish diabetes risk score for targeted screening. Diabetes Care. 2004;27(3):727–33.
20. Gray L, Taub N, Khunti K, Gardiner E, Hiles S, Webb D, et al. The Leicester Risk Assessment score for detecting undiagnosed type 2 diabetes and impaired glucose regulation for use in a multiethnic UK setting. Diabet Med. 2010;27(8):887–95.
21. Lee Y-h, Bang H, Kim HC, Kim HM, Park SW, Kim DJ. A simple screening score for diabetes for the Korean population. diabetes care. 2012;35(8):1723.
22. Mohan V, Deepa R, Deepa M, Somannavar S, Datta MA. A simplified Indian Diabetes Risk Score for screening for undiagnosed diabetic subjects. J Assoc Physicians India. 2005;53:759–763.
23. Rahman M, Simmons RK, Harding A-H, Wareham NJ, Griffin SJ. A simple risk score identifies individuals at high risk of developing type 2 diabetes: a prospective cohort study. Fam Pract. 2008;25(3):191–6.
24. Schulze MB, Hoffmann K, Boeing H, Linseisen J, Rohrmann S, Möhlig M, et al. An accurate risk score based on anthropometric, dietary, and lifestyle factors to predict the development of type 2 diabetes. Diabetes Care. 2007;30(3):510–5.
25. Chen L, Magliano DJ, Balkau B, Colagiuri S, Zimmet PZ, Tonkin AM, et al. AUSDRISK: an Australian type 2 diabetes risk assessment tool based on demographic, lifestyle and simple anthropometric measures. Med J Aust. 2010;192(4):197–202.
26. Sathish T, Kannan S, Sarma SP, Thankappan KRJPcd. Peer reviewed: screening performance of diabetes risk scores among Asians and Whites in rural Kerala, India. Prev Chronic Dis. 2013;10. :E37
27. Bernabe-Ortiz A, Smeeth L, Gilman RH, Sanchez-Abanto JR, Checkley W, Miranda JJ, et al. Development and validation of a simple risk score for undiagnosed type 2 diabetes in a resource-constrained setting. J Diabetes Res. 2016;2016:8790235-.
28. de León AC, Coello SD, Pérez MdCR, Medina MB, González DA, Díaz BB, et al. A simple clinical score for type 2 diabetes mellitus screening in the Canary Islands. Diabet Res Clin Pract. 2008;80(1):128–33.
29. Li J, Bergmann A, Reimann M, Bornstein S, Schwarz PJH, Research M. A more simplified Finnish diabetes risk score for opportunistic screening of undiagnosed type 2 diabetes in a German population with a family history of the metabolic syndrome. Horm Metab Res. 2009;41(02):98–103.
30. Dong J-j, Lou N-j, Zhao J-j, Zhang Z-w, Qiu L-l, Zhou Y, et al. Evaluation of a risk factor scoring model in screening for undiagnosed diabetes in China population. J Zhejiang Univ-Sci B. 2011;12(10):846.
31. Dugee O, Janchiv O, Jousilahti P, Sakhiya A, Palam E, Nuorti JP, Peltonen M. Adapting existing diabetes risk scores for an Asian population: a risk score for detecting undiagnosed diabetes in the Mongolian population. BMC Public Health. 2015;15(1):938.
32. Al Khalaf M, Eid M, Najjar H, Alhajry K, Thalib L. Screening for diabetes in Kuwait and evaluation of risk scores. East Mediterr Health J. 2010. 16(7):725–31.
33. Al-Lawati J, Tuomilehto JJDr, practice c. Diabetes risk score in Oman: a tool to identify prevalent type 2 diabetes among Arabs of the Middle East. 2007;77(3):438-44.
34. Carrillo-Larco RM, Aparcana-Granda DJ, Mejia JR, Barengo NC, Bernabe-Ortiz A. Risk scores for type 2 diabetes mellitus in Latin America: a systematic review of population-based studies. Diab Med. 2019;36(12):1573–84.
35. Tabaei BP, Herman WHJDC. A multivariate logistic regression equation to screen for diabetes: development and validation. Diabetes Care. 2002;25(11):1999–2003.
36. Gao W, Qiao Q, Pitkäniemi J, Wild S, Magliano D, Shaw J, et al. Risk prediction models for the development of diabetes in Mauritian Indians. Diabetes Med. 2009;26(10):996–1002.
37. Alebiosu OC, Familoni OB, Ogunsemi OO, Raimi T, Balogun WO, Odusan O, et al. Community based diabetes risk assessment in Ogun state, Nigeria (World Diabetes Foundation project 08-321). Indian J Endocrinol Metab. 2013;17(4):653.
38. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. Int J Surg. 2010;8(5):336–41.
39. Gudjinu HY, Sarfo B. Risk factors for type 2 diabetes mellitus among out-patients in Ho, the Volta regional capital of Ghana: a case–control study. BMC research notes. 2017;10(1):324.
40. Oguoma VM, Nwose EU, Skinner TC, Richards RS, Digban KA, Onyia IC, Anyasodor AE. Anthropometric indices: how they compare in screening of cardiometabolic risks in a Nigerian sub-population. Afr J Med Med Sci. 2016;45(1):91–8.
41. Skogberg N, Laatikainen T, Lilja E, Lundqvist A, Koponen P. Which anthropometric measures best indicate the risk for type 2 diabetes among migrants in Finland? Eur J Pub Health. 2017;27(suppl_3).
42. Agarwal G, Guingona MM, Gaber J, Angeles R, Rao S, Cristobal F. Choosing the most appropriate existing type 2 diabetes risk assessment tool for use in the Philippines: a case-control study with an urban Filipino population. BMC Public Health. 2019;19(1):1169.
43. Elizalde-Barrera CI, Rubio-Guerra AF, Lozano-Nuevo JJ, Olvera-Gomez JL. Triglycerides and waist to height ratio are more accurate than visceral adiposity and body adiposity index to predict impaired fasting glucose. Diabetes Res Clin Pract. 2019;153:49–54.
44. McGrath RT, Dryden JC, Newlyn N, Pamplona E, O'Dea J, Hocking SL, et al. Utility of the Hospital Admission Risk Programme diabetes risk calculator in identifying patients with type 2 diabetes at risk of unplanned hospital presentations. Intern Med J. 2018;48(10):1198–205.
45. Rauh SP, Rutters F, van der Heijden AA, Luimes T, Alssema M, Heymans MW, et al. External validation of a tool predicting 7-year risk of developing cardiovascular disease, type 2 diabetes or chronic kidney disease. J Gen Intern Med. 2018;33(2):182–8.
46. Zhang H, Wang C, Ren Y, Wang B, Yang X, Zhao Y, et al. A risk-score model for predicting risk of type 2 diabetes mellitus in a rural Chinese adult population: a cohort study with a 6-year follow-up. Diabetes Metab Res Rev. 2017;33(7).
47. Bould K, Scott S, Dunne S, Asimakopoulou K. Uptake of screening for type 2 diabetes risk in general dental practice; an exploratory study. Br Dent J. 2017;222(4):293–6.
48. Liu PJ, Ma F, Lou HP, Chen Y. Visceral adiposity index is associated with pre-diabetes and type 2 diabetes mellitus in Chinese adults aged 20-50. Ann Nutr Metab. 2016;68(4):235–43.
49. Khunti K, Gillies CL, Dallosso H, Brady EM, Gray LJ, Kilgallen G, Willis A, Zafar A, Davies MJ. Assessment of response rates and yields for two opportunistic tools for early detection of non-diabetic hyperglycaemia and diabetes (ATTEND). A randomised controlled trial and cost-effectiveness analysis. Diabetes Res Clin Pract. 2016;118:12–20.
50. Robinson C, Agarwal G, Nerenberg K. Validating the CANRISK prognostic model for assessing diabetes risk in Canada's multi-

ethnic population. Chronic diseases and injuries in Canada. 2011;32(1).
51. Xie J, Hu D, Yu D, Chen C-S, He J, Gu D. A quick self-assessment tool to identify individuals at high risk of type 2 diabetes in the Chinese general population. J Epidemiol Community Health. 2010;64(3):236–42.
52. Otero LM, Fong M, Papineau D, Thorne S, Zanetti ML. Testing a prediabetes screening approach for a Latin American population in Vancouver, Canada. J Nurs Healthc Chronic Illn. 2011;3(3):329–38.
53. Guo J, Shi Z, Chen JL, Dixon JK, Wiley J, Parry M. Translation and validation of the Canadian diabetes risk assessment questionnaire in China. Public Health Nurs. 2018;35(1):18–28.
54. Al-Lawati JA, Tuomilehto J. Diabetes risk score in Oman: a tool to identify prevalent type 2 diabetes among Arabs of the Middle East. Diabetes Res Clin Pract. 2007;77(3):438–44.
55. Al Khalaf MM, Eid MM, Najjar HA, Alhajry KM, Doi SA, Thalib L. Screening for diabetes in Kuwait and evaluation of risk scores. East Mediterr Health J. 2010;16(7):725–31.
56. Cabrera de León A, Coello SD, Rodríguez Pérez Mdel C, Medina MB, Almeida González D, Díaz BB, et al. A simple clinical score for type 2 diabetes mellitus screening in the Canary Islands. Diabetes Res Clin Pract. 2008;80(1):128–33.
57. Bindraban NR, van Valkengoed IGM, Mairuhu G, Holleman F, Hoekstra JBL, Michels BPJ, et al. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. BMC Public Health. 2008;8:271-.
58. Glümer C, Carstensen B, Sandbaek A, Lauritzen T, Jørgensen T, Borch-Johnsen K. A Danish diabetes risk score for targeted screening: the Inter99 study. Diabetes Care. 2004;27(3):727–33.
59. Griffin SJ, Little PS, Hales CN, Kinmonth AL, Wareham NJ. Diabetes risk score: towards earlier detection of type 2 diabetes in general practice. Diabetes Metab Res Rev. 2000;16(3):164–71.
60. Heikes KE, Eddy DM, Arondekar B, Schlessinger L. Diabetes risk calculator: a simple tool for detecting undiagnosed diabetes and pre-diabetes. Diabetes Care. 2008;31(5):1040–5.
61. Li J, Bergmann A, Reimann M, Bornstein SR, Schwarz PE. A more simplified Finnish diabetes risk score for opportunistic screening of undiagnosed type 2 diabetes in a German population with a family history of the metabolic syndrome. Horm Metab Res. 2009;41(2):98–103.
62. Pires de Sousa AG, Pereira AC, Marquezine GF, Marques do Nascimento-Neto R, Freitas SN, de CNRL, et al. Derivation and external validation of a simple prediction model for the diagnosis of type 2 diabetes mellitus in the Brazilian urban population. Eur J Epidemiol. 2009;24(2):101–9.
63. Saaristo T, Peltonen M, Lindström J, Saarikoski L, Sundvall J, Eriksson JG, Tuomilehto J. Cross-sectional evaluation of the Finnish Diabetes Risk Score: a tool to identify undetected type 2 diabetes, abnormal glucose tolerance and metabolic syndrome. Diab Vasc Dis Res. 2005;2(2):67–72.
64. Chaturvedi V, Reddy KS, Prabhakaran D, Jeemon P, Ramakrishnan L, Shah P, et al. Development of a clinical risk score in predicting undiagnosed diabetes in urban Asian Indian adults: a population-based study. CVD Prevention and Control. 2008;3(3):141–51.
65. Keesukphan P, Chanprasertyothin S, Ongphiphadhanakul B, Puavilai G. The development and validation of a diabetes risk score for high-risk Thai adults. J Med Assoc Thail. 2007;90(1):149–54.
66. Lee YH, Bang H, Kim HC, Kim HM, Park SW, Kim DJ. A simple screening score for diabetes for the Korean population: development, validation, and comparison with other scores. Diabetes Care. 2012;35(8):1723–30.
67. Pongchaiyakul C, Kotruchin P, Wanothayaroj E, Nguyen TV. An innovative prognostic model for predicting diabetes risk in the Thai population. Diabetes Res Clin Pract. 2011;94(2):193–8.
68. Ramachandran A, Snehalatha C, Vijay V, Wareham NJ, Colagiuri S. Derivation and validation of diabetes risk score for urban Asian Indians. Diabetes Res Clin Pract. 2005;70(1):63–70.
69. Xie J, Hu D, Yu D, Chen C-S, He J, Gu D. A quick self-assessment tool to identify individuals at high risk of type 2 diabetes in the Chinese general population. J Epidemiol Community Health. 2010;64(3):236–42.
70. Ruige JB, de Neeling JN, Kostense PJ, Bouter LM, Heine RJ. Performance of an NIDDM screening questionnaire based on symptoms and risk factors. Diabetes Care. 1997;20(4):491–6.

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CORRECTION

Correction to: A systematic review of diabetes risk assessment tools in sub-Saharan Africa

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The Author's and Editor's corrections were not modified in the original published proof.
The Original article has been corrected.

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REVIEW ARTICLE

The effects of formal nutrition education on anthropometric indices, lipid profile, and glycemic control of patients with type 2 diabetes: a systematic review and meta-analysis

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Abstract

Background Formal nutrition education improves diabetes management. We aimed to assess the effects of formal nutrition education on anthropometric indices, lipid profile, and glycemic control of patients with type 2 diabetes.

Methods Pubmed, Scopus, and Embase were searched. Randomized controlled trial design which evaluated nutritional education programs in adults with type 2 diabetes were selected.

Results Twenty-six publications were included in analysis. Formal nutrition education reduced glycated hemoglobin (− 0.72%; 95% CI: − 0.95, − 0.49), fasting blood glucose (− 13.40 mmol/L; 95% CI: − 22.47, − 4.34), total cholesterol (− 6.28 mmol/L; 95% CI: − 10.77, − 1.79), triacylglycerol (− 17.26 mmol/L; 95% CI: − 29.10, − 5.42), waist circumference (− 2.02 cm; 95% CI: − 3.11, − 0.92), diastolic blood pressure (− 2.92 mmHg; 95% CI: − 4.74, − 1.11), and systolic blood pressure (− 6.16 mmHg; 95% CI: − 11.03, − 1.28). However, no significant differences were observed in formal education for body mass index, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.

Conclusions Formal nutrition education in type 2 diabetic patients can improve fasting blood glucose levels, glycated hemoglobin, reduce total cholesterol, triacylglycerol, blood pressure, and waist circumference.

Keywords Socialization · Education · Nutrition · Diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders worldwide [1, 2] According to the World Health Organization (WHO), DM prevalence will increase from 4 to 5.40% in 1995 till 2035 [1]. Genetic and environmental risk factors affect incidence of diabetes [3]. The main complications of T2DM are microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (hypertension, hyperlipidemia, coronary artery disease, cerebral vascular disease, etc.). Generally, unhealthy lifestyle including physical inactivity and unhealthy dietary pattern is the main modifiable risk factor of T2DM [4].

DM decreases quality of life, rises premature death, and increases health care costs [5–8]. In addition, unlike developed countries that DM cases are predominantly older than 60 years, in developing countries, DM is most common in 20–59 years old adults, which can negatively affect the economic growth [5]. Education is necessary for diabetes care. Education socializes people regarding the expected behaviors for especial situations and people learn the expected values, norms, manners, styles, and behaviors [9, 10]. In other words, socialization teaches people what behaviors or tasks are expected of them. Especial socialization is needed for patient people including diabetic patients. Education, as a socialization factor, can be introduced formally or informally. Scientifically,

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formal education means using direct, rule-based, and organizational structures and informal education means using indirect, non-rule-based, and non-organizational structures. Formal methods focus on academic, schools, hospitals, clinics, and health centers education, while informal methods consist of web-based, phone, or printed-material educations [11–13]. The self-management use of web-based materials, networking-based and social media-based, which apply informally, is the most significant type of education among new types of education. The education (formal and informal) as a mechanism of socialization has important situation in individual and social behaviors and traits.

Nutritional education finds and addresses unhealthy dietary patterns and decreases sedentary behaviors. It is basic principal regarding self-management of diseases such as diabetes [11]. The most significant diabetic self-management education (DSME) models are the "group education" and the "individual education [14]." The basic method for DSME is face-to-face method [15]. "Face-to-face" education is one of the most common educational methods in health care systems. Patients have chance to ask their questions or discuss their issues. In addition, health care givers can change false information of patients and build an active relationship with them [14]. With group-based DSME, patients can meet each other and discuss their concerns about their health problems and consequences of diabetes. It increases diabetes information and quality of life [11, 16]. In other methods, dietary records, dietary consultation, and assessment can be performed efficiently by using internet and web-based platform. These methods increase patients' awareness about dietary intake and disease control [17].

Nine studies about formal education were included in the Cochrane review (2010); six studies compared face-to-face education with usual care and three of them compared face-to-face education with group education. Individual education for 6 to 12 months in comparison with usual care did not significantly attenuate glycemic indices, body mass index (BMI), blood pressure, or total cholesterol in T2DM patients. There were not any significant differences between individual education and group education regarding glycemic control, BMI, and systolic or diastolic blood pressure [18]. Although limited trials were available regarding the effects of web-based education on T2DM individuals, the available evidence suggested using web-based interventions improved nutritional knowledge and glycemic indices [19].

There is no consensus regarding the most effective model for the education of patients with type 2 diabetes educations. So, the aim of the present study was to assess the effectiveness of formal nutrition education compared with usual care on glycemic indices, lipid profiles, blood pressures, and anthropometric variables of type 2 diabetes patients.

Methods

Search strategy

A systematic literature search was conducted in PubMed, Scopus, and Embase up to November 2019. The following Medical Subjects and Headings (MeSH) terms and keywords were used: (1) "nutrition education"[Title/Abstract] OR "nutrition education"[MeSH Terms] OR "nutrition training"[Title/Abstract] OR "nutrition training"[MeSH Terms]; (2) "television"[Title/Abstract] OR "television"[MeSH Terms] OR "web"[Title/Abstract] OR "phone"[Title/Abstract] OR "press"[Title/Abstract] OR "newspaper"[Title/Abstract] OR "printed matter"[Title/Abstract] OR "university"[Title/Abstract] OR "university"[MeSH Terms] OR "academic"[Title/Abstract] OR "pre-university"[Title/Abstract] OR "health center"[Title/Abstract]; (3) "type 2 diabetes mellitus"[Title/Abstract] OR "type 2 diabetes mellitus"[MeSH Terms] OR "obesity"[Title/Abstract] OR "obesity"[MeSH Terms]; (4) 1 & 2 & 3. To find more relevant papers, the reference lists of related articles were checked. We included all relevant studies that had been published in English language.

Study eligibility

There was not any language restriction. Two different authors (AM, SMDR) reviewed and screened the title, abstract, and full text of included studies. Eligibility criteria were based on the PICOS. "population" was adults with type 2 diabetes mellitus, "intervention" was formal education, "Comparator" was a control group that received usual care, the standard of care was annual checkups or regular visits with health professionals, "outcomes" refer to all variables of serum blood glucose, lipid profiles, and anthropometric indices, and "study design" was randomized controlled trial studies. In other words, studies were included if they had randomized controlled trial design which evaluated nutritional education programs in adults with type 2 diabetes in comparison with usual care. Animal study, editorial/letter to editor, review article, studies that were not published in peer-reviewed journals such as abstracts from conference proceedings, dissertations, and master's theses, and designs other than randomized controlled trial studies with insufficient data were excluded.

Data extraction

Three authors (AM, SMDR, MHB) were responsible for extracting the data. The following data were extracted from each relevant paper: first author's information, study/

publication year, study design, sample size, intervention, control, study duration, age, sex, including family/friends, and outcomes. Means and standard deviations (SD) or standard errors (SE) of outcomes (FBS, HbA1c, total cholesterol (TC), LDL-C, HDL-C, triglyceride (TG), waist circumference (WC), and BMI) were also extracted for the effect size calculation.

Statistical analysis

We used percent (%), mmHg, mmol/L, kg/m^2, and centimeter (cm) as unit scale for all values of HbA1C, blood pressure, lipid profile, blood glucose, and waist circumference, respectively. We used changes in the mean and standard deviation (SD) to estimate the effect size. Mean difference was measured by subtracting the mean before- and after-intervention values for the intervention and control groups if we could not extract the effect size directly. SE was converted to SD for effect size calculation [20].

I-squared and chi-squared tests were used for heterogeneity assessment. In chi-squared test, alpha value of less than 0.1 declared significant heterogeneity. In *I*-squared test, values < 25% were considered low heterogeneity, 25 to 50% as moderate heterogeneity, and more than 50% were considered large amounts of heterogeneity. Random-effect model (I–V heterogeneity, no standard) was applied for calculating pooled effect size. Ninety-five percent confidence intervals were calculated for the weighted mean difference (WMD), and 0.05 or less were considered significant level. For assessing small-study effects, Begg's test, Egger's test, and funnel plot were used. To adjust the publication bias, "Trim and fill" analysis was used. All statistical analyses were done with Stata version 11.0 software (Stata Corporation).

Results

Study characteristics

Among 1356 papers, 81 full-text articles were assessed for inclusion and exclusion criteria (Fig. 1). Fifty-five articles were excluded after full-text screening: 29 studies on children, five studies on prevention, 16 studies with insufficient data, and five studies without control group. The PICOS criteria of eligible studies are described in Tables 1. The study duration varied from 8 to 192 weeks. The mean age of the participants ranged from 52.5 to 72.5 years. One study was performed on women, and 15 studies were applied on both genders. Finally, 20 studies, with 2422 participants in formal nutrition education, had sufficient data to be enrolled in the meta-analysis.

Meta-analysis results, formal nutrition education

The overall effect of formal education on FBS was − 13.40 mmol/L (95% CI: − 22.47, − 4.34; $p = 0.004$)) with significant heterogeneity ($I^2 = 63.3\%$, and $p = 0.005$) (Fig. 2). The overall effect of formal education on HbA1c was − 0.72% (95% CI: − 0.95, − 0.49; $p < 0.001$) with significant heterogeneity ($I^2 = 55.1\%$, and $p = 0.003$) (Fig. 2). The overall effect of formal education on TG was − 17.26 mmol/L (95% CI: − 29.10, − 5.42; $p = 0.004$) without significant heterogeneity ($I^2 = 0.0\%$, and $p = 0.617$) (Fig. 3). The overall effect of formal education on TC was − 6.28 mmol/L (95% CI: − 10.77, − 1.79; $p = 0.006$) without significant heterogeneity ($I^2 = 0.0\%$, and $p = 0.997$) (Fig. 3). The overall effect of formal education on LDL-C was − 1.80 mmol/L (95% CI: − 9.12, 5.51; $p = 0.629$) without significant heterogeneity ($I^2 = 0.0\%$, and $p = 0.845$) (Fig. 3). The overall effect of formal education on HDL-C was − 0.36 mmol/L (95% CI: − 2.39, 1.67; $p = 0.728$) without significant heterogeneity ($I^2 = 14.1\%$, and $p = 0.322$) (Fig. 3).

The overall effect of formal education on SBP was − 4.23 mmHg (95% CI: − 8.84, 0.39; $p = 0.073$) without significant heterogeneity ($I^2 = 36.7\%$, and $p = 0.162$). After excluding two studies according to sensitivity analysis (Cheskin LJ, et al. 2008; and Muchiri JW, et al. 2016) [23, 34], the overall effect of formal nutrition education on SBP changed (WMD: − 6.16 mmHg; 95% CI: − 11.03, − 1.28 without significant heterogeneity I^2: 33.6% and $p = 0.211$) (Fig. 4). The overall effect of formal education on DBP was − 2.92 mmHg (95% CI: − 4.74, − 1.11; $p = 0.002$) without significant heterogeneity ($I^2 = 0.0\%$, and $p = 0.545$) (Fig. 4).

The overall effect of formal education on BMI was − 0.58 kg/m^2 (95% CI: − 1.00, − 0.17; $p = 0.006$) without significant heterogeneity ($I^2 = 13.0\%$, and $p = 0.314$). After excluding a study according to sensitivity analysis (Gutschall MD, et al. 2009) [26], the overall effect of formal nutrition education on BMI changed (WMD − 0.40 kg/m^2; 95% CI: − 0.91, 0.10 without significant heterogeneity I^2: 4.9% and $p = 0.397$) (Fig. 4). The overall effect of formal education on WC was − 2.02 cm (95% CI: − 3.11, − 0.92; $p < 0.001$) without significant heterogeneity ($I^2 = 4.7\%$, and $p = 0.380$) (Fig. 4).

Publication bias

No evidence of publication bias was observed regarding the effects of formal education on blood glucose, lipid profile, blood pressure, and anthropometric indices (Figure S1). The Begg and Egger tests did not show publication bias for the effects of formal education on FBS ($p = 0.532$, $p = 0.424$), HBA1C ($p = 0.970$, $p = 0.732$), LDL ($p = 0.602$, $p = 0.489$), HDL ($p = 1.00$, $p = 0.777$),

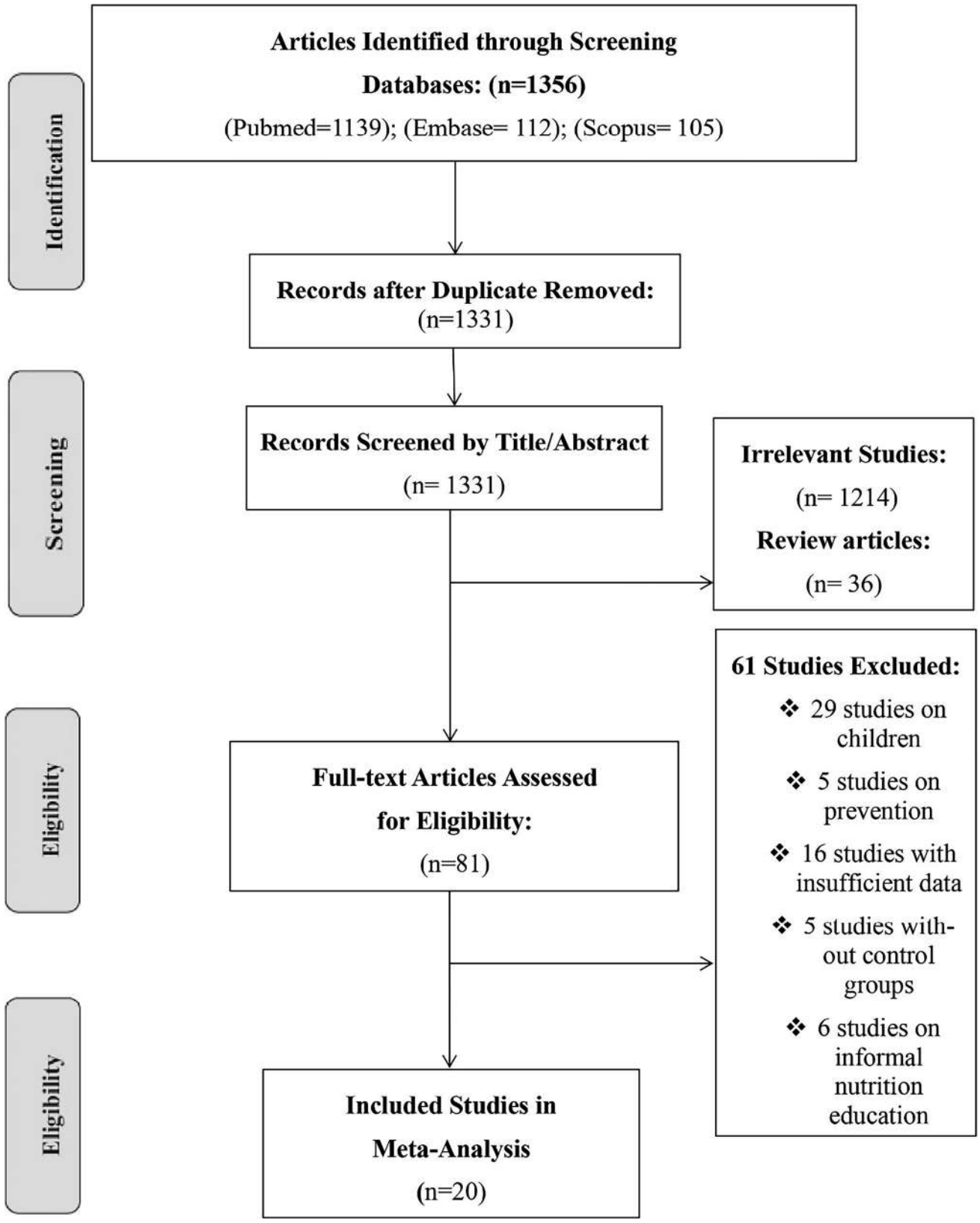


Fig. 1 Flow diagram of database searches and study selection

Table 1 Controlled clinical trials on formal nutritional education of type2 diabetic patients

Author and year	Sample size	Age (mean ± SD)	Population	Duration	Design	Intervention	Control	Including family/friends	Results
Beyazit, E. et al. (2011) [21]	50 (both)	52.5 ± 16	T2DM (insulin/hypoglycemic agents)	8 weeks 3 sessions; each one 30–40 min	RCT	Individualizing education	Usual care	Unclear	**At 2 months** 1. HbA1c (%) 2. BMI (kg/m^2) 3. BP (mmhg)
Brown, SA. et al. (2002) [22]	256 (both)	54 ± 8.25	T2DM	48 weeks 52 h over 12 months 12 weekly meetings + 14 biweekly sessions	RCT (un-blind)	Group education program Educator: nurse, dietitian, and community worker	Waiting list	Yes	**At 6 and 12 months** 1. HbA1c (%) 2. Fasting blood glucose (mg/dL) 3. Lipids (mg/dL) 4. BMI (kg/m^2) 5. Health beliefs 6. Diabetes knowledge (score)
Cheskin, LJ. et al. (2008) [23]	119 (both)		T2DM	86 weeks Every other week during 34-week weight loss phase + every 4 weeks during 52-week maintenance phase	RCT	Individualizing education and group education	Standard diet	Unclear	**At 34 and 86 weeks** 1. Weight (kg) 2. Waist circumference (cm) 3. Blood glucose (mmol/L) 4. Blood lipids (mmol/L) 5. BP (mmhg)
Deakin, TA. et al. (2003) [24]	314 (both)	61.55 ± 10.35	T2DM	56 weeks 12 h over 6 consecutive weeks (each one 2 h)	RCT (single blind)	Group education program Educator: diabetes educator	Routine treatment	Yes	**4 and 14 months** 1. HbA1c (%) 2. Blood pressure (mmHg) 3. Weight (kg) 4. BMI (kg/m^2) 5. Waist circumference (inch) 6. Lipid profile 7. Knowledge (score) 8. Self-management skills 9. Food frequency questionnaire 10. Treatment satisfaction 11. Quality of life 12. Empowerment score

Table 1 (continued)

Author and year	Sample size	Age (mean ± SD)	Population	Duration	Design	Intervention	Control	Including family/friends	Results
Domenech, MI. et al. (1995) [25]	79 (both)	52.9 ± 2.1	T2DM	48 weeks 6–8 h over 4 weekly sessions (each one 1.5–2 h)	RCT (unclear)	Group structured teaching/treatment program Educator: physician	Usual care	Yes	**12 months** 1. HbA1c (%) 2. Knowledge score (intervention group only) 3. Weight (kg) 4. Change in diabetes medication
Gutschall, MD. et al. (2009) [26]	109 (both)	59.2 ± 7.5	T2DM (non-insulin-dependent)	18 weeks 9 weekly sessions (each one 1.5–2 h)	RCT	Group education	Patients in the control group served as a wait list control condition	Unclear	**At 9 weeks and 18 weeks** 1. Weight (kg) 2. Waist circumference (cm) 3. BMI (kg/m^2) 4. FBS (mmol/L) 5. TAG (mmol/L)
Heller, SR. et al. (1988) [27]	75 (both)	56.45	T2DM	48 weeks 7.5 hs in total (4.5 h for 3 consecutive weeks + 1.5 h at both 3 and 6 months.)	RCT (unclear)	Group education Educator: diabetes specialist nurse and dietitian	Routine treatment Individual appointments with physician and dietitian at least at 3,6, and 12 months	Yes	**6 and 12 months** 1. HbA1c (%) 2. Blood glucose (mmol/L) 3. Weight loss (kg) 4. Diabetes knowledge
Holtrop, JS. et al. (2002) [28]	132 (F)	61.5	T2DM	24 weeks 9 h over 6 weekly sessions (each one 1–1.5 h.)	RCT (un-blind)	Group education Educator: trained lay health advisors	Routine treatment	Unclear	**6 months** 1. HbA1c (%) 2. BMI 3. Dietary habits 4. Beliefs 5. Stages of change
Kronsbein, P. et al. (1988) [29]	99 (both)	64 ± 8.5	T2DM	48 weeks 6–8 h over 4 weekly sessions (each one 1.5–2 h.)	RCT (unclear)	Group-structured treatment and teaching program (DTTP) Educator: paramedical staff (physician assistants)	Routine treatment while on waiting list	Unclear	**12 months** 1. HbA1c (%) 2. Weight (kg) 3. Knowledge (score) 4. Lipids (mmol/L) 5. Glucosuria 6. No oral hypoglycemic agents (%)

Table 1 (continued)

Author and year	Sample size	Age (mean ± SD)	Population	Duration	Design	Intervention	Control	Including family/friends	Results
Laitinen, JH. et al. (1993) [30]	86 (both)	53.2 ± 6.75	Obese T2DM (non-insulin-dependent)	48 weeks 6 sessions in total (every second month)	RCT	Individualized education	Usual education	Unclear	**At 3 and 15 months** 1. HbA1c (%) 2. FBS (mmol/L) 3. Weight (kg) 4. TC (mmol/L) 5. HDL-C (mmol/L) 6. TG (mmol/L)
Lozano, ML. et al. (1999) [31]	243 (both)	64.25	T2DM	96 weeks 90-min session for 2 consecutive days and repeated in year two	RCT (unclear)	Group education, health educational workshop Educator: nurses	Routine treatment	Unclear	**1 and 2 years** 1. HbA1c (%) 2. Blood glucose (mg/dL) 3. BMI (kg/m^2) 4.Knowledge 5. Self-monitoring
Mckibbin CL. et al. (2010) [32]	52 (both)	54 ± 8.65	Schizophrenia patients with DM	24 weeks 4 sessions (each one 1.5 h)	RCT	Group education, diabetes awareness and rehabilitation training (DART)	Usual care + information (UCI)	Unclear	**At 12 months** 1. HbA1c (%) 2. BMI (kg/m^2) 3. WC (cm) 4. Diabetes 5. Knowledge
Miller, CK. et al (2002) [33]	98 (both)	72.55 ± 4.2	Older adults with T2DM	10 weeks 10 weekly sessions (each one for 1.5–2 h)	RCT	Group education	Conventional care	Unclear	**At 10 weeks** 1. TC (mg/dL) 2. LDL (mg/dL) 3. HDL (mg/dL) 4. TG (mg/dL) 5. FBS (mg/dL) 6. HbA1c (%)
Muchiri JW, et al. (2016) [34]	82 (both)	58.5 ± 7.45	T2DM	48 weeks 8 weekly sessions (each one 2–2·5 h)	RCT (single blind)	Group nutrition education	Usual care	Unclear	**At 6 and 12 Months** 1. HbA1C 2. BP 3. Blood lipids 4. Dietary behaviors

Table 1 (continued)

Author and year	Sample size	Age (mean ± SD)	Population	Duration	Design	Intervention	Control	Including family/friends	Results
Pieber, TR. et al. (1995) [35]	94 (both)	64.65 ± 9.7	T2DM	24 weeks 6–8 h over 4 weekly sessions	RCT (unclear)	Group education, diabetes treatment and teaching program (DTTP) Educator: physician and office staff	Routine treatment with waiting list	No	**6 months** 1. HbA1c (%) 2.Diabetes knowledge (score) 3. BMI (kg/m^2) 4. Weight (kg) 5. Lipid profile (mmol/L) 6. Diabetes medication (no/day) 7. Blood pressure (mmHg)
Rickheim, PL. et al. (2002) [36]	170 (both)	52.25 ± 11	T2DM	24 weeks 7 h in total over 4 sessions	RCT (un-blind)	Group diabetes education program Educator: nurse and dietitian	Routine self-management education (4 individual sessions)	Unclear	**6 months** 1. HbA1c (%) 2. Weight (kg) 3. BMI (kg/m^2) 4. Diabetes knowledge (score) 5. Adjustment to diabetes (ATT19) 6. Quality of life 7. Activity levels (freq/duration) 8. Diabetes medication (% of participants taking)
Trento, M. et al. (1998) [37]	112 (both)	61.3	T2DM	48 weeks 4 sessions in total, every 3 months for 1 year (each one 60–70 min)	RCT (unclear)	Structured group education program Educator: two physicians and educationist	Routine treatment	Yes-optional	**1 year** 1. HbA1c (%) 2. FBG (mmol/L) 3. Weight (kg) 4. BMI (kg/m^2) 5. Knowledge (score) 6. Conduct (score) 7. Quality of life (score) 8. Treatment

Table 1 (continued)

Author and year	Sample size	Age (mean ± SD)	Population	Duration	Design	Intervention	Control	Including family/friends	Results
Trento, M. et al (2001) [38]	112 (both)	61.5	T2DM	96 weeks 8 sessions in total, every 3 months for 2 years (each one 1 h)	RCT (unclear)	Structured group education program Educator: two physicians and educationist	Routine treatment	Yes-optional	**2 years** 1. HbA1c (%) 2. FBG (mmol/L) 3. Weight (kg) 4. BMI (kg/m^2) 5. Knowledge (score) 6. Conduct (score) 7. Quality of life (score) 8. Treatment 9. Complications 10. Lipids (mmol/L)
Trento, M. et al (2002) [39]	112 (both)	61.5	T2DM	192 weeks 15 h over 4 years (every 3 months for 2 years + 7 sessions in year 3 + 4)	RCT (unclear)	Structured group education program Educator: two physicians and educationist	Routine treatment	Yes-optional	**4 years** 1. HbA1c (%) 2. FBG (mmol/L) 3. Weight (kg) 4. BMI (kg/m^2) 5. Lipids (mmol/L) 6. Blood pressure (mmHg) 7. Knowledge (score) 8. Conduct (score) 9. Quality of life (score) 10. Treatment 11. Complications
Zapotoczk, yH. et al. (2001) [40]	36 (both)	57.5 ± 9.8	T2DM	48 weeks 15 h over 10 monthly sessions (each one 1.5 h)	RCT (unclear)	Group education Educator: dietitian	Routine treatment (4 individual appointments)	Unclear	**12 months** 1. HbA1c (%) 2. Blood pressure (mmHg) 3. Lipid profile (mg/dL) 4. Weight (kg)

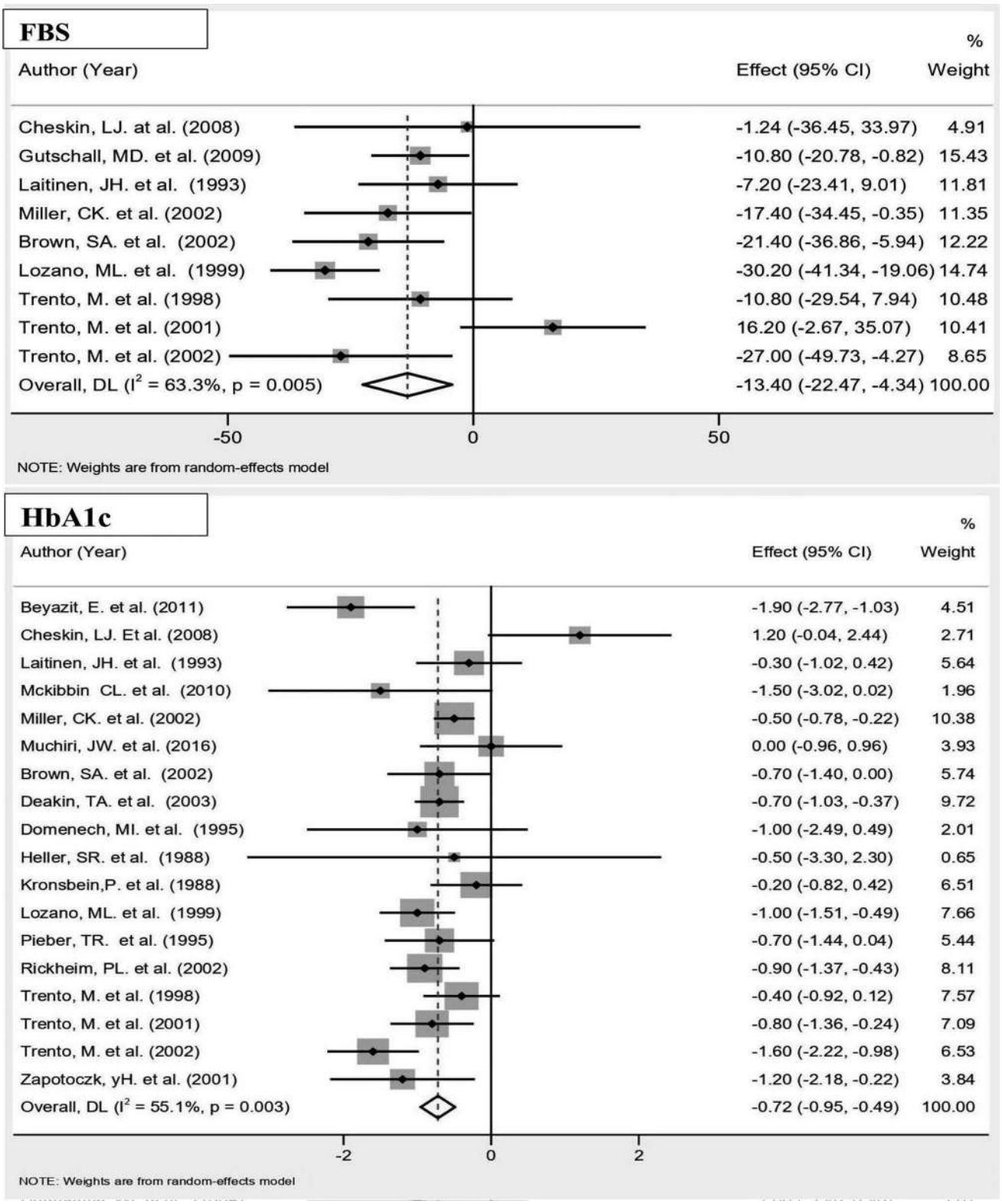
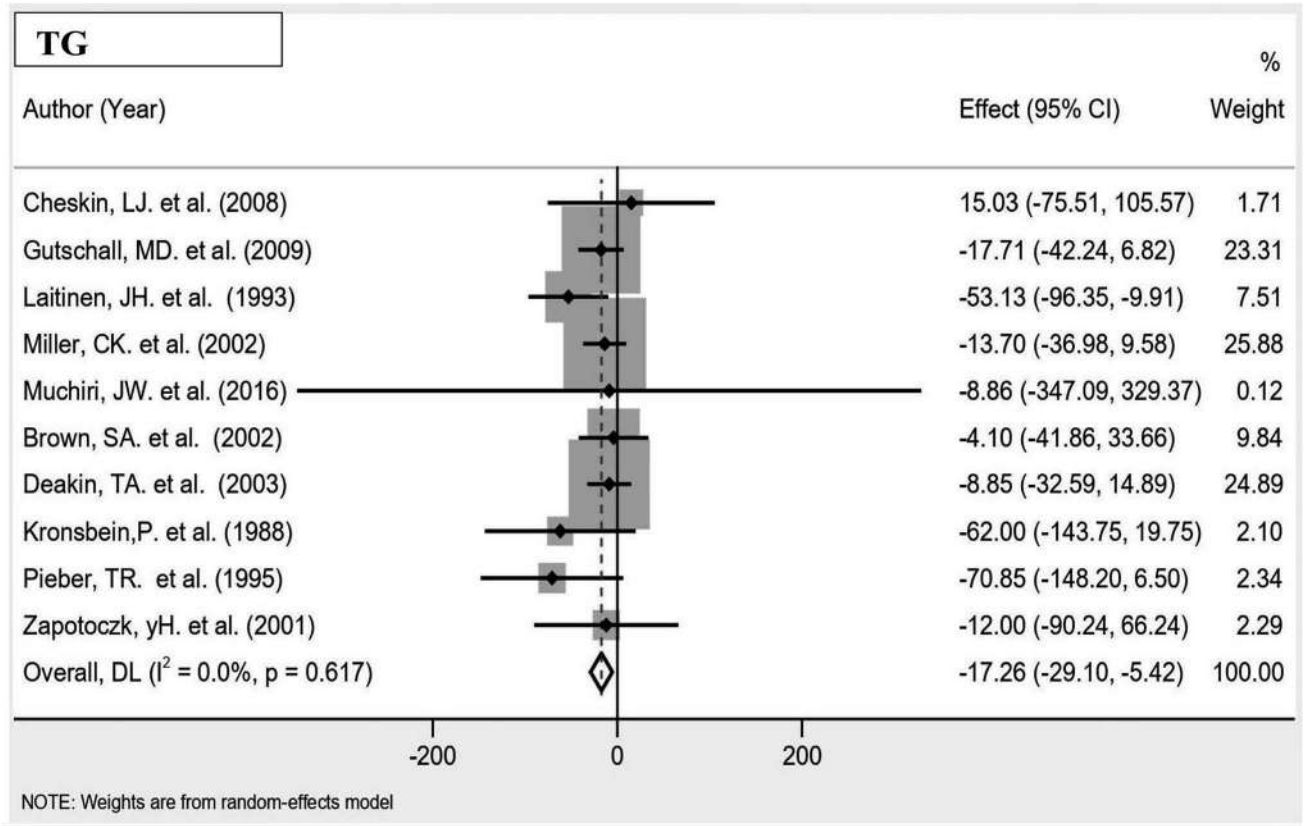


Fig. 2 Meta-analysis of the effect of formal nutrition education on fasting blood sugar and HbA1C in type 2 diabetic patients



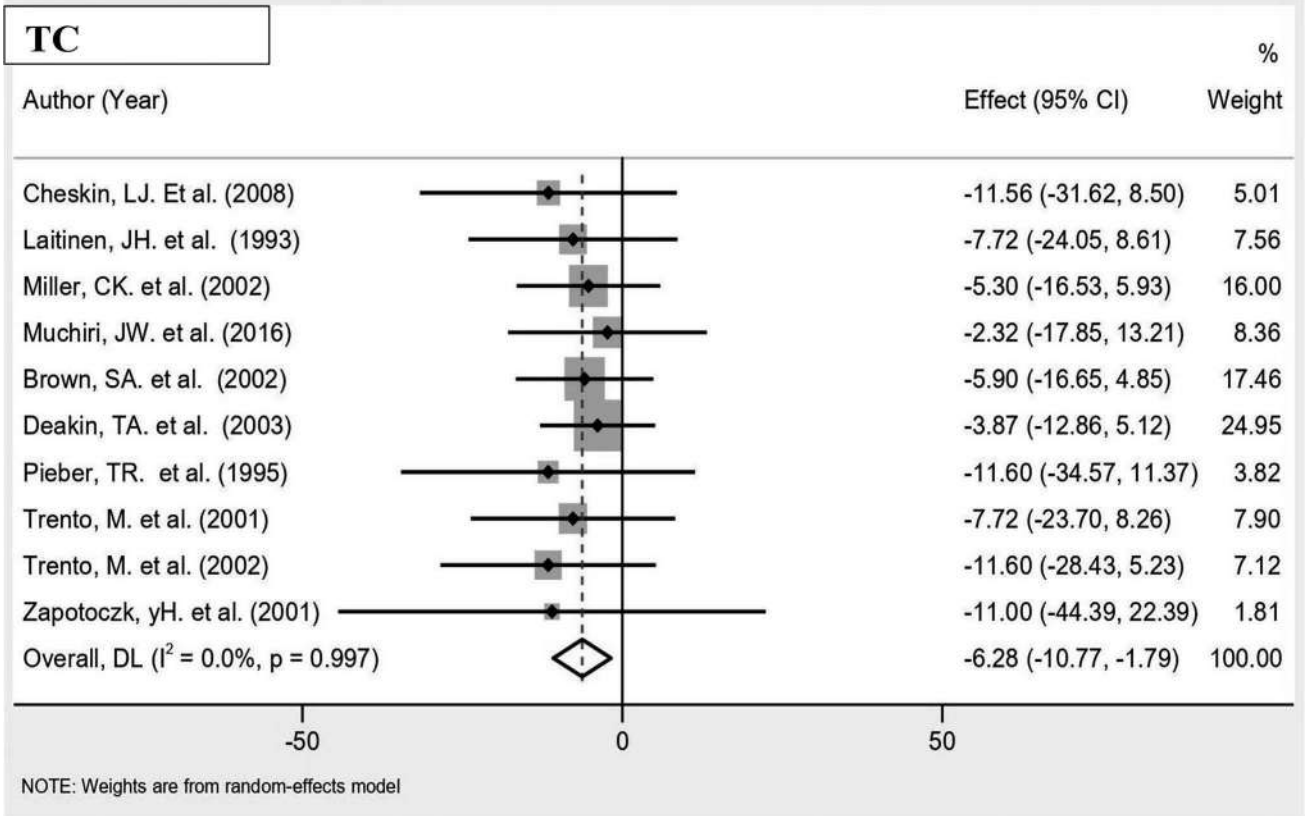


Fig. 3 Meta-analysis of the effect of formal nutrition education on blood lipids levels (LDL-C, HDL-C, TC, and TG) in type 2 diabetic patients

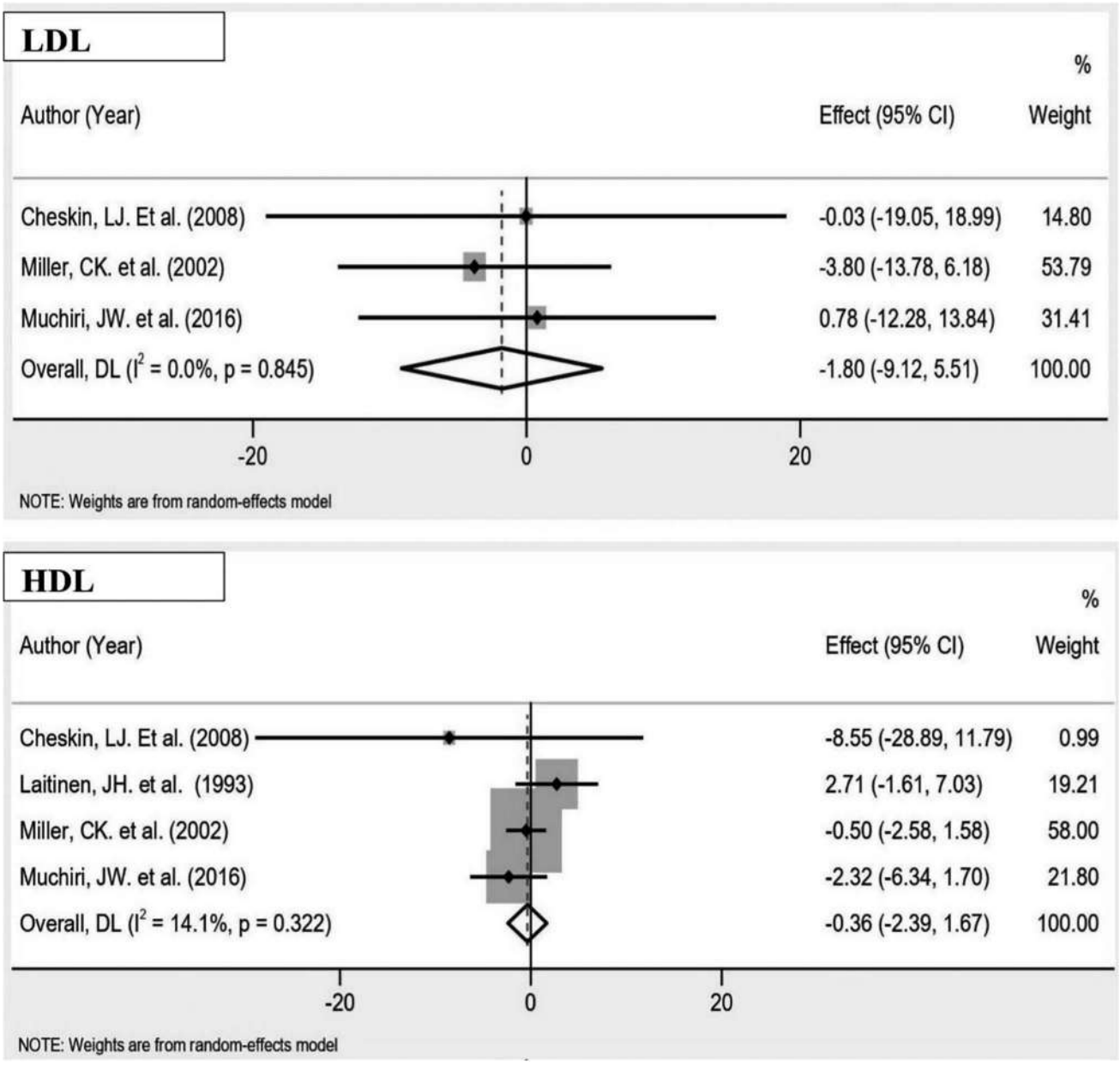


Fig. 3 (continued)

LDL ($p = 0.602$, $p = 0.489$), TG ($p = 0.531$, $p = 0.328$), SBP ($p = 0.573$, $p = 0.984$), DBP ($p = 0.452$, $p = 0.447$), BMI ($p = 0.246$, $p = 0.465$), and WC ($p = 1.00$, $p = 0.790$), respectively.

However, Begg and Egger tests were significant regarding the effect of formal education on TC ($p = 0.025$, $p = 0.017$). After publication bias adjustment, the pooled estimate of TC level did not change − 6.28 (95% CI: − 10.77, − 1.79), and there was low level of heterogeneity among the studies ($I^2 = 0.0\%$; $p = 0.997$).

Sensitivity analysis

To evaluate the effects of formal nutrition education on anthropometric and blood biomarkers of type 2 diabetic patients, we performed a sensitivity analysis according to the random-effects model. Results of sensitivity analysis showed that one study had no effect on results. However, the results revealed significant influence of one study on the pooled estimated effects of formal nutrition education on BMI (Gutschall MD, et al. 2009) [26] and SBP

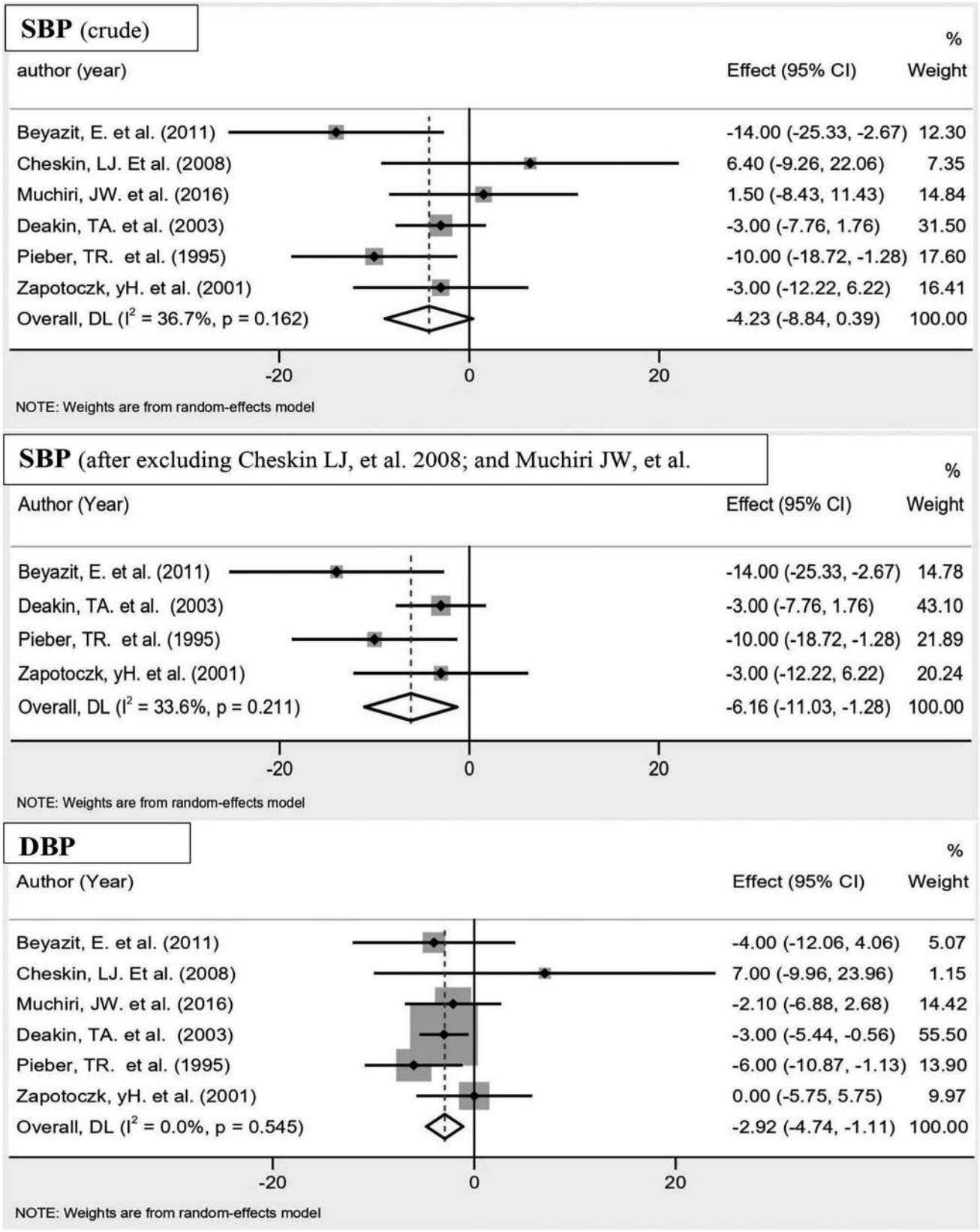


Fig. 4 Meta-analysis of the effect of formal nutrition education on blood pressures and anthropometric indices in type 2 diabetic patients

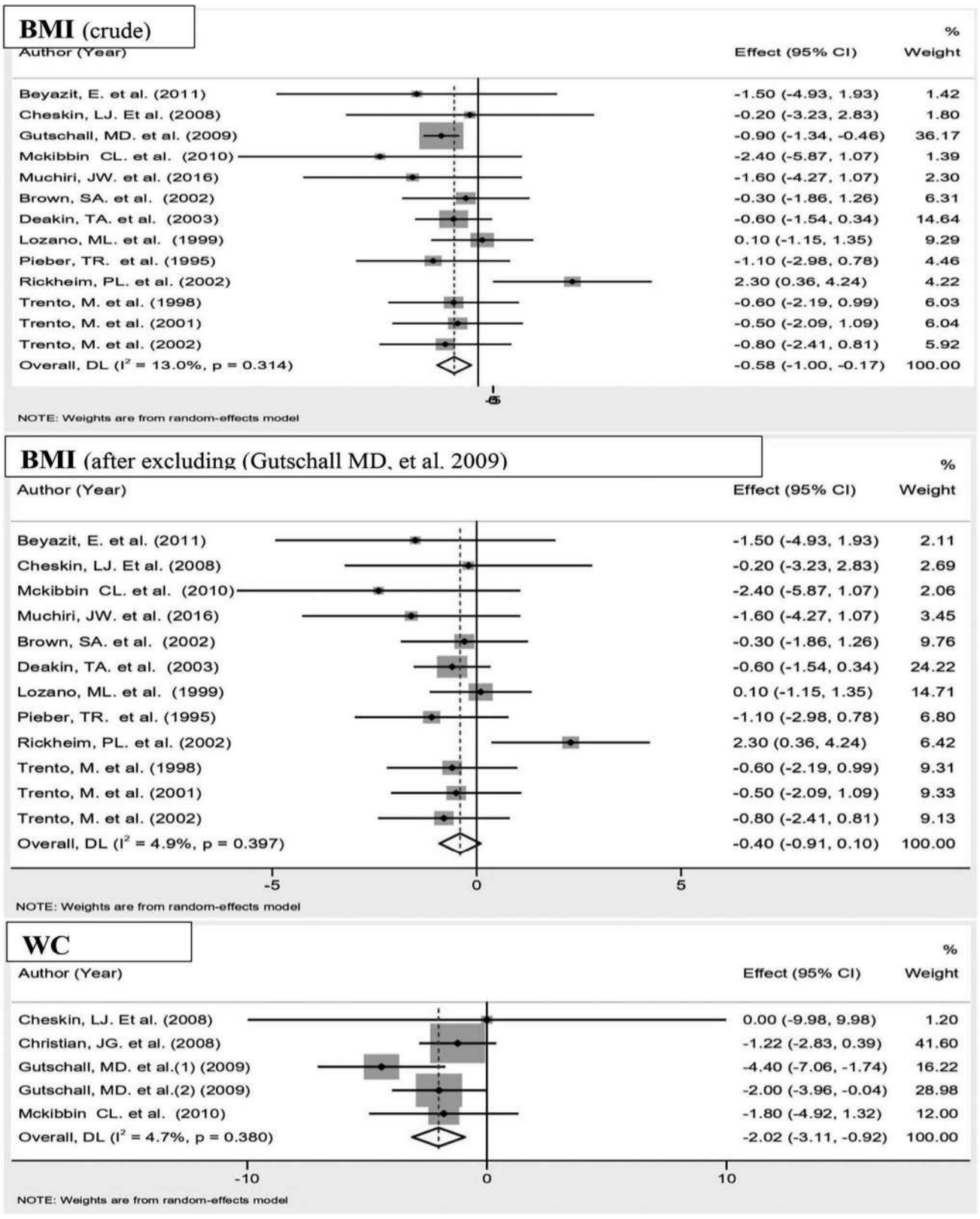


Fig. 4 (continued)

(Cheskin LJ, et al. 2008; and Muchiri JW, et al. 2016) [23, 34] (Figure S2).

Discussion

This meta-analysis suggests that formal education decreases the blood glucose, blood pressure, waist circumference, and lipid profiles especially total cholesterol and triacylglycerol, significantly.

Consistently, Chineyed et al. in 2013 suggested that although HbA1c decreased in all three groups of individual education, group education, or taking usual care, patients who had face-to-face education showed better glucose control than those who received group education by conversation map [17]. However, Li et al. in 2016 demonstrated that group education for diabetic patients was more effective on improving psychosocial metrics and 3-month HbA1c. Accordingly, the 3 months HbA1C was significantly lower in group education model than individual education model. However, the 6 months HbA1C did not have any statistically significant differences between groups [41]. Deakin et al. in 2003 had shown a significant 0.4% decrement in HbA1c for 4 months, and in 2005, they found that group-based education method reinforced diabetes control and knowledge of T2DM patients. [18, 24] Lozano et al. in a meta-analysis on 395 participants demonstrated the overall reduction of 1.4% in HbA1C, and a significant improvement in FBS by group education [31].

At 4–6 months, among three studies including 629 participants, there were no significant differences in total cholesterol levels among group education versus usual care groups [22, 24, 35]. At 12–14 months, TG levels were different between groups in three studies with 552 patients [22, 24, 40]. Studies on 628 patients showed 0.24 mmol/L descending in TG levels after group education program [22, 24, 35].

Conversely, the overall reduction in BMI was 0.2 kg/m^2 via group education without any heterogeneity between studies in three studies with overall 718 participants. But no statistically significant differences were observed for body weight [22, 35, 36]. Consistently, four studies with 566 participants did not show any significant effect of group-based diabetes education program on body weight or BMI. The overall reduction in body weight was 2.1 kg more than control group but difference was not statistically significant [24, 27, 35, 36].

Two studies showed that at 4 to 6 months, SBP decreased significantly about 5 mmHg, after group education [24, 35]. A trend towards 3 mmHg reduction in DBP was also observed. At 12–14 months, two studies demonstrated a small non-significant reduction of 3 mmHg in SBP after group education [24, 40].

Different results from different studies can be attributed to various methodological issues. Different educational contents in each subgroup (academic, health centers, hospital, or clinics); different educational targets (individual-based or group-based); variation in duration of each educational sessions, duration of the intervention, and involvement of families, friends, or none lead to different results. In addition, some prognostic factors including severity of the disease, different age groups, differences in blinding condition, and having different control groups can lead to controversy in the results. Most of the studies used routine treatments while the others used enhanced usual care, waiting list, and printed health education materials and computer-assisted is another reason for contradictory results.

This review has some strength. It includes the majority RCTs regarding the impact of nutritional education on T2DM adult patients, and it is the latest systematic review and meta-analysis in this topic. Other strengths include uncovering of biases like small-study effects, or defaults in the design, conduct, analysis, and interpretation. Nevertheless, the present review features some limitations. First, test condition bias is probable, since we used the data of published papers, and each patient's data were not available. Second, considering the small sample size of some of the relevant RCTs, significant metabolic changes associated might not have been detected. Third, in general, meta-analysis cannot improve the quality of the original studies, or cannot address the variability of patient populations, the quality of the data, and the potential for underlying biases.

Conclusion

Formal nutrition education can significantly improve total cholesterol, triacylglycerol, blood sugar, blood pressure, and waist circumference in type 2 diabetic patients. More research is needed based on these methods for decision-making. Totally, the results of this study showed that formal education as an important factor of socialization system of each society has important impact on the nutrition of type 2 diabetic patients, and consequently on their clinical indices. The improvement of formal nutrition education for diabetic patients and control the clinical indices of type 2 diabetic patients is advised.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-01023-6.

Author contribution AM and SMDR designed the research; SMDR and AM conducted the research; SMDR analyzed the data; SMDR wrote the paper; and MHB and SF edited the paper; AM had primary responsibility for final content. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Babazadeh T, Dianatinasab M, Daemi A, Nikbakht HA, Moradi F, Ghaffari-Fam S. Association of self-care behaviors and quality of life among patients with type 2 diabetes mellitus: Chaldoran County, Iran. Diabetes Metab J. 2017;41(6):449–56.
2. Mansoori Z, Ghaedi H, Sadatamini M, Vahabpour R, Rahimipour A, Shanaki M, et al. Downregulation of long non-coding RNAs LINC00523 and LINC00994 in type 2 diabetes in an Iranian cohort. Mol Biol Rep. 2018;45(5):1227–33.
3. Meybodi HR, Hasanzad M, Larijani B. Path to personalized medicine for type 2 diabetes mellitus: reality and hope. Acta Med Iran. 2017;55(3):166–74.
4. Asghari G, Ghorbani Z, Mirmiran P, Azizi F. Nut consumption is associated with lower incidence of type 2 diabetes: the Tehran Lipid and Glucose Study. Diabetes Metab. 2017;43(1):18–24.
5. Javanbakht M, Mashayekhi A, Baradaran HR, Haghdoost A, Afshin A. Projection of diabetes population size and associated economic burden through 2030 in Iran: evidence from microsimulation Markov model and Bayesian meta-analysis. PloS one. 2015;10(7):e0132505.
6. (NCD-RisC) NRFC. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4• 4 million participants. Lancet Diabetes Endocrinol. 2016;387:1513–30.
7. Waller K, Furber S, Bauman A, Allman-Farinelli M, van den Dolder P, Hayes A, et al. DTEXT - text messaging intervention to improve outcomes of people with type 2 diabetes: protocol for randomised controlled trial and cost-effectiveness analysis. BMC Public Health. 2019;19(1):262.
8. Keshavarz K, Lotfi F, Sanati E, Salesi M, Hashemi-Meshkini A, Jafari M, et al. Linagliptin versus sitagliptin in patients with type 2 diabetes mellitus: a network meta-analysis of randomized clinical trials. Daru. 2017;25(1):23.
9. Grusec JE, Hastings PD. The handbook of socialization: theory and research. New York and London: The Guilford Press; 2007.
10. Editors of Salem Press. The process of socialization. California: Salem Press; 2011.
11. Yang YS, Wu YC, Lu YL, Kornelius E, Lin YT, Chen YJ, et al. Adherence to self-care behavior and glycemic effects using structured education. J Diabetes Investig. 2015;6(6):662–9.
12. Gurusamy KS, Vaughan J, Davidson BR. Formal education of patients about to undergo laparoscopic cholecystectomy. Cochrane Database Syst Rev. 2014;2:CD009933.
13. Wong BM, Coffey M, Nousiainen MT, Brydges R, McDonald-Blumer H, Atkinson A, et al. Learning through experience: influence of formal and informal training on medical error disclosure skills in residents. J Grad Med Educ. 2017;9(1):66–72.
14. Reale R, Tumminia A, Romeo L, La Spina N, Baratta R, Padova G, et al. Short-term efficacy of high intensity group and individual education in patients with type 2 diabetes: a randomized single-center trial. J Endocrinol Invest. 2019;42(4):403–9.
15. Chinenye S, Young EE. Diabetes conversation map in Nigeria: a new socioeducational tool in diabetes care. Indian J Endocrinol Metab. 2013;17(6):1009–11.
16. Trento M, Passera P, Borgo E, Tomalino M, Bajardi M, Cavallo F, et al. A 5-year randomized controlled study of learning, problem solving ability, and quality of life modifications in people with type 2 diabetes managed by group care. Diabetes Care. 2004;27(3):670–5.
17. Chung LM, Law QP, Fong SS, Chung JW, Yuen PP. A cost-effectiveness analysis of teledietetics in short-, intermediate-, and long-term weight reduction. J Telemed Telecare. 2015;21(5):268–75.
18. Deakin T, McShane CE, Cade JE, Williams RD. Group based training for self-management strategies in people with type 2 diabetes mellitus. Cochrane Database Syst Rev (Online). 2005(2):CD003417. https://doi.org/10.1002/14651858.CD003417.pub2.
19. Ramadas A, Chan CKY, Oldenburg B, Hussein Z, Quek KF. Randomized-controlled trial of a web-based dietary intervention for patients with type 2 diabetes: changes in health cognitions and glycemic control. BMC Public Health. 2018;18(1):716.
20. Follmann D, Elliott P, Suh IL, Cutler J. Variance imputation for overviews of clinical trials with continuous response. J Clin Epidemiol. 1992;45(7):769–73.
21. Beyazıt E, Mollaoğlu M. Investigation of effect on glycosylated hemoglobin, blood pressure, and body mass index of diabetes intensive education program in patients with type 2 diabetes mellitus. Am J Mens Health. 2011;5(4):351–7.
22. Brown SA, Garcia AA, Kouzekanani K, Hanis CL. Culturally competent diabetes self-management education for Mexican Americans: the Starr County border health initiative. Diabetes Care. 2002;25(2):259–68.
23. Cheskin LJ, Mitchell AM, Jhaveri AD, Mitola AH, Davis LM, Lewis RA, et al. Efficacy of meal replacements versus a standard food-based diet for weight loss in type 2 diabetes a controlled clinical trial. Diabetes Educ. 2008;34(1):118–27.
24. Deakin TA, Cade JE, Williams DDR, Greenwood DC. Empowered patients: better diabetes control, greater freedom to eat, no weight gain! Diabetologia. 2003;46(2):A90.
25. Domenech MI, Assad D, Mazzei ME, Kronsbein P, Gagliardino JJ. Evaluation of the effectiveness of an ambulatory teaching/treatment programme for non-insulin dependent (type 2) diabetic patients. Acta Diabetol. 1995;32(3):143–7.
26. Gutschall MD, Miller CK, Mitchell DC, Lawrence FR. A randomized behavioural trial targeting glycaemic index improves dietary, weight and metabolic outcomes in patients with type 2 diabetes. Public Health Nutr. 2009;12(10):1846–54.
27. Heller SR, Clarke P, Daly H, Davis I, McCulloch DK, Allison SP, et al. Group education for obese patients with type 2 diabetes: greater success at less cost. Diabet Med. 1988;5:552–6.
28. Holtrop JS, Hickner J, Dosh S, Noel M, Ettenhofer TL. "Sticking to it—Diabetes Mellitus": a pilot study of an innovative behavior change program for women with type 2 diabetes. Am J Health Educ. 2002;33(3):161–6.
29. Kronsbein P, Mühlhauser I, Venhaus A, Jörgens V, Scholz V, Berger M. Evaluation of a structured treatment and teaching programme on non-insulin-dependent diabetes. Lancet. 1988;332(8625):1407–11.
30. Laitinen J, Uusitupa M, Siitonen O, Vanninen E, Pyörälä K. The maintenance of improved metabolic control after intensified diet therapy in recent type 2 diabetes. Diabetes Res Clin Pract. 1993;19(3):227–38.
31. Lozano ML, Armale MJ, Tena DI, Sanchez NC. The education of type-2 diabetics: why not in groups? Aten Primaria. 1999;23(8):485–92.
32. McKibbin CL, Golshan S, Griver K, Kitchen K, Wykes TL. A healthy lifestyle intervention for middle-aged and older schizophrenia patients with diabetes mellitus: a 6-month follow-Millerup analysis. Schizophr Res. 2010;121(1–3):203–6.
33. Miller CK, Edwards L, Kissling G, Sanville L. Nutrition education improves metabolic outcomes among older adults with diabetes mellitus: results from a randomized controlled trial. Prev Med. 2002;34(2):252–9.
34. Muchiri JW, Gericke GJ, Rheeder P. Effect of a nutrition education programme on clinical status and dietary behaviours of adults with

type 2 diabetes in a resource-limited setting in South Africa: a randomised controlled trial. Public Health Nutr. 2016;19(1):142–55.
35. Rickeim TR, Holler A, Siebenhofer A, Brunner GA, Semlitsch B, Schattenberg S, et al. Evaluation of a structured teaching and treatment programme for type 2 diabetes in general practice in a rural area of Austria. Diabet Med. 1995;12(4):349–54.
36. Rickheim PL, Weaver TW, Flader JL, Kendall DM. Assessment of group versus individual diabetes education: a randomized study. Diabetes Care. 2002;25(2):269–74.
37. Trento M, Passera P, Tomalino M, Pagnozzi F, Pomero F, Vaccari P, et al. Therapeutic group education in the follow-up of patients with non-insulin treated, non-insulin dependent diabetes mellitus. Diabetes Nutr Metab. 1998;11(3):212–6.
38. Trento M, Passera P, Tomalino M, Bajardi M, Pomero F, Allione A, et al. Group visits improve metabolic control in type 2 diabetes: a 2-year follow-up. Diabetes Care. 2001;24(6):995–1000.
39. Trento M, Passera P, Bajardi M, Tomalino M, Grassi G, Borgo E, et al. Lifestyle intervention by group care prevents deterioration of Type II diabetes: a 4-year randomized controlled clinical trial. Diabetologia. 2002;45(9):1231–9.
40. Zapotoczky H, Semlitsch B, Herzog G, Bahadori B, Siebenhofer A, Pieber TR, et al. A controlled study of weight reduction in type 2 diabetics treated by two reinforces. Int J Behav Med. 2001;8(1):42–9.
41. Li F, Yao P, Hsue C, Xu J, Lou Q. Impact of "Conversation Maps" on diabetes distress and self-efficacy of Chinese adult patients with type 2 diabetes: a pilot study. Patient Prefer Adherence. 2016;901:8–10.

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REVIEW ARTICLE

Association of the Pro12Ala gene polymorphism with treatment response to thiazolidinediones in patients with type 2 diabetes: a meta-analysis

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Abstract

Background/purpose of the study With the conflicting results on available studies regarding the association of the Pro12Ala polymorphism with the response of patients with type 2 diabetes mellitus (T2DM) to thiazolidinediones (TZDs), this meta-analysis was conducted to obtain more precise estimates.

Methods Relevant studies were searched in PubMed and Google Scholar and were selected according to the set inclusion criteria. Data were extracted and analyzed using both Review Manager 5.4.1 and Meta-Essentials. Pooled mean difference (MD), standardized mean difference (SMD), and odds ratios (ORs) and their 95% confidence intervals were computed to measure the association of the polymorphism with TZD treatment response.

Results Only four studies were included in this meta-analysis, with a sample size of 680. The pooled MD analysis results showed that FBG and HbA1c are significantly higher in the post-intervention group than in the pre-intervention group. No significant differences were noted between the genotypic groups (Pro12Pro vs. Pro12Ala and/or Ala12Ala). On the other hand, pooled OR analysis showed that T2DM that carry the PPARG gene variant are more likely to respond to TZD therapy.

Conclusion Based on the results of this meta-analysis, our findings suggest that the presence of the PPARG gene variant is associated with the response of patients with T2DM to TZD therapy. However, further studies are still needed to verify our claims.

Keywords Pro12Ala · PPARG gene · Thiazolidinediones · Type 2 diabetes · Meta-analysis

Introduction

Thiazolidinediones (TZDs) are a class of insulin sensitizers used in treating type 2 diabetes mellitus (T2DM), and these drugs are known to act by increasing the transactivation activity of the peroxisome proliferators activated receptors or PPARs [1]. The mechanism of action of these TZDs involves altering the transcription of genes influencing carbohydrate and lipid metabolism, resulting in changed amounts of protein synthesis. It is believed that TZDs reduce insulin resistance in adipose tissue, where PPARG or the PPAR gamma is predominant, and also in muscle and liver tissues [2]. Pioglitazone and rosiglitazone are the most common TZDs in the market; both are medications used to treat T2DM and are classified as agonists for the PPARG [3]. Pioglitazone improves glycemic control in people with T2DM by improving insulin sensitivity through its action at PPARG1 and PPARG2 [4]

The PPARG is a ligand-activated transcription factor located at 3p25, which plays an essential role in regulating lipid storage, fatty acid uptake, glucose uptake, and energy balance. It has a significant role in forming adipose tissue and the subcellular metabolism of the macrophage cells in the arterial wall [5]. The PPARG also activates genes involved in both glucose and insulin metabolism [6, 7]. Several single nucleotide polymorphisms (SNPs) in the PPARG have been

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associated with lipoprotein levels and insulin resistance. Among these polymorphisms, one significant SNP is the Pro12Ala that has been linked to lipoprotein levels and even insulin resistance of certain individuals [8–10]. This polymorphism causes the missense mutation CCA-GCA located at the exon 2 of the PPARG gene [11]. This results in the replacement of Proline to Alanine at the amino acid residue 12, hence the term Pro12Ala. Multiple studies have stated that the Pro12Ala polymorphism improves insulin sensitivity in humans [12, 13]. Individuals with at least one copy of Ala have lower BMI and higher insulin sensitivity. Also, having the Ala allele appeared to have a protective role against T2DM [14].

Certain studies show that patients with the Pro12Ala polymorphism respond better to TZD treatment [15–19]. However, results from various studies are still conflicting. Some studies emphasized that the Pro12Ala polymorphism in the PPARG gene has no significant effect on pioglitazone efficacy. The reaction to pioglitazone treatment is not dependent on the Pro12Ala polymorphism in the PPARG gene [20]. Hence, we performed this meta-analysis to obtain more precise estimates of the association of the Pro12Ala polymorphism in the PPARG gene with response to TZD therapy of patients with T2DM.

Methods

Literature search strategy, eligibility criteria, and study assessment

Articles were searched in PubMed and Google Scholar (title search only) using a combination of the following key search terms: "Pro12Ala" OR "*rs1801282*" OR "polymorphism" AND "pioglitazone" OR "rosiglitazone" OR "thiazolidinedione" AND "diabetes" as of September 26, 2021. No restrictions were applied to the date of publication. Titles and abstracts of the resulting studies were checked for eligibility. Only studies written in English were considered for this meta-analysis. Full text of the studies that passed the initial screening was collected and further assessed. Full-text articles were included in the meta-analysis if: (1) studies are conducted among T2DM patients; (2) studies that grouped their T2DM participants based on their Pro12Ala polymorphism genotype; and (3) studies that have pre- and post-intervention data for glycosylated hemoglobin (HbA1c) and fasting blood glucose (FBG) that are grouped based on their Pro12Ala polymorphism genotype. In this study, we defined treatment response as (1) changes in the post-intervention FBG and HbA1c between the genotypic groups (Pro12Pro group vs. Pro12Ala and/or Ala12Ala group); and (2) an increase in the number of responders to TZD based on the criteria set by Bluher et al. [20] as used by most included studies [20]. Briefly, TZD responders were defined as those with a > 15% decrease in HbA1c levels or > 20% decrease in FPG levels (or both) after TZD therapy. For the study design, articles classified as reviews, case reports, case studies, commentaries, and editorials were not included.

Data extraction

The following data were obtained from each included study: (i) first author's last name; (ii) year of publication; (iii) country where the study was conducted; (iv) method of SNP detection; (v) PPARG gene variants of the study groups; (vi) the total number of participants; (vii) the total number of participants per sex; (viii) mean age of the study groups; (ix) mean duration of diabetes in years; (x) duration of the TZD therapy; (xi) type of TZD therapy initiated; (xii) HbA1c (in %) and FBG (in mg/dL) data pre- and post-intervention in each genotypic group; and (xiii) number of TZD responders and non-responders across the study groups. For consistency and to preserve the original values of the HbA1c and FBG results, data obtained were no longer converted. Instead, only those with similar units were used for the analysis. All data needed were initially extracted by two of the authors. The other authors reviewed, verified, and counter-checked the extracted data and resolved any disagreements.

Study quality assessment

The quality of each included study was assessed using the Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group by the National Heart, Lung, and Blood Institute (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). Results obtained were described accordingly.

Data analysis

Pooled mean difference (MD), standardized mean difference (SMD), and odds ratios (ORs) computation were used in this study. The MD (for dependent groups), SMD (for independent groups), and the 95% confidence interval (CI) of the levels of HbA1c and FBG among patients with T2DM between the study groups (with and without the PPARG gene variant) were calculated and pooled. The protocol for analyzing the MDs/SMDs and ORs was adapted from previous researches done by the same author [21–23]. Computations of the pooled ORs and 95% CIs were done to measure the outcomes (responder or non-responder to TZD therapy) of an exposure (presence of the PPARG gene variant). The pooled MD, SMD, and OR estimates were determined either by the fixed- or random-effects model, depending on the presence of heterogeneity [24, 25]. The presence of heterogeneity was

Table 1 Characteristics of the included studies

First author and year	Country	Method of SNP detection	*N*	Overall mean age	Duration of diabetes in years	Duration of intervention in weeks	TZD therapy initiated	Criteria for TZD responders	No. of TZD responders	No. of TZD non-responders
Bluher, 2003	Germany	PCR	131	60.7	6.51	26	Pioglitazone monotherapy 45 mg/day	NA	NA	NA
Hsieh, 2010	Taiwan	PCR-RFLP	250	Responders = 57.4 Non-responders = 58.6	NA	24	Pioglitazone monotherapy 30 mg/day	Criteria by Bluher et al.	154	96
Kang, 2005	Korea	PCR	198	56.5	6.7	12	Rosiglitazone therapy 4 mg/day (no changes in previous medications)	Criteria by Bluher et al.	93	105
Namvaran, 2011	Iran	TaqMan	101	51.44	NA	12	Pioglitazone monotherapy 15 mg/day	Criteria by Bluher et al. as cited by Kang et al.	32[a]	69[a]

SNP, single nucleotide polymorphism; *N*, total number of participants; *TZD*, thiazolidinedione; *PCR*, polymerase chain reaction; *PCR-RFLP*, polymerase chain reaction-restriction fragment length polymorphism; *NA*, not applicable

[a] Values provided in the original article are in percentage and were converted to acquire the whole number.

examined using a chi-based Q test, and its degree was measured using I^2 statistics [26, 27]. All p-values (P^A) for association were two-sided with a significance threshold of < 0.05, whereas the p-value (P^H) for heterogeneity is set at < 0.10 due to the low power of the test [28]. All statistical analyses were done using both Review Manager ver. 5.4 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2014) and Meta-Essentials (Erasmus Research Institute of Management, 2017) [29]. The analysis for publication bias was no longer performed due to the limited number of eligible studies in this meta-analysis.

Results

Search results, characteristics, and quality of the included studies

The initial search resulted in 75 studies from both PubMed and Google Scholar. After screening the title and abstract, a total of 18 studies qualified for full-text assessment. Only four studies [15–17, 20] were included in this meta-analysis. No other study was obtained from checking the cited reference from each included text. The full summary of the literature search is presented in Fig. 1. As for the characteristics of the included studies, this is summarized in Table 1. Overall, a total sample size of 680 T2DM patients was included in this meta-analysis. All studies were conducted in various countries between the years 2003 and 2011. As shown in Table 1 as well, it can be noted that there is heterogeneity in the methodologies of the four studies in terms of the method of SNP detection, duration of intervention, and the TZD therapy initiated among the patients.

The risk of bias in each study could be judged as low in five out of 12 items, namely, study question; eligibility criteria and study population; enrollment of eligible participants; measurement of outcomes; and the use of appropriate statistical analysis, whereas items on study participants representative of clinical populations of interest, description of the intervention, and follow-up rate were indeterminable by the authors. Some studies did not report items on sample size, blinding of outcome accessors, and multiple outcome measures. Lastly, there was no mention that group-level interventions were adjusted for the individual-level outcomes.

FBG and HbA1c response of T2DM patients with the PPARG gene variant after TZD treatment

FBG and HbA1c data from the individual studies are summarized in Table 2. The analysis for the pooled difference using SMDs between the FBG and HbA1c levels between the various study groups is presented in Table 3. For this, three comparison models were done, namely, (M1) pre- and post-intervention

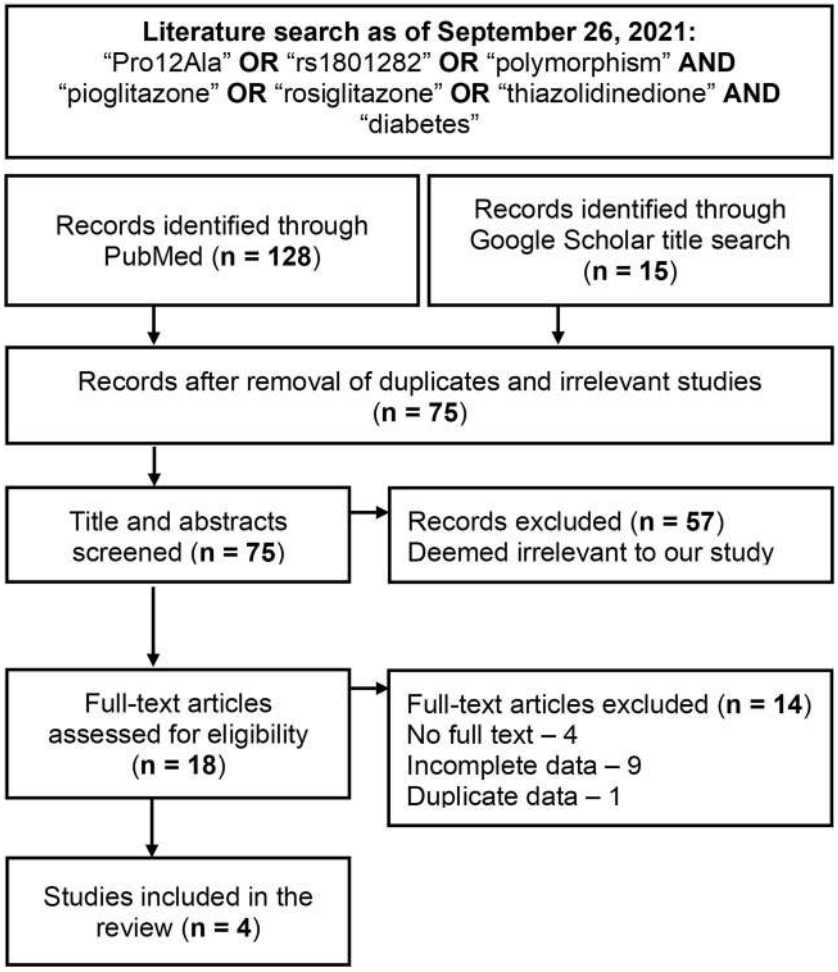

Fig. 1 Summary of the literature search

FBG and HbA1c between the groups with the wild-type (Pro12Pro) genotype, (M2) pre- and post-intervention FBG and HbA1c between the groups with the PPARG variant (Pro12Ala and/or Ala12Ala), and (M3) post-intervention FBG and HbA1c between the groups with wild-type genotype and PPARG variant.

Table 2 Biochemical parameters of T2DM patients per study across the different PPARG genotypes

	Wild type (Pro12Pro)						PPARG variant (Pro12Ala or Ala12Ala or 12Ala)					
	Pre-intervention			Post-intervention				Pre-intervention			Post-intervention	
First author	Mean	SD	*n*	Mean	SD	*n*	PPARG variant	Mean	SD	*n*	Mean	SD	*n*
FBG (mg/dL)													
Hsieh	185.8	58.2	197	142.6	42.5	197	12Ala	192.2	58.8	53	141.8	37.8	53
Kang	161.9	45	183	137.6	38.5	183	Pro12Ala	169.7	41.2	15	119.1	20.7	15
Namvaran	196.47	66.92	95	153.25	57.92	95	Pro12Ala	184.67	40.82	6	134.33	22.76	6
HbA1c (%)													
Bluher	8.92	1.2	110	7.7	1	110	Ala12Ala	8.84	0.9	5	7.34	1.4	5
							Pro12Ala	9.15	1.1	16	8.18	1.2	16
Hsieh	8.51	1.66	197	8.16	1.61	197	12Ala	8.32	1.55	53	7.78	1.25	53
Kang	8.12	1.51	183	7.55	1.25	183	Pro12Ala	8.36	1.5	15	6.95	0.66	15
Namvaran	9.22	2.06	95	8.24	1.74	95	Pro12Ala	9.93	2.57	6	9.1	1.53	6

PPARG, peroxisome proliferator-activated receptor gamma; *n*, number of participants; *FBG*, fasting blood glucose

Table 3 Summary of the MD and SMD analysis for the response of T2DM patients with the PPARG variant after TZD treatment

Parameter	Comparison	Test for heterogeneity			Test for association		
		I^2	P^H	AM	MD/SMD	95% CI	P^A
FBG (mg/dL)	Model 1	78%	0.01*	Random	−36.03	−50.21, −21.86	< 0.00001**
	Model 2	0%	1.00 NS	Fixed	−50.46	−64.10, −36.82	< 0.00001**
	Model 3	20%	0.29 NS	Fixed	−0.15	−0.40, 0.10	0.23 NS
HbA1c (%)	Model 1	83%	0.0004*	Random	−0.77	−1.19, −0.35	0.0003**
	Model 2	0%	0.43 NS	Fixed	−0.88	−1.26, −0.51	< 0.00001**
	Model 3	58%	0.05*	Random	−0.05	−0.44, 0.33	0.78 NS

FBG, fasting blood glucose; I^2, degree of heterogeneity; P^H, *p*-value for heterogeneity; *AM*, analysis model; *MD*, mean difference; *SMD*, standardized mean difference; *CI*, confidence interval; P^A, *p*-value for association; *NS*, not significant

Model 1: comparison of the analyte in the wild-type pre- (control) and post-intervention (experimental) groups using MD analysis

Model 2: comparison of the analyte in the PPARG variant pre- (control) and post-intervention (experimental) groups using MD analysis

Model 3: comparison of the analyte between the wild-type (control) and PPARG variant (experimental) group post-intervention using SMD analysis

*Significant at *p*-value of < 0.10

**Significant at *p*-value of < 0.05

Results of FBG for both M1 and M2 (MD: −50.46 to −36.03, 95% CI: −64.10, −21.86, P^A: < 0.00001) show that its levels are higher in the post-intervention group than the pre-intervention group with varying heterogeneity (I^2 = 0–78%, P^H = 0.01 to 1.00) of outcomes. On the other hand, results for HbA1c for both M1 and M2 (MD: −0.88 to −0.77, 95% CI: −1.26 to −0.35, P^A: < 0.00001 to 0.0003) also showed the same observations as the FBG results. Varying heterogeneity (I^2 = 0–83%, P^H = 0.004 to 0.43) of outcomes were also noted. No significant difference was observed for the M3 comparison for both FBG and HbA1c.

Association of the PPARG gene variant with TZD response

The association of the presence of the PPARG gene variant to the response of patients with T2DM to TZD was further determined using ORs analysis. Figure 2 shows the result of the fixed-effects model analysis. Based on the results, a significant association (OR: 2.69; 95% CI: 1.52 to 4.75; P^A = 0.0006) was observed with homogeneity (I^2 = 42%, P^H = 0.18) on the pooled outcome.

Discussion

Summary and interpretation of findings

Overall, the findings of this meta-analysis showed significant associations. For the SMD analysis, FBG and HbA1c levels were higher in the post-intervention group than in the pre-intervention groups. This means that TZD therapy was able to lower the levels of both markers after the intervention. However, when assessing the results of the wild-type group vs. the PPARG gene variant group, there was no significant difference in the reduction of both FBG and HbA1c in the post-intervention groups. On the other hand, for the OR analysis, a significant association was noted. The results suggest that those that carry the PPARG gene variant are more likely to respond to TZD therapy than those with the wild-type genotype.

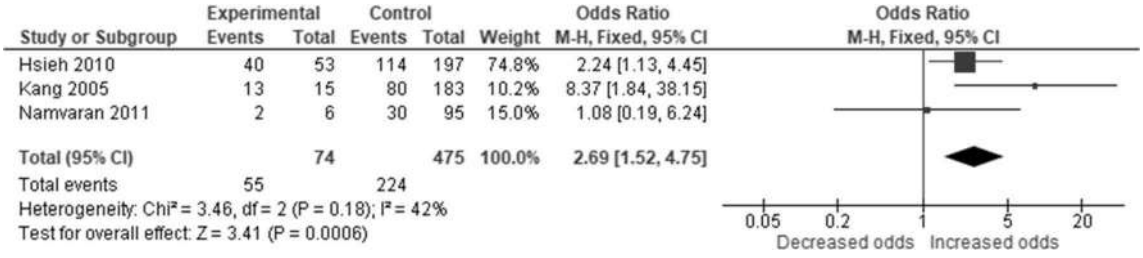

Fig. 2 Forest plot analysis for the association of the presence of the PPARG gene variant with TZD response

Findings from previous studies

Several researches have provided useful information on the possible mechanism through which the Pro12Ala polymorphism reduces the risk of T2DM [15–17, 20]. In the study of Bluher et al., the link between the Pro12Ala and the Pro12Pro variants in the PPARG gene and the pioglitazone response rate was investigated [20]. Results revealed that such PPARG genotype in position 12 did not affect the efficacy of pioglitazone in white patients with T2DM. Additional findings identified that initial fasting glucose of > 11.0 mmol/L, HbA1c value of > 9.0%, BMI of > 33 kg/m^2, and fasting C-peptide concentrations at baseline of > 2.5 pmol/L were the most common confounding factors for the responder frequency to pioglitazone treatment. Another study that aimed to establish the association between the Pro12Ala variant with pioglitazone response rate was done by Namvaran et al. [15, 30]. Here, multiple logistics regression was used and yielded no statistical correlation between the occurrence of the Pro/Pro or Pro/Ala variation and the frequency of response to pioglitazone treatment in the Iranian population. The considerable variations in insulin-to-glucose ratio before and after pioglitazone therapy, on the other hand, show that the Ala allele may have some protective effects.

Other studies that evaluated the probable link between the Pro12Ala polymorphism and TZD responsiveness in T2DM yielded contradictory results. According to Hsieh et al. [16], Chinese diabetes patients with the Pro12Ala and Ala12Ala genotypes of the PPAR-γ gene were more likely to respond to pioglitazone than the patients with the Pro12Pro genotype positively. These results disclose a link between Pro12Ala polymorphism and therapeutic responsiveness to pioglitazone in Chinese patients with T2DM. It was discovered that baseline FBG and triglyceride levels were related to pioglitazone response, while other parameters such as age, gender, and BMI were not. However, following repeated logistic regression analysis, the link between baseline FBG and triglyceride levels and response to pioglitazone treatment diminished. Kang et al. [17] concluded yet another investigation and found an association for another member of the TZD drug family — rosiglitazone. The study reported that Korean T2DM patients with the Pro12Ala genotype in the PPAR-γ gene had a better therapeutic response to rosiglitazone than patients with the Pro12Pro genotype. They concluded that the genetic variation in the PPARG gene can affect the response to rosiglitazone of patients with T2DM as shown in the significant reduction of FBG and HbA1c in the Pro12Ala group after treatment. Both studies were performed in Asian populations, suggesting that ethnic factors may be important. With the contradicting research findings and various factors that are yet to be considered, the question of the relationship between the Pro/Ala polymorphism and clinical response to pioglitazone treatment remains open.

Association of the Pro12Ala with TZD treatment response

The most well-known PPARG agonists are long and very long-chain fatty acids, their derivatives, and TZDs such as rosiglitazone and pioglitazone [31]. TZDs are diabetes medications that function as insulin sensitizers and treat T2DM [32]. TZDs stimulate PPARG, which modifies the transcription of insulin-sensitive genes, thereby enhancing insulin sensitivity in muscle and adipose tissues while decreasing insulin resistance in the liver and peripheral tissues [15]. This also controls glucose, lipid, and protein metabolism [33]. However, they are costly agents that have been linked to serious side effects. Clinical choices may benefit from genetic factors that predict individual patients' responses to TZDs [16].

The effects of several genetic variations of the PPAR gene on drug reactions in vitro were observed as well in previous studies [34], especially in TZD therapy [35]. These changes may induce variances in the efficacy of TZD therapy in clinical practice and may constitute a biological reason for a strong or poor response to TZD treatment [15]. Because of the high incidence of the Pro12Ala polymorphism, it is the most likely option to explain the potential link between PPAR and TZD response. Since adipose tissues exclusively express PPAR2, the metabolic effects of this SNP seem to be a result of alterations in the adipocytes, which leads to the release of many cytokines, such as adiponectin. Hence, since these adipocytokines are under the transcriptional control of PPAR, they may mediate the Pro12Ala effect on the pharmacologic action of TZDs. TZDs will increase adiponectin concentrations in the blood, improving insulin sensitivity and lowering blood glucose levels [36].

Limitations of the study

Even with the promising results, caution should still be taken when interpreting them and applying them clinically. Some of the study's limitations include (a) no recent publication on the topic, the latest study published was in 2011; (b) methodological heterogeneity wherein the method of SNP detection, duration of intervention, and the TZD therapy initiated among the patients substantially differ per study; (c) heterogeneity of some of the outcomes; (d) no representation of other ethnic groups in the study; (e) lack of standardization in the diagnostic tests used; (f) socioeconomic, sociodemographic, and the medical history of the participants were not controlled for; and (g) inconsistencies in the study groups. Other analyses in the study such as the association between the Pro12Ala polymorphism and the benefits of TZDs (e.g., effects on inflammation

and vascular changes) were no longer done since studies collected only focused on the effect of the SNP with treatment response. Also, other treatment response variables such as BMI, lipid profile, and insulin resistance were no longer studied since publications that contained such data are only two or below or there are inconsistencies in the unit of measurement used which could affect the overall results of the study. Lastly, because of the limited number of studies included, it is not possible to give the association of the gene polymorphism with pioglitazone and rosiglitazone separately.

Conclusion

To our knowledge, this is the first meta-analysis that investigated the association of the Pro12Ala SNP with TZD response in patients with T2DM. Overall, based on the results of the analysis, our findings suggest that the presence of the PPARG gene variant is associated with the response of patients with T2DM to TZD therapy. However, care should be taken when interpreting these findings since the exact mechanism of this association is still in question. Further controlled studies in various geographic locations and ethnic groups should be conducted. Also, studies that would look into the association of the polymorphism with the benefits offered by TZD should be done to further support this relationship.

Author contribution All authors have contributed substantially to collecting and analyzing the data and in writing and critically revising the manuscript.

Declarations

Ethics approval This article does not contain any studies with human or animal subjects.

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

References

1. Nanjan MJ, Mohammed M, Prashantha Kumar BR, Chandrasekar MJN. Thiazolidinediones as antidiabetic agents: a critical review. Bioorg Chem. 2018;77:548–67.
2. Hauner H. The mode of action of thiazolidinediones. Diabetes Metab Res Rev. 2002;18:S10–5.
3. Jia C, Huan Y, Liu S, Hou S, Sun S, Li C, Liu Q, Jiang Q, Wang Y, Shen Z. Effect of chronic pioglitazone treatment on hepatic gene expression profile in obese C57BL/6J mice. Int J Mol Sci. 2015;16:12213–29.
4. Smith U. Pioglitazone: mechanism of action. Int J Clin Pract Suppl. 2001.
5. Tyagi S, Gupta P, Saini A, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. J Adv Pharm Technol Res. 2011;2:236–40.
6. Alves MC, de Morais CC, Augusto EM, Abdalla DSP, Horst MA, Cominetti C. Polymorphisms in PPARG and APOE: relationships with lipid profile of adolescents with cardiovascular risk factors. Nutrire. 2017;42(1).
7. Chan KHK, Niu T, Ma Y, You NCY, Song Y, Sobel EM, Hsu YH, Balasubramanian R, Qiao Y, Tinker L, Liu S. Common genetic variants in peroxisome proliferator-activated receptor-γ (PPARG) and type 2 diabetes risk among women's health initiative postmenopausal women. J Clin Endocrinol Metab. 2013;98(3).
8. Stryjecki C, Peralta-Romero J, Alyass A, Karam-Araujo R, Suarez F, Gomez-Zamudio J, Burguete-Garcia A, Cruz M, Meyre D. Association between PPAR-γ 32 Pro12Ala genotype and insulin resistance is modified by circulating lipids in Mexican children. Sci Rep. 2016;6.
9. Johansson LE, Danielsson P, Norgren S, Marcus C, Ridderstråle M. Interaction between PPARG Pro12Ala and ADIPOQ G276T concerning cholesterol levels in childhood obesity. Int J Pediatr Obes. 2009;4(2):119–25.
10. Hsiao TJ, Lin E. The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor gamma (PPARG) gene in relation to obesity and metabolic phenotypes in a Taiwanese population. Endocrine. 2015;48(3):786–93.
11. Shanmuga Priya S, Sankaran R, Ramalingam S, Sairam T, Somasundaram L. Genotype phenotype correlation of genetic polymorphism of PPAR gamma gene and therapeutic response to pioglitazone in type 2 diabetes mellitus—a pilot study. J Clin Diagn Res. 2016;10(2):FC11–4.
12. Trombetta M, Bonetti S, Boselli ML, Miccoli R, Trabetti E, Malerba G, Pignatti PF, Bonora E, Del Prato S, Bonadonna RC. PPARG2 Pro12Ala and ADAMTS9 rs4607103 as "insulin resistance loci" and "insulin secretion loci" in Italian individuals. the GENFIEV study and the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) 4. Acta Diabetol. 2013;50(3):401–8.
13. Tellechea ML, Aranguren F, Pérez MS, Cerrone GE, Frechtel GD, Taverna MJ. Pro12Ala polymorphism of the peroxisome proliferator activated receptor-γ gene is associated with metabolic syndrome and surrogate measures of insulin resistance in healthy men. Circ J. 2009;73(11):2118–24.
14. Watanabe RM, Black MH, Xiang AH, Allayee H, Lawrence JM, Buchanan TA. Genetics of gestational diabetes mellitus and type 2 diabetes. Diabetes Care. 2007;30(Supplement 2):S134–40.
15. Namvaran F, Azarpira N, Rahimi-Moghaddam P, Dabbaghmanesh MH. Polymorphism of peroxisome proliferator-activated receptor γ (PPARγ) Pro12Ala in the Iranian population: relation with insulin resistance and response to treatment with pioglitazone in type 2 diabetes. Eur J Pharmacol. 2011;671(1–3):1–6.
16. Hsieh MC, der Lin K, Tien KJ, te Tu S, Hsiao JY, Chang SJ, Lin SR, Shing SJ, Chen HC. Common polymorphisms of the peroxisome proliferator-activated receptor-γ (Pro12Ala) and peroxisome proliferator-activated receptor-γ coactivator-1 (Gly482Ser) and the response to pioglitazone in Chinese patients with type 2 diabetes mellitus. Metab Clin Exp. 2010;59(8):1139–44.
17. Kang E, Park S, Kim H, Kim C, Ahn C, Cha B, Lim S, Nam C, Lee H. Effects of Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ2 gene on rosiglitazone response in type 2 diabetes. Clin Pharmacol Therap. 2005;78(2):202–8.
18. Zhang R, Wang J, Yang R, Sun J, Chen R, Luo H, Liu D, Cai D. Effects of Pro12Ala polymorphism in peroxisome proliferator-activated receptor-γ2 gene on metabolic syndrome risk: a meta-analysis. Gene. 2014;553:90–7.
19. Ramirez-Salazar M, Parez-Luque E, Fajardo-Araujo M, Garza SM, Malacara JM. Effect of the Pro12Ala polymorphism of the

PPARγ32 gene on response to pioglitazone treatment in menopausal women. Menopause. 2008;15:1151–6.
20. Blüher M, Lübben G, Paschke R. Analysis of the relationship between the Pro12Ala variant in the PPAR-γ2 gene and the response rate to therapy with pioglitazone in patients with type 2 diabetes. Diabetes Care. 2003;26(3):825–31.
21. Tiongco RE, Paragas NA, Dominguez MJ, Lasta SL, Pandac JK, Pineda-Cortel MR. ABO blood group antigens may be associated with increased susceptibility to schistosomiasis: a systematic review and meta-analysis. J Helminthol. 2018;11:1–10.
22. Pabalan N, Tiongco RE, Pandac JK, Paragas NA, Lasta S lo, Gallego N, Jarjanazi H, Pineda-Cortel MR. Association and biomarker potential of elevated serum adiponectin with nephropathy among type 1 and type 2 diabetics: a meta-analysis. PLoS ONE. 2018;13(12).
23. Tiongco RE, Cabrera FJ, Clemente B, Flake CC, Salunga MA, Pineda-Cortel MR. G276T polymorphism in the ADIPOQ gene is associated with a reduced risk of polycystic ovarian syndrome: a meta-analysis of Asian population. Taiwan J Obstet Gynecol. 2019;58(3):409–16.
24. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959;22(4):719–48.
25. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–88.
26. Lau J, JPA I, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med. 1997;127:820–6.
27. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ : Br Med J. 2003;327(7414):557–60.
28. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539–58.
29. Suurmond R, van Rhee H, Hak T. Introduction, comparison, and validation of Meta-Essentials: a free and simple tool for meta-analysis. Res Synth Methods. 2017;8:537–53.
30. Snitker S, Watanabe RM, Ani I, Xiang AH, Marroquin A, Ochoa C, Goico J, Shuldiner AR, Buchanan TA. Changes in insulin sensitivity in response to troglitazone do not differ between subjects with and without the common, functional Pro12Ala peroxisome proliferator–activated receptor-γ2 gene variant. Diabetes Care. 2004;27(6):1365–8.
31. Mirza AZ, Althagafi II, Shamshad H. Role of PPAR receptor in different diseases and their ligands: physiological importance and clinical implications. Eur J Med Chem. 2019;166:502–13.
32. Cariou B, Charbonnel B, Staels B. Thiazolidinediones and PPARγ agonists: time for a reassessment. Trends Endocrinol Metab. 2012;23(5):205–15.
33. Arnold Sv., Inzucchi SE, Echouffo-Tcheugui JB, Tang F, Lam CSP, Sperling LS, Kosiborod M. Understanding contemporary use of thiazolidinediones. Circulation: Heart Failure. 2019;12(6).
34. Cresci S. PPAR genomics and pharmacogenomics: implications for cardiovascular disease. PPAR Res. 2008;2008:1–11.
35. Aquilante CL. Pharmacogenetics of thiazolidinedione therapy. Pharmacogenomics. 2007;8(8):917–31.
36. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J Biol Chem. 1995;270(22):12953–6.

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CORRECTION

Correction to: Association of the Pro12Ala gene polymorphism with treatment response to thiazolidinediones in patients with type 2 diabetes: a meta-analysis

Johana Vallo[1] · Roselle Arbas[1] · Jan Erika Basilio[1] · Ivy Cayabyab[2] · Christian Noel Miranda[1] · Ma. Paulina Isabel Santos[1] · Louie Fernand Legaspi[3] · Raphael Enrique Tiongco[2]

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In Table 3, last column 4th row under P^A, it should be 0.0003**.

The Original article has been corrected.

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ORIGINAL ARTICLE

Evaluation of the reciprocal interaction between hepatic steatosis and type 2 diabetes: a comparative analysis with respect to anti-diabetic treatment, glycemic control, renal and hepatic function

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Abstract

Background This study aimed to evaluate reciprocal interaction between hepatic steatosis (HS) and type 2 diabetes (T2D) through comparative analysis of anti-diabetic treatment, glycemic control, and renal and hepatic function in T2D patients with versus without concomitant HS.

Methods A total of 102 T2D patients were included in this cross-sectional single-center study, and patients were divided into two groups including those with HS ($n=58$) and those without HS ($n=44$). Data on patient demographics, current anti-diabetic treatment, and serum levels for fasting blood glucose (FBG), HbA1c (%), urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lipids were recorded.

Results Diabetic patients with HS had younger age (59.6 ± 10.5 vs. 63.5 ± 14.5 years, $p=0.034$) and lower serum urea levels (28.5 (3–61) vs. 39 (14–138), $p=0.012$). Metformin (74.1% in patients with HS, 65.9% in patients without HS) was the most frequent anti-diabetic treatment in both groups with similar rate of glycemic control (HbA1c < 7%, 39.7%, and 40.9% of patients, respectively). HbA1c levels were positively correlated with serum urea ($r=0.308$, $p=0.042$), creatinine ($r=0.306$, $p=0.044$), and triglyceride ($r=0.358$, $p=0.017$) levels only in patients without HS. In patients with HS, no significant difference was noted in ALT and AST levels with respect to anti-diabetic regimen. Logistic regression analysis revealed that the presence of proteinuria (OR, 0.327, 95% CI 0.12 to 0.91, $p=0.032$) was associated with decreased likelihood of HS in T2D patients.

Conclusion In conclusion, our findings revealed no increase in the risk of poor glycemic control, dyslipidemia, or nephropathy pertaining to concomitant HS in T2D patients, as well as no difference in ongoing anti-diabetic treatments in terms of serum ALT and AST levels.

Keywords Type 2 diabetes · Hepatic steatosis · Anti-diabetic treatment · Glycemic control · Nephropathy · Transaminases

Introduction

NAFLD refers to a spectrum of liver damage that ranges from relatively benign hepatic steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis [1–3]. Being also considered as the hepatic manifestation of metabolic syndrome, NAFLD is a major risk factor for type 2 diabetes mellitus (T2D), and the two diseases frequently occur concomitantly with contribution of insulin resistance in the pathogenesis of both diseases [4–7].

Co-morbid NAFLD has been associated with significantly elevated risk for both macrovascular and microvascular complications in patients with T2D, particularly nephropathy and cardiopathy, while patients with NAFLD who also have T2DM are at an elevated risk for progression to fibrosis and NASH [5, 8–10]. In addition, the presence of NAFLD has been considered to be associated with increased likelihood of developing advanced chronic kidney diseases (CKD) [11] and with an increased risk for cardiovascular events [12]

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even in an advanced CKD cohort with high comorbidity [13, 14].

Therefore, the prognosis for patients with concomitant NAFLD and T2D is worsened due to the reciprocal influence of these two diseases that lead to increased risk for life-threatening sequela such as cardio-metabolic diseases and hepatocellular carcinoma, emphasizing the need for improved treatment options [4, 5, 9, 15].

This study was therefore designed to evaluate reciprocal interaction between hepatic steatosis (HS) and T2D through comparative analysis of anti-diabetic treatment, glycemic control, renal function, and hepatic function in T2D patients with versus without concomitant HS.

Materials and methods

Study population

A total of 102 T2D patients were included in in this cross-sectional single-center study, and patients were divided into two groups including those with HS ($n=58$) and those without HS ($n=44$), via ultrasound-based techniques for the diagnosis of HS. Adult patients (over 18 years of age) with T2D and available data on laboratory findings during their follow-up at internal medicine and endocrine clinics were included in the study.

Written informed consent was obtained from each patient following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Study parameters

Data on patient demographics (age, gender), current anti-diabetic treatment, blood biochemistry including fasting blood glucose (FBG, mg/dL), glycated hemoglobin (HbA1c, %), urea (mg/dL), creatinine (mg/dL), alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), cholesterol (mg/dL), triglyceride (mg/dL), low-density lipoprotein (LDL, mg/dL), and high-density lipoprotein (HDL, mg/dL) levels were recorded in patients with and without HS. Target HbA1c (< 7%) achievement rates were also recorded in both groups of diabetic patients.

Glycemic control rates were compared in patients with vs. without HS according to gender and treatment subgroups, while correlation of HbA1c levels with other laboratory parameters was analyzed in patients with vs. without HS. Glycemic and liver function test parameters were also evaluated with respect to anti-diabetic treatment in patients with HS.

Statistical analysis

Statistical analysis was made using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013). Chi-square ($\chi 2$) test and Fisher exact test were used for the comparison of categorical data, while numerical data were analyzed using Student's *t* test for variables with normal distribution and via Mann–Whitney *U* test for non-normally distributed variables. Correlation analysis was performed with Spearman correlation analysis. Data were expressed as mean ± standard deviation (SD), minimum–maximum, and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

Results

Patient demographics, laboratory findings, and treatment according to presence of HS

Diabetic patients with HS had younger age (59.6 ± 10.5 vs. 63.5 ± 14.5 years, $p=0.034$), lower serum urea levels (28.5 (3–61) vs. 39 (14–138) mg/dL, $p=0.012$), and higher serum AST (24.5 (9–117) vs. 17(7–43) U/L, $p<0.001$) and ALT (20 (10–58) vs. 18 (9–107) U/L, $p=0.019$) levels (Table 1).

Metformin (74.1% in HS group, 65.9% in patients without HS) was the most frequent anti-diabetic treatment in both groups, as followed by dipeptidyl peptidase-4 inhibitor (DPP4i; 40.9% and 29.3%, respectively) and insulin (34.1% and 27.6%, respectively). Median HbA1c levels were 7.3% in both HS and no-HS groups with achievement of glycemic control (HbA1c < 7%) in 39.7% and 40.9% of patients, respectively. No significant difference was noted in diabetes patients with vs. without HS according to gender, anti-diabetic treatment, and HbA1c levels (Table 1).

Glycemic control in T2D patients with vs. without HS in gender and treatment subgroups

No significant difference was noted in glycemic control achievement rates in patients with vs. without HS in gender and anti-diabetic treatment subgroups (Table 2).

Correlation of HbA1c with laboratory parameters in T2D patients with vs. without HS

HbA1c levels were positively correlated with serum urea ($r=0.308$, $p=0.042$), creatinine ($r=0.306$, $p=0.044$), and

Table 1 Patient demographics, laboratory findings, and treatments in T2D patients according to concomitant hepatic steatosis

			Hepatic steatosis		*p* value
			No ($n=44$)	Yes ($n=58$)	
Age (year)	Mean ± SD		63.5 ± 14.5	59.6 ± 10.5	**0.034**[1]
	Median (min–max)		67.5 (27–87)	59 (38–82)	
Gender, *n* (%)					
Male			13 (29.5)	20 (34.5)	0.753[2]
Female			31 (70.5)	38 (65.5)	
Anti-diabetic treatment, *n* (%)					
Metformin			29 (65.9)	43 (74.1)	0.494[2]
Pioglitazone			3 (6.9)	11 (19)	0.140[2]
DPP4i			18 (40.9)	17 (29.3)	0.312[2]
SGLT-2 inhibitor			7 (15.9)	8 (13.8)	0.987[2]
Insulin			15 (34.1)	16 (27.6)	0.624[2]
Laboratory findings, median (min–max)					
FBG (mg/dL)			138.5 (82–278)	125.5 (48–350)	0.591[1]
Urea (mg/dL)			39 (14–138)	28.5 (3–61)	**0.012**[1]
Creatinine (mg/dL)			0.8 (0.4–28)	0.8 (0.5–1.8)	0.191[1]
ALT (U/L)			17 (7–43)	24.5 (9–117)	**< 0.001**[1]
AST (U/L)			18 (9–107)	20 (10–58)	**0.019**[1]
Cholesterol (mg/dL), mean ± SD			218.4 ± 45.9	219.9 ± 46.6	0.871[3]
Triglyceride			146 (56–660)	155.5 (63–477)	0.646[1]
HDL (mg/dL)			47 (29–82)	47 (30–93)	0.761[1]
LDL (mg/dL), mean ± SD			132.8 ± 45.9	137.5 ± 42.5	0.593[3]
Proteinuria			13 (29.5)	7 (12.1)	0.051[2]
HbA1c (%)		Median(min–max)	7.3 (5.5–11.2)	7.3 (5.7–16.1)	0.903[1]
		At target (< 7%)	18 (40.9)	23 (39.7)	1.00[2]
		Not at target (≥ 7%)	26 (59.1)	35 (60.3)	

Values in bold indicate statistical significance ($p<0.05$)

DPP4i dipeptidyl peptidase-4 inhibitor; *SGLT2* sodium-glucose co-transporter-2; *FBG* fasting blood glucose; *HbA1c* glycated hemoglobin; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein

[1]Mann Whitney *U* test, [2]Yates continuity correction χ^2 test, [3]Student *t* test

triglyceride ($r=0.358$, $p=0.017$) levels only in patients without HS (Table 3).

Glycemic parameters and serum transaminases with respect to treatment in T2D patients with HS

In patients with HS, no significant difference was noted in ALT and AST levels with respect to anti-diabetic regimen. FBG and HbA1c levels were significantly higher in insulin-treated vs. insulin-naïve (193.5 (48–350) vs. 117 (71–266) mg/dL, $p<0.001$ and 10.1 (7.1–16.1) vs. 6.7 (5.7–10.5%, $p<0.001$, respectively) patients, in patients with vs. without DPP4i treatment (158 (114–321) vs. 117 (48–350) mg/dL, $p=0.002$ and 8.2 (6.4–12.8) vs. 6.8 (5.7–16.1)%, $p=0.002$, respectively), and in those without vs. with metformin therapy (162 (48–350) vs. 122 (71–321) mg/dL, $p=0.047$ and 8.3 (6.2–14.4) vs. 7 (5.7–16.1)%, $p=0.013$, respectively) (Table 4).

Multivariate logistic regression analysis for factors predicting the risk of HS

Logistic regression analysis revealed the decrease in urea (OR, 0.958, 95% CI 0.93 to 0.99, $p=0.005$) and proteinuria (OR, 0.327, 95% CI 0.12 to 0.91, $p=0.032$) but increase in ALT levels (OR, 1.078, 95% CI 1.03 to 1.13, $p=0.002$) to significantly predict the risk of HS in T2D patients (Table 5).

Discussion

Our findings revealed no significant difference in diabetic patients with and without HS in terms of selected anti-diabetic regiments and glycemic control, regardless of the gender, as well as no significant difference between ongoing anti-diabetic regimens in terms of serum transaminase levels. HbA1c levels were positively correlated with serum urea, creatinine, and triglyceride levels only in patients

Table 2 Glycemic control in patients with vs. without hepatic steatosis in gender and treatment subgroups

		Hepatic steatosis		*p* value
		Yes ($n=58$)	No ($n=44$)	
Males				
Glycemic control	Yes	10 (50)	7 (53.8)	1.00[1]
	No	10 (50)	6 (46.2)	
Females				
Glycemic control	Yes	13 (34.2)	11 (35.5)	1.00[1]
	No	25 (65.8)	20 (64.5)	
Metformin-treated patients				
Glycemic control	Yes	21 (48.8)	13 (44.8)	0.925[1]
	No	22 (51.2)	16 (55.2)	
Pioglitazone-treated patients				
Glycemic control	Yes	5 (45.5)	2 (66.7)	1.00[2]
	No	6 (54.5)	1 (33.3)	
DPP4i-treated patients				
Glycemic control	Yes	2 (11.8)	4 (22.2)	0.658[2]
	No	15 (88.2)	14 (77.8)	
SGLT2 inhibitor-treated patients				
Glycemic control	Yes	1 (12.5)	1 (14.3)	1.00[2]
	No	7 (87.5)	6 (85.7)	
Metformin alone				
Glycemic control	Yes	16 (72.7)	10 (76.9)	1.00[2]
	No	6 (27.3)	3 (23.1)	
Metformin plus other OADs				
Glycemic control	Yes	5 (23.8)	3 (18.8)	1.00[2]
	No	16 (76.2)	13 (81.3)	
Insulin-treated patients				
Glycemic control	Yes	0	1 (6.7)	0.484[2]
	No	16 (100)	14 (93.3)	

DPP4i dipeptidyl peptidase-4 inhibitor; *SGLT2* sodium-glucose co-transporter-2; *OAD* oral anti-diabetics

[1]Yates continuity correction, [2]Fisher exact test

Table 3 Correlations of HbA1c in patients with vs. without hepatic steatosis

		Correlation with HbA1c	
		Hepatic steatosis (−)	Hepatic steatosis (+)
Patient age	*r*	−0.062	0.312
	p	0.688	0.017
	n	44	48
FBG	*r*	0.673	0.732
	p	**< 0.001**	**< 0.001**
	n	44	58
Urea	*r*	0.308	0.077
	p	**0.042**	0.567
	n	44	58
Creatinine	*r*	0.306	0.023
	p	**0.044**	0.862
	n	44	58
ALT	*r*	−0.054	0.070
	p	0.730	0.603
	n	44	58
AST	*r*	−0.289	0.143
	p	0.057	0.285
	n	44	58
Cholesterol	*r*	0.062	0.048
	p	0.690	0.723
	n	44	58
Triglyceride	*r*	0.358	0.120
	p	**0.017**	0.371
	n	44	58
HDL	*r*	−0.065	−0.171
	p	0.673	0.198
	n	44	58
LDL	*r*	−0.006	0.015
	p	0.972	0.913
	n	44	58

Values in bold indicate statistical significance ($p<0.05$)

FBG fasting blood glucose; *HbA1c* glycated hemoglobin; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein

r correlation coefficient

Spearman correlation analysis

without HS, while serum urea levels were also significantly higher in diabetic patients without HS.

Although serum urea and creatinine are simple and useful biomarkers to assess kidney functions in diabetic patients, their correlation with HbA1c-based glycemic status has been addressed in a few studies among diabetics which also revealed conflicting data [16]. Accordingly, a statistically significant increase in serum creatinine and urea levels was reported in T2D patients as compared to the healthy control subjects [16], whereas some studies revealed no correlation of serum urea or creatinine levels with HbA1c levels or diabetes duration in T2D patients [16, 17], and others reported correlation of HbA1c positively with creatinine but negatively with blood urea level [18, 19].

Serum creatinine was reported to be significantly associated with different categories of impaired glucose regulation independent of known metabolic risk factors and gender [20], while our findings indicate the potential role of underlying liver functional status on the association of creatinine with impaired glycemic control in diabetic patients.

Besides, some authors suggested that NAFLD is associated with an increased incidence of chronic kidney disease in T2D patients, regardless of patient demographics, diabetes duration, glycemic control, lipid status, microalbuminuria, hypertension, and medications use [9].

Table 4 Glycemic parameters and transaminases with respect to treatment in T2D patients with hepatic steatosis

		T2D patients with hepatic steatosis			
Anti-diabetic treatment		FBG	HbA1c	ALT	AST
Metformin	No ($n=15$)	162 (48–350)	8.3 (6.2–14.4)	19 (10–117)	18 (10–42)
	Yes ($n=43$)	122 (71–321)	7 (5.7–16.1)	26 (9–89)	21 (13–58)
	p value	**0.047**	**0.013**	0.194	0.216
Pioglitazone	No ($n=47$)	127 (48–350)	7.4 (5.7–16.1)	23 (9–89)	19 (10–58)
	Yes ($n=11$)	118 (95–162)	7 (5.7–7.4)	26 (16–117)	21 (10–42)
	p value	0.242	0.084	0.493	0.897
DPP4i	No ($n=41$)	117 (48–350)	6.8 (5.7–16.1)	25 (9–117)	19 (10–58)
	Yes ($n=17$)	158 (114–321)	8.2 (6.4–12.8)	22 (10–82)	22 (10–49)
	p value	**0.002**	**0.002**	0.745	0.817
SGLT2 inhibitor	No ($n=50$)	122.5 (48–350)	7.1 (5.7–14.4)	26 (9–117)	20 (10–58)
	Yes ($n=8$)	156.5 (117–214)	8.8 (6.6–16.1)	20 (11–82)	19.5 (15–49)
	p value	-	-	-	-
Insulin	No ($n=42$)	117 (71–266)	6.7 (5.7–10.5)	26 (9–117)	20 (10–58)
	Yes ($n=16$)	193.5 (48–350)	10.1 (7.1–16.1)	20 (10–82)	20 (10–49)
	p value	**< 0.001**	**< 0.001**	0.454	0.760

Values in bold indicate statistical significance ($p<0.05$)

Data are expressed as median (min–max)

DPP4i dipeptidyl peptidase-4 inhibitor; *SGLT2* sodium-glucose co-transporter-2; *FBG* fasting blood glucose; *HbA1c* glycated hemoglobin; *ALT* alanine aminotransferase; *AST* Aspartate aminotransferase

Mann Whitney *U* test

Table 5 Logistic regression analysis for the factors predicting the risk of hepatic steatosis in T2D patients

	OR	95% CI (LB-UB)	p value
Gender	1.255	0.54–2.92	0.598
Age	0.974	0.943–1.007	0.119
Glycemic control	0.949	0.427–2.11	0.989
Metformin treatment	1.483	0.63–3.49	0.368
Pioglitazone treatment	3.199	0.84–12.26	0.090
DPP4 treatment	0.599	0.26–1.37	0.223
SGLT2 treatment	0.846	0.28–2.54	0.765
Insulin treatment	0.737	0.31–1.72	0.480
FBG	0.999	0.99–1.01	0.715
Urea	0.958	0.93–0.99	**0.005**
Creatinine	0.357	0.11–1.20	0.096
ALT	1.078	1.03–1.13	**0.002**
AST	1.012	0.98–1.06	0.324
Cholesterol	1.001	0.99–1.01	0.869
TG	0.999	0.996–1.003	0.743
HDL	1.006	0.97–1.04	0.727
LDL	1.002	0.99–1.01	0.589
HbA1C	1.062	0.87–1.30	0.557
Proteinuria	0.327	0.12–0.91	**0.032**

Values in bold indicate statistical significance ($p<0.05$)

DPP4i dipeptidyl peptidase-4 inhibitor; *SGLT2* sodium-glucose co-transporter-2; *FBG* fasting blood glucose; *HbA1c* glycated hemoglobin; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *OR* odds ratio; *CI* confidence interval; *LB* lower bound; *UB* upper bound

In fact, increased levels of serum creatinine and urea in T2D patients were reported not to correlate with duration of diabetes, and authors considered the long-term consequences of other factors such as aging, arterial hypertension, dyslipidemia, or obesity on kidney prior to development of hyperglycemia [16]. In the current study, significant positive correlation of serum creatinine and urea levels with HbA1c only in T2D patients without HS seems consistent in this regard, with higher serum urea levels and older age in this group of patients as compared to T2D patients with HS.

Our findings revealed no increase in the risk of poor glycemic control, dyslipidemia, or nephropathy pertaining to concomitant HS in T2D patients, as well as no difference among T2D patients with HS for serum transaminase levels according to ongoing anti-diabetic treatments.

NAFLD per se associates with increased risk of incident diabetes pointing to specific liver-related mechanisms [21, 22] as well as with increased risk of cardiovascular events even in an advanced CKD cohort with high comorbidity [23, 24]. In addition, the presence of T2D in NAFLD is considered to be associated with a 2.3- and 2.8-fold hazard ratio for overall and CVD-related mortality, respectively [25]. Notably, while insulin resistance, T2D, hyperlipidemia, and obesity are considered to be the common pathogenic mechanisms for both NAFLD and CKD [24], recent studies emphasize the variations in the role of NAFLD in T2D depending on the different subtypes of diabetes in terms of clinical and metabolic features, the insulin resistance in

particular [23, 26, 27]. Accordingly, the German Diabetes Study (GDS) reported that only the severe insulin-resistant diabetes cluster (SIRD) had increased prevalence of NAFLD and its progression along with higher risk of diabetic kidney disease and CVD [26].

Hence, the lack of any significant impact of concomitant liver disease on nephropathy along with identification of proteinuria as an independent risk factor for decreased likelihood of HS in T2D patients in the current study seems to be consistent with assessment of HS as a relatively benign and mild form of NAFLD, given that the severity of liver disease (raised hepatic transaminases and advanced fibrosis) was considered to predict the presence of impaired renal function [28]. However, findings from a meta-analysis also revealed that there is no association between the presence of T2D and impaired renal function in patients with NAFLD [29].

Hence, our findings seem to indicate no significant impact of concomitant NAFLD on diabetes course in terms of glycemic control and nephropathy as well as no difference in hepatic function with respect to ongoing anti-diabetic regimen in patients with T2D at the time of HS. However, given the elevated risk for progression to fibrosis and NASH in T2D patients [5], the likelihood of reciprocal deteriorative interaction between T2D and NAFLD seems more likely after progression to an advanced liver disease.

In this regard, given the consistently reported increased risk of diabetic microvascular (retinopathy, nephropathy) and macrovascular (cardiovascular disease) complications in T2D patients in the presence of concomitant NAFLD [5, 8–10], our findings support that the presence of NAFLD in T2D patients should alert clinicians to the coexistence of other chronic diabetic complications (including CKD and CVD) warranting evaluation and more intensive therapy as much as the risk for advancing liver disease [9].

Past studies revealed metformin, thiazolidinediones (TZDs), glucagon-like peptide-1 (GLP-1) analogs and statins to be safe options for the treatment of NAFLD/NASH in patients with concomitant T2DM, with slightly higher efficacy of metformin on histological markers associated with NAFLD, in relation to improved weight and metabolic profile [30, 31]. Accordingly, metformin was the most common anti-diabetic regimen in T2D patients, regardless of concomitant HS, supporting the consistently reported association of metformin treatment with improved transaminases in patients with NAFLD [4, 32]. Nonetheless, ın the current study, liver function parameters were similar with respect to type of ongoing anti-diabetic treatment in patients with HS, while metformin rather than DPP4i and insulin seems to be associated with better glycemic control in the presence of HS, possibly in relation to already advanced disease stage in patients initiating treatment intensification.

Certain limitations to this study should be considered. Firstly, due to cross-sectional single-center design of the present study, establishing any cause and effect relationships as well as generalizing our findings to overall diabetic population seems not possible. Second, lack of data on more advanced forms of NAFLD is another limitation which otherwise would extend the knowledge achieved in the current study.

In conclusion, our findings revealed no significant difference in diabetic patients with and without HS in terms of selected anti-diabetic regiments and glycemic control, regardless of the gender, as well as no significant difference between ongoing anti-diabetic regimens in terms of serum transaminase levels among T2D patients with HS. Considering nephropathy, HbA1c levels were positively correlated with serum urea and creatinine only in T2D patients without HS, which may be related to older age and higher serum urea levels in this group of patients as compared to those with HS. Future larger scale longitudinal studies in T2D patients with concomitant NAFLD that comparatively address the whole spectrum of liver disease and the entire diabetes-related microvascular and macrovascular complications are needed to elucidate the nature of the reciprocal interaction between T2D and NAFLD.

Availability of data and material Raw data can be obtained upon request to the corresponding author (teslime.ayaz@erdogan.edu.tr).

Declarations

Ethics approval The study was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Consent to participate Written informed consent was obtained from each patient following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Consent for publication The permission was obtained from the institutional ethics committee for the use of patient data for publication purposes.

Conflict of interest The authors declare no competing interests.

References

1. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002;346:1221–31.
2. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 2006;43(2 Suppl. 1):S99–112.
3. Clark JM, Diehl AM. Hepatic steatosis and type 2 diabetes mellitus. Curr Diab Rep. 2002;2:210–5.
4. Mills EP, Brown KPD, Smith JD, Vang PW, Trotta K. Treating nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a review of efficacy and safety. Ther Adv Endocrinol Metab. 2018;9:15–28.

5. Rhee EJ. Nonalcoholic fatty liver disease and diabetes: an epidemiological perspective. Endocrinol Metab (Seoul). 2019;34:226–33.
6. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: cause or consequence? J Hepatol. 2018;68:335–52.
7. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65:1038–48.
8. Targher G, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, et al. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. Diabetologia. 2008;51:444–50.
9. Targher G, Chonchol M, Bertolini L, Rodella S, Zenari L, Lippi G, et al. Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease. J Am Soc Nephrol. 2008;19:1564–70.
10. Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, et al. Nonalcoholic fatty liver disease is independently NAFLD and diabetes mellitus associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care. 2007;30:2119–21.
11. Dawwas GK, Liu X, Nguyen MH. Nonalcoholic fatty liver disease increases risk of incident advanced chronic kidney disease: a propensity-matched cohort study. J Intern Med. 2019;286:711–22.
12. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: a systematic review and meta-analysis. Sci Rep. 2016;6:33386.
13. Chinnadurai R, Ritchie J, Green D, Kalra PA. Nonalcoholic fatty liver disease and clinical outcomes in chronic kidney disease. Nephrol Dial Transplant. 2019;34:449–57.
14. Capone D, Vinciguerra M, Ragosta A, Citro V, Tarantino G. Troponin levels relate to CRP concentrations in patients with NAFLD on maintenance haemodialysis: a retrospective study. Adv Ther. 2020;37:3337–47.
15. Kang SH, Cho KH, Do JY. Non-alcoholic fatty liver disease is associated with low-grade albuminuria in men without diabetes mellitus. Int J Med Sci. 2019;16:285–91.
16. Chutani A, Pande S. Correlation of serum creatinine and urea with glycemic index and duration of diabetes in type 1 and type 2 diabetes mellitus: a comparative study. Nat J Physiol Pharm Pharmacol. 2017;7:914–9.
17. Karningsih TP, Mujianto B, Martini R. The Correlation between the levels of HbA1c with ureum and creatinine in patient with type 2 diabetes mellitus. Asian J Applied Sci. 2019;7:696–700.
18. Vaishnavi S, Patil S, Sontakke A, Mane D. Study of correlation between HbA1c and renal dysfunction in type-2 diabetes mellitus. Indian J Public Health Res Development. 2019;10:194–9.
19. Sivasubramanian V, Jetty K, Kumar SS. Correlation of HbA1c with urinary ACR, serum creatinine and eGFR in type-2 diabetes mellitus at Puducherry, South India. Int J Res Med Sci. 2019;7:1924–8.
20. Farasat T, Sharif S, Naz S, Fazal S. Significant association of serum creatinine with HbA1C in impaired glucose tolerant Pakistani subjects. Pak J Med Sci. 2015;31:991–4.
21. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care. 2018;41:372–82.
22. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015;62:S47-64.
23. Dewidar B, Kahl S, Pafili K, Roden M. Metabolic liver disease in diabetes - from mechanisms to clinical trials. Metabolism. 2020;111S:154299.
24. Zhang M, Lin S, Wang MF, Huang JF, Liu SY, Wu SM, et al. Association between NAFLD and risk of prevalent chronic kidney disease: why there is a difference between east and west? BMC Gastroenterol. 2020;20:139.
25. Stepanova M, Rafiq N, Younossi ZM. Components of metabolic syndrome are independent predictors of mortality in patients with chronic liver disease: a population-based study. Gut. 2010;59:1410–5.
26. Zaharia OP, Strassburger K, Strom A, Bönhof GJ, Karusheva Y, Antoniou S, et al. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. Lancet Diabetes Endocrinol. 2019;7:684–94.
27. Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a datadriven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018;6:361–9.
28. Nampoothiri RV, Duseja A, Rathi M, Agrawal S, Sachdeva N, Mehta M, et al. Renal dysfunction in patients with nonalcoholic fatty liver disease is related to the presence of diabetes mellitus and severity of liver disease. J Clin Exp Hepatol. 2019;9:22–8.
29. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, et al. Association of nonalcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med. 2014;11:e1001680.
30. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the study of liver diseases, American College of Gastroenterology, and the American Gastroenterological Association. Am J Gastroenterol. 2012;107:811–26.
31. Bhatt HB, Smith RJ. Fatty liver disease in diabetes mellitus. HepatoBiliary Surg Nutr. 2015;4:101–8.
32. Li Y, Liu L, Wang B, Wang J, Chen D. Metformin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Biomed Rep. 2013;1:57–64.

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ORIGINAL ARTICLE

Study on association of non-alcoholic fatty liver disease and serum vitamin A, E, and selenium levels in high-fat fed diet rats

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Abstract

Background Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of fatty liver disease, which occurs due to the accumulation of fat in the liver called steatosis.

Aim and objective In this study, the effect of high-fat diet on vitamin A and E and selenium and also the relationship between serum levels of triglyceride (TG) and vitamin A, as well as the hepatic levels of these two factors along with serum levels of vitamin E, selenium, and non-esterified fatty acids (NEFA) and its comparison with tri-acyl glycerol (TAG) of liver cells in rats fed a high-fat diet, were evaluated.

Material and methods Forty adult male Wistar rats were randomly divided into 2 equal groups of 20 animals, including the control and treatment groups fed with a high-fat diet. At the end of the study, serum and liver triglyceride levels, vitamin A, liver TAG, serum NEFA, serum vitamin E, and selenium were measured.

Result A significant decrease was observed in serum and liver vitamin A levels, serum levels of vitamin E, and selenium in the treatment group compared to the control group. Also, a significant increase was seen in serum and liver TG levels, TAG, and NEFA in the treatment group compared to the control groups ($p < 0.05$). The results of the present study suggested that increasing the TG, TAG, and NEFA levels caused a significant decrease in the amount of vitamin A and E and selenium levels in serum and hepatocytes.

Conclusion Finally, evaluation of serum and liver titers of vitamin A, E, and selenium at different time intervals with progressive changes in fatty liver (hepatitis, fibrosis, and cirrhosis) over a period of 1 month as well as pathological evaluation along with a serological examination of vitamin A and E and selenium in fatty liver is suggested for future studies.

Keywords NAFLD · Vitamin A · Vitamin E · High-fat diet · Rat

Introduction

Detoxification of drugs, excretion of substances caused by the destruction and renewal of red blood cells in the form of bile, production of coagulation factors, storage of sugar as glycogen, and regulation of sugar and fat metabolism are the most important roles of the liver in the body. Of course, the role of the liver in absorbing fat and defending against microbes and toxins absorbing fat and defending against microbes and toxins absorbed through food should not be ignored [1].

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of fatty liver disease, which occurs due to the accumulation of fat in the liver called steatosis, and the reason is not alcohol consumption [2]. This disease has become a major public health issue since it is increasingly occurring as a systemic disease in metabolic syndrome [3]. NAFLD leads to a wide range of liver diseases, including simple steatosis, non-alcoholic hepatitis (NASH) determined by fibrosis, and hepatocellular injury, which may progress to cirrhosis and hepatocellular carcinoma and so on [2–4] Complications of this disease that most patients show include obesity, insulin resistance, metabolic syndrome, type 2 diabetes, cardiovascular disease, dyslipidemia, and elevated triacylglycerol (TAG) and triglyceride (TG) levels

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[2, 5]. Accumulation of fat in the liver can cause cellular stresses such as oxidative stress, endoplasmic reticulum stress, and insulin resistance (IR) and inflammation[2, 5].

Vitamin A, which is a fat-soluble vitamin, is an important micronutrient that is essential for embryonic development, nerve cell growth, vision, reproduction, immune function, spermatogenesis, testosterone synthesis and release [6], glycolysis, adipogenesis and lipogenesis, cell differentiation and proliferation, triglyceride, and cholesterol metabolism [7]. Vitamin A has three physiological active forms (vitamins), namely retinol (alcohol), retina (aldehyde), and retinoic acid (acid), which are collectively known as retinoids [8].

Hepatic stellate cells (HSCs) are non-parenchymal cells that are found homogeneously throughout the peri-sinusoidal space of the Disse. Fifteen percent of liver cells belong to these cells. Microvascular pressure between the sinus Disse and the hepatic sinusoids and tensile pressure due to changes in sinus pressure are two types of mechanical pressure to which HSCs are exposed [9, 10]. Normally, stellate cells have no physiological activity and are a place to store vitamin A in the body [10]. The metabolic status of retinoic acid, retinol, and retinyl esters is closely relevant to HSC activation [10]. In a study by Saeed et al., it stated that vitamin A has a crucial role in NAFLD and the progression of liver disease, as well as it has a therapeutic potential [11]. During liver injury, HSCs lose their vitamin A storage capacity due to the secretion of myofibroblasts and extracellular matrix (ECM) components, including collagen type 1 and wound smooth muscle actin alpha. Activation of HSCs in NAFLD progression without investigation leads to irreversible liver ulcers and advanced fibrotic disease [12].

Vitamin E is an important fat-dissolving antioxidant in the chain in all cell membranes and protects against lipid peroxidation. Vitamin E can also act as a direct radical protector against superoxide and hydroxyl radicals. In the presence of fatty acids, fatty acid hydro-peroxide is produced by the free radical chain reaction, which causes cell damage [13]. Vitamin E reacts with lipid radicals to place electron-free radical pairs in a more stable vitamin E derivate. The role of vitamin E in inhibiting inflammation, cell adhesion, platelet aggregation, and smooth muscle cell proliferation has also been demonstrated in cell culture [14]. Oliveira et al. stated that vitamin E neither prevented the development of fatty liver nor reduced the oxidative stress in the rat model of NAFLD [15]. On the other hand, Pacana et al. indicated that vitamin E (RRR-α-tocopherol) is the only recommended treatment in NASH adults without diabetes or cirrhosis and with aggressive histology [16].

Selenium is an essential trace element associated with various functions involved in reproduction, fertility, and disease prevention [17]. While toxicity can be a serious threat when overdosed, observing a deficiency, which can lead to disease progression, slows growth. It exerts its three biological functions when combined in proteins as selenium cysteine residues, replacing the sulfur portion in cysteine. Most of the biological effects of selenium are caused by selenoproteins, and more than 30 selenoproteins (glutathione peroxidases, thioredoxin reductases, deiodinases, P selenoproteins, etc.) are expressed in mammals [18]. Selenium is mainly stored as glutathione peroxidase 1 (GPx1) in cytosol and red blood cells [14]. Mousavi et al. in a study showed that high-fat diet may result in low serum zinc and selenium levels rather than NAFLD in a rat model of NAFLD-fed high-fat diet [19].

Based on a review of past studies, it was found that a study related to the relationship between serum levels of TG and vitamin A as well as the liver levels of these two factors as well as serum levels of vitamin E, selenium, and unsaturated fatty acids and its comparison with TAG (triacyl glycerol) of liver cells in rats fed a high-fat diet, and also the effect of high-fat diet on vitamin A, E, and selenium has not been done yet, so this study was designed and done.

Material and methods

To prevent bias in the study, the different stages of the study were performed in a double-blind manner, meaning that the different stages of the experiment were planned so that neither the therapist nor the laboratory was aware of the experimental and control groups at any stage.

Animals

In this study, 40 adult male Wistar rats weighing 200 ± 20 g were used. The rats were kept in the experimental facility at 60% humidity and 23 ± 2 °C on a 12-h light–dark cycle. Storage conditions were the same for all groups, with the same diet and water freely available. After a week, the experiment began. Rats were randomly divided into 2 equal groups of 20: (1) control group and (2) treatment group with high-fat diet. In the treatment group, high-fat emulsion, 10 mg/kg daily at 8 am by gavage, was used according to Liang et al. (2006) method for one month to induce liver steatosis (Table 1) [20]. Standard food was used in the control group.

Sampling

At the end of the study, the rats were anesthetized with high doses of ketamine 60 mg/ kg (Alfasan, Germany) and xylazine 5 mg/kg (Alfasan, Germany), and 0.1 cc Heparin was injected into their heart.

Before sacrifice and after deep anesthesia, 3 cc blood was taken from the tail vein. Ten grams of liver samples was taken. The samples were transferred to the laboratory to

Table 1 Composition of high-fat emulsion for gavage to rats

Materials	Amount
Corn oil	400 g
Sucrose	150 g
Whole milk powder	80 g
Cholesterol	100 g
Sodium dioxycholate	10 g
Polysorbate 80	36.4 g
Propylene glycol	31.1 g
Multivitamin	2.5 g
Salt	10 g
Mixed minerals	1.5 g
Water	300 ml

measure serum triglyceride, liver triglyceride, serum vitamin A, and liver vitamin A, liver TAG, serum NEFA, serum vitamin E, and selenium levels.

Blood and tissue tests

Hepatocyte triglyceride was measured in the control and treatment groups according to Neri Frings method [21]. The amount of serum triglyceride, serum vitamin A, and hepatic vitamin A was measured by spectrophotometry using Randox kits (Randox, UK).

TAG levels in hepatocytes were measured by the Neri method [22], and in the serum of the studied rats, NEFA (non-esterified fatty acid) and vitamin E levels were measured by the HPLC method, and selenium was measured by atomic absorption spectrophotometry method.

Data analysis

Independent *T* test and Pearson correlation test (SPSS ver.22) were used to test differences among the control and treatment groups. The criterion for statistical significance was set at $p < 0.05$.

Results

Comparison of serum and liver vitamin A and triglyceride in study groups

According to the Table 2, the results show that there was a significant difference between serum triglyceride level ($p < 0.05$), serum vitamin A, liver vitamin A, and liver triglyceride levels ($p < 0.01$) in the study groups.

According to Table 3, it obtained a direct but insignificant relationship between liver triglyceride and serum vitamin A levels in the treatment group ($r = 0.264$ and $p = 0.432$) and an indirect relationship in the control group ($r = -0.195$ and $p = 0.805$). There was an inverse and significant relationship between liver triglyceride and liver vitamin A in the treatment group ($r = -0.685$ and $p = 0.020$) and also found a direct but insignificant relationship in the control group ($r = 0.493$ and $p = 0.407$). There was an indirect but insignificant relationship between liver triglyceride and serum triglyceride in the treatment group ($r = -0.559$ and $p = 0.074$) and in the control group obtained an indirect but insignificant relationship ($r = -0.034$ and $p = 0.966$). An indirect but insignificant relationship between serum triglyceride and serum vitamin A in the treatment group was obtained ($r = 0.228$ and $p = 0.500$) and in the control group an indirect, but insignificant relationship was found ($r = -0.605$ and $p = 0.395$). Also, there was an indirect but insignificant relationship between serum vitamin A and liver vitamin A in the treatment group ($r = -0.367$ and $p = 0.267$), and in the control group, an indirect but insignificant relationship was observed ($r = -0.690$ and $p = 0.310$).

Comparison of mean serum levels of vitamin E, selenium, and NEFA and hepatic TAG values of liver cells

Based on the data in Table 4, the difference observed in the mean serum vitamin E in the two groups is significant, meaning that the mean of vitamin E was different in the control and treatment groups and this amount was higher

Table 2 Comparison of serum and liver vitamin A and triglyceride in study groups

	Groups	Mean	Std. deviation	Std. error	*p* value
Serum vitamin A (µmol/l)	Treatment	26.54	0.93	0.28	0.001
	Control	33.25	1.70	0.85	
Serum triglyceride (mg/dl)	Treatment	54.00	3.28	0.99	0.036
	Control	49.75	2.50	1.25	
Liver vitamin A (µg/g tissue)	Treatment	21.72	1.27	0.38	0.001
	Control	28	1.41	0.70	
Liver triglyceride (µmol/g tissue)	Treatment	7.72	0.25	0.07	0.002
	Control	6.64	087	0.43	

Table 3 Relationship between the level of serum and liver vitamin A with liver triglyceride level in studied groups

	Liver triglyceride			
	Groups	Number	Pearson correlation	*p* value
Serum vitamin A	Treatment	20	0.264	0.432
	Control	20	−0.195	0.805
	Liver triglyceride			
Liver vitamin A	Treatment	20	−0.685	0.020
	Control	20	0.493	0.507
	Liver triglyceride			
Serum triglyceride	Treatment	20	−0.559	0.074
	Control	20	−0.034	0.966
	Serum vitamin A			
Serum triglyceride	Treatment	20	−0.228	0.500
	Control	20	−0.605	0.395
	Liver vitamin A			
Serum vitamin A	Treatment	20	−0.367	0.267
	Control	20	−0.690	0.310

in the control group ($p < 0.05$). According to Table 4, the observed difference in the mean serum selenium of both groups is significant, meaning that the mean selenium was different in the two groups and this value was higher in the control group ($p < 0.05$). Also, the mean value of NEFA was different in the two groups, and this value was higher in the treatment group ($p < 0.05$). The mean value of TAG was different in the two groups, and this value was higher in the treatment group ($p < 0.05$).

Evaluation of the relationship between mean TAG values of liver cells and mean serum levels of NEFA, vitamin E, and selenium

Based on the obtained data and according to Pearson correlation coefficient, it indicates the negative effect of TAG values of hepatocytes on the amount of serum vitamin E, which decreases with increasing TAG values of hepatocytes. Also, this coefficient indicates the negative effect of hepatocyte TAG on serum selenium, which decreases with increasing hepatocyte TAG. This coefficient indicates the positive effect of liver cell TAG on serum NEFA. Therefore, with increasing TAG of liver cells, the amount of NEFA increases.

Table 4 Comparison of the average of two by two variables in different groups

	Groups	mean ± SD	*p* value
Serum vit E (µg/dl)	Treatment	59.90 ± 3.90	($p < 0/05$)
	Control	86.80 ± 7.155	
Serum selenium (µg/ml)	Treatment	0.14 ± 0.31	($p < 0/05$)
	Control	1.09 ± 0.01	
Serum NEFA (µEq/dl)	Treatment	414.50 ± 18.05	($p < 0/05$)
	Control	216.40 ± 15.59	
Liver TAG (mg/g)	Treatment	48.70 ± 5.88	($p < 0/05$)
	Control	38 ± 1.58	

Discussion

In this study, serum and liver levels of vitamin A and their comparison with serum triglyceride and liver triglyceride levels in rats fed with high-fat diet were evaluated. The relationship between serum levels of vitamin E, selenium, NEFA, and hepatic TAG levels in the studied rats was also investigated. According to the results, we had a significant decrease in serum and liver vitamin A levels in rats in the treatment group compared to the control group. Also, we saw a significant increase in serum and liver triglyceride levels in rats in the treatment group compared to the control groups. On the other hand, in the treated group, a significant increase in TAG and NEFA was observed compared to the control group, and also a significant reduction in the serum levels of vitamin E and selenium was observed.

Accordant with the findings of the present investigation, a study which was done by Mahan et al. in 2008 showed that adding saturated fatty acids (animal oil, animal butter, margarine) to the diet of rats can cause fatty liver disease [23]. Excess vitamin A in the diet, which is more than the body needs immediately, is stored in the liver as retinol ester along with long-chain fatty acids [24]. The results of this study showed that with increasing serum hepatic triglyceride levels, serum and hepatic vitamin A levels are significantly reduced, which indicate that there is probably a relationship between these two parameters. Hepatic glucose and lipid metabolism are regulated by vitamin A metabolites at many different levels. Moreover, disease progression within the NAFLD spectrum to NASH, cirrhosis, and cancer is associated with declining circulating and hepatic retinol levels [25].

Contrary to the present study, an investigation conducted by Bravo et al. in 2012 on mice fed a high-fat diet (57% metabolizable energy as fat) for 18 weeks showed that feeding a high-fat diet caused elevated hepatic triglycerides and plasma insulin values, which promotes fatty liver and induces non-alcoholic fatty liver disease (NAFLD), while the results revealed the changes in vitamin E levels and a significant reduction in plasma glucose and vitamin A levels [26]. In a study which was done by Chaves et al. in 2014, serum retinol and also hepatic retinol levels were both analyzed in NAFLD patients. The result showed that hepatic retinol levels encountered an intense inverse relation with the grade of the disease,

which this result is consistent with the findings of the present study [27]. The possible mechanism of this contradiction may be the different causes of the disease or the grade of it. Retinol prevents the expression of PNPLA3 in HSC (hepatic satellite cells), while it is induced upon retinol depletion. In addition, PNPLA3 expression is induced upon HSC activation, and the PNPLA3-I148M variant further promotes fibrogenic features of HSC, including enhanced proliferation, migration, and expression of collagen type 1 alpha 1, pro-inflammatory cytokines, and chemokines alongside lower cellular retinol levels [28]. According to the mechanism, the cause of the development of liver damage in NAFLD due to vitamin A deficiency can be explained. Consistent with the result of the present study, another study which was done by Liu et al. in 2015 indicated that serum retinoic acid levels were shown to be inversely related to liver damage in NAFLD. In addition, at RA concentration and RXR-α levels were inversely associated with liver triglyceride content in the disease [29]. In another study on vitamin A deficiency, rats have been shown an elevation in free cholesterol and triglyceride levels in the hearts and total cholesterol in the aorta [30, 31].

A study which was done by Zamin et al. in 2010, found that fatty liver model rats had reduced hepatic levels of vitamin E and methionine, which was consistent with our study [32]. On the other hand, a study by Ateşşahin et al., in 2005 showed that NAFLD could cause the reduction of vitamin E and selenium levels in serum and the liver by increasing oxidative stress [33]. In a study, Sanyal et al. demonstrated that vitamin E has a beneficial effect on the treatment of non-alcoholic steatohepatitis in adults. They also stated that vitamin E was effective by improving the antioxidant system [34]. The result of a study by Schäfer et al. (2004) showed that dietary selenium levels increase the amount of fat in liver cells and decrease serum selenium and vitamin E levels by increasing the TAG of liver cells which is consistent with the results of our study [35]. In a study by Tzanetakou et al. (2012), 24 weeks of high cholesterol diet in rats increased total serum cholesterol and decreased serum levels of vitamin E and selenium. Consistent with this result, in our study, a high-fat diet reduced serum levels of vitamin E and selenium [36]. Vitamin E has powerful antioxidant properties. On the other hand, vitamin E increases the production of transforming growth factor-b, which plays a major role in fibrosis and increases mRNA-procollagen-I expression in stellate cells. Vitamin E can decrease inflammation, which is so important in NAFLD, by reducing TNF-a and decreasing interleukin 1, 6, and 8 expressions. Because of its antioxidant effects, its possible role in NAFLD is being studied in recent years. These antioxidant properties of vitamin E are widely being used in several clinical settings such as cardiovascular diseases, stroke prevention, prevention of dementia, and prevention and treatment of steatohepatitis [37].

The results of the present study showed that increasing the triglyceride levels of hepatocytes caused a significant decrease in the amount of vitamin A in hepatocytes. Also, with increasing the triglyceride values of hepatocytes, the serum levels of vitamin A decreased insignificantly. Also, based on the present findings, it can be concluded that the incidence of NAFLD increases the TAG values of hepatocytes and serum NEFA and decreases the serum levels of vitamin E and selenium. Suano de Souza et al., in a study on overweight/obese school children, showed that non-alcoholic fatty liver disease causes micronutrient deficiencies like vitamin A [38]. Another study, Botella-Carretero et al., showed that serum retinol and alpha-tocopherol are inversely correlated with body mass index. They also stated that serum retinol is inversely associated with serum concentrations of transaminases in non-alcoholic fatty liver disease patients [39]. Chaves et al., in another study, stated that there is an association between liver retinol and the degree of NAFLD [27].

Finally, evaluation of serum and liver titers of vitamin A, E, and selenium at different time intervals with progressive changes in fatty liver (hepatitis, fibrosis, and cirrhosis) over a period of 1 month as well as pathological evaluation along with a serological examination of vitamin A and E and selenium in fatty liver is suggested for future studies.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval In this study, all ethical considerations and working protocols on laboratory animals were approved by the Ethical Committee of Laboratory Animal Rights of the Research Center of the Islamic Azad University of Tabriz

References

1. Washabau RJ, Meyer HP, Rothuizen J, Lidbury JA, Steiner JM, Richter K, et al. Liver. Canine feline gastroenterol [Internet]. Elsevier; 2013 [cited 2020 Aug 27]. p. 849–957. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161409/
2. Jiang W, Guo M-H, Hai X. Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. World J Gastroenterol [Internet]. 2016;22:10180. Available from: http://www.wjgnet.com/1007-9327/full/v22/i46/10180.htm
3. Jegatheesan P, Beutheu S, Freese K, Waligora-Dupriet A-J, Nubret E, Butel M-J, et al. Preventive effects of citrulline on Western diet-induced non-alcoholic fatty liver disease in rats. Br J Nutr [Internet]. 2016;116:191–203. Available from: https://www.cambridge.org/core/product/identifier/S0007114516001793/type/journal_article

4. Sigala B, McKee C, Soeda J, Pazienza V, Morgan M, Lin C-I, et al. Sympathetic nervous system catecholamines and neuropeptide y neurotransmitters are upregulated in human nafld and modulate the fibrogenic function of hepatic stellate cells. Targher G, editor. PLoS One [Internet]. 2013;8:e72928. Available from: https://dx.plos.org/10.1371/journal.pone.0072928
5. Schuppan D, Schattenberg JM. Non-alcoholic steatohepatitis: pathogenesis and novel therapeutic approaches. J Gastroenterol Hepatol [Internet]. 2013;28:68–76. Available from: http://doi.wiley.com/10.1111/jgh.12212
6. Clagett-Dame M, Knutson D. Vitamin A in reproduction and development. nutrients [Internet]. 2011;3:385–428. Available from: http://www.mdpi.com/2072-6643/3/4/385
7. Hill GE, Johnson JD. The vitamin A–redox hypothesis: a biochemical basis for honest signaling via carotenoid pigmentation. Am Nat [Internet]. 2012;180:E127–50. Available from: https://www.journals.uchicago.edu/doi/10.1086/667861
8. Jeyakumar S, Vajreswari A. Vitamin A as a key regulator of obesity & its associated disorders: evidences from an obese rat model. Indian J Med Res [Internet]. 2015;141:275. Available from: http://www.ijmr.org.in/text.asp?2015/141/3/275/156554
9. Yi S-H, Zhang Y, Tang D, Zhu L. Mechanical force and tensile strain activated hepatic stellate cells and inhibited retinol metabolism. Biotechnol Lett [Internet]. 2015;37:1141–52. Available from: http://link.springer.com/10.1007/s10529-015-1785-5
10. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev [Internet]. 2008;88:125–72. Available from: https://www.physiology.org/doi/10.1152/physrev.00013.2007
11. Saeed A, Dullaart R, Schreuder T, Blokzijl H, Faber K. Disturbed vitamin A metabolism in non-alcoholic fatty liver disease (NAFLD). Nutrients [Internet]. 2017;10:29. Available from: http://www.mdpi.com/2072-6643/10/1/29
12. Trasino SE, Tang X-H, Jessurun J, Gudas LJ. A retinoic acid receptor β2 agonist reduces hepatic stellate cell activation in nonalcoholic fatty liver disease. J Mol Med [Internet]. 2016;94:1143–51. Available from: http://link.springer.com/10.1007/s00109-016-1434-z
13. Weiss WP, Hogan JS, Wyatt DJ. Relative bioavailability of all-rac and RRR vitamin E based on neutrophil function and total α-tocopherol and isomer concentrations in periparturient dairy cows and their calves. J Dairy Sci [Internet]. 2009;92:720–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022030209703789
14. Chauhan SS, Celi P, Ponnampalam EN, Leury BJ, Liu F, Dunshea FR. Antioxidant dynamics in the live animal and implications for ruminant health and product (meat/milk) quality: role of vitamin E and selenium. Anim Prod Sci [Internet]. 2014;54:1525. Available from: http://www.publish.csiro.au/?paper=AN14334
15. Oliveira CPMS, da Costa Gayotto LC, Tatai C, Nina BI Della, Lima ES, Abdalla DSP, et al. Vitamin C and vitamin E in prevention of nonalcoholic fatty liver disease (NAFLD) in choline deficient diet fed rats. Nutr J [Internet]. 2003;2:9. Available from: http://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-2-9
16. Pacana T, Sanyal AJ. Vitamin E and nonalcoholic fatty liver disease. Curr Opin Clin Nutr Metab Care [Internet]. NIH Public Access; 2012 [cited 2021 Jul 5];15:641–8. Available from: /pmc/articles/PMC4984672/
17. Hefnawy AEG, Tórtora-Pérez JL. The importance of selenium and the effects of its deficiency in animal health. Small Rumin Res [Internet]. 2010;89:185–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0921448809003149
18. Rayman MP. Selenium and human health. Lancet. 2012.
19. Mousavi SN, Faghihi A, Motaghinejad M, Shiasi M, Imanparast F, Amiri HL, et al. Zinc and selenium co-supplementation reduces some lipid peroxidation and angiogenesis markers in a rat model of NAFLD-Fed high fat diet. Biol Trace Elem Res [Internet]. 2018;181:288–95. Available from: http://link.springer.com/http://link.springer.com/10.1007/s12011-017-1059-2
20. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. Eur J Pharmacol [Internet]. 2006;543:116–22. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0014299906005449
21. Malihe P, Tahora S, Farzad A. Alterations in serum lipid, lipoprotein and visceral abdominal fat pad parameters of hypercholestrolemic guinea pigs in response to short term garlic consumption. African J Biotechnol [Internet]. 2010;9:7930–3. Available from: http://academicjournals.org/journal/AJB/article-abstract/90DB65119313
22. Neri BP, Frings CS. Improved method for determination of triglycerides in serum. Clin Chem [Internet]. Oxford Academic; 1973 [cited 2020 Aug 27];19:1201–2. Available from: https://academic.oup.com/clinchem/article/19/10/1201/5676688
23. Mahan LK, Escott-Stump S. Krause's food and nutrition therapy. Krause's Food Nutr. Ther. 2008.
24. O'Byrne SM, Blaner WS. Retinol and retinyl esters: biochemistry and physiology. J Lipid Res [Internet]. 2013;54:1731–43. Available from: http://www.jlr.org/lookup/doi/http://www.jlr.org/lookup/doi/10.1194/jlr.R037648
25. Trasino SE, Tang X-H, Jessurun J, Gudas LJ. Obesity leads to tissue, but not serum vitamin a deficiency. Sci Rep. 2015;5:15893.
26. Bravo E, Palleschi S, Rossi B, Napolitano M, Tiano L, D'Amore E, et al. Coenzyme Q metabolism is disturbed in high fat diet-induced non alcoholic fatty liver disease in rats. Int J Mol Sci [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2012 [cited 2020 Aug 5];13:1644–57. Available from: /pmc/articles/PMC3291983/?report=abstract
27. Chaves GV, Pereira SE, Saboya CJ, Spitz D, Rodrigues CS, Ramalho A. Association between liver vitamin A reserves and severity of nonalcoholic fatty liver disease in the class III obese following bariatric surgery. Obes Surg [Internet]. 2014;24:219–24. Available from: http://link.springer.com/10.1007/s11695-013-1087-8
28. Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. Hepatology. 2017;
29. Liu Y, Chen H, Wang J, Zhou W, Sun R, Xia M. Association of serum retinoic acid with hepatic steatosis and liver injury in nonalcoholic fatty liver disease. Am J Clin Nutr [Internet]. 2015;102:130–7. Available from: https://academic.oup.com/ajcn/article/102/1/130/4564323
30. Vega VA, Anzulovich AC, Varas SM, Bonomi MR, Giménez MS, Oliveros LB. Effect of nutritional vitamin A deficiency on lipid metabolism in the rat heart: Its relation to PPAR gene expression. Nutrition. 2009;
31. Gatica L V., Vega VA, Zirulnik F, Oliveros LB, Gimenez MS. Alterations in the lipid metabolism of rat aorta: effects of vitamin A deficiency. J Vasc Res. 2006;
32. Zamin Jr I, Mattos AA de, Mattos ÂZ de, Coral G, Santos D, Rhoden C. The vitamin E reduces liver lipoperoxidation and fibrosis in a model of nonalcoholic steatohepatitis. Arq Gastroenterol [Internet]. 2010;47:86–92. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-28032010000100015&lng=en&tlng=en
33. Ateşşahin A, Yilmaz S, Karahan I, Pirinçci I, Taşdemir B. The effects of vitamin E and selenium on cypermethrin-induced oxidative stress in rats ahmet. Turkish J Vet Anim Sci. 2005;29:385–91.
34. Sanyal AJ, Chalasani N, Kowdley K V., McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med [Internet].

2010;362:1675–85. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0907929
35. Schäfer K, Kyriakopoulos A, Gessner H, Grune T, Behne D. Effects of selenium deficiency on fatty acid metabolism in rats fed fish oil-enriched diets. J Trace Elem Med Biol. 2004.
36. Tzanetakou IP, Doulamis IP, Korou L-M, Agrogiannis G, Vlachos IS, Pantopoulou A, et al. Water soluble vitamin E administration in wistar rats with non-alcoholic fatty liver disease. Open Cardiovasc Med J. 2012;
37. Cankurtaran M, Kav T, Yavuz B, Shorbagi A, Halil M, Coskun T, et al. Serum vitamin-E levels and its relation to clinical features in nonalcoholic fatty liver disease with elevated ALT levels. Acta Gastroenterol Belg. 2006;69:5–11.
38. Suano de Souza, Silverio Amancio, Saccardo Sarni, Sacchi Pitta, Fernandes, Affonso Fonseca, et al. Non-alcoholic fatty liver disease in overweight children and its relationship with retinol serum levels. Int J Vitam Nutr Res [Internet]. 2008;78:27–32. Available from: https://econtent.hogrefe.com/doi/10.1024/0300-9831.78.1.27
39. Botella-Carretero JI, Balsa JA, Vázquez C, Peromingo R, Díaz-Enriquez M, Escobar-Morreale HF. Retinol and α-tocopherol in morbid obesity and nonalcoholic fatty liver disease. Obes Surg [Internet]. 2010;20:69–76. Available from: http://link.springer.com/10.1007/s11695-008-9686-5

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ORIGINAL ARTICLE

Prevalence of dysglycemia and its associations with age and body mass index among community dwelling adults in a developing country

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Abstract

Introduction Dysglycemia includes prediabetes and diabetes. We aimed to study the prevalence of dysglycemia, and its associations with age and body mass index (BMI) among community dwelling adults in Sri Lanka.

Methods The prevalence of dysglycemic state (FPG > 100 mg/dL) and its associations with age and BMI in males and females were estimated. The association between gender and glycemic status in different BMI ranges and age groups were estimated. The optimal cut-off points of BMI to determine the risk of dysglycemia in both genders were calculated.

Results Prevalence of prediabetes and diabetes of females were 25.3% and 16.4% and of males were 26.2% and 17.4% respectively. Dysglycemia showed a significant positive correlation with age in both genders and a significant positive correlation with BMI in males ($p < 0.05$). Aging (OR = 1.05, CI 1.02–1.08, $p < 0.001$) and increasing BMI (OR = 1.10, CI 1.05–1.15, $p < 0.001$) of males and aging (OR = 1.04, CI 1.02–1.06, $p < 0.001$) of females are significantly associated with dysglycemia. The optimal cut-off point of BMI for males was 22.86 kg/m^2 (sensitivity 76.6%, specificity 53.9%) to determine the risk of dysglycemia.

Conclusions Four out of ten adults in the screened population were dysglycemic. An increase in BMI is significantly associated with dysglycemic status in males compared to females. The recommended cut-off value of BMI as 23 kg/m^2 for South Asian population to categorize overweight individuals has an adequate sensitivity to recognize dysglycemic adult males but not the females in this community.

Keywords Dysglycemia · Body mass index · Fasting plasma glucose · Obesity · Prevalence

Introduction

Dysglycemia in the form of either pre-diabetes or diabetes mellitus (DM) is a leading metabolic abnormality which affects individuals in the developing as well as in developed countries [1]. The early stage of dysglycemia is termed as pre-diabetes and includes individuals with impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG). The global prevalence of pre-diabetes is rising rapidly and is estimated that number of people with pre-diabetes in the age group of 20–79 years is 352 million in 2017. The numbers are predicted to reach 587 million by the year 2045 [2]. A substantial majority of these people live in low- and middle-income countries. According to the International Diabetes Federation (IDF) data, the prevalence of IGT is 3.0% in people aged 20–79 years in the South East Asia region [2]. People with pre-diabetes have a high risk of developing DM and its associated comorbidities especially cardiovascular diseases [2]. The prevalence of DM is increasing globally and the IDF has estimated as 415 million adults suffer from DM at present and this number will reach to 642 million by 2040 [3]. Notably, the number of individuals living in the South East Asian region with DM has risen sharply during the past few years. The IDF has estimated that 6.8–10.8% of the adult population is living with DM in the South East Asian countries [3]. Environmental changes, behavioral

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patterns, and lifestyle changes of people resulted in escalating rates of development of DM in this region [4, 5].

According to the recent estimates of IDF, prevalence of diabetes among adults in Sri Lanka is 8.6% [2]. Subsequent problems due to high prevalence of DM have become a major health care burden in Sri Lanka. Therefore, screening of individuals for dysglycemia which includes both categories of prediabetes and diabetes seems important and cost effective practice to prevent or delay the occurrence of hyperglycemia associated complications. The commonly available tools for screening dysglycemic state are fasting plasma glucose (FPG) concentration, oral glucose tolerance test (OGTT) and percentage of glycated hemoglobin (HbA_{1C}). The American Diabetes Association (ADA) has defined the individuals who have pre-diabetes as IFG measured by FPG level 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L), or IGT measured by 2-h values in the OGTT test of 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) [6]. Even though pre-diabetes status is defined using HbA_{1C} as 6.0 to 6.5% range, it fails to identify a considerable number of patients who have IFG and/or IGT. Individuals with FPG concentration $\geq$ 126 mg/dL ($\geq$ 6.9 mmol/L), OGTT $\geq$ 200 mg/dL ($\geq$ 11.0 mmol/L), and HbA_{1C} level $\geq$ 6.5% are classified as patients with DM [6]. According to the recent findings, HbA_{1C} cutoff values for diabetes vary across different ethnicities and geographical regions compared to FPG and OGTT cutoff values. Studies carried out in different settings have proposed several cutoff values for HbA_{1C} [7, 8] and for Sri Lankans, it was reported as $\geq$ 6.3% according to a local study [9].

Epidemiological studies have revealed that increasing age and body mass index (BMI), prevalence of dyslipidemia, hypertension, and family history of DM are important and well-established risk factors associated with dysglycemia. Among these factors, increasing age and BMI were considered in the present study. A recent study has found several associations between increasing BMI and dysglycemia operating via proinflammatory cytokines (tumor necrosis factor and interleukin-6), insulin resistance, deranged fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress [10]. The BMI, calculated as weight (kg) divided by height square (m^2), is the most common method of assessing overweight and obesity in routine clinical practice. The World Health Organization (WHO) has defined the cut-off points of BMI to classify overweight and obesity as 25 kg/m^2 and 30 kg/m^2 respectively based on the epidemiological evidence investigating associations with mortality and morbidity [11]. The evidences on these cut-off points originate from white populations, and the level of risk associated with the classification of overweight and obesity vary across racial groups. Based on this, an expert committee of the WHO has recommended revised cut-off points to classify overweight and obesity as 23 kg/m^2 and 27.5 kg/m^2 respectively for the Asian population [12]. However, further evidences suggest that these revised cut-off points exert some limitations at the determination of risk of some epidemic diseases in Asians [13, 14].

Previous studies revealed the association of dysglycemia and its associated comorbidities and mortality with increasing age [15, 16]. Factors such as increasing insulin resistance and impaired pancreatic islet cell function are linked to the increased prevalence of dysglycemia with aging [17]. The age-related increase in insulin resistance is associated with adiposity, sarcopenia, and physical inactivity which has also been identified as risk factors for dysglycemia [18]. Furthermore, vitamin D deficiency is one of the factors accelerating insulin resistance, obesity and DM [19–21]. The ADA recommends to screen individuals having high BMI with other risk factors and all others aged $\geq$ 45 years for dysglycemia at a minimum of three-year intervals using one of the screening tools such as FPG, OGTT, or HbA_{1C} [17].

Even though the associations of risk factors with dysglycemia and the gender differences are well established, whether the association of dysglycemia with increasing age and BMI is similar in both genders has not been explored previously in the Sri Lankan population. Based on all these, we aimed to study the gender wise prevalence of glycemic status, its associations with age and BMI and to determine an optimal cutoff of BMI to assess the risk of dysglycemia in the Sri Lankan population.

Materials and methods

Study design

This study was a community based cross-sectional study conducted in randomly selected divisional secretariat areas of semi urban localities in Southern Sri Lankan city of Galle, during February 2018 to September 2019. A cluster sampling method was used to recruit study subjects. Prior to the commencement of the study, the study was clearly explained to the study population by the researchers.

Study population and study sample

The present study was the first phase of a clinical trial designed to test the efficacy of a herbal drug in newly diagnosed individuals with type 2 diabetes mellitus. To assess the prevalence of newly diagnosed type 2 diabetes mellitus, considering a confidence limit of 95%, expected

prevalence of newly diagnosed type 2 diabetes mellitus as 10% and a precision of 0.05, the required sample size was 138. Five divisional secretariat areas were randomly selected to enroll the participants. With an estimated design effect of 1.5 for cluster sampling, 207 individuals from each selected divisional secretariat area were required. Assuming that the prevalence of type 2 diabetes mellitus in each of those selected areas was different, the minimum sample required for the study was 1035. Considering 20% of non-responders and screen failures, a minimum of 1250 subjects were required. In the present study, a total number of 1691 individuals were invited to participate.

Five divisional secretariat areas were randomly selected from the total of 19 divisions following the simple random sampling method using excel based random number generation. A number of seven "Grama Niladhari" divisions (divisions similar to wards or villages) were selected randomly from each of the selected divisional secretariat areas following the simple random sampling method. An average number of 48 individuals (belong to age group of 30–60 years) were recruited for the present study from each of selected "Grama Niladhari" division. One eligible individual was selected randomly from one household. A random starting point was identified and the households on either side of the road in a randomly determined direction were visited to identify and select eligible individuals until the required numbers from each "Grama Niladhari" division were being enrolled. Patients with previously diagnosed diabetes both type 1 and type 2, and who were on long term steroids for chronic disorders and pregnant women were excluded from the study.

Detection of indices

Demographic and anthropometric parameters such as age, gender, height and weight were collected from all participants. Weight was measured using a portable scale without shoes and height was measured using a height bar in standing position without shoes while keeping the shoulder in erect position. Subjects with a BMI below 18.5 kg/m^2 were classified as underweight, between 18.5 and 22.9 kg/m^2 as normal, between 23.0 and 24.9 kg/m^2 as overweight, and equal or larger than 25.0 kg/m^2 were classified as obese.

Overnight fasting (8–10 h) venous blood samples were collected from each participant. Collected blood samples were stored in an ice bath till the delivery to laboratory. The separated plasma samples were stored at − 80° C until analyzed. FPG concentration was estimated by glucose oxidase method. Dysglycemia was diagnosed based on the ADA recommended criteria [6]. FPG concentration < 5.6 mmol/L and ≥ 5.6 mmol/L were considered normoglycemia and dysglycemia respectively [6]. FPG concentration between 5.6 mmol/L to 6.9 mmol/L and ≥ 6.9 mmol/L were considered pre-diabetes and diabetes respectively.

Statistical analysis

Data were analyzed using the version 25.0 of Statistical Package for Social Sciences (SPSS) software. The continuous variables were presented as mean ± SD while the categorical data were expressed as percentages. Normality of the data sets was checked using Kolmogorov–Smirnov test. Descriptive data of both genders were compared using independent sample t-test. The correlation between FPG values and anthropometric measures of age and BMI were assessed in both genders through the linear correlation analysis. Binary logistic regression was used to calculate odds ratio for the association of age and BMI (independent variables) with dysglycemia as the dependent variable in both genders. The chi-square test was used to assess the association between gender and glycemic status in each of BMI categories and age groups. Receiver operating characteristic (ROC) curves were developed for BMI values of both genders separately as a risk factor of dysglycemia. The optimal cut-off point of BMI was determined using Youden index (maximum [sensitivity + specificity − 1]) [22]. $p \leq 0.05$ was considered statistically significant.

Results

Although 1691 individuals were invited for the screening programs, only 1334 individuals participated with a non-response rate of 21.1%. The eligible population consisted of 1120 individuals with 803 (71.7%) females and 317 (28.3%) males. Figure 1 shows the flow diagram of the recruitment of eligible participants for the present study.

Mean age, BMI and FPG concentration of female subjects were 45.7 ± 9 years, 26.8 ± 6.4 kg/m^2 and 5.9 ± 2.1 mmol/L respectively. Mean age, BMI, and FPG concentration of male subjects were 44.5 ± 8.1 years, 24.8 ± 5.5 kg/m^2, and 6.0 ± 2.2 mmol/L respectively (Table 1).

Of the total number of females, 468 (58.3%) were normoglycemic, 203 (25.3%) were pre-diabetic, and 132 (16.4%) were detected to have diabetes. Among the male population, 179 (56.5%) individuals were normoglycemic, 83 (26.2%) were pre-diabetic, and 55 (17.4%) were detected to have diabetes. The gender wise prevalence of glycemic status diagnosed by FPG concentration is summarized in Table 2.

Glycemic status diagnosed by FPG concentration showed a significant positive correlation with the age in both genders and a significant positive correlation with BMI in males (Table 3).

Regression analysis revealed that increase in age and BMI in the whole population are significantly associated with

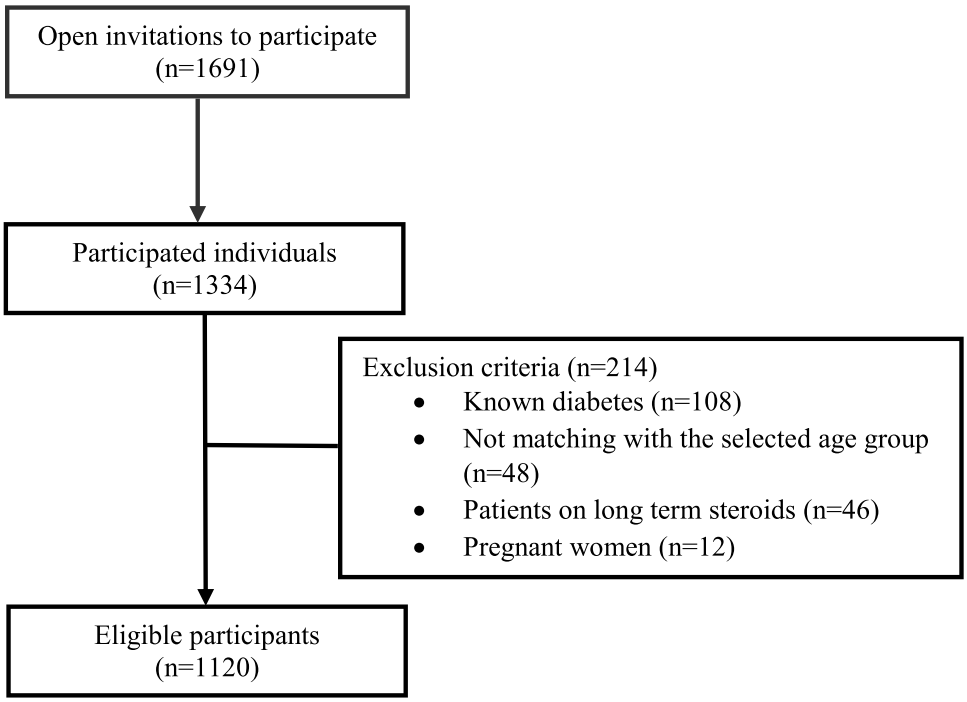

Fig. 1 Flow diagram for the recruitment of eligible participants

Table 1 Descriptive statistics of the participants

	Female		Male		*p* value
	Mean	SD	Mean	SD	
Height (cm)	149.4	10.9	161.6	11.0	0.000
Weight (kg)	57.0	10.6	64.2	12.1	0.000
BMI (kg/m^2)	26.8	6.4	24.8	5.5	0.095
Age (years)	45.7	8.7	44.6	7.9	0.037
FPG (mmol/L)	5.9	2.1	6.0	2.2	0.450

BMI, body mass index; *FPG*, fasting plasma glucose

Table 2 Prevalence of glycemic status according to gender

Glycemic status	Number of females	%	Number of males	%
Normoglycemia	468	58.3	179	56.5
Pre diabetes	203	25.3	83	26.2
Diabetes	132	16.4	55	17.4

dysglycemia (Table 4). When considering the gender wise associations, increase in age in both genders and increase in BMI only in males were significantly associated with dysglycemia (Table 4).

As shown below, the prevalence of normoglycemia decreased and the prevalence of dysglycemia increased with rising BMI and age in both genders (Figs. 2 and 3).

Results of the chi-square test showed that there is a significant difference between gender and glycemic status in normal weight (X^2 (1) = 7.663, $p = 0.006$) and obese (X^2 (1) = 7.476, $p = 0.006$) categories. However, there is no association between gender and glycemic status diagnosed by FPG concentration in any of the considered age groups.

Figure 4 shows the ROC curve of BMI to assess the risk of dysglycemia for both genders separately. The area under the ROC curve (AUC) of females was not-significant (0.516 ± 0.021, $p = 0.43$) whereas ROC curve for males was significant (0.651 ± 0.031, $p = 0.000$) in assessing the risk of dysglycemia by means of BMI. The optimal cut-off value of BMI for males was 22.86 kg/m^2 (sensitivity 76.6%, specificity 53.9%) to determine the risk of dysglycemia.

Table 3 Gender wise correlations between glycemic status vs age and BMI

	Females		Males	
	Age	BMI	Age	BMI
FPG	$r = 0.158$ $p = 0.000$	$r = 0.003$ $p = 0.932$	$r = 0.160$ $p = 0.004$	$r = 0.162$ $p = 0.004$

BMI, body mass index; *FPG*, fasting plasma glucose

Table 4 Study population associations of age and BMI with dysglycemia

	Variable	OR	95% CI	*p* value
Whole population	Age	1.04	1.03–1.06	0.000
	BMI	1.03	1.01–1.05	0.006
Females	Age	1.04	1.02–1.06	0.000
	BMI	1.01	0.99–1.03	0.474
Males	Age	1.05	1.02–1.08	0.000
	BMI	1.10	1.05–1.15	0.000

BMI, body mass index

Discussion

Results of the present study revealed high prevalence of dysglycemia in the community and the gender specific associations of two major risk factors namely increasing age and BMI. The cutoff value of FPG ≥ 5.6 mmol/L (100 mg/ dL) was used to define dysglycemia as the value is well established universally with considerable specificity and sensitivity. One of the important findings of the present study is almost four out of ten adults suffer from dysglycemia either in the form of pre-diabetes or diabetes. Further, the prevalence of pre-diabetes (26.2%) as well as diabetes (17.4%) is higher in males than that of females as 25.3% and 16.4% respectively. Although there are no previously reported facts on the prevalence of dysglycemia, a higher prevalence of diabetes in men (14.2%) than in women (13.5%) in four provinces in Sri Lanka by involving of individuals between 35 and 65 years of age has been reported [23]. Similar observations were made in the studies carried out in different settings [24, 25] and is suggested that high prevalence of DM in males than in females is due to gender specific differences in visceral fat accumulation [24]. However, the exact mechanism related to the higher prevalence of dysglycemia in males than in females is still unclear and further investigations are warranted. Our study is the most recent population-based study conducted with an aim of estimating of the prevalence of dysglycemia. With respect to the preexisted prevalence of diabetes mellitus in Sri Lanka [23], the present study clearly indicated a dramatic increment in newly diagnosed cases of diabetes mellitus in Galle, as one of the rapidly growing cities in Sri Lanka. The spatial structure of Galle is being subjected to changes over the time by expanding the growth towards outer city. This scenario has made significant changes in the city structure as well as in the lives of resident individuals and as a result

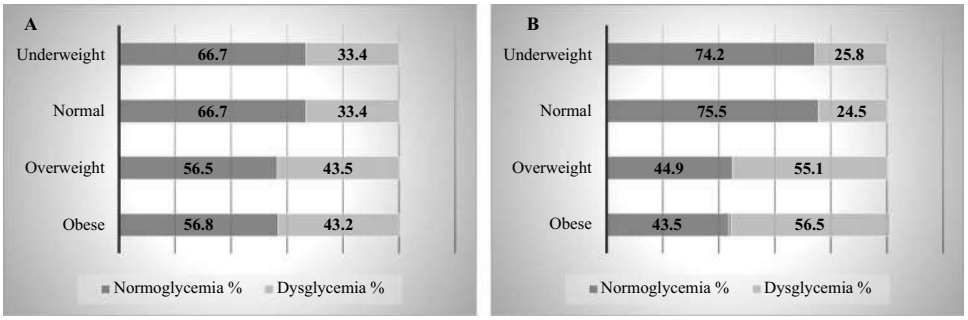

Fig. 2 Prevalence of glycemic status according to BMI in **A** females **B** males

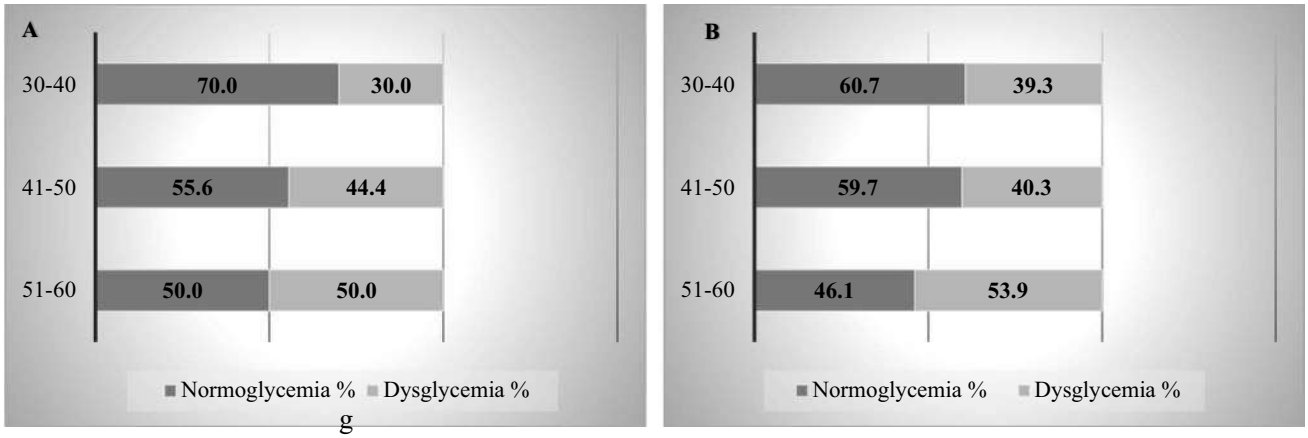

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Fig. 3 Prevalence of glycemic status according to age in **A** females **B** males

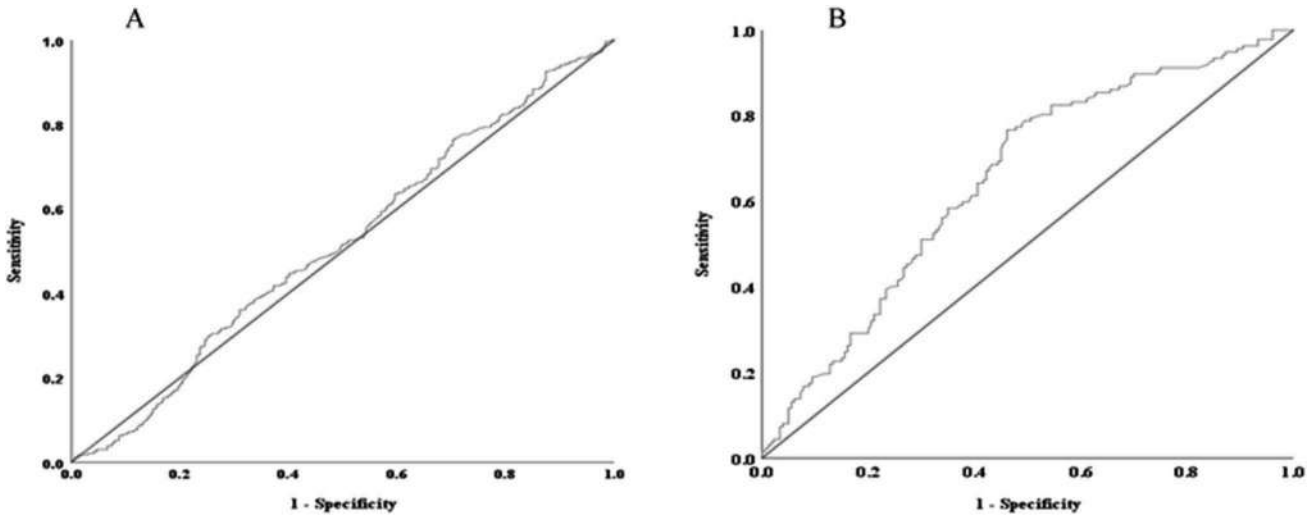

Fig. 4 ROC curve for BMI as the risk of dysglycemia in **A** females **B** males

the individuals are on unhealthy diet, physical inactivity etc. All these facts might contribute for this increment of high prevalence of newly detected cases of dysglycemia and further studies are warranted on this concern. In contrast, a high prevalence of dysglycemia was seen in females than in males in a study conducted in rural, urban and plantation sector in Kalutara district, Western province, Sri Lanka by involving 1300 adults between aged of 35 to 64 years [22].

Findings of the present study also revealed that FPG concentration was significantly and positively correlated with age in both genders whereas significant association with BMI was seen only in males. Furthermore, the binary logistic regression analysis revealed that increase in age of both genders and BMI of males are significantly associated with dysglycemia. Although the development of dysglycemia is multifactorial, insulin resistance and decrement of the pancreatic insulin secretion are well reported pathogenetic mechanisms associated with dysglycemia. Age is the one of the most important factors which affects these mechanisms. Development of insulin resistance with increasing age of population has also been demonstrated using hyperinsulinemic-euglycemic clamp method [26] and responsible factors for the reduced insulin effectiveness with aging have been summarized as increased abdominal fat mass, decreased physical activity, mitochondrial dysfunction, hormonal changes, increased oxidative stress and inflammation [27]. Indeed, the prevalence of dysglycemia has increased rapidly with aging in the past decades and management of dysglycemia in elders is very complicated as they commonly have several co-existing health issues that could affect the overall management of patients.

Over the past decades, progressive increase in body weight among individuals in developed countries than in the low- or middle-income developing countries has been reported [28]. A recent trend of similar weight gain among individuals is highlighted in developing countries as well [29]. The mean BMI values of females (26.8 ± 6.4 kg/m^2) and males (24.8 ± 5.5 kg/m^2) found in our study were in the range of obese and overweight respectively. These values suggest a possible interplay of genetic factors, westernized lifestyle, physical inactivity etc. among Sri Lankans as a nation living in a developing country of South East Asian region. According to the classification of BMI, our results revealed that the prevalence of dysglycemia is increased with an increment in BMI in both genders. Moreover, the present study disclosed that there is a significant difference between the gender and glycemic status in normal weight and obese categories. The male population with normal weight has the lowest prevalence of dysglycemia (24.5%). However, in the obese category, males have the highest prevalence of dysglycemia (56.5%). This might be due to their careless behavior in the management of early stage of dysglycemia because of their occupational activities and their habits including smoking and addiction to intake of alcohol. In fact, males are often the breadwinners of the family and hence more likely to be employed than females in the South East Asian countries including Sri Lanka. These factors also influence the inclusion of a smaller number of males than females in the community screening programs as in the present study. However, further research is warranted to rectify this finding.

As it was important to determine the optimal cut-off points of BMI to assess the risk for dysglycemia, ROC curves were drawn for both genders separately. According to the results, cut-off value for BMI in males was 22.86 kg/m^2 with 76.6% sensitivity and 53.9% specificity. The AUC value for BMI (0.651) in ROC analysis unveiled that

BMI > 22.86 kg/m^2 could be used as an acceptable clinical parameter to determine the risk of dysglycemia among adult males in this community. The observed BMI cut-off point of 22.86 kg/m^2 is very close to the WHO recommended revised cut-off value for overweight (23 kg/m^2) among the South Asian population. However, since the AUC value of BMI for females is almost 0.5 (0.51), this was not further subjected to estimate sensitivity or specificity.

The main strength of this study was its community-based design to detect previously undetected dysglycemia and the study of gender wise differences in association of age and BMI with dysglycemia in a developing South East Asian country during an era of an obesity pandemic. Our findings highlight the rising burden of both dysglycemia and obesity among adults in a developing country in this region. It also strengthens the use of recommended cut-off value of 23 kg/m^2 for the South Asian region to categorize overweight individuals especially the males in this population. However, even with higher representation of females (72%) in the study sample, similar conclusion for females could not be arrived from our findings. We hope the findings would stimulate further studies to determine the possibility of distinct gender-based association of obesity and dysglycemia in this community. Without analyzing the results of such large-scale studies, it would be premature to predict the necessity to adopt separate, gender specific BMI cut-off values to categorize overweight and obesity as in the case of using separate cut-off values of waist circumference for defining central obesity for males and females. Conducting the study in a limited geographical locality in Southern Sri Lanka, the presence of relatively high female representation in the study sample and the use of FPG test results instead of the gold standard OGTT to screen dysglycemia are the main limitations of this study.

Conclusions

The prevalence of dysglycemia either in the form of prediabetes or diabetes in the male population (43.6%) is higher than that of the female population (41.7%) in this cohort. An increase in age in both genders and BMI in males is significantly associated with dysglycemia. The cut-off value of BMI > 22.86 kg/m^2 could be used as an acceptable clinical parameter to determine the risk of dysglycemia by means of overweight among the male population in Sri Lanka. Hence, the WHO recommended cut-off point of 23 kg/m^2 is further corroborated for the male population in Sri Lanka in assessing the risk of dysglycemia by means of overweight. Based on the finding of this study, it is important to implement measures to control overweight and obesity more vigorously in males to reduce the onset of dysglycemia.

Declarations

Ethical considerations Ethical clearance for the study was granted by the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka (14.06.2017:3.9). Written informed consent was obtained from each one of the participants who underwent screening.

Conflict of interest Authors have declared that they have no conflicts of interest.

References

1. Tabish SA. Is diabetes becoming the biggest epidemic of the twenty-first century? Int J Health Sci. 2007;1(2):5–8.
2. IDF. International Diabetes Federation Atlas. 2017.
3. IDF. International Diabetes Federation Atlas. 2015.
4. Sierra GN. The global pandemic of diabetes. AJDM. 2009;17(11):4–8.
5. Teixeira-Lemos E, Nunes S, Teixeira F, Reis F. Regular physical exercise training assists in preventing type 2 diabetes development: focus on its antioxidant and anti-inflammatory properties. Cardiovasc Diabetol. 2011. https://doi.org/10.1186/1475-2840-10-12.
6. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. Diabetes Care. 2018;41(1):13–27.
7. Ghazanfari Z, Haghdoost AA, Alizadeh SM, Atapour J, Zolala F. A comparison of HbA_{1c} and fasting blood sugar tests in general population. Int J Prev Med. 2010;1(3):187–94.
8. Lai YJ, Lee ST, Kuo SC, Chou P. Comparison of fasting plasma glucose and glycated hemoglobin for diagnosing diabetes in a Taiwanese population. Metab Syndr Relat Disord. 2015;13(5):203–7.
9. Herath HMM, Weerarathna TP, Dahanayake MU, Weerasinghe NP. Use of HbA1c to diagnose type 2 diabetes mellitus among high risk Sri Lankan adults. Diab Met Syndr Clin Res Rev. 2017;11(4):51–5.
10. Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW, et al. Obesity and type 2 diabetes: what can be unified and what needs to be individualized? J Clin Endocrinol Metab. 2011;96(6):1654–63.
11. World Health Organization (1998) Obesity: preventing and managing the global epidemic. Report on a WHO consultation on obesity
12. World Health Organization Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet (London, England). 2004;363(9403):157–63.
13. Razak F, Anand SS, Shannon H, Vuksan V, Davis B, Jacobs R, Teo KK, McQueen M, Yusuf S. Defining obesity cut points in a multiethnic population. Circulation. 2007;115(16):2111–8.
14. Jafar TH, Chaturvedi N, Pappas G. Prevalence of overweight and obesity and their association with hypertension and diabetes mellitus in an Indo-Asian population. CMAJ. 2006;175(9):1071–7.
15. Kalyani RR, Golden SH, Cefalu WT. Diabetes and aging: unique considerations and goals of care. Diabetes Care. 2017;40(4):440–3.
16. Kaewput W, Thongprayoon C, Varothai N, Sirirungreung A, Rangsin R, Bathini T, et al. Prevalence and associated factors of hospitalization for dysglycemia among elderly type 2 diabetes patients: a nationwide study. World J Diabetes. 2019;10(3):212–23.

17. Kirkman MS, Briscoe VJ, Clark N, Florez H, Haas LB, Halter JB, et al. Diabetes in older adults. Diabetes Care. 2012;35(12):2650–64.
18. Amati F, Dubé JJ, Coen PM, Stefanovic-Racic M, Toledo FG, Goodpaster BH. Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care. 2009;32(8):1547–9.
19. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. J Biomed Biotechnol. 2012. https://doi.org/10.1155/2012/634195.
20. Cândido F, Bressan J. Vitamin D: link between osteoporosis, obesity, and diabetes? Int J Mol Sci. 2014;15(4):6569–91.
21. Li YX, Zhou L. Vitamin D deficiency, obesity and *diabetes*. Cell Mol Biol. 2015;61(3):35–8.
22. De Silva AP, De Silva SHP, Haniffa R, Liyanage IK, Jayasinghe S, Katulanda P, et al. Inequalities in the prevalence of diabetes mellitus and its risk factors in Sri Lanka: a lower middle income country. Int J Equity Health. 2018. https://doi.org/10.1186/s12939-018-0759-3.
23. Wijewardene K, Mohideen MR, Mendis S, Fernando DS, Kulathilaka T, Weerasekara D, et al. Prevalence of hypertension, diabetes and obesity: baseline findings of a population based survey in four provinces in Sri Lanka. Ceylon Med J. 2005;50(2):62–70.
24. Nordström A, Hadrévi J, Olsson T, Franks PW, Nordström P. Higher prevalence of type 2 diabetes in men than in women is associated with differences in visceral fat mass. J Clin Endocrinol Metab. 2016;101(10):3740–6.
25. Rothney MP, Catapano AL, Xia J, Wacker WK, Tidone C, Grigore L, et al. Abdominal visceral fat measurement using dual-energy X- ray: association with cardiometabolic risk factors. Obesity. 2013;21(9):1798–802.
26. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol Endocrinol Metab. 1979;237(3):214–23.
27. Goulet ED, Hassaine A, Dionne IJ, Gaudreau P, Khalil A, Fulop T, et al. Frailty in the elderly is associated with insulin resistance of glucose metabolism in the postabsorptive state only in the presence of increased abdominal fat. Exp Gerontol. 2009;44(11):740–4.
28. Chiriboga DE, Ma Y, Li W, Olendzki BC, Pagoto SL, Merriam PA, et al. Gender differences in predictors of body weight and body weight change in healthy adults. Obesity. 2008;16(1):137–45.
29. Shukla HC, Gupta PC, Mehta HC, Hébert JR. Descriptive epidemiology of body mass index of an urban adult population in western India. J Epidemiol Community Health. 2002;56(11):876–80.

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ORIGINAL ARTICLE

Pre-diabetes and it's predictors in Abia State, Eastern Nigeria

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Abstract

Background and objectives The prevalence of diabetes mellitus (DM) is rising in sub-Saharan Africa, including Nigeria. A previous study in Abia State, Nigeria, showed a high prevalence of diabetes, with no significant difference in urban and rural communities. This study aimed at investigating the prevalence and risk factors for impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), which represent reversible and preventable early signs of DM.

Subjects, materials, and methods A cross-sectional comparative study of 2800 adult residents of Abia State, comprising equal number of urban and rural respondents. Interviewer-administered semi-structured questionnaires were used for data collection. Fasting blood glucose was performed for all the respondents, while oral glucose tolerance test (OGTT) was done for 2424 respondents, comprising 1117 urban residents and 1307 rural residents. Data was analyzed using SPSS version 20.

Results Mean age of the respondents was 48.54 ± 13.24 years: rural $= 54.23 \pm 14.26$ years and urban $= 42.85 \pm 13.24$ years, $p < 0.001$. Male to female ratio was 1:2.5 ($p < 0.001$). Pre-diabetes (IFG and IGT) was observed in 6.3% of the respondents, comprising 3.9% in urban and 8.7% in rural residents, $p < 0.001$. The prevalence of IFG and IGT was 4.7% and 12%, respectively, $p < 0.001$. Independent predictors of pre-diabetes included abnormal waist circumference (WC), hypertension, and daily intake of fruits and vegetables in the urban area, while in the rural area, they included hypertension and abnormal WC.

Conclusion The prevalence of pre-diabetes is high in Abia State, with a higher burden among rural residents. Hypertension and abnormal WC are significant predictors of pre-diabetes in Abia State. Daily consumption of fruits/vegetables in processed forms may be associated with an increased risk of pre-diabetes.

Keywords Pre-diabetes · Urban · Rural · Abia · Nigeria

Introduction

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) also known as pre-diabetes pre-date the onset of DM [1,2]. When identified early, the progression of IFG/IGT to diabetes can be reduced by 25–60%, with adequate intervention, mainly lifestyle interventions aimed at weight reduction and with the use of metformin [1,2]. A previous community-based study in Abia State revealed an overall prevalence of DM of 3.6%, with no significant difference between the urban and rural communities studied (4.4% and 3.0%, respectively) High alcohol intake, low physical activity, and obesity were significant associations with DM in the study [3].

Urbanization with the adoption of western lifestyles in developing countries has led to the abandonment of the healthier traditional lifestyles, characterized by regular vigorous physical activities and subsistence on high-fiber diet, rich in vegetables and fruits, with a resultant increase in the prevalence of diabetes and other non-communicable diseases (NCDs) [4,5]

The health systems in many countries including Nigeria are not equipped to meet the increasing challenges of diabetes and other NCDs care, hence the need for preventing or delaying their development. Pre-diabetes represents early warning sign of diabetes, which is largely reversible with

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appropriate interventions. This study is aimed at investigating the prevalence and predictors of pre-diabetes (IFG and IGT) in Abia State. Knowledge of this will help in planning appropriate preventive measures as well as measures to halt or delay its progression to diabetes.

Methodology

The study was carried out in Abia State, one of the 36 states of the Federal Republic of Nigeria, located in the eastern part of the country. It has three senatorial districts, namely Abia north, Abia south, and Abia central, and two main cities, namely Umuahia, the capital city, and Aba, the commercial city [6]. It also has 17 local government areas and an approximate population of 2.8 million, with males forming 46.6% and females 53.4% [6]. The predominant tribe is Igbo, and the major source of income is from agriculture and commerce [6]. The study was cross-sectional comparative, involving adults, aged 18 and above, who had lived in Abia State for at least 2 years. Exclusion criteria included being very ill, having a mental illness, pregnancy, or being in the puerperium.

The sample formula for comparison of proportions between two groups, [7] was used to estimate a minimum sample size of 1382 for each group, using the previous prevalence of diabetes for rural and urban areas in Abia State, of 3.0% and 4.4%, respectively [3]. This was rounded off to 1550 for each group, to take care of 10% attrition rate. A total sample size of 3100 was calculated; however, a total of 2800 respondents were studied, comprising equal number of urban and rural residents.

A multistage (3 stage) cluster random sampling method was used to select the respondents.

1st stage: From a list of all the 14 LGAs that house the rural communities in Abia State, a simple random technique (balloting) was used to select Isiala Ngwa North LGA. Similarly, from the 3 LGAs that house the major urban cities in Abia State, a simple random technique (balloting) was used to select Aba North LGA.

2nd stage: All the administrative wards in the selected LGAs were listed (12 in Aba North and 10 in Isiala Ngwa North). A simple random technique (balloting) was used to select Ama-asaa ward (ward 01) in the rural area and Eziama (ward 01) in Aba North.

3rd stage: Catchment villages from the selected ward in the rural LGA (Ama-asaa) were listed. Five villages (Umueke-Amachi, Umuogele-Amachi, Umunkolo-Amachi, Usaka-Umuofor, and Umuosonyike) were selected by simple random technique, and they formed the unit of selection in the rural area. Catchment areas From Eziama ward in Aba North LGA were listed. Umungasi and Brass were selected by simple random technique. Brass, Umungasi, and their environs (Faulk's road and Umungasi-Abayi road) formed the unit of selection in the urban area. Community mobilization was done via announcements by town and market announcers and religious organizations in the selected areas, as well as house to house and street to street announcements. Recruitment of respondents was done centrally at Amachi-Nsulu health center and Usaka-Umuofor town hall in the rural area, while in the urban area, it was done at Umungasi town hall and Abayi town hall. Recruited respondents who gave consent to participate in the study were selected consecutively, until the required sample size was obtained.

An interviewer-administered semi-structured questionnaire (modified WHO screening tool for non-communicable diseases) was used to collect the respondents' data [8]. The questionnaires were pre-tested before use. Copies of the pre-tested tool were made and administered to all consenting respondents.

Recruitment and selection of the respondents were done on the first day of contact with them. Socio-demographics, medical history, and physical examination were obtained for each of them on the first day, while fasting blood glucose (FBG) tests were done on the following day (2nd day), using pre-calibrated and pre-tested Accu-chek glucometers and finger pricked blood samples. Respondents who were not previously diagnosed to have diabetes mellitus and with IFG or FBG within the normal range, following the initial blood glucose test, had an oral glucose tolerance test (OGTT) done on the same day.

Weight (Wt) in kg and height (Ht) in cm were obtained using a seca weighing scale and stadiometer [8]. Body mass index (BMI) was calculated by dividing the weight (Wt) in kg by the square of the height (Ht^2) in meters, viz. $BMI = Wt/Ht^2$ (in kg/m^2). All values were taken to the nearest one decimal place [8]. Waist circumference (WC) and hip circumference (HC) in centimeters (cm) were measured, using a flexible inelastic tape to the nearest 0.1 cm [9]. Blood pressure (BP) was measured using Accoson mercury sphygmomanometer (standard cuff: 15 × 55 cm). A resting systolic BP of 140 mmHg and above and/or a diastolic BP of 90 mmHg and above were regarded as hypertension. Respondents taking antihypertensive drugs were also regarded as hypertensive, even if their BP were below these cutoff points [8].

The OGTT was done by preparing a glucose solution using 82.5 g Allenbury's glucose D (an equivalent of 75 g anhydrous glucose), which was dissolved in 200 ml of clean drinking water and given to each eligible respondent to drink. Blood glucose was measured 2 h after intake of the glucose drink, with the use of the same Accu-chek glucometer used to estimate the fasting blood glucose level. This was recorded as the 2-h post-glucose load value. A fasting blood glucose value of 110 to 125 mg/dL (6.1 to 6.9 mmol/L) was termed impaired fasting glucose (IFG). A 2-h value between 140 and 199 mg/dL (7.8–10.9 mmol/L) was termed

impaired glucose tolerance. A value lower than 140 mg/dL (7.8 mmol/L) was taken as normal, while those ≥ 200 mg/dL (11.1 mmol/L) were regarded as diabetes [8].

Data generated from the study was cleaned and analyzed using SPSS version 20 (SPSS Inc. Chicago, IL, USA) statistical software. Mean ± standard deviation was computed for normally distributed continuous variables. Proportions of the categorical variables were computed. Differences in anthropometric and clinical characteristics between urban and rural as well as pre-diabetes were analyzed using Student's *t* test. The association of pre-diabetes with age, anthropometric parameters (BMI, WC, HC, WHR), and blood pressure was determined using multiple logistic regression. *p* value ≤ 0.05 was taken as statistically significant. Results were presented in a chart and tables.

Ethical approval for the study was obtained from the Abia State Ministry of Health ethical committee. Permission was also sought from the community leaders before the study was commenced. The study was carried out from January to March 2017.

Results

The study aimed at investigating the prevalence and predictors of pre-diabetes in Abia State, Nigeria. A total of 2800 respondents, comprising 1400 urban and 1400 rural residents, were studied. Oral glucose tolerance test (OGTT) was performed for 2424 respondents, comprising 1117 urban residents and 1307 rural.

The mean age ± SD of the respondents in the study was 48.54 ± 14.89 years, urban = 42.85 ± 13.24 years, and rural = 54.23 ± 14.26 years, $p < 0.001$. There were more female respondents in both the urban and the rural areas, with a ratio of 3:1.2 for urban and 3:1 for rural. The age distribution of the respondents is shown in Fig. 1. In the rural area, 961 (68.6%) of the study respondents were farmers, while in the urban area, 850 (60.7%) were engaged in business activities for their livelihood. Other socio-demographic characteristics are as shown in Table 1. Hypertension, overweight, obesity, and abnormal waist circumference are significantly more prevalent among the urban respondents, while abnormal waist-hip ratio is significantly more observed in the rural area as shown in Table 2.

Pre-diabetes (IFG and IGT) was observed in 6.3% (176) of the respondents in this study, with urban constituting 54 (3.9%) and rural 122 (8.7%). Pre-diabetes was significantly

Table 1 Socio-demographic characteristics of the respondents

	Urban *n* (%)	Rural *n* (%)	χ^2	*p* value
Occupation				
Farming	159 (11.4)	961 (68.6)	998.164	< 0.001*
Fishing	0 (0.0)	12 (0.9)		
Business	850 (60.7)	303 (21.6)		
Housewife	48 (3.4)	11 (0.8)		
Retired	41 (2.9)	31 (2.2)		
Civil servant	280 (20.0)	80 (5.7)		
Student	22 (1.6)	2 (0.2)		
Marital status				
Single	324 (23.1)	57 (4.1)	356.515	< 0.001*
Married	939 (67.1)	926 (66.1)		
Widowed	125 (8.9)	417 (29.8)		
Separated	12 (0.9)	0 (0.0)		
Educational level				
No formal	109 (7.8)	85 (6.1)	1963.076	< 0.001*
Primary	242 (17.3)	1005 (71.8)		
Secondary	681 (48.6)	284 (20.3)		
Tertiary	368 (26.3)	26 (1.8)		

*p** significant, *BMI* body mass index, *WHR* waist-hip ratio, *WC* waist circumference

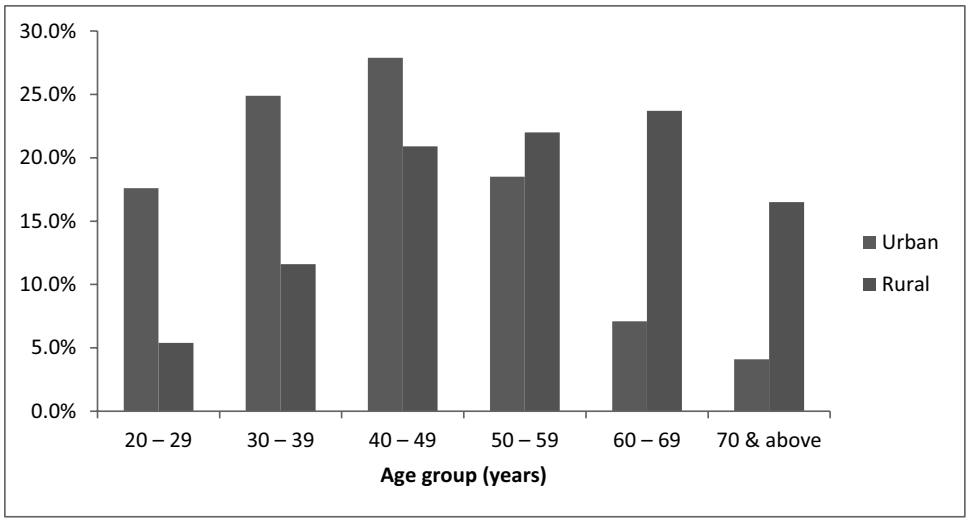

Fig. 1 Age distribution of the respondents

Table 2 Clinical characteristics of the respondents

	Urban *n* (%)	Rural *n* (%)	χ^2	*p* value
Hypertension				
Yes	568 (40.6)	857 (61.2)	119.354	< 0.001*
No	832 (59.4)	543 (38.8)		
BMI				
Normal	493 (35.2)	650 (46.4)	149.478	< 0.001*
Underweight	7 (0.5)	94 (6.7)		
Overweight	502 (35.9)	430 (30.7)		
Obesity	398 (28.4)	226 (16.1)		
WC				
Abnormal	459 (32.8)	289 (20.6)	52.720	< 0.001*
Normal	941 (67.2)	1111 (79.4)		
WHR				
Abnormal	682 (48.7)	1294 (92.4)	633.606	< 0.001*
Normal	718 (51.3)	106 (7.6)		

*p** significant, *BMI* body mass index, *WC* waist circumference, *WHR* waist-hip ratio

more prevalent in the rural area, when compared with urban ($p < 0.001$, OR = 5.10, 95%CI = 3.244–8.029). Impaired fasting glucose alone was observed in 2.4% of the respondents, with urban having 1.3% and rural 3.4%, while IGT alone was observed in 5.7% of the respondents, with urban having 4.3% and rural 7.7%.

Obesity was present in 78 (44.3%) of those with pre-diabetes, while 89 (60.6%) had waist circumference above normal. Obesity, abnormal WC, abnormal WHR, and hypertension are significant associations with pre-diabetes as shown in Table 3. Age greater than 45 and daily consumption of fruits and vegetables are significantly associated with pre-diabetes in the urban area, while in the rural area, lack of physical activity is associated with pre-diabetes as shown in Table 4. Independent predictors of pre-diabetes include hypertension, abnormal WC, and daily intake of fruits/vegetables in the urban areas, while in the rural areas, it is hypertension and abnormal WC as shown in Table 5.

Discussion

The study aimed at determining the prevalence and predictors of pre-diabetes in rural and urban communities of Abia State, Nigeria. A high prevalence of pre-diabetes was observed in this study. Previous studies in Nigeria and other parts of the world have reported varying prevalence of IFG and IGT, with, however, similar risk factors which include family history of diabetes, increasing age, increased BMI, high WC, high WHR, and hypertension [10–18]. These factors have been shown to cause varying degrees of insulin resistance, with resultant pancreatic dysfunction and DM [19]. Ejike et al. reported a prevalence of IFG of 1.1%, in Umudike, a sub-urban town in Abia State, with female sex and markers of obesity (high BMI, increase WHR and WC) being significant associations [10]. Enang et al. reported a prevalence of IFG and IGT of 9% and 20%, respectively, in a study carried out in Calabar, Nigeria [11], while Nwatu et al. in their own study in Enugu, Nigeria, reported a prevalence of IFG and IGT of 9.3% and 15.8%, respectively, and pre-diabetes of 21.5%, with age, hypertension, and physical inactivity being

Table 3 Association of pre-diabetes with some clinical characteristics of the respondents

	Pre-diabetes		*p* value	OR	95%CI for OR
	Yes *N* = 176 *n* (%)	No *N* = 2624 *n* (%)			
BMI					
Normal	54 (30.7)	1089 (41.5)			
Underweight	8 (4.5)	93 (3.5)	0.162	1.735	0.802–3.755
Overweight	36 (20.5)	896 (34.1)	0.339	0.810	0.527–1.247
Obesity	78 (44.3)	546 (20.8)	< 0.001*	2.881	2.006–4.137
WC					
Abnormal	89 (50.6)	659 (25.1)	< 0.001*	3.050	2.241–4.152
Normal	87 (49.4)	1965 (74.9)			
WHR					
Abnormal	143 (81.2)	1833 (69.9)	0.002*	1.870	1.269–2.755
Normal	33 (18.8)	791 (30.1)			
Hypertension					
Yes	134 (76.1)	1444 (55.0)	< 0.001*	2.607	1.828–3.718
No	42 (23.9)	1180 (45.0)			

*p** significant, *BMI* body mass index, *WC* waist circumference, *WHR* waist-hip ratio, *FHxDM* family history of diabetes

Table 4 Association between sociodemographic, lifestyle characteristics of respondents and pre-diabetes in urban and rural areas

	Pre-diabetes				
	Yes *n* (%)	No *n* (%)	*p* value	OR	95%CI for OR
Urban					
Age (years)					
≥46	31 (5.5)	533 (94.5)	0.010*	2.06	1.19–3.56
<46	23 (2.8)	813 (97.2)			
Family hx of DM					
Yes	7 (4.0)	166 (96.0)	0.890	1.059	0.471–2.381
No	47 (3.8)	1180 (96.2)			
Tobacco use					
Yes	51 (3.8)	1290 (96.2)	0.618	0.738	0.223–2.437
No	3 (5.1)	56 (94.9)			
Alcohol use					
Yes	54 (4.0)	1294 (96.0)	NA	NA	NA
No	0 (0.0)	52 (100.0)			
Daily intake of F/V					
Yes	22 (6.4)	321 (93.6)	0.006*	2.195	1.258–3.832
No	32 (3.0)	1025 (97.0)			
Lack of phys. activity					
Yes	42 (3.9)	1048 (96.1)	0.989	0.955	0.517–1.915
No	12 (3.9)	298 (96.1)			
Rural					
Age (years)					
<46	28 (7.1)	368 (92.9)	0.172	0.737	0.475–1.143
≥46	94 (9.4)	910 (90.6)			
Family hx of DM					
Yes	8 (9.8)	74 (90.2)	0.730	1.142	0.537–2.428
No	114 (8.6)	1204 (91.4)			
Tobacco use					
Yes	118 (8.9)	1215 (91.1)	0.418	1.530	0.547–4.277
No	4 (6.0)	63 (94.0)			
Alcohol use					
Yes	118 (9.1)	1185 (90.9)	0.106	1.530	0.547–4.277
No	4 (4.1)	93 (95.9)			
Daily intake of F/V					
Yes	13 (5.8)	213 (94.2)	0.088	0.596	0.329–1.080
No	109 (9.3)	1065 (90.7)			
Lack of phys. activity					
Yes	18 (14.5)	106 (85.5)	0.018*	1.91	1.12–3.28
No	104 (8.2)	1172 (91.8)			

*p** significant, *Family hx of DM* family history of diabetes mellitus, *F/V* fruits and vegetables, *NA* not applicable

Table 5 Multivariate analysis showing predictors of pre-diabetes in urban and rural areas

			95%CI for OR	
	p value	OR	Lower	Upper
Urban				
Hypertension	0.032	1.965	1.060	3.643
WC (abnormal)	<0.001*	4.127	2.039	8.356
Underweight	0.183	1.698	0.778	3.705
Age group (<46)	0.058	0.565	0.314	1.019
Daily intake of F/V	0.003*	2.382	1.341	4.231
Rural				
Age group (<46)	0.752	0.928	0.582	1.478
Hypertension	0.027*	1.730	1.063	2.814
WC (abnormal)	<0.001*	3.598	2.216	5.841
Underweight	0.441	1.397	0.597	3.267
Physical activity	0.072	0.598	0.341	1.046

*p** significant, *WC* waist circumference, *F/V* fruits and vegetables

significant predictors [12]. These are higher than the prevalence of IFG and IGT observed in our study, which may be a reflection of the rising trend in diabetes and its risk factors in the study areas. An earlier rural community screening for DM and its risk factors in Anambra state, Eastern Nigeria, by Mezie et al., showed that 8.2% of the participants had impaired fasting glucose (IFG), with male gender, age, and hypertension being significantly associated with IFG [13]. Nyenwe et al. reported a lower prevalence rate of IGT of 2.2%, in Port-Harcourt, about two decades prior to this study, with obesity, high WHR, family history of diabetes, and increasing age being factors associated with diabetes and pre-diabetes [14]. The study was done more than a decade ago and may not reflect the current prevalence of IGT in the region studied. The predictors of pre-diabetes observed in our study (abnormal WC and hypertension) are similar to what was observed in previous studies [10–18].

Differences in geographical location and rapid progression from pre-diabetes to diabetes may explain the lower prevalence of IFG and IGT observed in our study, when compared with recent studies in other parts of the country. Chiwanga et al., in Uganda, reported a prevalence of pre-diabetes of 13.8%, with family history of diabetes and hypertension, being significant associations [17]. Thorpe et al. in New York city reported a much higher prevalence of IFG of 23.5%, with older age and low income being significant associations [18]. Despite the variations in the reported prevalence of IFG and IGT from different studies, the prevalence observed in this study falls within what the International Diabetes Federation (IDF) estimated (4.5–12.1%) [5]. Type 2 diabetes mellitus has insidious onset, often preceded by a long period of insulin resistance, and in resource poor countries, it is recognized late, with various short- and long-term complications [20]. This preceding period of insulin resistance represents the pre-diabetes state (IFG and IGT), which is largely preventable and reversible through lifestyle modifications [1,2]. The presence of both IFG and IGT in an individual represents more marked insulin resistance at the liver and skeletal muscles [21]. Pre-diabetes has variable natural history,

with approximately 25% progressing to diabetes, 50% remaining in their abnormal glycemic state, and 25% reverting to normal glucose tolerance over an observational period of 3–5 years [22,23]. Individuals who are older, overweight, and have other diabetes risk factors are more likely to progress [22,23]. It is worrisome that IGT which is a more sensitive predictor of cardiovascular risk than IFG is higher in this study. This study suggests that the prevalence of IFG, IGT, and even DM may be rising in Abia State. Rapid urbanization, increasingly sedentary lifestyles, and unhealthy eating habits are factors that may contribute largely to the increased prevalence of pre-diabetes. The prevalence of pre-diabetes in this study is significantly higher in the rural area when compared to urban. This is in contrast to previous reports from communities in Nigeria, where a higher prevalence of pre-diabetes and diabetes was reported in urban communities when compared with rural [15,16]. It is worrisome to note that a previous community-based study in Abia State, conducted 6 years earlier, showed no urban–rural difference in the prevalence of DM [3]. This may be a reflection of an ongoing epidemiologic transition. In developing countries with low-income settings, diabetes is often postulated to be a disease of the rich, but with increasing economic development and lifestyle transition among all groups, diabetes now affects the poor also [24,25]. Ramchandra et al. in India found no significant urban–rural difference in the prevalence of IGT (8.7% and 7.8%, respectively), which was thought to be due to rapid conversion of IGT to DM in the urban community [26]. Alberts et al. reported a high prevalence of DM of 8.8% in a study conducted a few years ago in a poor rural black community of South Africa [27]. More recently, a community-based study in Uganda and Tanzania, two sub-Saharan African countries, showed an overall prevalence of diabetes of 10.1% which was highest among rural Ugandan residents (16.1%) compared to teachers in Tanzania (8.3%) and peri-urban Ugandan residents (7.6%) [17]. The prevalence of pre-diabetes was also high in that study, with family history of diabetes and hypertension being significant associations [17]. Another large community survey in China reported a higher prevalence of pre-diabetes and diabetes in rural communities, when compared to urban provinces (30.5%, 6.0%, and 10.5%, respectively) [28]. Similarly, a higher prevalence of DM was reported recently in a rural community of Ghana by Kweku et al. [29]. This trend is worrisome, since pre-diabetes is a risk factor for the development of type 2 DM, which was previously thought to be less prevalent in rural sub-Saharan countries, when compared with urban. The reasons that may account for the higher prevalence in the rural areas include increasing age, higher life expectancy, hypertension, variations in dietary habits, and influence of proximity of rural areas to major urban centers.

The association of daily physical activity with a reduction in the frequency of pre-diabetes observed only in the rural area may be due to the type of physical activities rural dwellers engage in, which include farming, trekking and use of bicycles as a means of transportation. Physical activities especially moderate intensity exercises have been shown to be beneficial in the prevention and delaying of diabetes in at risk individuals [1,2]. The association of daily intake of fruits and vegetables with pre-diabetes observed in the urban areas in this study is at variance with previous studies, where daily consumption of fruits and vegetables is linked with a reduction in the prevalence of pre-diabetes and diabetes [30–33]. This may be due to the consumption of the fruits and vegetables in processed forms, instead of eating them in the raw form. Overconsumption of fruits and vegetables may occur when processed prior to consumption. There may also be additives like sugars in the processed fruits/vegetables. The assessment of fruits and vegetables consumption in this study was via self-report by the respondents; also, the pattern of consumption and the quantity consumed were not assessed for. However, the majority of the fruit vendors in urban communities of Abia State is observed to display their goods in blended forms of a cocktail of fruits. It is likely that the majority of the fruit and vegetable consumption by the respondents was done in this form. Intake of fruits and vegetables in processed forms has been linked with the development of diabetes, due to its high sugar and low fiber content [34,35]. Fruits have also been found to contain relatively high levels of fructose, which is linked with insulin resistance and pancreatic dysfunction [36].

Previous studies have linked daily consumption of fruits and vegetables with a reduction in the prevalence of pre-diabetes and diabetes and other range of diseases as it has both a high antioxidant and fiber content and relatively low-energy density and glycemic index [30–33]. The consumption of fruits and vegetables in those studies was done in the raw (unprocessed) forms.

Regular consumption of alcohol is associated with an increased risk of pre-diabetes in this study. Though the quantities and types of alcohol consumed were not assessed for in this study, previous studies have demonstrated that chronic heavy consumption of alcohol is an independent predictor of diabetes as it is linked to insulin resistance and beta cell dysfunction [37,38]

Conclusion and Recommendation

In conclusion, there is a high burden of pre-diabetes among rural residents of Abia State, Nigeria, similar to what has been observed in studies from other parts of the world, including sub-Saharan Africa [17,27–29]. Daily consumption of fruits and vegetables in processed forms may be linked with an increased risk for pre-diabetes. When providing counselling on fruits and vegetable consumption, emphasis should

be made on consuming them in the raw (unprocessed forms) to derive their health benefits. More frequent community-based education on lifestyle changes and screening for diabetes is recommended in Abia State, Nigeria.

References

1. Knowler WC, Barrett-Conner E, Fowler SE, Hammon RF, Lachin JM, Walker EA, et al. The diabetes prevention program research group: reduction in the incidence of type 2 iabetes with lifestyle intervention or metformin. N Engl J Med. 2002;346:393–403.
2. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijav V. Indian diabetes prevention programme (IDPP): the indian diabetes prevention programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49:289–97.
3. Okpechi IG, Chukwuonye II, Tiffin N, Madukwe OO, Onyeonoro UU, Umeizudike TI.et al. Blood pressure gradients and cardiovascular risk factors in urban and rural populations in Abia State South Eastern Nigeria using the WHO STEPwise approach. PLoS ONE 8: e73403. https://doi.org/10.1371/journal.pone.0073403
4. Dirk LC, Henrik F, David LM, Beatrice K, Inge T, Michael KB, et al. Prevalence of glucose intolerance and associated risk factors in rural and urban populations of different ethnic groups in Kenya. Diabetes Res Clin Pract. 2009;84:303–10.
5. International Diabetes Federation: IDF diabetes atlas.7th ed, Brussels, Belgium: International Diabetes Federation, 2015. http://www.eatlas.idf.org. Accessed on 20 Mar 2016
6. Data obtained from the official portal of Abia State Government, Nigeria. 2016. Available at https://www.abiastate.gov/ng/state-profile/historyofabiastate/org. Accessed 13 Oct 2016
7. Sundar Rao PSS, Richard J. Introduction to biostatistics and research methods simplified. 5th ed: PHI Learning Private Limited.2012
8. WHO STEP wise approach to surveillance- Instrument v.3.1 5–16
9. National Heart, Lung and blood institute. US department of health and human services. www.nhibi.nih.gov/guidelines/obesity. Accessed 13th June,2015
10. Ejike CE, Uka NK, Nwachukwu S. Diabetes and pre diabetes and correlates with measures of obesity. Afr J Biochem Res. 2015;9:55–60.
11. Enang OE, Otu AA, Essien OE, Okpara H, Fasanmade OE, Ohwovoriole AE, et al. Prevalence of dysglycaemia in Calabar. A cross sectional observational study among residents of Calabar, Nigeria. Epidemiology/Health service research. BMJ open diab Res care. 2014;2:e000032.
12. Nwatu CB, Ofoegbu EN, Unachukwu CN, Young EE, Okafor CI, Okoli CE. Prevalence of pre-diabetes and associated risk factors in a rural Nigerian community. Int J Diabetes Dev Ctries. 2013;36(2). https://doi.org/10.1007/s13410-015-0401-5.
13. Mezie-Okoye MM. Diabetes in older adults: experience from a rural community in south-east Nigeria. African J Diabetes Med. 2013;21:45–8.
14. Nyenwe EA, Odia OJ, Ihekwaba AE, Babatunde S. Type 2 diabetes in adult Nigerians: a study of its prevalence and risk factors in Port Harcourt, Nigeria. Diabetes Res Clin Pract. 2003;62:177–85.
15. Sabir AA, Isezuo SA, Ohwovoriole AE. Dysglycaemia and its risk factors in an urban Fulani population of Northern Nigeria. West Afr J Med. 2011;30:325–30.
16. Sabir A, Ohwovoriole A, Isezuo S, Fasanmade O, Abubakar S, Iwuala S. Type 2 diabetes mellitus and its risk factors among the rural Fulanis of northern Nigeria. Ann Afr Med. 2013;12:217–22.
17. Chiwanga FS, Njelekela MA, Diamond MB, Banjunirwe F, Guwatudde D, Nankya-Mutyoba J et al. Urban and rural prevalence of diabetes and pre-diabetes and risk factors associated with diabetes in Tanzania and Uganda. *Glob Health Action.* 2016; 9: https://doi.org/10.3402/gha.v9.3144
18. Thorpe LE, Upadhyay UD, Chamany S, Garg R, Mandel-Ricci J, Kellerman S, et al. Prevalence and control of diabetes and impaired fasting glucose in New York city. Diabetes Care. 2009;32:57–62.
19. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev. 1995;75:473–86.
20. Azevedo M, Alla S. Diabetes in sub-saharan Africa: Kenya, Mali, Mozambique, Nigeria, South Africa and Zambia. Int J Diabetes Dev Ctries. 2008;28:101–8.
21. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R et al. Impaired fasting glucose and impaired glucose tolerance: Implications for care. IN: Diabetes care. American Diabetes Association ;2007. Page 753–759,
22. Shaw JE, Zimmet PZ, de Courten M, Dowse GK, Chitson P, Gareeboo H, et al. Impaired fasting glucose or impaired glucose tolerance: what best predicts future diabetes in Mauritius? Diabetes Care. 1999;22:399–402.
23. Stern MP, Williams K, Haffner SM. Identification of persons at high risk for type 2 diabetes mellitus: do we need the oral glucose tolerance test? Ann Intern Med. 2000;136:575–81.
24. Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes Relat Metab Disord. 2004;28:S2–9.
25. Goldstein J, Jacoby E, del Aguila R, Lopez A. Poverty is a predictor of non-communicable disease among adults in Peruvian cities. Prev Med. 2005;41:800–6.
26. Ramachandran A, Mary S, Yamuna A, Murugesan N, Snehalatha C. High prevalence of diabetes and cardiovascular risk factors associated with urbanization in India. Diabetes Care. 2008;31:893–8.
27. Alberts M, Urdal P, Steyn K, Stensvold I, Tverdal A, Nel JH, et al. Prevalence of cardiovascular diseases and associated risk factors in a rural black population of South Africa. Eur J Cardiovasc Prev Rehabil. 2005;12:347–54.
28. Zhao M, Lin H, Yuan Y, Wang F, Xi Yang, Wen LM, et al. Prevalence of pre-diabetes and its associated risk factors in rural areas of Ningbo, China. Int J Environ Res Public Health. 2016;13:808–12.
29. Kweku M, Nyavor P, Bani F, Axame WK, Owusu R, Takramah W, et al. Prevalence and awareness of type 2 diabetes among urban and rural traders in Holwe municipality, Ghana. Int J Clinical Case. 2017;1:64–77.
30. Y. Wu, D. Zhang*, X. Jiang, W. Jiang Fruit and vegetable consumption and risk of type 2 diabetes mellitus: a dose-response meta-analysis of prospective cohort studies. *Nutrition, Metabolism & Cardiovascular Disease*s 2015; 25:140-147
31. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, et al. Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J Am Diet Assoc. 2009;109:414–21.
32. Carter P, Gray LJ, Troughton J, Khunti K, Davies MJ. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. BMJ 2010;341. c4229. Published 2010 Aug 18. doi:https://doi.org/10.1136/bmj.c4229
33. Ceriellona. Oxidative stress and glycaemic regulation. Metabolism 2000; 49:27–29
34. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruits, vegetables and fruit juices and risk of diabetes in women. Diabetes care. 2008;31:1311–7.
35. Wu-Qing H, Ying Lu, Ming Xu, Huang Jing, Yi-Xiang Su, Zhang Cai-Xia. Excessive fruit consumption during the second trimester

is associated with increased likelihood of gestational diabetes mellitus: a prospective study. Sci Rep. 2017;7:43620. https://doi.org/10.1038/srep43620.
36. Richard JJ, Mark SS, Yuri S, Takahiko N, Daniel IF, Duke-Hee K, et al. Potential role of sugar(fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney diseases and cardiovascular disease. Am J Clin Nutr. 2007;86:899–906.
37. Hodge AM, Dowse GK, Collinqs VR, Zimmet PZ. Abnormal glucose tolerance and alcohol consumption in three populations at high risk of non-insulin-dependent diabetes mellitus. Am J Epidemiol. 1993;137:178–89.
38. Wannamethee SG, Shaper AG, Perry IJ, Alberti KG. Alcohol consumption and the incidence of type 2 diabetes. J Epidemiol Community Health. 2002;56:542–8.

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ORIGINAL ARTICLE

Validation of RSSDI therapeutic wheel with clinical experience of Indian physicians

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Abstract

Background The Research Society for Study of Diabetes in India (RSSDI) has developed country-specific guidelines focusing on patient-centric approach for effective management of type 2 diabetes mellitus(T2DM) depending on modifiable and non-modifiable risk factors and pre-existing complications (ABCDEFG).

Methods We conducted a multicenter, retrospective real world study ($n=780$) to validate the use of RSSDI therapeutic wheel by using individually preferred guidelines and clinical expertise of Indian physicians.

Result In elderly population (> 60 years), most commonly prescribed oral hypoglycemic agents (OHA) were DPP-4i (91.23%), metformin (74.74%), sulfonylureas (66.49%), and SGLT-2i (56.18). In patients with BMI > 30 kg/m^2 ($n=133$), order of preference was metformin (92.48%), DPP-4i (34.58%), and SGLT-2i (34.58%). In patients with eGFR 30–45 ml/min/1.73^2 ($n=32$), metformin (81.25%), DPP-4i (40.63%), and SGLT-2i (40.63%) were prescribed. Analysis of prescription pattern according to baseline glycemic status (n = 380) shows most commonly prescribed OHA in patients with HbA1c > 10% ($n=144$) were metformin ($n=144$, 100%), sulfonylureas ($n=75$, 52.08%), insulin ($n=74$, 51.38%), and DPP-4i ($n=71$, 50%). We observed increasing use of DPP-4i (43.33% for < 6 months to 51.69% for > 10 years), SGLT-2i (30% for < 6 months, 34.74% for > 10 years), insulin (20% for < 6 months to 46.61% for > 10 years), and AGI (6.66% for < 6 months to 12.71% for > 10 years) with increasing duration of diabetes. Most commonly prescribed drugs in T2DM with CVD were metformin (81.30%), DPP-4i (49.59%), and insulin (34.96%). In patients with limited financial status, metformin (67.70%), sulfonylureas (45.07%), and insulin (39.43%) were commonly used. The findings from prescriptions prescribed after identifying the most important factors (ABCDEFG) helped in obtaining better glycemic control among these subjects. This validated the suggestions of RSSDI therapeutic wheel.

Conclusion Physicians can adopt RSSDI therapeutic wheel for simple patient centric and effective management of T2DM.

Keywords Type 2 diabetes mellitus · RSSDI therapeutic wheel · Age · BMI · Chronic kidney disease

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Introduction

Diabetes is one of the huge global health issues of the twenty-first century. Type 2 diabetes mellitus (T2DM) accounts for 90–95% of cases of diabetes diagnosed in adults and is associated with increased risk of microvascular, macrovascular cardiovascular complications, and premature deaths. The prevalence of T2DM has increased consistently over the past years. It is estimated that over 463 million individuals are suffering with T2DM globally, while around 50% of patients remain undiagnosed [1]. India ranks second in the world after China for the highest number of diabetes cases. International Diabetes Federation (IDF, 2019) reported 77 million diabetic individuals in India with prevalence of 10.4% and has anticipated this number to reach 134.2 million by 2045 [2]. Recent International guidelines such as American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) have recommended Individualization of T2DM management according to different patients' factors [3]. Despite these efforts, diabetes management practices in India remain suboptimal [4]. This could be partly due to diverse religions, cultures, languages, food habits, lifestyles, Asian Indian Phenotype, and reduced metabolic capacity associated with higher susceptibility to diabetes [5, 6].

Adopting country-specific guidelines can improve the success of treatment outcomes in diabetes. The Research Society for the Study of Diabetes in India (RSSDI) therefore published the clinical practice recommendations for the management of T2DM in 2015, thereafter updated every 2nd year which can significantly impact holistic diabetes care. The recommendations by RSSDI were specifically designed considering the diverse cultural and socioeconomic background of Indians [7]. RSSDI has developed therapeutic wheel which is a patient-centric approach also called as ABCD (EFGH) approach for diabetes management. However, the utility and practicability of this therapeutic wheel need to be validated by clinical experience of Indian physician and diabetologist. We conducted a study to validate the use of RSSDI therapeutic wheel by using individually preferred algorithms based on personal clinical expertise.

Methods

We conducted a multicenter, retrospective real-world study in 5 centers across India (Guwahati, Kolkata, Thiruvananthapuram, Nagpur, and Ahmedabad). The data between the year 2018 and 2019 were retrospectively collected for the factors determining the selection of antidiabetic drugs: A, age; B, BMI; C, CKD; D, duration of diabetes; E, established CVD; F, finance; G, glycemic status; H, hypoglycemia, along with the therapy prescribed to the patients by physicians from these centers using the standard of care and clinical judgement. These data were then compared with the preferred diabetes management from the therapeutic wheel suggested by the RSSDI guidelines (2018).

Statistical analysis

Demographic details were analyzed by descriptive statistics, while other data were analyzed by using inferential statistics in MS Excel V.2016. Categorical variables were expressed as numbers and percentages.

Results

We analyzed the data of 780 patients of type 2 diabetes mellitus from 5 centers across India (Guwahati, Kolkata, Thiruvananthapuram, Nagpur and Ahmedabad) for validation of parameters of RSSDI therapeutic wheel—age, BMI, CKD, duration of diabetes, established CVD, finance status, and hypoglycemia.

A-Age

Majority of patients belonged to age 30–60 years (71.79%), while 24.87% and 3.33% of patients belonged to age group of > 60 years and < 30 years, respectively. Out of 24.87% of elderly population (> 60 years) in our study, most commonly prescribed antidiabetic drugs were DPP-4 inhibitors (91.23%), which was followed by metformin (74.74%), sulfonylureas (66.49%), and SGLT-2 inhibitors (56.18). However, in age group of 30–60 years (out of 71.79%), the order of prescribed antidiabetic drugs were metformin (79.28%), alpha glucosidase inhibitors (42.69%), DPP-4 inhibitors (42.32%), and sulfonylureas (39.10%) (Table 1).

B-BMI

We analyzed the prescription pattern according to body mass index (BMI) categories ($n = 753$). In our study, majority of patients were obese with BMI > 25 kg/m^2 ($n = 505$, 66.93%). In BMI category 25–29.99 kg/m^2, Metformin was most commonly prescribed antidiabetic drug (84.90%), followed by DPP-4 inhibitors (46.09%) and SGLT-2 inhibitors (19.94%). Similar pattern of prescription was observed in patients with BMI > 30 kg/m^2 (Table 2).

Table 1 Antidiabetic drug prescription pattern according to age

	Age group (in years)		
	< 30 (*n* = 26)	30–60 (*n* = 560)	> 60 (*n* = 194)
Metformin (M)	20 (76.92)	444 (79.28)	145 (74.74)
Sulfonylureas (SU)	7 (26.92))	219 (39.10)	129 (66.49)
DPP-4 inhibitors (D)	8 (30.76)	237 (42.32)	177 (91.23)
Pioglitazone (P)	0	71 (12.67)	58 (29.89)
SGLT-2 Inhibitors (Sg)	4 (15.38)	89 (15.89)	109 (56.18)
Alpha glucosidase inhibitors (A)	1 (3.84)	38 (42.69)	54 (18.51)
Glinides (GL)	0	7 (1.25)	10 (5.15)
GLP analogues (G)	0	3 (0.53)	10 (5.15)
Insulin (I)	6 (23.07)	100 (17.85)	56 (28.86)

Values are expressed as number (percentage), *n* = 780

Table 2 Antidiabetic drug prescription pattern according to BMI

	BMI (in kg/m^2)				
	< 18.5 (*n* = 9)	18.5–22.9 (*n* = 100)	23–24.9 (*n* = 140)	25–29.9 (*n* = 371)	> 30 (*n* = 133)
Metformin	3 (33.33)	68 (68)	(73.57)	(84.90)	123 (92.48)
Sulfonylurea (SU)	5 (55.55)	53 (53)	49 (35)	34 (09.16)	18 (13.53)
DPP4 inhibitors (D)	2 (22.22)	39 (39)	62 (44.28)	171 (46.09)	46 (34.58)
Pioglitazone (P)	2 (22.22)	33 (33)	18 (12.85)	30 (8.08)	13 (9.77)
SGLT2 inhibitors (Sg)	1 (11.11)	6 (6)	19 (13.57)	74 (19.94)	46 (34.58)
Alpha glucosidase inhibitors (A)	0	5 (5)	7 (5.00)	41 (11.05)	9 (6.76)
Glinides (GL)	0	0	3 (2.14)	5 (1.34)	4 (3.00)
GLP analogues (G)	0	0	0	7 (1.88)	4 (3.00)
Insulin	4 (44.44)	31 (31)	25 (17.85)	41 (11.05)	4 (3.00)

Values are expressed as number (percentage). *BMI* body mass index, *n* = 753

C-CKD

We could analyze the data of 309 patients, majority of which belonged to e-GFR categories of > 60–90 ml/min/1.73^2 (*n* = 136, 44.01%) and 45–60 ml/min/1.73^2 (*n* = 73, 23.65%). When the use of antidiabetic drugs was analyzed according to kidney function (eGFR), metformin (100%) was most commonly used antidiabetic drug which was followed by DPP-4inhibitors (62.5%) sulfonylureas (59.55%) and SGLT-2 inhibitors (37.5%) in e-GFR category of > 60–90 ml/min/1.73^2 (*n* = 136). In eGFR of 45–60 ml/min/1.73^2 (*n* = 73), order of prescription was metformin (79.45%), DPP-4 inhibitors (54.79), and SGLT-2 inhibitors (34.24). In eGFR of 30–45 ml/min/1.73^2 (*n* = 32), metformin (81.25%), DPP-4 inhibitors (40.63%), and SGLT-2 inhibitors (40.63%) were most commonly prescribed drugs. In advance CKD with e-GFR < 30 ml/min/1.73^2 (*n* = 44), DPP-4 inhibitors (75%) and insulin (52.27%) were most commonly prescribed antidiabetic drugs (Fig. 1).

D-Duration of diabetes, E- Established CVD

We could analyze the data of 311 patients of T2DM for drug prescription pattern according to duration of diabetes and established cardiovascular disease (CVD).Around 38% of patients had more than 10 years of duration of diabetes, while 26.68, 25.72 and 9.64% of patients had duration of diabetes of 6 months-5 years, 5–10 years, and < 6 months respectively. We observed increasing use of DPP-4 inhibitors (43.33% for < 6 months to 51.69% for > 10 years), SGLT-2 inhibitors (30% for < 6 months to 34.74% for > 10 years), insulin (20% for < 6 months to 46.61% for > 10 years), and AGI (6.66% for < 6 months to 12.71% for > 10 years) with increasing duration of diabetes. In contrasts, the use of sulfonylureas (36.66% for < 6 months to 20.03% for > 10 years) and pioglitazone (33.33% for < 6 months to 12.71% for > 10 years) was reported to decrease with advancing duration of diabetes (Fig. 2). Our study reported 123 (15.76%) of established cardiovascular disease (CVD) cases. Most commonly prescribed drug in T2DM patients

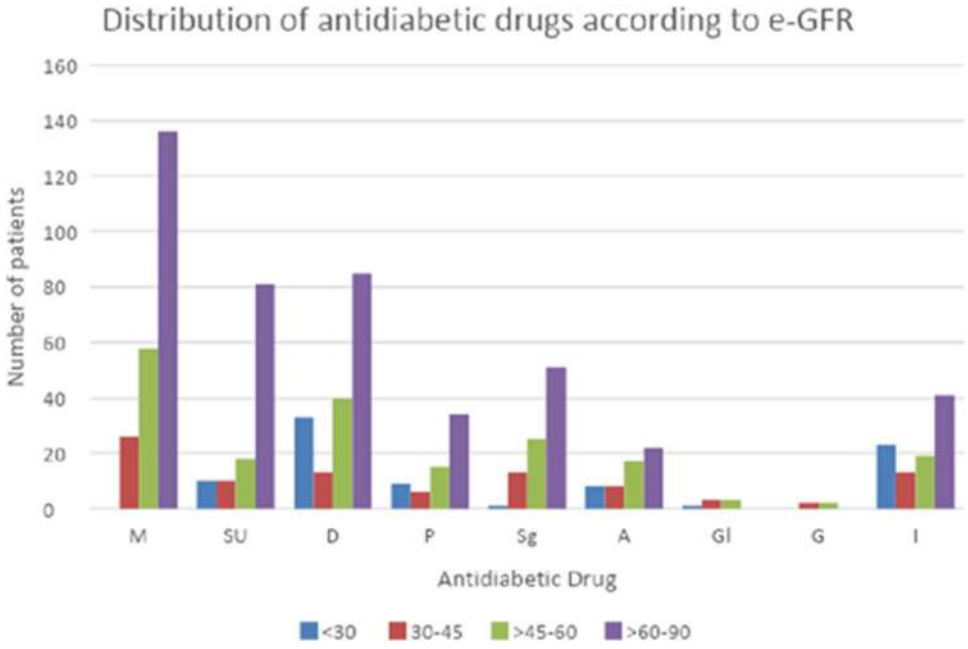

Fig. 1 Distribution of the antidiabetic drugs according to eGFR. *M* metformin, *SU* sulfonylureas, *D* DPP-4 inhibitors, *P* pioglitazone, *Sg* SGLT-2 inhibitors, *A* alpha glucosidase inhibitors, *GL* glinides, *G* GLP-1 analogues, *I* insulin, Unit of eGFR is ml/min/1.72 m^2; $n=309$

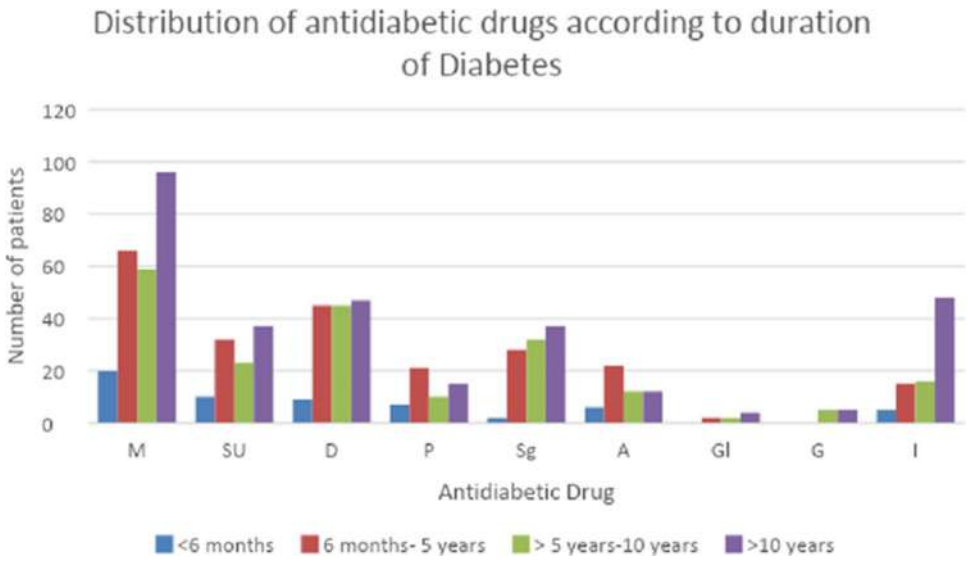

Fig. 2 Distribution of the antidiabetic drugs according to duration of diabetes. *M* metformin, *SU* sulfonylureas, *D* DPP-4 inhibitors, *P* pioglitazone, *Sg* SGLT-2 inhibitors, *A* alpha glucosidase inhibitors, *GL* glinides, *G* GLP-1 analogues, *I* insulin; $n=311$

with CVD were metformin (81.30%), DPP-4 inhibitors (49.59%), and insulin (34.96%), while SGLT-2 inhibitors and GLP-1 analogues were used only in 18.70% and 7.31% respectively (Table 3).

Glycemic status

When we analyzed the prescription pattern according to baseline glycemic status ($n=380$), most commonly prescribed antidiabetic drugs in T2DM patients with HbA1c > 10% ($n=144$) were metformin ($n=144$, 100%), followed by sulfonylureas ($n=75$, 52.08%), insulin (n = 74, 51.38%), and DPP-4 inhibitors ($n=71$, 50%). In HbA1c categories of 7–10% ($n=179$), most commonly prescribed antidiabetic drugs following metformin ($n=179$, 100%) were DPP-4 inhibitors ($n=92$, 61.11%), and sulfonylureas ($n=88$, 63.88%) (Fig. 3).

Finance

We analyzed the data of 214 patients for prescription pattern according to financial status of patients. Most commonly prescribed drug in patients with adequate financial status were metformin (82.51%), DPP-4 inhibitors (60.13%), SGLT-2 inhibitors (44.76%), and insulin (32.16%). In patients with limited financial status, metformin (67.70%),

Table 3 Antidiabetic drug prescription pattern according to established CVD

Sr. No	Anti-diabetic drug	No. of patients (%)
1	Metformin (M)	100 (81.30)
2	Sulfonylureas (SU)	31 (25.20)
3	DPP-4 inhibitors (D)	61 (49.59)
4	Pioglitazone (P)	7 (5.69)
5	SGLT-2 inhibitors (Sg)	27 (18.70)
6	Alpha glucosidase inhibitors (A)	10 (8.13)
7	Glinides (Gl)	7 (5.69)
8	GLP analogues (G)	9 (7.31)
9	Insulin (I)	43 (34.96)

$n = 123$, Values are expressed as number (percentage), *CVD* cardiovascular disease

sulfonylureas (45.07%), and insulin (39.43%) were commonly used antidiabetic drugs (Fig. 4).

Discussion

Lifestyle modifications and monotherapy with oral hypoglycemic agent metformin are generally considered first-line intervention for glycemic control. As the disease progresses, β cell function continues to decline in T2DM patients who require effective glycemic control. Most often, the efficacy of monotherapy decreases after a few years of treatment, resulting in ineffective glycemic control, and does not prevent the progression of disease, which requires an additional agent

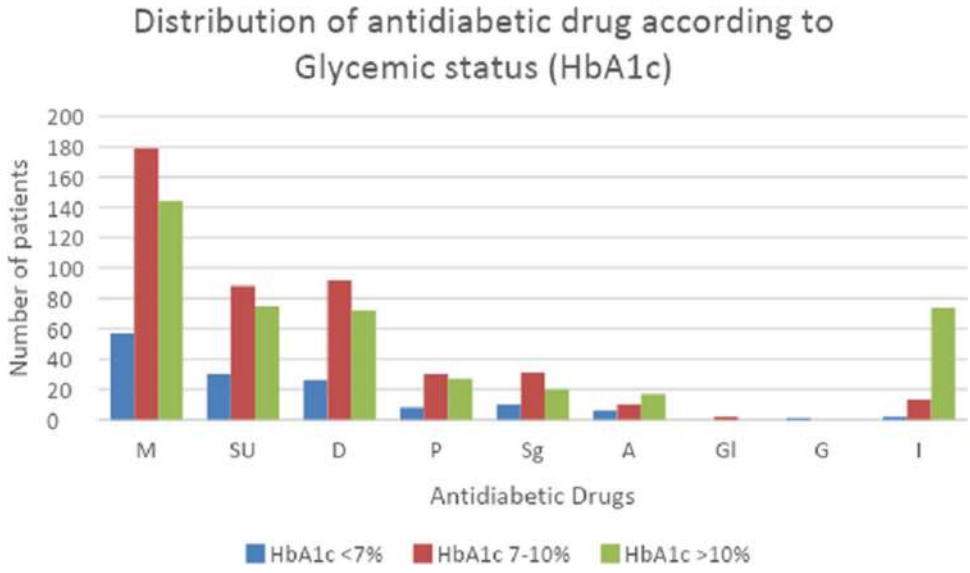

Fig. 3 Distribution of the antidiabetic drugs according to the glycemic status (HBA1c). *M* metformin, *SU* sulfonylureas, *D* DPP-4 inhibitors, *P* pioglitazone, *Sg* SGLT-2 inhibitors, *A* alpha glucosidase inhibitors, *GL* glinides, *G* GLP-1 analogues, *I* insulin, HbA1c is expressed in percentage (%); $n = 380$

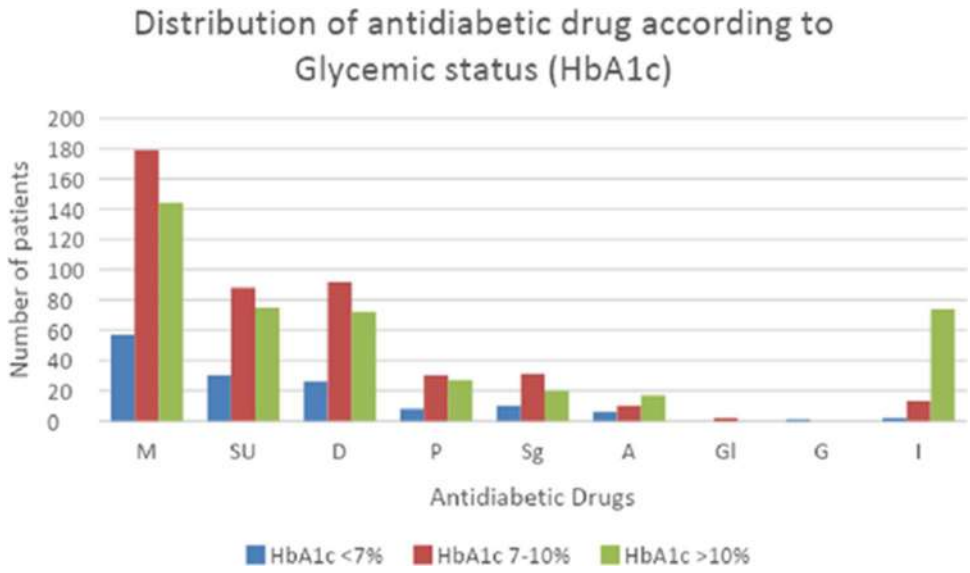

Fig. 4 Distribution of the antidiabetic drugs according to financial status. *M* metformin, *SU* sulfonylureas, *D* DPP-4 inhibitors, *P* pioglitazone, *Sg* SGLT-2 inhibitors, *A* alpha glucosidase inhibitors, *I* insulin, *A* adequate financial status, *L* Limited financial status, $n = 211$

for effective glycemic control. For the successful management of T2DM, there arises a need for combination therapy with agents having complementary mechanisms of action [8, 9]. Choice of any antidiabetic agent should be taken into account with regard to the patients' general health status and associated medical conditions. RSSDI guidelines have recommended patient-centric approach, which is also called RSSDI therapeutic wheel for selecting antidiabetic drugs after metformin in Indian patients of T2DM. This therapeutic wheel has recommended the combination of metformin and one of the treatment options based on patients age (A), BMI (B), CKD (C), duration of diabetes (D), established CVD (E), financial condition (F), glycemic status (G), and hypoglycemia concern (H). This recommendation is also called ABCD (EFGH) approach for individualizing pharmacotherapy for patients of T2DM [7]. We conducted retrospective analysis to validate the utility of RSSDI therapeutic wheel (2018) with clinical practice of Indian healthcare practitioner (Fig. 5).

The Global Burden of Disease Study reported higher prevalence of diabetes in elderly population with age > 60 years (16.1–19%) compared with younger [10]. The underlying pathophysiology of diabetes in these patients is intensified by the direct effects of aging on metabolic regulation [11]. Careful consideration to goal-directed glycemic management can help avoid complications of uncontrolled hyperglycemia and hypoglycemia in these patients [12]. In elderly patients of T2DM, metformin is recommended as the initial oral medication in addition to lifestyle management similar to that of the younger population [7, 11]. eGFR-adjusted doses of DPP-4 inhibitors may be a suitable addition to metformin for elderly patients to avoid hypoglycemia and weight gain. Multiple clinical studies supported the Gliptins as efficacious and safe with no tolerability issue when used as add-on therapy in elderly patients with T2DM [13–16]. Using DPP-4 inhibitor in elderly patients with T2DM combined with post-stroke mild cognitive impairment can lower blood glucose and improve cognitive ability by improvement of Aβ gathering and reduction in inflammatory response [17]. A clinical study- GENERATION has shown similar glycemic efficacy and safety saxagliptin and glimepiride in elderly T2DM patients [18]. Also multiple studies have found beneficial efficacy of insulin without increasing the risk of hypoglycemia or greater total direct healthcare costs in elderly population [19]. Although AGI are recommended in elderly population by RSSDI due to moderate efficacy and minimal side effects like hypoglycemia, gastrointestinal (GI) safety of these drugs needs to be monitored in

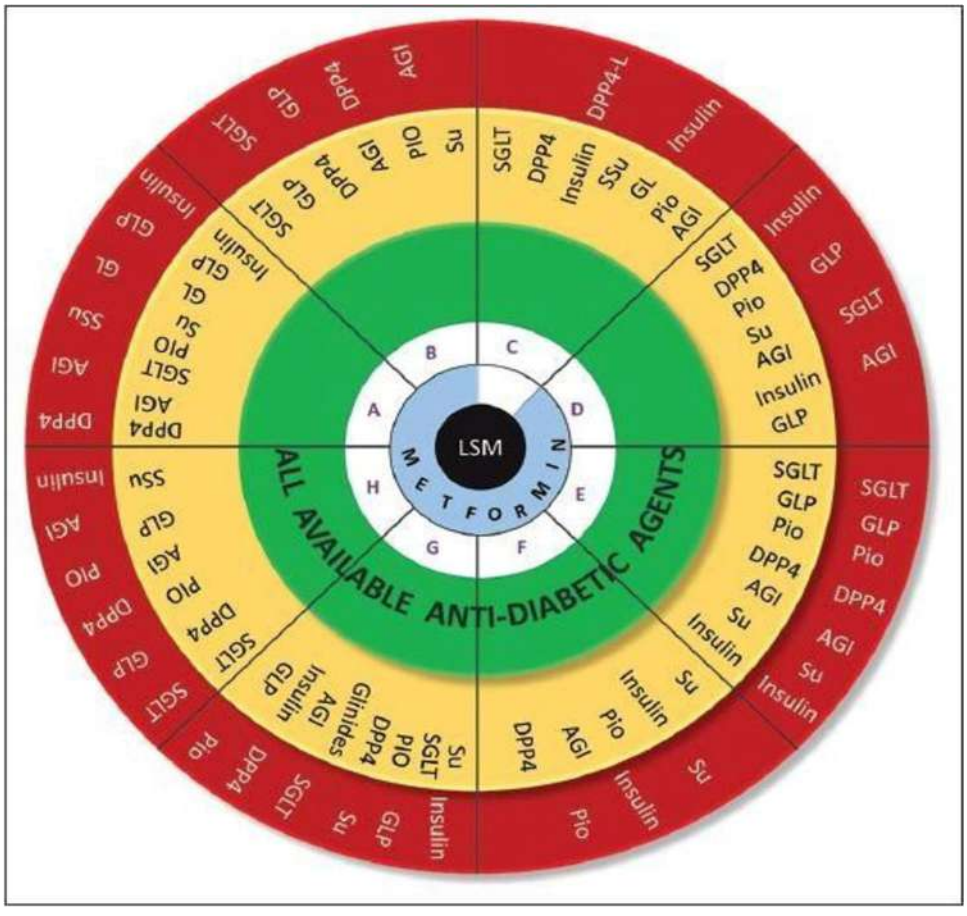

Fig. 5 RSSDI- ESI therapeutic wheel

elderly patients of diabetes [20]. Based on these evidences, RSSDI therapeutic wheel has recommended DPP-4 inhibitors, short-acting sulfonylureas (SU), AGI, and insulin after first-line treatment of metformin [7]. Our findings are in line with RSSDI recommendation as DPP-4 inhibitors (91.23%), metformin (74.74%), and SU (66.49%) were most commonly used antidiabetic drugs. However, insulin (28.86%) and AGI (18.51%) were less frequently used in elderly population in our study. This might be due to safety concerns of these classes of drugs (hypoglycemia with insulin, GI safety with AGI) in elderly patients.

While prescribing treatments for overweight or obese patients with T2DM, providers should first consider antidiabetic medications which cause either weight neutrality or weight loss. In those T2DM patients with higher BMI, RSSDI therapeutic wheel recommends GLP-1 analogues, SGLT-2 inhibitors (associated with weight loss), and AGI and DPP-4 inhibitors (weight neutral) [7]. In our study with BMI category of > 30 kg/m^2, the order of preference was metformin (92.48%), DPP-4 inhibitors (34.58%), and SGLT-2 inhibitors (34.58%). Reason for non-preference of GLP-1 analogues in patients with higher BMI in this study could be non-compliance to GLP-1 analogues injectable therapy by patients.

In patients of renal impairment with reduced e-GFR, DPP-4 inhibitors can be safely administered with dose adjustment. In fact, linagliptin and teneligliptin do not require any dose adjustment in renal disease [21]. Insulin, preferably short-acting insulin, may be used in any stages of renal insufficiency with reduced dose at failing e-GFR [22]. Repaglinide may be used across all stages of renal insufficiency [23]. SGLT-2 inhibitors has shown improvement of renal outcomes in T2DM patients with CKD [24]. On the background of these data, RSSDI therapeutic wheel recommends SGLT-2 inhibitors, DPP-4 inhibitors, insulin, and glinides in patients of T2DM with CKD [7]. In patients with eGFR of 30–45 ml/min/1.73^2 ($n = 32$) in our study, metformin (81.25%), DPP-4 inhibitors (40.63%), and SGLT-2 inhibitors (40.63%) were most commonly prescribed drugs. In advance CKD with e-GFR < 30 ml/min/1.73^2 ($n = 44$), DPP-4 inhibitors (75%) and insulin (52.27%) were most commonly prescribed antidiabetic drugs. These findings are in line with RSSDI therapeutic wheel.

Glycemic durability of antidiabetic drug should be taken into consideration while selecting the drug for the long-term management of diabetes mellitus [25]. With advancing duration of T2DM, RSSDI therapeutic wheel has recommended to use DPP-4 inhibitors, pioglitazone, insulin, and SGLT-2 inhibitors in addition to metformin because of durable glycemic efficacy of these antidiabetic drugs [7]. We observed increasing use of DPP-4 inhibitors (43.33% for < 6 months to 51.69% for > 10 years), SGLT-2 inhibitors (30% for < 6 months to 34.74% for > 10 years), and insulin (20% for < 6 months to 46.61% for > 10 years) with increasing duration of diabetes. In contrasts, the use of sulfonylureas (36.66% for < 6 months to 20.03% for > 10 years) and pioglitazone (33.33% for < 6 months to 12.71% for > 10 years) was reported to decrease with advancing duration of diabetes. This prescription pattern supports the RSSDI therapeutic wheel (except for pioglitazone).

RSSDI therapeutic wheel has recommended SGLT-2 inhibitors, DPP-4 inhibitors, insulin, and pioglitazone in patients of established CVD where lesser options are available [7]. Our findings are in line with these recommendation as metformin, DPP-4 inhibitors, SGLT-2 inhibitors, and insulin were prescribed in T2DM patients with established CVD. Lesser preference to pioglitazone in T2DM patients with CVD could be because of risk of associated heart failure with pioglitazone [26].

Metformin, sulfonylureas and insulin were commonly used antidiabetic drugs in patients with limited financial status observed in our study which are as per the recommendation by therapeutic wheel [7]. We were not able to collect the data for hypoglycemia as it is subjective most of the time. The use of SGLT2i and glinides has been observed to be in much limited numbers in spite of the recommendations at the time of the retrospective data collection. This could be attributed to the limitations of financial status of the patients and/or the choice of insulin above glinide prescription for the prescribing HCP.

Conclusion

ABCD (EFGH) or RSSDI therapeutic wheel is a patient-centric approach for the management of T2DM in routine clinical practice in India. Most of our findings of antidiabetic drug prescription are in consistency with RSSDI therapeutic wheel. Physicians and healthcare professionals should adopt the RSSDI therapeutic wheel for effective management of T2DM.

Declarations

Conflict of interest None declared.

References

1. Pinchevsky Y, Butkow N, Raal FJ, Chirwa T, Rothberg A. Demographic and clinical factors associated with development of type 2 diabetes: a review of the literature. Int J Gen Med. 2020;13:121.
2. International Diabetes Federation. *IDF Diabetes Atlas, 9th edn.* Brussels, Belgium: 2019. Available at: http://www.diabetesatlas.org. Access on 27th Aug 2020

3. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ, Buse JB. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2018;61(12):2461–98.
4. Ramachandran A, Jain SM, Mukherjee S, Phatak S, Pitale S, Singh SK, Agrawal N, Majumdar A, Deshpande N, Jhulka S, Minakshisundaram S. Suboptimal glycemic control among subjects with diabetes mellitus in India: a subset analysis of cross-sectional wave-7 (2016) data from the International Diabetes Management Practices Study (IDMPS). Ther Adv Endocrinol Metab. 2020;11:2042018820937217.
5. Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol. 2016;12(6):357–70.
6. Wells JC, Pomeroy E, Walimbe SR, Popkin BM, Yajnik CS. The elevated susceptibility to diabetes in India: an evolutionary perspective. Front Public Health. 2016;4:145.
7. Bajaj S. RSSDI clinical practice recommendations for the management of type 2 diabetes mellitus 2017. Int J Diabetes Dev Countries. 2018;38(1):1–15.
8. Vijayakumar TM, Jayram J, Cheekireddy VM, Himaja D, Teja YD, Narayanasamy D. Safety, efficacy, and bioavailability of fixed-dose combinations in type 2 diabetes mellitus: a systematic updated review. Curr Ther Res. 2017;84:4–9.
9. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin. 2004;88(4):787–835.
10. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, Mukhopadhyay S, Thomas N, Bhatia E, Krishnan A, Mathur P. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. Lancet Glob Health. 2018;6(12):e1352–62.
11. LeRoith D, Biessels GJ, Braithwaite SS, Casanueva FF, Draznin B, Halter JB, Hirsch IB, McDonnell ME, Molitch ME, Murad MH, Sinclair AJ. Treatment of diabetes in older adults: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2019;104(5):1520–74.
12. Umpierrez GE, Pasquel FJ. Management of inpatient hyperglycemia and diabetes in older adults. Diabetes Care. 2017;40(4):509–17.
13. Barnett AH, Huisman H, Jones R, von Eynatten M, Patel S, Woerle HJ. Linagliptin for patients aged 70 years or older with type 2 diabetes inadequately controlled with common antidiabetes treatments: a randomised, doubleblind, placebo-controlled trial. The Lancet. 2013;382(9902):1413–23.
14. Strain WD, Lukashevich V, Kothny W, Hoellinger MJ, Paldánius PM. Individualised treatment targets for elderly patients with type 2 diabetes using vildagliptin add-on or lone therapy (INTERVAL): a 24 week, randomised, double-blind, placebo-controlled study. The Lancet. 2013;382(9890):409–16.
15. Chien MN, Lee CC, Chen WC, Liu SC, Leung CH, Wang CH. Effect of sitagliptin as add-on therapy in elderly type 2 diabetes patients with inadequate glycemic control in Taiwan. Int J Gerontol. 2011;5(2):103–6.
16. Strain WD, Agarwal AS, Paldánius PM. Individualizing treatment targets for elderly patients with type 2 diabetes: factors influencing clinical decision making in the 24-week, randomized INTERVAL study. Aging (Albany NY). 2017;9(3):769.
17. Xue J, Wang C, Pan C, Xing H, Xu L, Chen X, Wang X, Wang N. Effect of DPP-4 inhibitor on elderly patients with T2DM combined with MCI. Exp Ther Med. 2020;19(2):1356–62.
18. Schernthaner G, Durán-Garcia S, Hanefeld M, Langslet G, Niskanen L, Östgren CJ, Malvolti E, Hardy E. Efficacy and tolerability of saxagliptin compared with glimepiride in elderly patients with type 2 diabetes: a randomized, controlled study (GENERATION). Diabetes Obes Metab. 2015;17(7):630–8.
19. Lee P, Chang A, Blaum C, Vlajnic A, Gao L, Halter J. Comparison of safety and efficacy of insulin glargine and neutral protamine hagedorn insulin in older adults with type 2 diabetes mellitus: results from a pooled analysis. J Am Geriatr Soc. 2012;60(1):51–9.
20. Josse RG, Chiasson JL, Ryan EA, Lau DC, Ross SA, Yale JF, Leiter LA, Maheux P, Tessier D, Wolever TM, Gerstein H. Acarbose in the treatment of elderly patients with type 2 diabetes. Diabetes Res Clin Pract. 2003;59(1):37–42.
21. Penno G, Garofolo M, Del Prato S. Dipeptidyl peptidase-4 inhibition in chronic kidney disease and potential for protection against diabetes-related renal injury. Nutr Metab Cardiovasc Dis. 2016;26(5):361–73.
22. Foundation NK. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. Am J Kidney Dis. 2012;60(5):850–86.
23. Ioannidis I. Diabetes treatment in patients with renal disease: is the landscape clear enough? World J Diabetes. 2014;5(5):651.
24. Kluger AY, Tecson KM, Lee AY, Lerma EV, Rangaswami J, Lepor NE, Cobble ME, McCullough PA. Class effects of SGLT2 inhibitors on cardio renal outcomes. Cardiovasc Diabetol. 2019;18(1):99.
25. Kim KJ, Choi JH, Kim KJ, An JH, Kim HY, Kim SG, Kim NH. Determinants of long-term durable glycemic control in new-onset type 2 diabetes mellitus. Diabetes Metab J. 2017;41(4):284–95.
26. Giles TD, Miller AB, Elkayam U, Bhattacharya M, Perez A. Pioglitazone and heart failure: results from a controlled study in patients with type 2 diabetes mellitus and systolic dysfunction. J Cardiac Fail. 2008;14(6):445–52.

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ORIGINAL ARTICLE

E-health initiatives for screening and management of diabetes in rural Rajasthan

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Abstract

Background and aims Mobile or electronic health technology (e-health) initiatives are being employed in various health programmes for disease monitoring. Very few such studies have been conducted in India and other developing countries. We planned this study to assess the feasibility and usefulness of e-health intervention for health workers as accredited social health activist (ASHA) in screening and management of diabetes.

Methods We developed a web-based application for use on a portable device (tablet) to screen and diagnose non-communicable diseases like diabetes, provide health education focused on diet and physical exercise and promote adherence to therapies. ASHA workers were recruited in two villages of Rajasthan and trained to use this technology.

Results With the use of e-health initiatives, among populations above 18 years, we found 1.19% (29/2430) diabetics, 44.82% (13/29) newly detected and 2.84% (69/2430) prediabetics. The prevalence of prediabetes decreased over a period of time in the population under follow-up. Also, could start 37.93% diabetics on treatment due to constant follow-up, referral and monthly specialist visits to the village.

Conclusion Training ASHA worker in e-health is feasible and can assist in screening for diseases. This technology facilitates lifestyle and diet modifications and leads to better adherence to therapies by repeated sessions of one-to-one health education. In our study area, we found low levels of diabetes and prediabetics, which can be due to low waist circumference, low BMI and W/H ratio and high MET.

Keywords Diabetes · Prediabetes · E-health · App · ASHA · Rural

Introduction

E-health refers to the use of information and communication technology within healthcare environments. It is mostly used to describe health initiatives where the Internet is used to deliver health information, data or services. It also includes initiatives on mobile phones, tablets and other m-health initiatives as well [1]. Virtualization, cloud-based software and software as a service are mature technologies, which developing countries like India are adopting fast [2].

In a vast country like India, the majority of the population residing in rural areas have low access to proper healthcare. Also, epidemiological data is inaccessible and undependable [3], thus affecting various preventive programmes. The utilization of Information and Communication Technologies (ICT) as in e-health can improve reach quality healthcare, and also enhance the quality of health-related data, as seen in other developing countries [4, 5].

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The Global Burden of Disease Study shows a growing number of chronic disease patients due to a considerable rise in life expectancy [6]. Thus, authorities are focusing to decrease this growing load by the use of technology to help outpatient monitoring and self-aid [7].

In a recent review of developing countries' health systems, it has been concluded that web-based electronic health record systems and m-health has contributed to enormous health advances in countries like Bangladesh [8, 9]. In developed countries, e-health improves the efficiency and effectiveness of healthcare delivery, while in emerging and developing countries, it provides access to basic healthcare to people, usually facing poverty [10].

A major benefit of e-health is it can be administered to a large section of the population in a short duration. It decreases the time required for analyzing a large amount of data in comparison to the paper-based data collection system. This is especially helpful in the case of non-communicable diseases since these involve multiple follow-ups [11].

To provide accessible and affordable healthcare to the rural population at their doorstep, we have a female Accredited Social Health Activist (ASHA) via National Rural Health Mission, in every village of India. They act as a link between the community and the public health system. As ASHA may be an eight pass or more, many times they do not properly maintain the data and many times these data are lost due to improper maintenance of files.

An e-health initiative, Swasthya Slate, has also been developed by the Public Health Foundation of India; it can do 33 tests and has attachments like BP apparatus, glucometer, urine analyzer etc. to give on-the-spot and accurate results [12].

This study is an attempt to see the changes a technologically equipped grass-root level health worker can do in bringing down the burden of diabetes in rural India. Through this study, we aim to use e-health in screening and provide timely intervention for diabetes and its predisease state i.e. prediabetes (PD). We used conventional methodologies like glucometer to diagnose patients for the above-mentioned disease states and used e-health initiatives in surveillance and management of these disease states.

Methods

This prospective observational cohort study was funded by Rajasthan University of Health Sciences, Jaipur.

Location

Two villages having difficult approaches and inadequate health services were identified:

Lakhesra village, 30 km from our Medical College under Luniyawas PHC (primary health centre), Agra Road, Jaipur District; and

Kapurwala village, 25 km from our Medical College under Dadiya PHC on Diggi-Malpura Road, Jaipur District.

Study population

The total population covered under this project was around 4000. We identified and contacted ASHAs of the concerned villages. Before the start of the study, ethics approval was taken from Institutional Ethics Committee for conducting this study.

Study conduct

The study was conducted in three phases in a time period of 1 year.

(i) Vanguard phase (1 month): All aspects of the protocol were further refined and tailored, development of an android application for tablet, development of training module for ASHA, final training and standardization of methods.
(ii) Recruitment phase (3 months): We numbered all the households starting from the entry point of the village consecutively while recruiting the households. Initial cross-sectional analysis using baseline data was initiated.
(iii) Follow-up phase (8 months): Follow-up of all diabetic, prediabetic subjects (above 18 years of age) every month, for up to 1 year of the beginning of the project (i.e. 8 months), to document any changes in their health profile and educate them about their diet and physical activity, increase awareness among them about complications of these disease states through videos in our app and our monthly specialist visits and also do a follow-up on them for the compliance of these. Also, making them aware of the need for regular treatment and removing their myths about the disease and treatments and tracking their compliance with treatment.

E-health application (Health Smart App)

An android-based application was developed, which was used on tablets. It had some pop-up videos for information and education on the relevant problem. These pop-up videos automatically appeared for the health education of the participants as soon as the app identified the risk, e.g. for smokers, alcoholics, high-risk pregnancies, incomplete immunization, also those related to family planning and sanitation. Pop-up

videos would also spring up on a high BMI (obesity), W/H ratio, high blood pressure, high blood sugar (diabetes) and for those diagnosed with anemia.

There were various types of questionnaires in the application.

Questionnaires consisted of various parts:
Community (village) Profile Questionnaire
Family level forms

- Family Census—records demographic information, tobacco use, education and morbidities in all inhabitants of the household.
- Household Questionnaire—covers domains related to house structure, amenities, access to water and sanitation

Individual-level forms/measurements

- Records information about lifestyle-related diseases, maternal and child health, different morbidities.
- Physical measures: blood pressure (above 18 years), height, weight, heart rate, waist circumference.
- Blood sample: for blood sugar determination (fasting) by a glucometer and Hb estimation by hemoglobin colour scale strips.

The health worker (ASHA) was provided with tablets which were GPS, Skype and 3G equipped, which enabled them to conduct real-time data collection with visualisation and real-time processing of information at the central location (RUHS College of Medical Sciences, Jaipur). Automatic data transfer and report generation at the central location (RUHS College of Medical Sciences, Jaipur) were the vital characteristics of this application.

All subjects were followed up every month, for up to 1 year of the beginning of the project (i.e. 8 months), to document any changes in the health profile of all the participants. For those households being recruited in the first month, the follow-up started from the consecutive month and so on.

The work plan of ASHA

ASHA was trained for 2 weeks to use the tablet. She was trained to do entry in the tablet, using questionnaires in it, and to take physical measurements like BP, height, weight, measuring blood pressure, blood sugar through glucometer etc. She collected baseline data (3 months) and then did monthly follow-up. ASHA tracked changing lifestyles, risk factors and screening of non-communicable diseases (NCDs) using periodic standardized data collection in the two villages. She provided health education to subjects through pop-up videos in the app.

Blood sugar determination (fasting) (above 18 years) was done by glucometer by glucose oxidase method as per WHO and was interpreted. In the case of fasting plasma glucose (FPG), > 126 mg/dl, a repeat test was conducted [13].

Regular follow-up was done by ASHA for those who were at high risk (hypertension, diabetes mellitus, anaemia) in the form of BP monitoring, blood sugar estimation and hemoglobin estimation. For diabetics and prediabetics, monthly blood sugar estimation was done; also, health education through pop-up videos was done. ASHA referred such patients to the first referral unit (FRU). We also organised specialist visits once a month to the village itself, to provide treatment, and health education to the patients individually there. Tracking of these newly diagnosed cases and old cases for regular treatment and follow-up was done by ASHA. Reporting of any new event in the household in the form of morbidity, mortality and birth was done on a daily basis. Supervision and monitoring of ASHA by the coordinating team was done weekly. We trained and retrained the ASHAs to use these e-health initiatives to empower them in providing better services at the grass-root level to the people and also be able to screen the population for various NCDs and provide timely referrals so that we can control the occurrence of these diseases.

Data collection

The data was transferred directly to the central computer, and reports on various aspects were generated automatically on a daily basis. This helped us to capture any deviation from normal in the health status of the community and family.

Statistical analysis

Statistical analysis was done using Excel, and analysis was done in the form of percentage, mean, standard deviation, confidence intervals and tests of significance. A significance level of 0.05 was regarded for the interpretation of the analysis.

Results

A total of 825 families, 405 in Lakhesra and 420 in Kapurwala, were surveyed, using e-health initiatives through our health smart app on tablets by ASHAs, covering a total population of 3853 (1807 and 2046 at Lakhesra and Kapurwala respectively) in 3 months; they were then followed up for 8 months. Out of this total population surveyed, we took a population above 18 years (2430 individuals making for about 63% of the total population) for blood sugar measurement.

Demographic characteristics of the studied participants showed the maximum number of participants in the age group 25–35 years (28.9%) followed by those in the 18–25 years age group (25.8%). Also, there was almost an equal proportion of males and females (Table 1).

We found 1.19% diabetics (29/2430), although there were only 55.2% (16/29) known diabetics, and 44.8% (13/29) were newly detected. The mean blood sugar level of diabetics was 159.31 mg/dl ± 38.92. We also found 2.8% (69/2430) prediabetics.

At the end of our follow-up, we could bring down the blood sugar levels of 30/69 (43.5%) prediabetics to the normal range. The mean blood sugar level for prediabetics was 116.17 mg/dl ± 4.66, while after follow-up of 8 months, blood sugar levels of prediabetics decreased to 106.42 mg/dl ± 14.09. This difference in blood sugar level at 95% confidence interval (95% CI 6.21–13.28) was found to be highly significant (*p* less than 0.0001). Although no change was seen in the number of diabetics, we could start 11/29 (37.9%) on treatment, while 14/29 (48.3%) were already on treatment.

Discussion

The current research has tried to highlight the importance and practical utility of e-health initiatives in reaching out to the remotest villages of India to provide healthcare, in form of a simplified tablet-based application that can be used by village-level health functionaries to store data, provide health education and get timely reminders to visit patients. It will be a boon to the second-most densely populated nation of the world. The current study has emphasized the application of e-health in the Indian healthcare system. A total of 825 families, 405 in Lakhesra and 420 in Kapurwala, were surveyed using e-health initiatives through our health smart app used on tablets by ASHAs, covering a total population of 3853 (1807 and 2046 at Lakhesra and Kapurwala respectively). Out of this total population surveyed, we focused on population above 18 years of age (2430 individuals making for about 63% of the total population) for blood sugar measurement. Most of the studies conducted to determine the burden of the above-mentioned diseases have included this age group [14–16].

Table 1 Demographic characteristics of participants

Age group no. (%)	
18–25 years	627 (25.8)
25–35 years	703 (28.9)
35–45 years	485 (19.9)
45–60 years	389 (16.0)
≥ 60 years	226 (9.3)
Total	2430 (100.0)
Gender no. (%)	
Male	1239 (51.0)
Female	1191 (49.0)
Total	2430 (100)

Prediabetes

An ICMR-funded study [17] showed 15% of people in the state to be prediabetic, 14% in rural and 17% in the urban sector. When compared to other rural studies in India, we found a lower prevalence of prediabetes 2.8% in our study area, which is also lower as compared to that reported by Gaur et al. [18] which is 6.15%, Anjana et al. [19] 8.3%, 12.8%, 8.1% and 14.6% in rural areas of TN, Maharashtra, Jharkhand and Chandigarh, respectively [19] and 13.5% in rural Tamil Nadu by Balagopal et al. [20], indicating regional disparities and lower prevalence of prediabetics in our area.

Diabetes

As per the World Health Organization (WHO), diabetes global prevalence among people (over 18 years) is 8.5% (2014), while in India, the number is estimated to be 72.96 million cases. The prevalence in urban and rural India ranges from 10.9 to 14.2% and 3.0 to 7.8% respectively among 20 years and above population (INDIAB Study).

When compared to other rural studies in India using WHO criteria, we found a lower prevalence of diabetes (1.2%), which is also lower as compared to that reported by Balagopal et al. [20], Anjana et al. [19], Paliwal et al. [21] and Little et al. [14]. A study conducted in Northwestern Rajasthan (2004) in the RAICA community suggested the association of camel milk with a low prevalence of diabetes [22].

We also observed relatively low BMI and WHR and low waist circumference among adults in our study area. Also, the MET (metabolic equivalent of task) of the adults ranged from moderate to vigorous levels.

All these together in our area may be a cause of low levels of diabetes and prediabetes.

Approximately half (44.82%) of individuals with diabetes were undiagnosed previously or newly diagnosed. The ratio of known to newly diagnosed diabetes was similar to a study by Anjana et al. [19] (48% undiagnosed) and 56.4% by Little et al. [14], but lower than a study by Misra et al. [23] (25% undiagnosed). This shows low awareness of diabetes amid the study population and variation in the coverage of screening programmes.

Post intervention

We could bring down the blood sugar levels of prediabetics (43.5%), but there was no change in the number of diabetics, due to constant follow-up of these patients by ASHAs and through health education through videos in our app and also health education related to their diet and physical activity during our monthly specialist visits. For diabetics, we could start 11/29 (37.9%) on treatment while 14/29 (48.3%) were already on treatment. In keeping with other lifestyle modification studies as Balagopal et al. [20] in a rural village, reported a decrease in fasting blood sugar levels by 11% among prediabetes and 25% among diabetics. Ramachandran et al. [24] used SMS for the intervention and showed a decrease in diabetes incidence. Reduction in HbA1c using e-health initiatives like m-health has been reported by many authors in India recently [25–27]. In a metanalysis done in 13 different studies in China on mobile-health intervention to improve diabetes care and self-management, there was a moderate effect on glycemic control after intervention [28].

Our intervention proved that e-health initiatives are feasible and may be effective for screening and management of diabetes in a rural population. Moreover, studies based on e-health at different sites should evaluate their cost-effectiveness and replicability.

Limitation of our study

We covered only two villages that are located nearby the capital of Rajasthan. Also, the duration of follow-up was relatively short (8 months only). We can plan a study with longer follow-up to be able to draw more authentic conclusions. This article will serve as a seed for further research in the said subject to cover more villages and tribal areas and even urban areas with e-health initiatives.

Declarations

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

Ethics approval The protocol was approved by the institutional ethics committee of the medical college.

Informed consent After taking written informed consent from the head of all households, all individuals of the village were recruited and underwent detailed baseline assessments.

References

1. True vault [Internet]. E-health. Available at: https://www.truevault.com/ehealth. Accessed 10 Oct 10 2020.
2. E-health for India- path trodden and the vista beyond. Health management. 2014; 14(3). Available at: https://healthmanagement.org/c/healthmanagement/issuearticle/ehealth-for-india-path-trodden-and-the-vista-beyond. Accessed 10 Oct 2020.
3. Jha P, Gajalakshmi V, Gupta PC, Kumar R, Mony P, Dhingra N, RGI-CGHR Prospective Study Collaborators, et al. Prospective study of one million deaths in India: rationale, design, and validation results. PLoS Med. 2006;3(2):e18.
4. Lewis T, Synowiec C, Lagomarsino G, Schweitzer J. E-health in low- and middle-income countries: findings from the Center for Health Market Innovations. Bull World Health Organ. 2012;90(5):332–40.
5. Piette JD, Lun KC, Moura LA Jr, Fraser HSF, Mechael PN, Powell J, et al. Impacts of e-health on the outcomes of care in low- and middle-income countries: where do we go from here? Bull World Health Organ. 2012;90:365–72.
6. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, Global Burden of Disease Study 2013 Collaborators, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800.
7. Castelnuovo G, Mauri G, Simpson S, Colantonio A, Goss S. New technologies for the management and rehabilitation of chronic diseases and conditions. Biomed Res Int. 2015;2015:180436.
8. Balabanova D, Mills A, Conteh L, Akkazieva B, Banteyerga H, Dash U, et al. Good health at low cost 25 years on: lessons for the future of health system strengthening. Lancet. 2013;381:2118–33.
9. Adams AM, Ahmed T, El Arifeen S, Evans TG, Huda T. Reichenbach Lancet Bangladesh Team. Innovation for universal health coverage in Bangladesh: a call to action. Lancet. 2013;382(9910):2104–11.
10. Lewis J, Ray P, and Liaw S-T. Recent Worldwide Developments in e-health and m health to more effectively manage cancer and other chronic diseases – a systematic review yearbook of medical informatics. 2016;(1):93.
11. De Jongh T, Gurol-Urganci I, Vodopivec-Jamsek V, Car J, Atun R. Mobile phone messaging for facilitating self-management of long-term illnesses. Cochrane Database Syst Rev. 2012;12:CD007459.
12. DNA. Battery of 33 tests, packed in Dr Kanav Kahol's Swasthya Slate. 2015 Nov 27. Available at: dnaindia.com/health/report-battery-of-33-tests-packed-in-dr-kanav-kahol-s-swasthya-slate-2149478 Accessed on: 10 Oct 2020.
13. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–97.
14. Little M, Humphrites S, Patel K, Dodd W, Dewey C. Factors associated with glucose tolerance, pre-diabetes and type 2 diabetes in a rural community of South India: a cross-sectional study. Diabetol Metab Syndr. 2016;8:21.
15. Kumar S K, Singh Z, Purty A J, Kar M, Vedapriya D R, Mahajan P, et al. Diabetes prevalence and its risk factors in a rural area of Tamil Nadu. Indian J Community Med. 35(3):396–399.
16. Subramanian H, Soudarssanane MB, Jayalakshmy R, Thiruselvakumar D, Navasakthi D, Sahai A, et al. Non-pharmacological interventions in hypertension: a community-based cross-over randomized controlled trial. Indian J Community Med. 2011;36:191–6.
17. TNN. 15% people in Rajasthan at risk of diabetes: a study. The Times of India. 2019. Available at: https://m.timesofindia.com/city/jaipur/15-people-in-rajasthan-at-risk-of-diabetes-study/amp_articleshow/72047007.cms. Accessed 10 Oct 2020
18. Gaur VK, Ranga AK. Statistical analysis of the rural area of Bikaner. IJAER. 2018;13:16756–7.

19. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, ICMR–INDIAB Collaborative Study Group, et al. Prevalence of diabetes and prediabetics (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-India DIABetes (ICMR-INDIAB) study. Diabetologia. 2011;54(12):3022–7.
20. Balagopal P, Kamalamma N, Patel TG, Misra R. A community-based diabetes prevention and management education program in a rural village in India. Diabet Care. 2008;31:1097–104.
21. Paliwal HP, Capoor S, Yadav A, Chaturvedi S. Study of prevalence and awareness of diabetes mellitus in rural-urban population in and around Jaipur [Rajasthan] conducted at a tertiary centre. 2017. IOSR-JDMS. 2017;16:91–94.
22. Agrawal RP, Singh G, Nayak KC, Kochar DK, Sharma RC, Beniwal R. Prevalence of diabetes in camel milk consuming RAICA rural community of North-Western Rajasthan. Int J Diab Dev Countries. 2004;24.
23. Misra R, Misra A, Kamalamma N, Vikram NK, Gupta S, Sharma S, et al. Difference in prevalence of diabetes, obesity, metabolic syndrome and associated cardiovascular risk factors in a rural area of Tamil Nadu and an urban area of Delhi. Int J Diabetes Dev Countries. 2011;31:82–90.
24. Ramachandran A, Snehalatha C, Ram J, Selvam S, Simon M, Nanditha A, et al. Effectiveness of mobile phone messaging in the prevention of type 2 diabetes by lifestyle modification in men in India: a prospective, parallel-group, randomised controlled trial. Lancet Diabetes Endocrinol. 2013;1:191–8.
25. Kleinman NJ, Shah A, Shah S, Phatak S, Viswanathan V. Improved medication adherence and frequency of blood glucose self-testing using an m-health platform versus usual care in a multisite randomized clinical trial among people with type 2 diabetes in India. Telemed J E-health. 2017;23(9):733–40.
26. Jha S, Dogra S, Yadav A, et al. A prospective observational study to assess the effectiveness of electronic health (E-health) and mobile-health (M-health) platform versus conventional care for the management of diabetes mellitus. Int J Diabetes Dev Ctries. 2016;36:529–34.
27. Goruntla N, Mallela V, Nayakanti D. Impact of pharmacist-directed counseling and message reminder services on medication adherence and clinical outcomes in type 2 diabetes mellitus. J Pharm Bioallied Sci. 2019;11(1):69–76.
28. Cui M, Wu X, Mao J, Wang X, Nie M. T2DM self-management via smartphone applications: a systematic review and meta-analysis. PLoS ONE. 2016;11(11):e0166718.

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ORIGINAL ARTICLE

A valid self-help tool to measure the role of spousal support in the care of persons with diabetes mellitus

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Abstract

Objective Family members, especially the spouse, could reinforce or discourage lifestyle/behavioral change to manage one's diabetes. This study was done to describe and validate a scale that measured the spouse's support specific to the management of diabetes.

Method A questionnaire that embodies various domains of self-care was developed and administered to both the patient and their spouse. The total score was used to validate the scale, using glycemic control (HbA1c) as the criterion. Various measures of validity with their respective 95% CI were calculated.

Results There were 249 eligible couples. Mean age of those with diabetes was 57.4 ± 11.3 years. Duration of diabetes ranged from less than a year to 40 years, with a mean duration of 12.0 ± 8.3 years.

The spousal support score was negatively correlated to HbA1c ($r = -0.25$, $p < 0.0001$) and had a significant discriminatory power (AUC: 0.62 (0.55–0.69)) based on ROC curve. Those who scored greater than 13 were considered to be supportive based on Youden's Index J. Using this cut-off, the likelihood ratio was significantly high [LR + (95% CI): 1.7 (1.4–2.2)] and those who had poor support from their spouses had 2.4 times the odds of having uncontrolled diabetes compared to those with good spousal support [OR (95% CI):2.4 (1.3–4.5)], irrespective of other factors. The internal consistency of the questions used for this multidimensional scale was appropriately low (Cronbach's alpha = 0.67). Self-care was constant across groups.

Conclusion Being clearly associated with glycemic control, the Spousal Support Scale can be used in the clinic for health education and as a self-assessment tool by the couple.

Keywords Type 2 diabetes · Spousal Support Scale (SSS) in diabetes · Social support · Health behavior · Adherence · Exercise · Diet · Self-management · Family

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Abbreviations

T2 DM	Type 2 diabetes mellitus
AADE	American Association of Diabetes Educators
ROC	Receiver operating curve
SSS	Spousal Support Scale
SCS	Self-Care scale
LR	Likelihood ratio

Introduction

Living with diabetes can be extremely frustrating. Maintaining and controlling diabetes requires a strict diet plan and exercise routines as well as medications. Support systems at various levels are necessary in order to handle the challenges involved in the management of the same. In addition to the treating physician, a multi-disciplinary team involving nurses, dieticians, and physiotherapists, along with diabetes

educators [1], is required. Also, implementing Electronic Health Records with disease registries and real-time clinical guideline support [2]; shifting the disease management from tertiary care hospitals to primary health centres; using standardized treatment protocol, and consistent follow-up and patient education by trained personnel [3]; using text messaging services via mobile phones [4]; and using health video blogs (vlogs) [5] are all various means of support.

These ideas have been successful in some people groups. However, most often they have not been able to show consistent and significant change in glycemic control. Moreover, the shift away from the acute care model is not usually appreciated by the patient [6]. Reminders via mobile phones are useful for medication adherence but may not be sufficient for lifestyle changes [7]. Therefore, other forms of support such as engaging communities [8] or peers with diabetes who are themselves non-professionals [9] or family members, friends and spouses or partners [10] to provide social support were explored. Of these the most support was perceived to come from the spouse or partner, especially by women [11].

Since it was noted that families, including the spouse, could reinforce or discourage lifestyle/behavioral change to manage diabetes care [12], we hypothesized that spousal support can be measured and developed into a scale. The components of this scale would highlight areas of neglect, and the score would be an indicator of glycemic control which can be used as a self-help tool. For this we first developed a scale based on the seven behaviors recommended by the American Association of Diabetes Educators (AADE) for Self-Care and then validated the same. Unlike other studies that measured the quality of support [13], we assessed the spouse's behavioral support specific to the management of diabetes.

Methods

This was a cross-sectional study, done among patients who attended an integrated diabetes clinic of the endocrinology department, at a tertiary care teaching institute of Kerala over 6 months, from August 2019 to February 2020.

Adults with diabetes who have a spouse or partner living with them and consenting for the study were included. However, those with major comorbidity like malignancy or end-stage organ dysfunction were excluded. Similarly, patients or spouse of a patient who is wheelchair or bed bound, and patients with extreme financial difficulty, acute illness, or other stressor that could affect an interview were excluded.

The minimum required sample size to be able to validate a tool with a likelihood ratio [14] whose lower bound of the 95% confidence interval is greater than 1 is 108 couples under the following assumptions: sensitivity of 50%, specificity of 50%, pre-test probability of 66.7%. That is, the ratio of those with uncontrolled to controlled diabetes is 2:1. However, all patients who fulfilled the criteria during the 6-month study period were included ($n = 249$ couples).

Thorough history and clinical examination were performed by the first author. Baseline investigations were done at an accredited in-house lab. Glycemic control was measured using HbA1c. A value of 8.0% or higher was considered uncontrolled diabetes. Though HbA1c of $< 6.5\%$ indicates acceptable control as per ADA recommendation [15] and a value $< 7\%$ is considered appropriate, having a strict target will also put a person with diabetes at risk of hypoglycemia, particularly in those who are elderly [16]. Hence, we considered an HbA1c value of $< 8\%$ as desirable in this study.

Patients and their spouses were interviewed during their routine visit to the hospital. A questionnaire was used to measure self-care and spousal support.

Tool development

This multidimensional scale developed to measure the role of day to day management of diabetes (by the patient and spouse), addressed all seven behaviors recommended by the American Association of Diabetes Educators (AADE) for Self-Care. However, the questions were not asked one domain at a time. Rather, the information was elicited during the health education session in a conversational style.

The choice of questions to elicit these behaviors and the weight given to each domain by adding more questions to certain domains and less to others were based on the clinical experience of a group of Endocrinologists, Clinical Pharmacists, and Diabetes Nurse Educator, to ensure content validity. These questions were based on well-established norms of diabetes care and addressed the various aspects of self-care and support.

The seven behaviors and the choice of questions to elicit each behavior are described below. We included only the simplest or most basic characteristic of each behavior as we have ample evidence that even small lifestyle changes would have an effect on glycemic control.

- Healthy coping: positive coping behaviors such as showing concern and being able to talk/discuss issues related to diabetes openly in the home could reduce stress [13] which in turn may lead to better glycemic control. Two weightage points were given to this behavior.
- Healthy eating: a weightage of 2 was given for this behavior. A 3 meal 3 snack diet pattern and regular meal time was considered sufficient to cause a change in glycemic control [17] by preventing hypoglycemia which in turn would prevent binge eating causing fluctuating blood glucose levels (roller-coaster effect).

- Being active: only the regularity of exercising was considered for this behavior, hence got only 1 weightage point. The effect of physical activity on blood sugar will last about 24 h [18]. Exercise helps to decrease insulin resistance.
- Taking medication: rather than the type of medication used we considered timely medication important as glycemic control can be maintained if there is regularity in medication [19–21], physical activity, and food intake. Only 1 weightage point was given for this behavior.
- Monitoring: monitoring blood glucose is the best way to manage diabetes; therefore, 3 weightage points were given to this behavior. Thus, 3 questions were used to elicit this behavior, one related to home based monitoring, another related to frequency of monitoring using home based monitors, and third one related to HbA1c. A conscientious person, either the patient or the spouse, can use a tool such as a glucometer at home to adjust food intake and medication/insulin use to bring about glycemic control [22–25].
- Reducing risk: learning to reduce the risk of developing other complications is very important as once other comorbidities set in, glycemic control will be given less importance [26]. Therefore, 3 weightage points were given for this behavior. Quarterly doctor's visit, being able to talk to the doctor about the disease, and being aware of the complications of diabetes have been shown to reduce the risk of developing these complications [27].
- Problem solving: the major problem of those with poor glycemic control is handling episodes of hypoglycemia. Therefore, knowing what to do in those situations is a sure indicator of both self-care and support as studies have shown that this is the major cause of anxiety in diabetic patients [28]. One weightage point was given to this behavior.

A verbal frequency type scale with thirteen questions each for Self-Care practices and Support was created. Potential answers being 'yes /always', 'sometimes', and 'no /never'.

Four questions each, related to insulin administration, storage and refill were created to be used if applicable. However, these values were not added to the Self-Care or Support scores. Two questions were used to assess the patient's opinion of spousal support and the remaining three were related to HbA1c, other stressors if any etc.

Thus, a total of 39 questions were asked to the couple, of which only 13 each formed the Self-Care scale and the Spousal Support Scale respectively. All negative answers (no/never) were scored as zero and positive answers (yes/always) as two. When an answer was given as 'sometimes', it was scored as one. Questions were formulated in such a way that all positive answers were the desirable answers, thus making a higher score more desirable. The sum of the scores of the set of thirteen questions was used to calculate the Self-Care Score and the Support Score.

Statistical analysis

The following statistical analysis was done to test the validity of the scale: Pearson's correlation coefficient to measure the linear relationship of the above scores with HbA1c, an indicator of blood glucose control; area under the curve of a receiver operating curve (ROC) to test the discriminatory power of the Support Score; Youden's Index J to find the optimal cut-off associated with uncontrolled diabetes. Prevalence odds ratio with 95% confidence interval was calculated to estimate the odds of uncontrolled diabetes using this cut-off. Validity was measured using likelihood ratio and 95% confidence interval. Cronbach's alpha was calculated to check the internal consistency of the scale.

Ethical considerations

This study (Ref No. IEC/2019/03/75) was reviewed by the Institutional Ethics Committee of our institution which is registered under the Drugs Control General of India (Reg. No. ECR/1098/Inst/KL/2018). No incentive was given to the study subjects to participate.

Results

Among the 719 patients who presented with diabetes during the study period, 249 patients were eligible as per the inclusion and exclusion criteria. Among them 165 (69.0%) had uncontrolled diabetes (HbA1c > 8.0), 125 (50.2%) were on insulin, and 135 (54.2%) were men. Mean age was 57.4 ± 11.3 years. Duration of diabetes ranged from less than a year to 40 years, with a mean duration of 12.0 ± 8.3 years.

The distribution of consistent self-care practices and spousal support based on the seven behaviors of self-care is shown in Table 1, where the answer, 'yes /always' to any question was taken as consistent behavior.

The distribution of Self-Care practices and Spousal Support specific to insulin usage is shown in Table 2.

Two questions were added to assess the patient's opinion of support extended by their spouse. Only 16 (6.4%) believed their spouse supported them to be active. However, 236 (94.8%) believed their spouses supported regular visits to the doctor.

Validity of support scale

The internal consistency of the questions used for this multidimensional scale was 0.67 as measured by Cronbach's alpha.

Table.1 Distribution of consistent Self-Care practices and Spousal Support

Domain	Self-care ($N=249$) n (%)	Support ($N=249$) n (%)
Healthy coping		
Are you concerned about your/your spouse's blood sugar reading?	152 (61.0)	216 (86.8)
Do you discuss/read about DM at home?	132 (53.0)	134 (53.8)
Healthy eating		
Do you follow/encourage eating at a regular time	11 (4.4)	142 (57.0)
Do you follow/encourage 3 Meal-3 Snack diet Pattern?	35 (14.1)	72 (28.9)
Being active		
Do you/encourage to exercise regularly?	37 (14.9)	16 (6.4)
Taking medication		
Do you take/remind to take medicines on time?	182 (73.1)	227 (91.2)
Monitoring		
Do You check/remind to check HbA1c every 3 months?	24 (9.6)	39 (15.7)
Do You check/ help to check Blood Glucose at home?	147 (59.0)	130 (52.2)
Do You check/remind to check Blood Glucose at least twice per week?	90 (36.1)	172 (69.1)
Reducing risk		
Do you go/accompany your spouse to the hospital for check-up regularly?	131 (52.6)	242 (97.2)
Are you able to ask questions about the illness to your doctor?	222 (89.2)	231 (92.8)
Do you know about complications of diabetes?	76 (30.5)	80 (32.1)
Problem solving		
Do you know what to do at the time of hypoglycemia?	167 (67.1)	10 (4.0)

Table.2 Distribution of consistent Self-Care practices of insulin usage and Spousal Support

	Self-care ($n=125$), n (%)	**Support ($n=125$), n (%)**
Do you store/encourage to store insulin in refrigerator?	105 (84.0)	28 (22.4)
Do you refill/encourage to refill on time?	92 (73.6)	57 (45.6)
Do you self-administer/help administer insulin?	87 (69.6)	45 (36.0)
Do you know where insulin must be administered?	44 (35.2)	47 (37.6)

Spousal involvement scale included two parts: one on self-care practices and the other on support. Interestingly, increase in self-care score was associated with a slight decrease in HbA1C ($r=-0.15$, $p=0.02$) and an increase in support score also was associated with a slight decrease in HbA1C ($r=-0.26$, $p=<0.0001$) as shown in Fig. 1.

On comparing the average self-care score for those with controlled diabetes, defined as ≤ 8% HbA1c, and those without, the difference was not statistically significant ($p=0.31$). However, the average score for support was significantly different ($p=0.0012$). That is, those with controlled diabetes had a significantly higher support score.

The support score also had a significant discriminatory power as shown by the area under the ROC curve of 0.62 (0.55–0.69) (see Fig. 2). The optimal cut-off using Youden's Index J was at 13. Those with scores greater than 13 were considered to have good support. Using this cut-off, those who had poor support from their spouses had 2.4 times the odds of having uncontrolled diabetes than those with good spousal support [POR (95% CI): 2.4 (1.3–4.5)], irrespective of other factors. The only other independent factor was age at diagnosis [POR (95% CI): 2.4 (1.02–5.4)]. The interaction of these two factors was not significant. Neither were other factors such as gender, education, and income significantly different for those with and without controlled diabetes.

Using the same cut-off, the likelihood ratio, another measure of validity, was significantly high [LR + (95% CI): 1.7 (1.4–2.2)]. The results of both measures of validity are shown in Table 3.

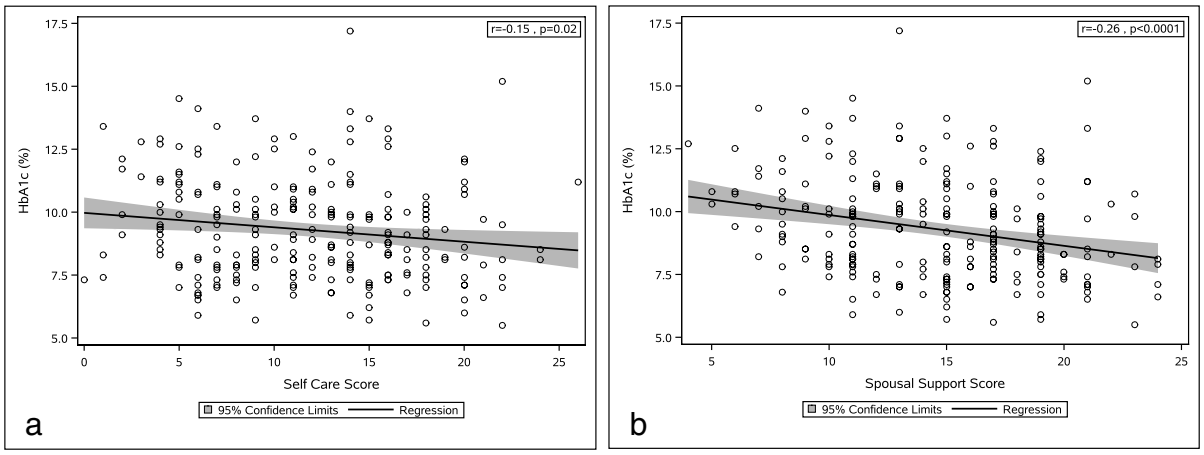

Fig. 1 Linear inverse correlation between HbA1c (%) and **a** Self-care score and **b** Spousal Support score

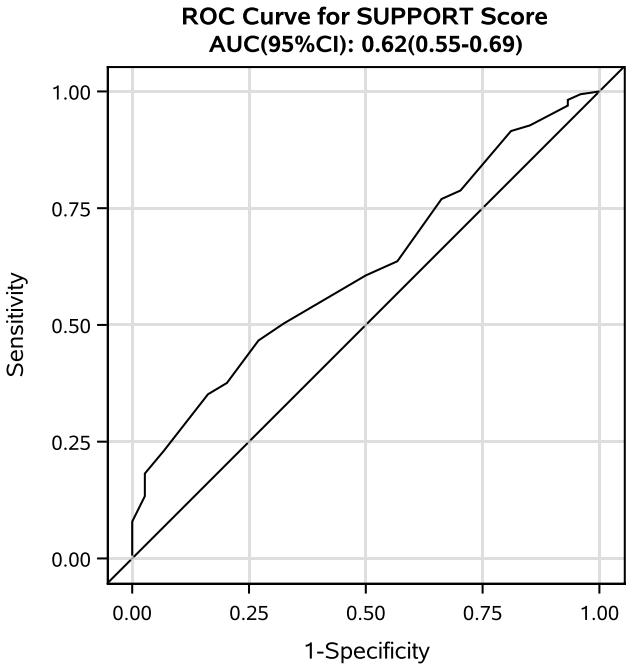

Fig. 2 Area under the curve displays discriminatory power of Spousal Support Scale

Table.3 Tests of validity of Support score

Likelihood ratio (95% CI)	1.7 (1.4–2.2)
Prevalence odds ratio (95% CI)	2.4 (1.3–4.5)

Discussion

A systematic approach is required for successful diabetes care. To this end, awareness of diabetes-related complications and self-care among those living with diabetes has been advocated widely. However, glycemic control still seems to be evasive. Of the various efforts to manage diabetes, various forms of social support seem to have some effect. Spousal support, as indicated by various studies mentioned earlier, seems to be the most sustainable and the best way forward. Therefore, to harness spousal support, we developed a scale that is specific to diabetes care behaviors. The objective was to arrive at a scale which not only points out areas where support is low but also acts as a self-help tool which would be a good indicator of glycemic control, even if not diagnostic in nature. We have considered both content validity and criterion validity to establish that Spousal Support Scale (SSS) is a valid tool for the above mentioned purpose.

Content validity

The SSS includes all the domains recommended by the American Association of Diabetic Educators (AADE) for Self-Care. Relevant questions reflecting each domain were included to assess self-care based on the clinical experience of the panel of experts. The number of questions per domain was also decided based on the experience of the panel of experts. A matching question to elicit supportive behavior formed the support scale. The appropriately low Cronbach alpha of 0.67 validates the multi-dimensionality of the scale.

Areas of poor support were easily identified using the SSS, as described below.

Maximum support was seen in risk reduction by accompanying the spouse for doctor's appointments (97.2%) and by asking the doctor for any clarifications regarding the disease (92.8%) followed by reminding to adhere to medication (91.2%) and showing concern over the blood glucose readings (86.8%).

As expected, adherence to anti-diabetes agents is associated with a decrease in HbA1c [29]. However, worldwide, adherence rates for medication for diabetes range from as low as 38.5% to as high as 93.1% [30]. The high proportion of spouses who support not only to adhere to medication but also for regular check-ups and involvement in blood glucose reading and in talking to the doctor is promising.

However, more support could have been given in biweekly blood glucose monitoring (69.1%); eating on time (57.0%); proactive acquisition of knowledge on DM (53.8%); and checking blood glucose at home (52.2%).

Although the benefits of routine glucose monitoring are well documented, it may not always be feasible in resource-poor settings. Even when the patient can afford such intense monitoring, these newer technologies and devices may seem challenging. Moreover, decreasing vision and mobility also adds to the burden of monitoring glucose levels and as complications increase, patients tend to concentrate more on the complications alone, ignoring glycemic control. We have noticed that our patients prefer to check blood glucose levels in a laboratory at infrequent intervals rather than overcome the above hurdles. Therefore, this is one area where spousal support becomes essential. Taking the responsibility of monitoring at home or just reminding to monitor will definitely go a long way.

Five areas where support was very low included being aware of complications (32.1%), encouraging diabetic diet, as in portion control and avoiding simple sugars (28.9%), quarterly monitoring of HbA1c (15.7%), exercising (6.4%), and knowing what to do in case of hypoglycemia (4.0%).

In the event of severe hypoglycemia, a person suffering from it may not be able to help themselves and will need external help to recover without sequelae. People living with diabetes have expressed feelings of fear and anxiety and have themselves articulated the need to make others in their social circle aware of hypoglycemia and of their own need during such events [31]. Therefore, it is imperative that the spouse knows about hypoglycemia and its immediate management. However, in this study, the least support was seen in this area.

Adhering to an exercise regimen in the presence of complications such as neuropathy or periarthritis becomes tedious with increasing age. Social support has been shown to be a significant factor in increasing or sustaining exercise adherence in general [32, 33].

Prior studies have shown that lack of support is associated with non-adherence to proper diet [34]. Diabetes-related complications such as neuropathy and renal failure can be prevented easily by maintaining good glycemic control by both monitoring HbA1c every quarter and by adhering to proper diabetic diet. Therefore, support from the spouse in diet control, sustained exercise, and regular monitoring would be even more effective than any other form of social support.

Similarly, the support offered for insulin users in administering, storing, refilling insulin, and the knowledge of where the insulin has to be administered was very low. This is another area where the role of spouse may help improve glycemic control. If a spouse or partner can also be present with the patient when skill training on insulin administration is imparted, this ought to help.

As much as diabetes education and external support programmes are appropriate for people with diabetes, support of family, particularly the spouse in the care of diabetes, plays a significant role in improving glycemic control. This simple tool that we created can be easily used by the physician to find out areas where more support can be given by the spouse. While administering this tool, a comprehensive diabetes education can be achieved as it stresses on several details of managing diabetes every day. Moreover, the value of the tool is enhanced, since this tool's validity in predicting glycemic control is quite reasonable, as shown by various measures of validity, discussed below.

Criterion validity

A non-invasive and non-pharmaceutical intervention like care has long been known to improve the condition of a patient. However, such care is often not quantified or measured nor is their effect on cure or disease management ever measured. We sought to do the same, not only to identify specific areas of neglect but also to check if it is associated with glycemic control.

The total score obtained by just adding the score of these various domains was correlated with HbA1c. As support increased, the HbA1c decreased, with a correlation coefficient of -0.26. Although the value of the coefficient is low, the inverse relationship indicated by the negative sign and the linear relationship indicated by the low p value (<0.05) shows that this scale is associated with glycemic control. However, the low value of the coefficient clearly indicates that this tool cannot be used as a surrogate for more accurate diagnostic tools. Since the purpose of this study, as mentioned above, was not to develop a tool to diagnose diabetes but a tool that would measure effective support, the inverse and linear relationship supports the criterion validity of the tool.

Using a score of 13 as cut-off, based on Youden's Index J after drawing a ROC curve, the likelihood ratio of 1.7 also validates the use of the tool [35]. A value greater than 1 is sufficient to validate the tool. A much higher value would make the scale more diagnostic. However, that is not the purpose of the tool. Other measures of validity such as positive (PPV) and negative predictive value (NPV) of a scale are dependent on the prevalence of the condition and therefore

could vary according to the population tested and therefore not included here. Similarly measures such as sensitivity and specificity are used for diagnostic tools and hence not included either.

Although the area under the curve (AUC) of 0.62 (0.55–0.69) is modest, the scale is able to discriminate clearly as shown by AUC being greater than 0.50 and as seen from the ROC curve which does not cross the line of 'no discrimination' at any point. In other words, this scale is not ambiguous at any point. Since the purpose of the scale was not to diagnose diabetes, but to measure effective support, the fact that the scale clearly discriminates those who had uncontrolled diabetes from those who did not, validates the use of the scale.

In our study population, the average level of self-care was the same for all and was not associated with glycemic control. However, those who maintained a good control of their blood glucose were 2.4 times more likely to have supporting spouses (with a score greater than 13) than those who did not maintain glycemic control [POR (95% CI): 2.4 (1.3–4.5)]. Factors such as gender, education, and income were not significantly different for those with and without controlled diabetes. The only other independent factor associated with glycemic control was age at diagnosis. Since almost 20% were diagnosed before the age of 35, we used 35 years as a cut-off. We found that those diagnosed before the age of 35 were 2.4 times more likely to have uncontrolled diabetes than those who were diagnosed later. However, the interaction term was not significant, indicating that the association between spousal support and glycemic control as measured using this scale is not affected by age at diagnosis. Neither it is affected by other socioeconomic factors such as gender, education, and income.

Based on all the measures of criterion validity tested above, it is clear that spousal support as measured by this scale is modestly associated with glycemic control. This tool therefore is both an educational tool as well as a measure of effective spousal support. It can also be used by the couple themselves as a self-help tool, to manage their illness by having a rough idea of glycemic control and figure out areas that need attention which would lead to a better quality of life. Other scales to measure support are either unidimensional such as for exercise only [36] or a measure of perceived support from friends and family [37, 38] unlike the SSS which is multidimensional and addressed directly to the spouse. Moreover, it is a short tool that can easily be used in the clinic, unlike the others.

This study was conducted in Kerala, a southern state of India, which has a very high cumulative incidence of diabetes (21.9%) as per a recent prospective cohort study [39]. It is much higher than any other Indian state despite the fact that the health sector is well established in Kerala, with an efficient primary health care network. Eventually, the onus of looking after one's health lies primarily on the individual, indicating the need to strengthen the capacity to self-manage diabetes care at home. Moreover, in places like India, financial freedom of the woman is limited and dependence on the spouse for her medical needs is the norm. That is, spousal support is essential even to keep medical appointments or refill medications. Therefore, roping in the husband for diabetes care may show improvement in glycemic control. Further studies that follow up these patients after introducing them to this scale are required to prove the effectiveness of the scale as a self-help tool.

Limitations

This study included only those who came regularly with their spouse. Therefore, those with very poor support were inadvertently excluded. However, had we included them, the importance of spousal support in maintaining glycemic control would have been only further emphasized. Another limitation is that since we used HbA1c value of 8% as adequate control, comparing with other studies is not easy. However, this target was chosen, as mentioned above, since a majority of our patients were elderly and so having a strict target could put them at risk of hypoglycemia. Finally, the characteristics of the spouse were not collected. We examined them and included only those who were physically and mentally able to give support.

Conclusion

The Self-Care Scale and the Spousal Support Scale can be used to measure self-care and spousal support. The Spousal Support Scale is a good tool, not only to point out areas of deficiency in support by the treating team, but also for the couple themselves to self-assess their supportive skills — as higher score is associated with better glycemic control. It should be noted that this tool was not created as a surrogate for definitive diagnosis of diabetic control but only to measure effective spousal support.

Author contribution JNJ contributed with the idea and concept of spousal support in the care of diabetes. AMJ contributed to study conception and design, preparation of proforma, interpretation of data, manuscript writing, proof reading and final approval of the article. MJ contributed to data collection, and final approval of the article. AKF contributed to making the questionnaire, compiling data, data entry, editing and writing of the article, and final approval of the article. AD contributed to statistical analysis, interpretation of data, manuscript writing, proofreading, and final approval of the article. This manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

Declarations

Conflict of interest The authors declare no conflict of interests.

Ethical clearance This study was approved by the institutional review board as well as the ethics committee. IEC STUDY No: IEC/2019/03/75.

References

1. Campbell DJ, Sargious P, Lewanczuk R, McBrien K, Tonelli M, Hemmelgarn B, et al. Use of chronic disease management programs for diabetes: in Alberta's primary care networks. Can Fam Physician. 2013;59(2):e86-92.
2. Ciemins EL, Coon PJ, Fowles JB, Min SJ. Beyond health information technology: critical factors necessary for effective diabetes disease management. J Diabetes Sci Technol. 2009;3(3):452–60.
3. Every B. Better for ourselves and better for our patients: chronic disease management in primary care networks. Healthc Q. 2007;10(3):70–4.
4. Güner TA, Coşansu G. The effect of diabetes education and short message service reminders on metabolic control and disease management in patients with type 2 diabetes mellitus. Prim Care Diabetes. 2020;14(5):482–7.
5. Huh J, Liu LS, Neogi T, Inkpen K, Pratt W. Health vlogs as social support for chronic illness management. ACM Trans Comput Hum Interact. 2014;21(4).
6. Wilson PM, Brooks F, Procter S, Kendall S. The nursing contribution to chronic disease management: a case of public expectation? Qualitative findings from a multiple case study design in England and Wales. Int J Nurs Stud. 2012;49(1):2–14.
7. Yasmin F, Ali L, Banu B, Rasul FB, Sauerborn R, Souares A. Understanding patients' experience living with diabetes type 2 and effective disease management: a qualitative study following a mobile health intervention in Bangladesh. BMC Health Serv Res. 2020;20(1):29.
8. Plumb J, Weinstein LC, Brawer R, Scott K. Community-based partnerships for improving chronic disease management. Prim Care. 2012;39(2):433–47.
9. Johansson T, Keller S, Winkler H, Ostermann T, Weitgasser R, Sönnichsen AC. Effectiveness of a peer support programme versus usual care in disease management of diabetes mellitus type 2 regarding improvement of metabolic control: a cluster-randomised controlled trial. J Diabetes Res. 2016;2016:3248547.
10. Rosland AM, Piette JD. Emerging models for mobilizing family support for chronic disease management: a structured review. Chronic Illn. 2010;6(1):7–21.
11. Primomo J, Yates BC, Woods NF. Social support for women during chronic illness: the relationship among sources and types to adjustment. Res Nurs Health. 1990;13(3):153–61.
12. Henry SL, Rook KS, Stephens MA, Franks MM. Spousal undermining of older diabetic patients' disease management. J Health Psychol. 2013;18(12):1550–61.
13. Fisher L, Chesla CA, Skaff MM, Gilliss C, Mullan JT, Bartz RJ, et al. The family and disease management in Hispanic and European-American patients with type 2 diabetes. Diabetes Care. 2000;23(3):267–72.
14. Simel DL, Samsa GP, Matchar DB. Likelihood ratios with confidence: sample size estimation for diagnostic test studies. J Clin Epidemiol. 1991;44(8):763–70.
15. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009;32(1):193–203.
16. Sardar P, Udell JA, Chatterjee S, Bansilal S, Mukherjee D, Farkouh ME. Effect of intensive versus standard blood glucose control in patients with type 2 diabetes mellitus in different regions of the world: systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc. 2015;4(5).
17. Asif M. The prevention and control the type-2 diabetes by changing lifestyle and dietary pattern. J Educ Health Promot. 2014;3:1.
18. Adams OP. The impact of brief high-intensity exercise on blood glucose levels. Diabetes Metab Syndr Obes. 2013;6:113–22.
19. Pladevall M, Williams LK, Potts LA, Divine G, Xi H, Lafata JE. Clinical outcomes and adherence to medications measured by claims data in patients with diabetes. Diabetes Care. 2004;27(12):2800–5.
20. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998;21(9):1414–31.
21. Schectman JM, Nadkarni MM, Voss JD. The association between diabetes metabolic control and drug adherence in an indigent population. Diabetes Care. 2002;25(6):1015–21.
22. Ziegler R, Heidtmann B, Hilgard D, Hofer S, Rosenbauer J, Holl R, et al. Frequency of SMBG correlates with HbA1c and acute complications in children and adolescents with type 1 diabetes. Pediatr Diabetes. 2011;12(1):11–7.
23. Rosenstock J, Davies M, Home PD, Larsen J, Koenen C, Schernthaner G. A randomised, 52-week, treat-to-target trial comparing insulin detemir with insulin glargine when administered as add-on to glucose-lowering drugs in insulin-naive people with type 2 diabetes. Diabetologia. 2008;51(3):408–16.
24. Garber AJ. Treat-to-target trials: uses, interpretation and review of concepts. Diabetes Obes Metab. 2014;16(3):193–205.
25. Polonsky WH, Fisher L, Schikman CH, Hinnen DA, Parkin CG, Jelsovsky Z, et al. Structured self-monitoring of blood glucose significantly reduces A1C levels in poorly controlled, noninsulin-treated type 2 diabetes: results from the Structured Testing Program study. Diabetes Care. 2011;34(2):262–7.
26. Pentakota SR, Rajan M, Fincke BG, Tseng CL, Miller DR, Christiansen CL, et al. Does diabetes care differ by type of chronic comorbidity?: an evaluation of the Piette and Kerr framework. Diabetes Care. 2012;35(6):1285–92.
27. Bamashmus MA, Gunaid AA, Khandekar R. Regular visits to a diabetes clinic were associated with lower magnitude of visual disability and diabetic retinopathy-a hospital-based historical cohort study in yemen. Diabetes Technol Ther. 2009;11(1):45–50.
28. Fidler C, Elmelund Christensen T, Gillard S. Hypoglycemia: an overview of fear of hypoglycemia, quality-of-life, and impact on costs. J Med Econ. 2011;14(5):646–55.
29. Capoccia K, Odegard PS, Letassy N. Medication adherence with diabetes medication: a systematic review of the literature. Diabetes Educ. 2016;42(1):34–71.
30. Krass I, Schieback P, Dhippayom T. Adherence to diabetes medication: a systematic review. Diabet Med. 2015;32(6):725–37.
31. Brown JB, Valliere Y, McLachlan C, Reichert SM, Webster-Bogaert S, Ratzki-Leewing A, et al. Beyond the Sick Role: The many roles of adults with type 1 and type 2 diabetes in the management of hypoglycemia-the InHypo-DM Study, Canada. Can J Diabetes. 2020;44(7):657–62.
32. Niedermeier M, Herzog S, Kopp-Wilfling P, Burtscher M, Kopp M. Is the effect of physical activity on quality of life in older adults mediated by social support? Gerontology. 2019;65(4):375–82.
33. Oka RK, King AC, Young DR. Sources of social support as predictors of exercise adherence in women and men ages 50 to 65 years. Womens Health. 1995;1(2):161–75.

34. Aggarwal B, Liao M, Allegrante JP, Mosca L. Low social support level is associated with non-adherence to diet at 1 year in the Family Intervention Trial for Heart Health (FIT Heart). J Nutr Educ Behav. 2010;42(6):380–8.
35. McGee S. Simplifying likelihood ratios. J Gen Intern Med. 2002;17(8):646–9.
36. Noroozi A, Ghofranipour F, Heydarnia AR, Nabipour I, Shokravi FA. Validity and reliability of the social support scale for exercise behavior in diabetic women. Asia Pac J Public Health. 2011;23(5):730–41.
37. Naderimagham S, Niknami S, Abolhassani F, Hajizadeh E, Montazeri A. Development and psychometric properties of a new social support scale for self-care in middle-aged patients with type II diabetes (S4-MAD). BMC Public Health. 2012;12:1035.
38. La Greca AM, Bearman KJ. The diabetes social support questionnaire-family version: evaluating adolescents' diabetes-specific support from family members. J Pediatr Psychol. 2002;27(8):665–76.
39. Vijayakumar G, Manghat S, Vijayakumar R, Simon L, Scaria LM, Vijayakumar A, et al. Incidence of type 2 diabetes mellitus and prediabetes in Kerala, India: results from a 10-year prospective cohort. BMC Public Health. 2019;19(1):140.

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ORIGINAL ARTICLE

The role of pharmacists in diabetes management in Abu Dhabi, United Arab Emirates

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Abstract

Objective To evaluate the services rendered by pharmacists, including the education offered, and the counseling barriers that are involved in providing necessary pharmaceutical care to patients with diabetes.

Methods A cross-sectional survey was conducted among a representative sample of licensed pharmacists from Abu Dhabi. A self-administered questionnaire was designed and validated through pilot study and consisted of the demographics, counseling services, education provided, and barriers involved in providing pharmaceutical care to patients with diabetes. A total of 68 questions (20 closed-ended and 48 open-ended), of which the closed-ended questions used a 5-point Likert-type scale to evaluate responses (1 = never, 2 = rarely, 3 = sometimes, 4 = usually, 5 = always). The data obtained were analysed using the Statistical Package for Social Sciences (SPSS®) program. Inferential statistics (chi-square test) was used to identify any significant differences between the responses of the participants regarding certain statements in the questionnaire with a p-value of < 0.05

Results The response rate was 60%, and the results indicated that over 35% of the pharmacists surveyed were actively involved in providing basic services to patients with diabetes. More than half of the respondents (45–75%) made a positive contribution towards educating patients on the role of medication in diabetic management. Less than 30% of pharmacists *always* provided counseling services on diabetes-associated complications. Almost 40% of the pharmacists *always* provided necessary insights relating to the need for a healthy lifestyle and living choices. Time constraints (89%) and workload (89.7%) represent the main barriers to providing diabetic care. Chi-square test was performed to determine the proportional differences in response patterns between gender, years of work experience, and average number of patients/customers per day. This is then compared with education services which seems to be differed from expected individual distribution indicating positive correlation.

Conclusions A positive impact seems to be reflected on diabetes care management, and thus, a collaborative approach between the pharmacist and practitioners is a welcome move.

Keywords Pharmacists' counseling · Pharmacist-led management · Glycemic control · Outcome

Introduction

Diabetes mellitus disorder results in a state of hyperglycemia caused by defects in insulin secretion, insulin action, or both [1]. Being a progressive metabolic disease, diabetes mellitus needs to be managed properly to avoid microvascular and macrovascular complications, which lead to chronic morbidity/mortality [2].

The International Diabetes Federation (IDF) in 2011 stated that United Arab Emirates (UAE) ranks tenth in the prevalence of disease and shares 18.8% global burden of diabetics which is approximated to rise to 21.6% by the year 2030 [3]. The number of patients with diabetes in the Middle East and North

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Africa (MENA) region was recorded to be more than 39 million in the year 2017.

Diabetes care is a lifelong procedure requiring therapeutic management, strict medical adherence, and lifestyle modification [4]. Communication between the physician, pharmacist, and patient is crucial for effective application of therapy [5, 6]. The role of a pharmacist has evolved from being just a medicine provider to a social influencer or guide for the patients. The World Health Organization developed a "seven-star concept" stating the necessity for pharmacists to play a role far bigger than just selling drugs [7]. Community pharmacies are easily accessible to the local population where the pharmacist can play a major role in educating the patients with diabetes, thereby improving their health and quality of life [5]. Although community pharmacies are quite popular among the general public, yet adherence to traditional pharmacy practices has rendered community pharmacies unreliable in some cases. Furthermore, these pharmacies suffer due to huge workload, shortage of time, lack of qualified staff, and limited support from physicians and management [8, 9]. New initiatives are currently being undertaken in UAE, which include employing licensed pharmacists in hospitals and engaging pharmacy colleges in standardization of the pharmacy policies [8, 9].

The pharmacist can contribute in diabetes management by screening patients for diabetes, assessing patients' health status, medical compliance, referring patients to other healthcare professionals, and monitoring the outcomes. A pharmacist educating patients with diabetes should preferably be a certified diabetes educator, market savvy, possess effective communication skills, and be able to devote time and effort [10].

In order to get the certification for diabetes educator, the pharmacist requires at least 1000 h of experience in providing disease-state management for diabetic patients and successful completion of an examination [10]. To better educate patients, pharmacists are rampantly working in ambulatory care clinics and community health centers, thereby improving medication outcomes for diabetic patients [11].

The aim of the current research is to encompass the counseling services provided by pharmacists related to glycemic control by checking glucose levels and Hb1Ac cut off and to counsel on signs and symptoms of hypoglycemia, use of insulin pen, and side effects of oral hypoglycemics and insulin pen. The current research also assembles the information regarding health living choices and barriers to provide pharmaceutical care to patients.

Methods

Study design and population

This is a cross-sectional study design which was conducted among the licensed pharmacists employed in community pharmacies, private, semi-government, and government hospitals in Abu Dhabi and Al Ain city, UAE. The contact details of the pharmacist including their emails were obtained from Department of Health–Abu Dhabi registered pharmacists.

A total of 3348 qualified pharmacists were invited via email with information pertaining to survey, stating its voluntary nature with freedom to decline to participate or to withdraw from research at any point of time. The contact details of the researchers were also included for any possible queries arising during the completion of survey. Participants were assured that their confidentiality and anonymity be strictly maintained.

A mere 500 licensed pharmacists who accepted the invitation were included initially. This was followed by a reminder email for the non-responders every week from July to the end of September 2018.

Sample size The sampling groups were classified as community pharmacists, private hospital pharmacists, semi-government pharmacists, and government hospital pharmacists. *Raosoft© Research Solutions* [12] was used to calculate the sample size, using a 95% confidence level with 5% margin of error. From a total population size of 3348 pharmacists, a minimum of around 300 responses was needed for statistical analysis. A conservative estimated response rate of 15% was used in the survey.

Inclusion and exclusion criteria The inclusion criterion was pharmacist holding a valid license and exclusion criteria involved all trainee pharmacists or pharmacist practicing outside Abu Dhabi.

After receiving the responses, the data were exported into Microsoft Excel spread sheet on a secure computer. Survey responses were kept confidential and used only for the purposes of completing this study.

Study instrument

A self-administered questionnaire was designed after thorough literature search [13–16] and consisted of the following sections: demographics, counseling services, education provided, and barriers involved in providing pharmaceutical care to patients with diabetes. The questionnaire consisted of six sections, containing a total of 68 questions (20 closed-ended and 48 open-ended), of which the closed-ended questions used a 5-point Likert-type scale to evaluate responses (1 = never, 2 = rarely, 3 = sometimes, 4 = usually, 5 = always).

The first section highlighted the demographic details and contained 15 questions, on age, gender, position, location, education, number of patients with diabetes seen daily, method of dispensing, and specialized training in diabetic

care. Section II-A included five questions, based on the pharmacist's willingness to provide additional care and their education around blood glucose meters. Section II-B contained eleven questions to identify willingness of pharmacists to educate patients with diabetes on signs, causes, and treatment of hypoglycemia, medication adherence, refill history, time of administration, adverse effects, use and storage of syringes, and insulin pens. Section II-C contained nine questions on the pharmacists' ability to provide counseling on the management of comorbidities, such as cardiovascular disease and high blood pressure. Section II-D contained six questions, on the pharmacists' willingness to provide additional education around immunization, diet, exercise, smoking cessation, and healthy lifestyle. The final section examined the pharmacist- and pharmacy-related barriers and patient-related barriers encountered. Any additional obstacles encountered by the pharmacists were also specified. The questionnaire was designed in English language and was subjected to face validity and content validity.

Instrument validation

Face validity was done by subjecting to five experts in the field and their comments were considered.

The content validity ratio (CVR) was computed for all questionnaire items. Items with a CVR score of 0.7 or more were considered acceptable, while items that had a CVR of less than 0.7 were revised. Moreover, the content validity index (CVI) was calculated to be 0.74, which indicated acceptable content validity of the entire questionnaire. The reliability of the questionnaire was assessed by computing Cronbach's α, for which a value of 0.935 was obtained, indicating acceptable internal consistency for research purpose. Finally, the validated version of the questionnaire was piloted with 20 subjects to ensure relevance and clarity. The pilot sample was excluded from the final sample.

The pilot study findings conform to the adjustments to the items and to the rating scale and were incorporated into the finalized questionnaire. The survey was further revised by two academic experts before administering it to the target population.

Ethics approval

Al Ain University Ethical Review Committee approved the study and approval letter was obtained to allow the researcher to distribute and collect the questionnaires. The participation of the pharmacist in this study was entirely voluntary and without compensation. Before data collection, they were informed about the purpose of the survey and that the administration and completion of questionnaire would be done with their consent. All participants signed the informed consents. Anonymity of respondents was preserved in the study.

Data analysis

The data were analysed using Statistical Package for the Social Sciences (SPSS, version 20, Chicago, IL, US). The internal reliability of the survey was measured using Cronbach's alpha. Descriptive statistics and frequency distributions were calculated from the responses received. Descriptive statistics was also used to determine the most prominent barriers faced by pharmacists in providing care to patients with diabetes and the proportion of each group of respondents who replied to each statement in the questionnaire. Inferential statistics (Chi-Square Test) was used to identify any significant differences between the responses of the participants regarding certain statements in the questionnaire with a p-value of < 0.05.

For open-ended questions, simple content analysis was applied to infer the differences in between the responses.

Results

Response rate

The survey questionnaire was sent to 500 pharmacists who agreed to participate in the survey, and 300 completed responses were received. The response rate was calculated as 60%.

If more than two questions were left unanswered in a submission, it was considered an incomplete response. Twelve such surveys were eliminated to keep all the parameters of survey response same.

In order to complete the 300 participants, follow-up was performed with non-responders and therefore the researchers achieved the target sample.

Description of the study population

The 300 respondents referred above have been categorized in Table 1. Female pharmacists (60.7%, $n = 182$), pharmacists belonging to the age group 30–40 years (59.3%, $n = 178$), were in majority. From the total respondents, 92.7% were pharmacists ($n = 278$), while 7.3% were clinical pharmacist ($n = 22$). Nearly three-fourths of the respondent pharmacists graduated with a bachelor's degree (71%; $n = 213$); 36% had practice experience of 5–10 years ($n = 108$) who worked alone with no collaboration.

In terms of pharmacy type, community pharmacists show the highest response rate of 37.3% ($n = 112$). With regard to location, two-thirds of the survey responses were from pharmacists practicing in Abu Dhabi (56.7%, $n = 170$). In terms

Table 1 Demographic characteristics of the study population

Parameter	*N* (%)
Gender	
Male	118 (39.3)
Female	182 (60.7)
Age	
20–29	98 (32.7)
30–40	178 (59.3)
> 40	24 (8)
Counselor position	
Pharmacist	278 (92.7)
Clinical pharmacist	22 (7.3)
Level of education	
Bachelor	213 (71)
Master	54 (18)
Pharm D	31 (10.3)
PhD	2 (0.7)
Languages spoken	
Arabic	258 (86)
English	42 (14)
Years of work experience:	
Less than 1 year	30 (10)
1–5 years	90 (30)
6–10 years	108 (36)
More than 10 years	72 (24)
Pharmacy type	
Community	112 (37.3)
Private hospital	99 (33)
Government hospital	70 (23.3)
Semi-government hospital	19 (6.3)
Pharmacy location area	
Abu Dhabi	170 (56.7)
Al Ain	130 (43.3)
Counseling area	
Available	243 (81)
Not available	57 (19)
The average number of patients/customers per day	
Below 100	164 (54.7)
100–200	46 (15.3)
201–400	48 (16)
> 400	42 (14)
Diabetic patient seen on daily bases	
< 10	119 (39.7)
10–29	88 (29.3)
30–50	37 (12.3)
> 50	56 (18.7)
Have you had specialized training in dealing with diabetic patient counseling	
Yes	103 (34.3)
No	197 (65.7)

of the average number of patients visiting the pharmacy per day, 54.7% of the pharmacists were from pharmacies that filled fewer than 100 prescriptions per day. Notably, 103 (34.3%) of the respondents had specialized training in dealing with counseling of patients with diabetes.

A positive attitude was expressed towards educating the patients on the use of glucometers, a suitable schedule for blood glucose testing, interpretations, and counseling on current treatment targets for blood glucose (41.3% $n = 124$; 39.3% $n = 118$; 36.7% $n = 110$, respectively) (Table 2).

Approximately 30% of pharmacists expressed willingness to educate patients with diabetes on the causes, signs and symptoms, and management of hypoglycemia ($n = 98$, 88, 90, respectively). Slightly more than one-fourth of the pharmacists reported reviewing the drug refill histories of patients to uncover poor medication adherence. As expected, over 50% of the pharmacists were engaged primarily in routine activities, such as drug handling and storage (Table 2).

The results of the study show less involvement of the pharmacists in offering education on complications associated with diabetes. For example, 23.7% of the respondents reported providing recommendations on appropriate drug therapy, 20.7% reported educating patients on the risk of cardiovascular diseases, 29% reported counseling diabetes patients on taking care of the feet, and only 22% reported offering regular screening of neuropathic pain and retinopathy. All participants reportedly had access to patients' profiles and the ability to order blood lipid profile tests, but less than a fourth routinely educated patients regarding eye exams, cardiovascular problems, kidney function screening, foot care, and setting the recommended targets (Table 2).

It was observed that 39.3%, 39.7%, and 41.7% of the total respondents reported educating their patients on the importance of a healthy balanced diet, exercise, and maintenance of healthy lifestyle, respectively (Table 2). The respondents affirmed that their employers routinely offer smoking cessation programs, but only 36.3% of the pharmacists were reportedly involved in explaining the impact of smoking on patient health (Table 2). Less than a quarter of the pharmacists reported educating patients with diabetes on the significance of annual immunizations (Table 2).

Nearly 90% of the participants revealed that time constraints and workload were the major challenges limiting their ability to deliver quality services in the management of diabetes. Around 90% of the pharmacists reported a lack of time as well as workload being the major impediments in attending patients whereas 49% reported lack of reimbursement and motivation which account as barriers to provide good counseling to patients. Nearly half of the pharmacists reported patient hesitation in discussing their diseases while 44% explained lack of privacy in the pharmacy as the major reason for the communication barrier (Table 3).

Table 2 Education services

Variable	Never *N* (%)	Rarely *N* (%)	Sometimes *N* (%)	Usually *N* (%)	Always *N* (%)
Section II-A Blood glucose and HbA1c of pharmacists towards providing additional care to patients with diabetes mellitus					
Counsel on the use of a blood glucose meter including how to obtain a blood sample	20 (6.7)	27 (9)	62 (20.7)	67 (22.3)	124 (41.3)
Counsel on the appropriate times to check blood glucose sample	22 (7.3)	36 (12)	65 (21.7)	59 (19.7)	118 (39.3)
Counsel on the current treatment targets for blood glucose	21 (7)	42 (14)	65 (21.7)	62 (20.7)	110 (36.7)
Counsel on the interpretation of A1C results	42 (14)	61(20.3)	82 (27.3)	58 (19.3)	57 (19)
Provide drug therapy recommendations to the physician to help the patient reach blood glucose targets	56 (18.7)	57 (19)	64 (21.3)	46 (15.3)	76 (25.3)
Section II-B Diabetes medication					
Counsel on signs and symptoms of hypoglycemia	7 (2.3)	50 (16.7)	77 (25.7)	68 (22.7)	98 (32.7)
Identify possible causes of hypoglycemia	12 (4)	44 (14.7)	87 (29)	69 (23)	88 (29.3)
Provide recommendations for treatment of hypoglycemia	12 (4)	47 (15.7)	92 (30.7)	59 (19.7)	90 (30)
Review the patient's drug refill history to identify poor adherence	27 (9)	61 (20.3)	78 (26)	51 (17)	83 (27.7)
Conduct a drug history (including prescription medications over-the-counter products, herbal products)	24 (8)	39 (13)	80 (26.7)	59 (19.7)	98 (32.7)
Describe potential adverse effects of each oral anti-diabetic drug	12 (4)	45 (15)	101 (33.7)	53 (17.7)	89 (29.7)
Describe the appropriate time to administer oral anti-diabetic drug	3 (1)	15 (5)	44 (14.7)	57 (19)	180 (60)
Counsel on the appropriate use of syringes and needles	7 (2.3)	31 (10.3)	64 (21.3)	65 (21.7)	133 (44.3)
Counsel on the appropriate use of insulin pens	4 (1.3)	22 (7.3)	60 (20)	56 (18.7)	158 (52.7)
Counsel on the appropriate storage of insulin	1 (0.3)	11 (3.7)	34 (11.3)	40 (13.3)	214 (71.3)
Counsel on appropriate insulin administration (mixing insulin, injection technique, timing of injection, rotation of sites)	14 (4.7)	34 (11.3)	56 (18.7)	53 (17.7)	143 (47.7)
Section II-C Comorbid diseases management					
Providing education on cardiovascular disease-associated risk factors	22 (7.3)	59 (19.7)	81 (27)	76 (25.3)	62 (20.7)
Provide education on the importance of controlling blood pressure in diabetes	11 (3.7)	45 (15)	74 (24.7)	66 (22)	104 (34.7)
Provide drug therapy recommendations to the physician to help the patient reach blood pressure targets	48 (16)	59 (19.7)	78 (26)	44 (14.7)	71 (23.7)
Provide education on the importance of regular screening of nephropathy (e.g., type of test, where to get the test, how often to test)	37 (12.3)	63 (21)	79 (26.3)	60 (20)	61 (20.3)
Review the patient's drug profile to identify drugs that are renally cleared	48 (16)	61 (20.3)	73 (24.3)	49 (16.3)	69 (23)
Provide education on the importance of regular screening for neuropathic pain	33 (11)	56 (18.7)	86 (28.7)	59 (19.7)	66 (22)
Provide education on the importance of regular screening for retinopathy	40 (13.3)	62 (20.7)	79 (26.3)	55 (18.3)	64 (21.3)
Counsel on good foot care techniques	25 (8.3)	50 (16.7)	85 (28.3)	56 (18.7)	84 (28)
Provide education on the importance of diabetes foot care	23 (7.7)	50 (16.7)	87 (29)	53 (17.7)	87 (29)
Section II-D Healthy living choices					
Provide education about the importance of immunization for influenza and pneumococcal pneumonia in diabetes	52 (17.3)	80 (26.7)	77 (25.7)	46 (15.3)	45 (15)
Provide basic information on diet as it relates to diabetes management	10 (3.3)	32 (10.7)	69 (23)	71 (23.7)	118 (39.3)
Provide basic information on exercise as it relates to diabetes management	12 (4)	35 (11.7)	75 (25)	59 (19.7)	119 (39.7)
Providing education on smoking cessation for smoker	23 (7.7)	42 (14)	74 (24.7)	52 (17.3)	109 (36.3)
Providing education on healthy life style and determine healthy choices	10 (3.3)	29 (9.7)	70 (23.3)	66 (22)	125 (41.7)
Providing education on immunization	44 (14.7)	69 (23)	79 (26.3)	61 (20.3)	47 (15.7)

More than half of the respondents reported lack of patient motivation (57.3%) and lack of knowledge in the pharmacists role (66.7%), lack of reimbursement (49.7%), and inadequate health insurance (55%) as contributing factors to patients' decision to reach out to pharmacists for treatment of their disease (Table 3).

A chi-square test of independence was performed to determine proportional differences in response patterns between demographic characteristics as mentioned in Table 1. The test indicated that gender and education services provided to patients with diabetes were significant ($p = 0.036$). It was also observed that male pharmacists were more likely to show an interest in providing education services related to diabetes than their female counterparts ($p = 0.036$). Pharmacists with more than 10 years of experience appeared more likely to provide education services

Table 3 Summary of barriers

Variable	YES *N* (%)	NO *N* (%)
Barriers of providing pharmaceutical care to diabetic patients: pharmacist and practice site–related factors		
Time constraint	267 (89)	33 (11)
Lack of training	194 (64.7)	106 (35.3)
Lack of pharmacy management support	144 (48)	156 (52)
Workload	269 (89.7)	31 (10.3)
Lack of motivation	147 (49)	153 (51)
Lack of reimbursement	149 (49.7)	151 (50.3)
Lack of patient appreciation	145 (48.3)	155 (51.7)
Communication barriers	133 (44.3)	167 (55.7)
Lack of private counseling area	131 (43.7)	169 (56.3)
Incomplete patient history or profile needed for assessment	213 (71)	87 (29)
Lack of support from patient physician	178 (59.3)	122 (40.7)
Barriers of providing pharmaceutical care to diabetic patients: patients-related factors		
Lack of motivation	172 (57.3)	128 (42.7)
Financial reasons	144 (48)	156 (52)
Level of education	191 (63.7)	109 (36.3)
Waiting time	258 (86)	42 (14)
Lack of knowledge about pharmacist roles	199 (66.3)	101 (33.7)
Patient age	170 (56.7)	130 (43.3)
Communication barriers	172 (57.3)	128 (42.7)
Lack of referral from patient physician	209 (69.7)	91 (30.3)
Inadequate health insurance	165 (55)	135 (45)

to patients with diabetes as compared to others ($p = 0.044$). Pharmacists visited by fewer patients per day appeared more likely to provide education services than those pharmacists who were visited by a high number of patients, in which case, the pharmacist would be more likely to resort to traditional practice ($p = 0.018$) (Table 4).

Discussion

This work is one of the few studies to discuss about the pharmacists generalized positive attitude towards diabetic care management—their activities, knowledge, and attitude towards the patients with diabetes in UAE. Despite the positive behavior of the community pharmacist in providing routine services such as patient counseling, they rarely offered services associated to the management of comorbidities. The key finding and the utmost barrier to offer services were reported to be the visualization of pharmacist as medical dispenser only by physicians and patients.

The current research reported that pharmacists in UAE are not actively involved in counseling diabetic patients towards application and treatment of blood glucose levels which is comparable to the findings made by various studies [13–16]. The study also reported that pharmacists in UAE lack active engagement; as a member of a multidisciplinary team, many of them did not show proactive behavior in offering drug therapy-based recommendations to physicians. These findings are in concordance with the findings of El Hajj et al. [15].

The current research reflected that the pharmacist were willing to deliver basic services such as appropriate drug administration counseling and the usage of insulin to the patients but their willingness to provide hypoglycemia related services was less. In contrast to that, a study from Kuwait by Al-Haqan et al. [16] reported that male pharmacists offered hypoglycemia management services more frequently than females. This may be related to fewer number of female pharmacists that generally work in community pharmacies in the Mediterranean region.

The study shows that only few respondents (pharmacists) were bound to be counseling patients on diabetes-associated complications due to insufficient time, inadequate reimbursement, and lack of defined scope of a pharmacist's ability in the eyes of the patient [3]. Since pharmacies in UAE remain open for long hours, pharmacists can be approachable easily to provide patient care without further delay (if required) [14]. The confidence levels of UAE pharmacists towards providing diabetes care

Table 4 Correlation between demographics and educational services

Gender	**Education services provided to patient with diabetes**					**Total**	**Significant chi-square *P* value .036**
	Never ***N*** **(%)**	**Rarely** ***N*** **(%)**	**Sometimes** ***N*** **(%)**	**Usually** ***N*** **(%)**	**Always** ***N*** **(%)**		
Male	0 (0)	8 (6.8)	36 (30.5)	41 (34.7)	33 (28)	118	
Female	5(2.8)	24 (13.2)	67 (36.8)	55 (30.2)	31 (17)	182	
Years of work experience	**Education services provided to patient with diabetes Total**					**Total**	**Significant chi-square *P* value .044**
	Never ***N*** **(%)**	**Rarely** ***N*** **(%)**	**Sometimes** ***N*** **(%)**	**Usually** ***N*** **(%)**	**Always** ***N*** **(%)**		
Less than 1 year	4 (13.2)	7 (23.2)	6 (20)	5 (16.6)	8 (27)	30	
1–5 years	2 (2.2)	10 (11.1)	27 (30)	23 (25.6)	28 (31.1)	90	
6–10 years	2 (1.8)	12 (11.1)	38 (35.2)	23 (21.3)	33 (30.6)	108	
More than 10 years	1 (1.4)	3 (4.2)	27 (37.6)	11 (15.3)	30 (41.7)	72	
The average number of patients	**Education services provided to patient with diabetes Total**					**Total**	**Significant chi-square *P* value .018**
	Never ***N*** **(%)**	**Rarely** ***N*** **(%)**	**Sometimes** ***N*** **(%)**	**Usually** ***N*** **(%)**	**Always** ***N*** **(%)**		
Below 100	1 (0.6)	21 (12.8)	36 (22)	60 (36.6)	46 (28)	164	
100–200	3 (6.5)	6 (13)	12 (26.1)	13 (28.3)	12 (26.1)	46	
201–400	1 (2.1)	5 (10.4)	21 (43.8)	13 (27)	8 (16.7)	48	
>400	8 (19.5)	3 (7.3)	12 (29.3)	10 (24.4)	8 (19.5)	41	

could be related to their educational qualifications being acquired from different pharmacy schools [15]. It is pertinent that pharmacists should provide specialized training, such as workshops and certification programs followed by practice-based diabetes training program to offer adequate primary care services to their patients [17, 18].

Although clinical practice guidelines mentioned immunization in diabetes (influenza and pneumonia vaccine administration), pharmacists reported ignorance in guiding patients on the significance of immunizations [19].

The community pharmacists reported improvement in offering regular screening of neuropathic pain and retinopathy which is higher than previously reported research [20]. Only few pharmacists reported that they routinely educate patients regarding eye exams, cardiovascular problems, kidney function screening, foot care, and set recommended targets for their testing.

Research findings from Qatar and Kuwait clearly highlighted that pharmacists promoted the importance of diet and exercise as non-pharmacological approaches to their patients [15, 16]. This is in concordance with the findings of present research done in UAE.

While most of the pharmacists were supported by the management, still time constraints and workload were highlighted as major barriers in providing sufficient care to patients. This is in line with the work of Shatnawi and Latif [14] in which similar barriers were cited to provide optimum patient care services in diabetes.

The current research reported positive correlation between the number of patients visits and provision of educational services as fewer than 100 customers/day placed pharmacist at ease to provide counseling services in comparison to more than 100 customers/day where the pharmacists be able to use traditional dispensing approach.

Having many patients encourage the pharmacists to have a detailed discussion with each one of them; however, a long waiting time prompts the patients to defer treatment which can be easily avoided by scheduling appointments [14], increasing pharmacy staff and technicians, upgrading to electronic health records, providing private areas for counseling in pharmacies, and promoting pharmacist-based services [15].

Simpson et al. [21] reported that pharmacists who are certified diabetes educators (CDEs) receive additional fees, have positive vision towards diabetes, and offered increased number of services in diabetes management as compared to non-specialized community pharmacists. This visible difference between speciality trained CDEs and non-specialized pharmacists suggests the incorporation of diabetic management training programs into existing pharmacists' curriculum. Such training program will encourage more community pharmacists to undertake CDE training courses to help improve diabetes care and more inclination to serve their patients. Therefore, that for successful integration of a healthy lifestyle, regular reminders from sources like healthcare team are necessary to improve quality of life in patients with diabetes mellitus. Last but not the least, UAE

being a region of multiethnicities need to have different intervention programs towards the identification of prevention of diabetes progression as different ethnicities have various other risk factors and prevalence statistics [22].

Conclusion

The current research reported pharmacists' willingness towards detailed management of diabetes-related comorbidities. Nevertheless, the limited services they provide elicited the concept that the pharmacist role must be vital to multidisciplinary team approach and mutual collaboration among all the stakeholders is vital for the implementation of a patient-centered care. It is imperative to remind the general public and physicians that pharmacists are trusted and easily approachable healthcare professional who are confident enough to provide management towards diabetes-related comorbidities.

Limitations

The major limitation of the current research is that the sample is recruited from Abu Dhabi and Al Ain city, and henceforth, it is suggested to include all seven Emirates to extrapolate the findings.

Author contribution FD conceptualized the project, and performed data analysis and manuscript development.

AE contributed to data collection, analysis, interpretation, and manuscript development.

SHJ contributed in interpretation and manuscript development.

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval Al Ain University Ethics Committee COP/302/2018.

Consent to participate The participation of the pharmacists in this study was entirely voluntary and without compensation. Before data collection, they were informed about the purpose of the survey and that the completion and submission of the questioner would be taken on their consent. All participants signed the informed consents. Anonymity of respondents was preserved in the study.

References

1. Alhyas L, McKay A, Majeed A. Prevalence of type 2 diabetes in the States of the co-operation council for the Arab States of the Gulf: a systematic review. PLoS ONE. 2012;7(8):e40948.
2. Shareef J, et al. Evolving role of clinical pharmacist in the management of diabetes mellitus. Int J Res Med Sci. 2015;3(6):1305–8.
3. Sulaiman N, et al. High prevalence of diabetes among migrants in the United Arab Emirates using a cross-sectional survey. Sci Rep. 2018;8:6862.
4. Lindenmeyer A, et al. Interventions to improve adherence to medication in people with type 2 diabetes mellitus: a review of the literature on the role of pharmacists. J Clin Pharm Ther. 2006;31:409–19.
5. Hughes D, et al. The role of the pharmacist in the management of type 2 diabetes: current insights and future directions. Integr Pharm Res Prac. 2017;6:15–27.
6. Abduelkarem A, Sackville M. Changes of some health indicators in patients with type 2 diabetes: a prospective study in three community pharmacies in Sharjah, United Arab Emirates. Libyan J Med. 2009;4:29–38.
7. WHO. New tool to enhance role of pharmacists in health care. Available from: http://www.who.int/mediacentre/news/new/2006/nw05/en/index.html. Accessed 30 May 2018
8. Kheir N, et al. Pharmaceutical care in the Arabic-speaking Middle East: literature review and country informant feedback. Avicenna. 2013;2:1–9.
9. Rayes IK, et al. Perception of community pharmacists toward their current professional role in the healthcare system of Dubai, United Arab Emirates. Saudi Pharm J. 2015;23:235–40.
10. Campbell R. Role of the pharmacist in diabetes management. Am J Health Syst Pharm. 2002;59(9):S18–21. https://doi.org/10.1093/ajhp/59.suppl_9.s18.
11. Smith M. Pharmacists' role in improving diabetes medication management. J Diabetes Sci Technol. 2009;3(1):175–9. https://doi.org/10.1177/193229680900300120.
12. Calculator, Raosoft. Sample size. 2004. Raosoft. http://www.raosoft.com/samplesize.html[Accessed 29 April 2018].
13. Younis WS, et al. Pharmacists' attitudes towards diabetes and their involvement in diabetes education. Ann Pharmacother. 2001;35:841–5.
14. Shatnawi A, Latif DA. A qualitative assessment of West Virginia pharmacist activities and attitude in diabetes management. J Eval Clin Pract. 2017;23(3):586–92.
15. El Hajj MS, et al. Diabetes care in Qatar: a survey of pharmacists' activities, attitudes and knowledge. Int J Clin Pharm. 2018;40:84–93.
16. Al-Haqan AA, et al. Pharmacists attitudes and role in diabetes management in Kuwait. Med Princ Pract. 2017;26(3):273–9.
17. Schapansky LM, Johnson JA. Pharmacists' attitudes toward diabetes. J Am Pham Assoc. 2000;40:371–7.
18. Anderson RM, et al. A comparison of the diabetes-related attitudes of health care professionals and patients. Patient Educ Couns. 1993;21:41–50.
19. The American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2017;40(suppl 1):S1-132.
20. van Hecke O, Austin SK, Khan RA, Smith BH, Torrance N. Neuropathic pain in the general population: a systematic review of epidemiological studies. Pain. 2014;155(4):654–62.
21. Simpson SH, et al. Survey of pharmacist activities and attitudes in diabetes management. Can Pharm J (Ott). 2009;142:128–34.
22. Hamoudi R, Sharif-Askari NS, Sharif-Askari FS, Abusnana S, Aljaibeji H, Taneera J, Sulaiman N. Prediabetes and diabetes prevalence and risk factors comparison between ethnic groups in the United Arab Emirates. Sci Rep. 2019;9(1):1–7.

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ORIGINAL ARTICLE

Evaluation of treatment strategies and pregnancy outcome among GDM twin versus GDM singleton pregnancy

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Abstract

Objective To compare the treatment strategies and pregnancy outcomes between women with gestational diabetes mellitus carrying twin and singleton pregnancies.

Method We conducted a retrospective chart review of women with singleton and twin pregnancy; those visited Sunil's Diabetes Care n' Research Centre, Nagpur, and Gupte Hospital Pune, between 2006 and 2019, for the treatment of gestational diabetes mellitus (GDM). Propensity scores for baseline characteristics were used to assemble a matched-pairs cohort of women with twin and singleton pregnancies.

Results A subset of 132 singleton cases was obtained from 801 records that matched with 44 cases of twin pregnancy, with a ratio of 3:1. Treatment modalities, insulin dose, and mode of delivery differed insignificantly between the two groups. Univariate analysis showed significantly higher risk of Premature Baby Unit (PBU) admission > 48 h (OR 2.687; 95% CI 1.064–6.784; $p = 0.0364$) and small for gestational age newborns (OR 5.286; 95% CI 2.826–9.887; $p < 0.0001$) in twin pregnancies, and lower risk of premature birth (OR 0.225; 95% CI 0.127–0.402; $p < 0.0001$) and large for gestational age newborns (OR 0.239; 95% CI 0.068–0.841; $p < 0.0257$). In a multiple regression analysis, twin pregnancies were found to be the strongest predictors of small for gestational age infant (OR 3.960; 95% CI 1.928–8.135; $p < 0.0001$).

Conclusion Although treatment strategies and insulin doses differ insignificantly between gestational diabetes mellitus women carrying twin and singleton pregnancies, their treatment should be planned to achieve glycemic goals.

Keywords Gestational diabetes mellitus · Twin pregnancy · Insulin doses · Neonatal outcome · Perinatal death

Introduction

Gestational diabetes mellitus (GDM) incidence is increasing globally due to the increasing prevalence of obesity among women of reproductive age and advancing maternal age [1]. Indian women have a higher predisposition to develop diabetes in pregnancy possibly due to genetic predisposition. A community-based study showed a GDM prevalence

Highlights

- The treatment strategies and adverse pregnancy outcomes were compared between twin and singleton gestation in India
- Treatment modalities, insulin dose, and mode of delivery differed insignificantly between the two groups.
- Women with GDM and twin pregnancies were at 3.96 times higher risk for having small for gestational age infant.

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of 13.9% in India [2]. The average twin pregnancy rate in developing countries is 13.1 per 1000 or one twin birth in 76.3 births. India has a twinning rate of 8 per 1000 or one in 125 births. Twin pregnancies represent 0.8% of all pregnancies in India [3]. Some of the studies have shown a higher incidence of GDM in twin gestations versus singleton [4–6].

It is an established fact that GDM is associated with maternal and neonatal complications in singleton pregnancies [7–9]. However, the findings are inconsistent about the impact of GDM in twin pregnancies. Some studies have reported an increased risk of preeclampsia, cesarean delivery, neonatal hypoglycemia, neonatal respiratory complications, and admission to the neonatal intensive care unit (NICU) in twin pregnancies because of the higher baseline risk of prematurity and hypertensive complications [10–18]. While few reported that GDM does not increase the risk of adverse pregnancy outcomes in women with twins [19]. Also, good glycemic control in GDM twin pregnancies was not necessarily associated with better clinical outcomes [20].

Currently, the same glucose targets are applied to all women with GDM and there is no evidence that glucose targets for twin pregnancies are different from singleton pregnancies. Some of the studies have observed that women with GDM twin pregnancies were less likely to have a family history of diabetes; this may infer that their future risk for developing diabetes may be different. Data are conflicting on insulin therapy in twin gestation. One study showed that GDM twin pregnancies were less likely to require insulin therapy. The reasons for this are unclear [21]. Another study has shown that twin pregnancy in type 1 diabetes requires twice the dose of insulin as that of singleton pregnancy [22].

Presently, there is little evidence to guide the management of GDM twin pregnancies. Data from South Asia on GDM twin pregnancy is rare. Moreover, only a limited number of studies have used GDM singleton pregnancies as a control group. Thus, the current study aimed to test the hypothesis that treatment strategies and adverse pregnancy outcome is likely to differ in twin compared to singleton gestation.

Material and methods

Diagnosis of GDM was made by a single-step fasting and 2-h 75-g oral glucose tolerance test (OGTT). Subjects were considered to have GDM if the 2-h post glucose plasma glucose values were ≥ 140 mg/dL, based on the WHO definition [23]. Subjects with pre-existing diabetes, pregnancies complicated by genetic or structural fetal anomalies, and those with clinically suspected pre-gestational diabetes, where hyperglycemia was diagnosed for the first time during pregnancy, but glycemic status suggestive of pre-existing diabetes was excluded. The maternal history and clinical characteristics of selected subjects were assessed. Demographic, anthropometry, biochemical, and treatment-related data were collected from diagnosis till delivery. As there is no central registry on GDM in India, fetal outcome data were collected from attending obstetrician and was correlated with management strategies.

In this study, Bad obstetric history (BOH) is defined as the occurrence of three or more consecutive spontaneous abortions before 20 weeks of gestation from the last menstrual period [24]. Large for gestational age (Macrosomia) is defined as a birth weight of more than 3.5 kg as per Indian recommendations, while low birth weight is defined as a birth weight of less than 2.5 kg [25–27]. The WHO growth chart was used to identify the low birth weight and large for gestational age (LGA) infants [28]. Neonatal hypoglycemia is defined as a blood glucose concentration of less than 40 mg% in term infants during the first 48 h of life [29]. Preterm birth is defined as any birth before 37 completed weeks of gestation or fewer than 259 days since the first day of the woman's last menstrual period (LMP) [30]. The neonatal period is referred to as the first 28 days following birth, and the neonatal intensive care unit (NICU) admission of more than 48 h was considered for evaluation. Neonatal death is defined as the death of a live-born baby during the first 28 days of life (0–27 days) [31]. Perinatal mortality has been used to include deaths that might somehow be attributed to obstetric events, such as stillbirths and neonatal deaths in the first week of life [31].

Statistical analysis

The qualitative factors like parity, bad obstetric history, family history of DM, history of GDM, mode of delivery, and GDM treatment were expressed in terms of frequencies and percentages. The quantitative variables such as age, pre-pregnancy BMI, 2 h Post Glucose Plasma Glucose (PGPG) were described in terms of mean and standard deviation. The qualitative variables were compared between singleton and twin pregnancy groups using Pearson's Chi-square test, while the quantitative variables were compared using Student's *t* test. The logistic regression was used to quantify the risk of various adverse neonatal outcomes in twin pregnancies with reference to singleton pregnancies. Further, multiple logistic regression was used to evaluate the risk after adjusting with maternal clinical parameters between the two groups. All the statistical analyses were performed using R-3.6.1. The significance level is considered as a p value less than 0.05.

Results

The incidence of twin pregnancy in women with GDM was found to be 2.01% in the studied population. A subset of 132 singleton cases was obtained from 801 records that matched

with 44 cases of twin pregnancy, with a ratio of 3:1. The descriptive statistics of the singleton and twin groups are shown in Table 1. The twin group had a higher proportion of primipara mothers ($p = 0.0007$), while the singleton group had a higher incidence of bad obstetric history ($p = 0.0003$). There was no significant difference in maternal age, pre-pregnancy BMI, a family history of DM, and prior GDM between the twin and singleton groups.

Table 1 also provides a comparison of treatment strategy and mode of delivery in two study groups. Regarding the GDM treatment, there was no significant difference in diet and the rate of insulin requirement between the twin and singleton groups. Among those requiring insulin therapy, the mean insulin dose in singleton pregnancy at diagnosis was 13.70 ± 12.23 units/day which increased to 24.42 ± 18.90 units/day at delivery, while in twin pregnancy it was 17.35 ± 15.73 units/day at diagnosis, which increased to 27.30 ± 19.57 units/day. The mean insulin dose between the two groups also differed insignificantly. Likewise, there was no significant difference in the mode of delivery between the twin and singleton groups. Further, the number of live births in the singleton group was comparable to that in the twin group respectively [129/132 (97.72%) vs. 86/88 (97.72%)].

Univariate analysis showed a lower risk of preterm delivery (< 37 weeks) (OR, 0.225; CI, 0.127–0.402; $p < 0.0001$) and large for gestational age (LGA) infants (OR, 0.239; CI, 0.068–0.841; $p = 0.0257$) in twin pregnancies (Table 2). Also, the risk of having small for gestational age infants was about five times (OR, 6.00; CI, 2.826–9.887; $p < 0.0001$), and the likelihood of PBU admission for > 48 h was more than 2.5 times in twin pregnancies (OR, 2.687; CI, 1.064–6.784; $p = 0.0364$). After adjustment for confounders (i.e., age of mother, BOH, family history of DM, 2 h PGPG, GDM treatment, and mode of delivery) by multivariate analysis, the risk of delivering small for gestational age infants was 3.96 times higher in twin group (adjusted odds ratio 3.960; 95% CI, 1.928–8.135; $p = 0.0001$).

Discussion

Many studies have compared GDM and non-GDM twin pregnancies, but only a few have compared twin and singleton pregnancies in women with GDM [1]. In the current study, we aimed to test the hypothesis, that the treatment

Table 1 Baseline characteristics and GDM treatment in singleton and twin pregnancies

Factor	Pregnancy		*p* value
	Singleton ($n = 132$)	Twin ($n = 44$)	
Age (in years) [mean ± SD]	28.98 ± 3.97	29.09 ± 4.07	0.8723 (NS)*
Primipara [no. (%)]			
No	74 (56.06)	11 (25)	0.0007 (S)†
Yes	58 (43.94)	33 (75)	
Bad obstetric history [no. (%)]			
No	87 (65.91)	42 (95.45)	0.0003 (S)†
Yes	45 (34.09)	2 (4.55)	
Family history of DM [no. (%)]			
No	47 (35.61)	19 (43.18)	0.4721 (NS)†
Yes	85 (64.39)	25 (56.82)	
Pre-pregnancy BMI [mean ± SD]	24.29 ± 4.56	23.75 ± 5.33	0.5473 (NS)*
2 h PGBG [mean ± SD]	163.36 ± 42.75	155.35 ± 41.78	0.3292 (NS)*
Past history of GDM [no. (%)]			
No	128 (96.97)	43 (97.73)	0.9999 (NS)†
Yes	4 (3.03)	1 (2.27)	
GDM treatment			
Diet	47 (35.61)	19 (43.18)	0.4721 (NS)
Insulin	85 (64.39)	25 (56.82)	
Mode of delivery			
FTND	15 (11.36)	1 (2.27)	0.1301 (NS)
LSCS	117 (88.64)	43 (97.73)	
Number of live babies	129	86	-

FTND full-term normal delivery, *LSCS* lower segment cesarean section, *Obtained using Pearson's Chi-square test; *NS* non-significant, *PGBG* Post Glucose Blood Glucose

Table 2 Unadjusted and adjusted risk of various neonatal outcomes in twin pregnancies as compared to singleton pregnancies

Neonatal outcome	Group	Odds ratio [95% CI; p value*]	
		Unadjusted	Adjusted‡
Premature birth (< 37 weeks)			
	Singleton	1.000	1.000
	Twin	0.225 [0.127–0.402; < 0.0001 (HS)]	0.495 [0.080–3.056; 0.4489 (NS)]
Large for gestational age			
	Singleton	1.000	1.000
	Twin	0.239 [0.068–0.841; 0.0257 (S)]	0.360 [0.093–1.387; 0.1377 (NS)]
Appropriate for gestational age			
	Singleton	1.000	-
	Twin	0.353 [0.201–0.619; 0.3526 (NS)]	
Small for gestational age			
	Singleton	1.000	1.000
	Twin	5.286 [2.826–9.887; < 0.0001 (HS)]	3.960 [1.928–8.135; 0.0001 (S)]
Neonatal hypoglycemia			
	Singleton	1.000	-
	Twin	0.647 [0.263–1.727; 0.4112 (NS)]	
Hyperbilirubinemia			
	Singleton	1.000	-
	Twin	0.241 [0.029–2.040; 0.1918 (NS)]	
NICU admission > 48 h			
	Singleton	1.000	1.000
	Twin	2.687 [1.064–6.784; 0.0364 (S)]	2.215 [0.704–6.968; 0.1737 (NS)]
Perinatal mortality			
	Singleton	1.000	-
	Twin	0.488 [0.096–2.477; 0.3869 (NS)]	

‡Adjusted with parameters like age of mother, bad obstetric history, family history of DM, 2 h PGBG, GDM treatment, mode of delivery;*Obtained using Fisher's exact test; *HS* highly significant, *S* significant, *NS* non-significant

strategies and pregnancy outcomes are likely to differ in twin pregnancies compared to a matched cohort of singleton pregnancies in women with GDM. Our results indicate that GDM women with twin or singleton gestation differed insignificantly in their treatment strategies and insulin doses. Insulin requirements need not be proportional to the number of fetuses. Regression analysis found a significant association between the twin pregnancies complicated by GDM and the decreased incidence of premature birth and LGA birth. However, the odds of having premature baby unit (PBU) admission > 48 h and SGA births were higher in twin pregnancies compared to singleton pregnancies.

GDM women carrying twins had a higher risk of cesarean delivery and preterm birth as compared with women without GDM [32]. Ooi et al. [21] have shown that twin pregnancies were less likely to require insulin therapy. Other studies have shown that the need to start insulin therapy among GDM twin pregnancies varied from 11–36% [33, 34]. Being tertiary care referral centers, higher number of pregnant women with GDM required insulin therapy to achieve target glycemic control in our study. However, the difference was statistically insignificant in diet or insulin therapy to GDM between twin and singleton pregnancies. The results are in line with a previous study reporting no significant difference between the proportion of women requiring insulin and the average daily insulin doses in twin and singleton pregnancies associated with GDM and carbohydrate intolerance [35]. The possible explanation for such findings may be the increased utilization of glucose in twin pregnancies due to two fetuses and the higher maternal basal metabolic rate. A study by Fox et al. has revealed that the insulin dose requirement need not be proportional to the number of fetuses, rather the treatment could be planned just to achieve glycemic control in GDM patients throughout the gestation period, even in twin pregnancy [20]. Though not GDM, a retrospective study by Callesen et al. on 15 twins versus 108 singleton pregnancies in women with type 1 diabetes have demonstrated that they require higher insulin, and the weekly increase in insulin dose between 14 and 27 weeks was doubled compared with singleton pregnancies [22]. This was not observed in our GDM cohort.

The mode of twin delivery depends on their presentation. Vaginal birth is usually preferred when both the twins are in vertex presentation and not associated with any risk factors. In 2011, a systematic review and meta-analysis involving 18 articles (39, 571 twin sets) found no difference in second twin outcomes between vaginal and cesarean delivery when both the twins are in vertex position [36]. A single-center retrospective study by Poulain et al. found no association between twin pregnancies complicated by glucose intolerance and an increased risk of macrosomia or cesarean section [37]. In our study, the mode of delivery by cesarean section was higher in the twin pregnancy group compared to the singleton group; however, the difference was statistically insignificant. This is in agreement with the previous studies reporting a higher rate of cesarean sections in women with GDM and twin pregnancies. In 2016, a prospective observational study reported that the preference for cesarean delivery was higher in twin pregnancies [38]. The presence of non-vertex position for one or both twins and/or some maternal or fetal complications may be the cause of the high cesarean delivery rate in twin pregnancies.

A study from South India has reported BOH in about 39% of women with GDM [39]. However, another study from North India has reported previous perinatal losses in about 15% of GDM mothers [40]. In this chart review, only 2 (4.55%) women in the twin group were identified to have BOH compared to 45 (34.09%) women in the singleton group. This may be attributed to more number of primipara in the twin pregnancy versus singleton group.

In this review, we found twin babies at risk of > 48 h NICU stay with OR of 2.69. In 2015, Foeller et al. conducted a study using US birth data (from 2006 to 2009) to compare neonatal outcomes between twin pregnancies with diabetes (16,562 GDM and 2137 preexisting diabetic) and without diabetes [41]. Similar to our study, Foeller et al. noticed an increased risk of prolonged ventilation (> 6 h) and NICU admission in diabetic twin pregnancies [41]. However, Hiersch et al. compared the women with or without GDM for pregnancy outcomes in singleton and twin pregnancies and reported no higher risk of admission to NICU, and hypoglycemia in twin infants of women with GDM [32]. We also found insignificant differences in neonatal hypoglycemia and perinatal mortality in both groups. We did not find any case of congenital malformation in our study cohort, as all of them were cases of GDM.

Earlier publications have stated that the risk of fetal growth restriction remains high in women with twin pregnancies. Similarly, we found a 5.29 times greater risk of SGA infants in twin pregnancies. This is due to the decreasing growth rate in twins relative to that of singletons starting from the 30th week of gestation onwards, it is rather unlikely to have LGA fetuses in twin pregnancies with GDM [42]. Similarly, in our study, we found a lower risk for LGA infants in twin pregnancies (OR, 0.239; 95% CI, 0.068–0.841; $p = 0.0257$). A retrospective cohort study from Japan has reported an extremely lower risk of LGA and a higher risk of low birth weight in GDM twins compared to singletons. This might be attributed to the limited maternal resources that are being shared by multiple fetuses [43].

Studies have shown a significantly higher risk of preterm delivery in twin pregnancies versus singleton pregnancies and no association between prematurity and GDM [44]. In contrast, we found a lower risk of preterm delivery (< 37 weeks) among women carrying twin pregnancies (OR, 0.225; CI, 0.127–0.402; $p < 0.0001$). In 2006, Cho et al. comparing perinatal outcomes of twin pregnancies in women with or without GDM found no differences [45]. They concluded that well-controlled GDM may not increase perinatal complications in twin pregnancies. Our observations were similar to this study.

Strengths and limitations

Data on twin GDM from South East Asia are rare as most of the studies have compared GDM twin pregnancy with non-GDM singleton or non-GDM twin pregnancies. This study is probably the first Indian and rare from South East Asia, which has compared GDM twin with GDM singleton gestation. Propensity score-based matching of baseline features has been hardly used earlier in such studies. The treatment (either insulin or diet plan) administered to these patients in two groups was also captured during the analysis and the resultant in terms of neonatal outcomes was compared between the groups. The limitations of this study are its retrospective nature, the scanty data available for comparing insulin therapy in twin GDM versus singleton GDM, and data is captured from only two centers; however, being both tertiary care diabetes institutes, we deal with the scattered population (high-risk complicated pregnancies) across the central and western part of India.

Conclusion

GDM women with twin or singleton gestation differed insignificantly in their treatment strategies and insulin doses. Insulin requirements need not be proportional to the number of fetuses. Treatment should be planned to achieve glycemic goals, even in twin pregnancy.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval After obtaining the approval from Institutional Ethics Committee, we conducted a retrospective chart review of women with singleton and twin pregnancy, those visited Sunil's Diabetes Care n' Research Centre, a tertiary care center for diabetes in Nagpur, Central India, and Gupte Hospital Pune, West India, for the treatment of gestational diabetes mellitus from the year 2006 to 2019.

References

1. Mcgrath RT, Hocking SL, Scott ES, et al. Outcomes of twin pregnancies complicated by gestational diabetes: a meta-analysis of observational studies. J Perinatol. 2017;37:360–8.
2. Seshiah V, Balaji V, Balaji MS, et al. Pregnancy and diabetes scenario around the world: India. Int J Gynaecol Obstet. 2009;104:S35–8.
3. Smits J, Monden C. Twinning across the developing world. PLoS One. 2011;6(9):e25239.
4. Weissman A, Drugan A. Glucose tolerance in singleton, twin and triplet pregnancies. J Perinat Med. 2016;44:893–7.
5. Retnakaran R, Shah BR. Impact of twin gestation and fetal sex on maternal risk of diabetes during and after pregnancy. Diabetes Care. 2016;39:e110–1.
6. Corrado F, Caputo F, Facciolà G, Mancuso A. Gestational glucose intolerance in multiple pregnancy. Diabetes Care. 2003;26(5):1646. https://doi.org/10.2337/diacare.26.5.1646.
7. Hiersch L, Yogev Y. Impact of gestational hyperglycemia on maternal and child health. Curr Opin Clin Nutr Metab Care. 2014;17:255–60.
8. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008; 358:1991–2002.
9. Aviram A, Guy L, Ashwal E, et al. Pregnancy outcome in pregnancies complicated with gestational diabetes mellitus and late preterm birth. Diabetes Res Clin Pract. 2016;113:198–203.
10. Rauh-Hain JA, Rana S, Tamez H, et al. Risk for developing gestational diabetes in women with twin pregnancies. J Matern Fetal Neonat Med. 2009;22:293–9.
11. Simoes T, Queiros A, Correia L, et al. Gestational diabetes mellitus complicating twin pregnancies. J Perinat Med. 2011;39:437–40.
12. Foeller ME, Zhao S, Szabo A, et al. Neonatal outcomes in twin pregnancies complicated by gestational diabetes compared with non-diabetic twins. J Perinatol. 2015;35:1043–7.
13. Buhling KJ, Henrich W, Starr E, et al. Risk for gestational diabetes and hypertension for women with twin pregnancy compared to singleton pregnancy. Arch Gynecol Obstet. 2003;269:33–6.
14. Francisco C, Wright D, Benko Z, et al. Competing-risks model in screening for pre-eclampsia in twin pregnancy by maternal characteristics and medical history. Ultrasound Obstet Gynecol. 2017;50:501–6.
15. Rouse DJ. Twins are not a per se indication for cesarean delivery. Obstet Gynecol. 2017;129:974–5.
16. Gonzalez Gonzalez NL, Goya M, Bellart J, et al. Obstetric and perinatal outcome in women with twin pregnancy and gestational diabetes. J Matern Fetal Neonat Med. 2012;25:1084–9.
17. Guillen MA, Herranz L, Barquiel B, et al. Influence of gestational diabetes mellitus on neonatal weight outcome in twin pregnancies. Diabet Med. 2014;31:1651–6.
18. Lai FY, Johnson JA, Dover D, et al. Outcomes of singleton and twin pregnancies complicated by pre-existing diabetes and gestational diabetes: a population-based study in Alberta, Canada, 2005–11. J Diabetes. 2016;8:45–55.
19. Okby R, Weintraub AY, Sergienko R, et al. Gestational diabetes mellitus in twin pregnancies is not associated with adverse perinatal outcomes. Arch Gynecol Obstet. 2014;290:649–54.
20. Fox NS, Gerber RS, Saltzman DH, et al. Glycemic control in twin pregnancies with gestational diabetes: are we improving or worsening outcomes? J Matern Fetal Neonatal Med. 2016;29:1041–5.
21. Ooi S, Wong VW. Twin pregnancy with gestational diabetes mellitus: a double whammy? Diabetes Care. 2018;41:e15–6.
22. Callesen NF, Ringholm L, Stage E, Damm P, Mathiesen ER. Insulin requirements in type 1 diabetic pregnancy: do twin pregnant women require twice as much insulin as singleton pregnant women? Diabetes Care. 2012;35(6):1246–8.
23. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15:539–53.
24. Lakshmi KC. Immunological causes of bad obstetric history. J of Evidence Based Med & Hlthcare. 2014;1:2086–99.
25. Paul VK, Deorari AK, Singh M. Management of low birth weight babies. In: Parthasarathy A, editor. IAP Textbook of Pediatrics. 2nd ed. New Delhi: Jaypee Brothers; 2002. p. 60.
26. Mahalakshmi MM, Bhavadharini B, Maheswari K, et al. Comparison of maternal and fetal outcomes among Asian Indian pregnant women with or without gestational diabetes mellitus: a situational analysis study (WINGS-3). Indian J Endocr Metab. 2016;20:491–6.
27. Disorders related to short gestation and low birth weight, not elsewhere classified; P07; Chapter XVI Certain Conditions Originating in the Perinatal Period; International statistical classification of diseases and related health problems- 10th revision (ICD-10), Fifth edition, 2016, 3 v. WHO Library Cataloguing-in-Publication Data.
28. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and Development; WHO Library Cataloguing-in-Publication Data; ISBN 92 4 154693 X; NLM Classification: WS 103; World Health Organization 2006.
29. Hussain K, Blankenstein O, De Lonlay P, et al. Hyperinsulinaemic hypoglycaemia: biochemical basis and the importance of maintaining normoglycaemia during management. Arch Dis Child. 2007;92(7):568–70.
30. Soon BT. The global action report on preterm birth. Geneva: World Health Organization. 2012.
31. World Health Organization. Neonatal and perinatal mortality : country, regional and global estimates. World Health Organization. 2006. https://apps.who.int/iris/handle/10665/43444
32. Hiersch L, Berger H, Okby R, et al. Gestational diabetes mellitus is associated with adverse outcomes in twin pregnancies. Am J Obstet Gynecol. 2019;220(102):e1-8.
33. Poulain C, Duhamel A, Garabedian C, et al. Outcome of twin pregnancies associated with glucose intolerance. Diabetes Metab. 2015;41:387–92.
34. Dinham GK, Henry A, Lowe SA, et al. Twin pregnancies complicated by gestational diabetes mellitus: a single centre cohort study. Diabet Med. 2016;33:1659–67.

35. Schwartz DB, Daoud Y, Zazula P, et al. Gestational diabetes mellitus: metabolic and blood glucose parameters in singleton versus twin pregnancies. Am J Obstet Gynecol. 1999;181(4):912–4.
36. Rossi AC, Mullin PM, Chmait RH. Neonatal outcomes of twins according to birth order, presentation and mode of delivery: a systematic review and meta-analysis. BJOG. 2011;118(5):523–32. https://doi.org/10.1111/j.1471-0528.2010.02836.x.
37. Poulain C, Duhamel A, Garabedian C, et al. Outcome of twin pregnancies associated with glucose intolerance. Diabetes Metab. 2015;41(5):387–92.
38. Jhaveri RR, Nadkarni TK. Perinatal Outcome of Second Twin with Respect to Mode of Delivery: An Observational Study. J Clin Diagn Res. 2016;10(12):QC26–QC28. https://doi.org/10.7860/JCDR/2016/21688.9112
39. Shridevi A, Prabhudev P, Bhovi MR. A clinical study of prevalence of gestational diabetes mellitus and associated risk factors at a tertiary care centre in Karnataka, India. Int J Reprod Contracept Obstet Gynecol. 2017;4(6):1840–5.
40. Kalra P, Kachhwaha CP, Singh HV. Prevalence of gestational diabetes mellitus and its outcome in western Rajasthan. Indian J Endocrinol Metab. 2013;17(4):677–80. https://doi.org/10.4103/2230-8210.113760.
41. Foeller ME, Zhao S, Szabo A, Cruz MO. Neonatal outcomes in twin pregnancies complicated by gestational diabetes compared with non-diabetic twins. J Perinatol. 2015;35(12):1043–7.
42. Alexander GR, Kogan M, Martin J, et al. What are the fetal growth patterns of singletons, twins, and triplets in the United States? Clin Obstet Gynecol. 1998;41(1):115–25.
43. American College of Obstetricians and Gynecologists Committee on Practice Bulletins--Obstetrics. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 30, September 2001 (replaces Technical Bulletin Number 200, December 1994). Gestational diabetes. Obstet Gynecol. 2001;98(3):525–38. Retraction in: Obstet Gynecol. 2013;122(2 Pt 1):405.
44. Minakami H, Kosuge S, Fujiwara H, et al. Risk of premature birth in multifetal pregnancy. Twin Res. 2000;3(1):2–6.
45. Cho HJ, Shin JS, Yang JH, et al. Perinatal outcome in twin pregnancies complicated by gestational diabetes mellitus: a comparative study. J Korean Med Sci. 2006;21:457–9.

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ORIGINAL ARTICLE

The Notch pathway regulates KLF4 in podocyte injury induced by high glucose

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Abstract

Background Diabetic nephropathy (DN) is a common complication of diabetes. The Notch pathway plays an important role in podocyte injury and decreases the expression of podocyte-specific proteins in DN. However, the exact mechanisms are still elusive.

Objective The purpose of this study was to investigate the effect of the Notch pathway on podocyte injury by regulating Krüppel-like factor 4 (KLF4) in high glucose (HG)-induced podocytes.

Methods The expression of the Notch pathway and KLF4 was regulated in HG-induced podocytes. Western blotting was used to detect the protein expression of Notch1, Notch intracellular domain 1 (NICD1), KLF4, nephrin, and integrin β1. The mRNA levels of Notch1, KLF4, nephrin, and integrin β1 were determined by real-time polymerase chain reaction.

Results HG increased the expression of Notch1 and NICD1 in podocytes and inhibited the expression of KLF4 ($p < 0.05$ or $p < 0.01$). Inhibition of the Notch pathway by DAPT increased the KLF4, nephrin, and integrin β1 levels in HG-induced podocytes ($p < 0.01$). Downregulation of KLF4 expression by KLF4-siRNA inhibited the expression of nephrin and integrin β1 in HG-induced podocytes ($p < 0.01$).

Conclusion The Notch pathway may regulate the expression of nephrin and integrin β1 through KLF4 in HG-induced podocytes.

Keywords Diabetic nephropathy · High glucose · Podocyte · Notch pathway · Krüppel-like factor 4

Introduction

Diabetic nephropathy (DN), a common complication of diabetes, is one of the main causes of end-stage renal failure. It is of great significance for the majority of diabetic patients to explore its pathogenesis and seek effective prevention and treatment. Podocytes attach to the outside of the glomerular basement membrane (GBM) and are an important part of the glomerular filtration barrier (GFB). Most studies have shown that podocyte injury plays a major role in proteinuria and glomerulosclerosis in DN [1].

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The Notch pathway plays a critical role in kidney development and disease. The binding between ligand and receptor leads to a conformational change in the Notch receptor, which releases the Notch intracellular domain (NICD) by γ-secretase. Then, the NICD enters the nucleus and activates the transcription of downstream genes [2]. A γ-secretase inhibitor (DAPT) can inhibit the activity of γ-secretase, reduce the production of NICD, and then inhibit the activation of the Notch pathway [3]. The mechanism of the Notch pathway in podocyte injury in DN has been confirmed. The increased expression of NICD1 induces podocyte apoptosis through the activation of p53 and causes proteinuria and glomerulosclerosis [4]. We also found that the activation of the Notch pathway under high glucose (HG) conditions induces podocyte apoptosis and decreases the expression of podocyte-specific proteins [3, 5].

Krüppel-like factor (KLF), a transcription factor of eukaryotic zinc finger protein, participates in the regulation of various biological processes of cells, including proliferation, differentiation, growth, and development [6, 7]. In

recent years, the role of KLF4 in glomerular disease has been gradually studied. KLF4 plays a renoprotective role in diabetic kidney disease, which is associated with the activation of podocyte autophagy and is involved in the mTOR signaling pathway [8]. Inhibition and overexpression of the Notch pathway changes KLF4 expression and goblet cell differentiation in the mouse intestine, which indicated that the relationship between the Notch pathway and KLF4 expression was key to promote intestinal tumorigenesis [9]. Whether the Notch pathway regulates KLF4 expression in podocyte injury in DN has not been confirmed. In this study, podocytes were cultured with HG to assess the expression of the Notch pathway and KLF4. Subsequently, we suppressed Notch pathway to investigate the expression of KLF4 and podocyte-specific proteins, further explored the mechanism of podocyte injury in DN and provided a basis for the prevention and treatment of DN.

Materials and methods

Cell line and reagents

Conditionally, immortalized mouse podocytes were purchased from the Cell Resource Center, Peking Union Medical College, Beijing, China. RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco-BRL (Gaithersburg, MD, USA). D-glucose and γ-secretase inhibitor (DAPT) were purchased from Sigma (St. Louis, MO, USA). γ-Interferon was purchased from Peprotech (Rocky Hill, NJ, USA). Rabbit polyclonal antibodies against Notch1 and NICD1 were purchased from Cell Signaling Technology (Danvers, MA, USA). A rabbit polyclonal antibody against KLF4 was purchased from Proteintech (Chicago, IL, USA). Rabbit polyclonal antibodies against nephrin and integrin β1 were purchased from Abcam Technology (Cambridge, MA, USA). A rabbit anti-β-actin polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipofectamine 2000 and TRIzol reagent were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). The real-time polymerase chain reaction (PCR) system was obtained from Promega (Madison, WI, USA).

Cell culture

Mouse podocytes were first cultured in an incubator at 33 °C in 5% CO_2 with RPMI-1640 containing 10% FBS and 10 U/ml γ-interferon to induce proliferation. After the proliferative process, the cells were cultured in RPMI-1640 without γ-interferon in an incubator at 37 °C in 5% CO_2 for 14 days to induce differentiation. After the podocytes had grown to 75–85% confluence, the cells were synchronized in serum-free RPMI-1640 medium for 24 h. Podocytes were cultured to assess the expression of KLF4, Notch1, and NICD1 with HG (30 mmol/L D-glucose) for 0, 12, 24, 48, and 72 h. Then, we used DAPT to inhibit activation of the Notch pathway. Podocytes were divided into three groups: normal glucose group (NG, 5.5 mmol/L D-glucose), HG group, and HG + DAPT (1 μmol/L) group. Transient transfections of podocytes with nonspecific small interfering RNA (NS-siRNA) and KLF4-siRNA (Santa Cruz Biotechnology) were performed with Lipofectamine 2000 according to the manufacturer's instructions.

Western blotting

Total protein was extracted from podocytes and assessed by the Coomassie Protein Assay Reagent (Sigma-Aldrich). A total of 30 μg protein was separated on SDS–polyacrylamide gel and then transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% fat-free dry milk at 37 °C for 1 h, the membranes were incubated overnight at 4 °C with rabbit anti-Notch1, NICD1, KLF4, nephrin, integrin β1, and β-actin polyclonal antibodies. After washing with Tris-buffered saline with Tween-20 (TBST), the membrane was incubated with a goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody and then scanned using the Odyssey Fc System (LI-COR; Amersham, Piscataway, NJ, USA). The intensity of Western blot bands was measured using the UVP Image Station Lab Works 4.5 software (Upland, CA, USA). Protein expression was normalized relative to β-actin.

Real-time PCR

Total RNA was extracted from podocytes and reverse transcribed into cDNA. The cDNA was prepared on an ABI 7500 real-time PCR system with the following conditions: 95 °C for 30 s and 40 cycles of amplification (95 °C for 5 s, 60 °C for 30 s). Two oligonucleotide primers were used for each gene as follows: Notch1, 5′-GTG GAT GAC CTA GGC AAG TCG-3′ and 5′-GTC TCC TCC TTG TTG TTC TGC A-3′; KLF4, 5′-TTC CAA CTC GCC GCC AAC CCA CC-3′ and 5′-TTG ATG TCC GCC AGG TTG TTG AA-3′; nephrin, 5′-GCT CAG GGA AGA CAG CAA CA-3′ and 5′-GAT AGA GCC CAG AAG CCT CG-3′; integrin β1, 5′-TCG ATC CTG TGA CCC ATT GC-3′ and 5′-AAC AAT TCC AGC AAC CAC GC-3′; and 18S, 5′-GTA ACC CGT TGA ACC CCA TT-3′ and 5′-CCA TCC AAT CGG TAG TAG CG-3′. The mRNA levels of each gene were normalized to 18S and analyzed using the relative standard curve method of analysis/ΔC_t method of analysis.

Statistical analysis

SPSS 17.0 software was used for statistical analysis. One-way analysis of variance (ANOVA) was used for the multiple-group comparisons. Statistical significance was considered at $p < 0.05$.

Results

Expression of Notch pathway molecules and KLF4 in HG-induced podocytes

The protein and mRNA levels of Notch1 increased gradually in HG-induced podocytes in a time-dependent manner ($p < 0.05$ or $p < 0.01$, Fig. 1a-c). After HG stimulation of podocytes, NICD1 protein expression increased starting at 12 h, peaked at 48 h and then decreased at 72 h ($p < 0.01$, Fig. 1a and b). HG decreased the protein and mRNA levels of KLF4 in a time-dependent manner and decreased gradually from 12 to 72 h ($p < 0.01$, Fig. 1a-c). HG activated the Notch pathway and reduced KLF4 expression in podocytes.

Notch pathway inhibition changed the expression of KLF4, nephrin, and integrin β1 in HG-induced podocytes

Compared with the NG group, the Notch1 protein and mRNA levels were increased in podocytes stimulated by HG at 48 h ($p < 0.01$, Fig. 2a-c). The use of DAPT did not inhibit the increase in Notch1 protein and mRNA expression in HG-induced podocytes ($p > 0.05$). HG dramatically increased NICD1 protein expression in podocytes for 48 h. DAPT reduced the expression of NICD1 protein in HG-induced podocytes ($p < 0.01$, Fig. 2a and b). HG stimulation decreased the KLF4, nephrin, and integrin β1 protein and mRNA levels in podocytes compared with NG for 48 h. After DAPT inhibited the activation of the Notch pathway, the KLF4, nephrin, and integrin β1 protein and mRNA levels

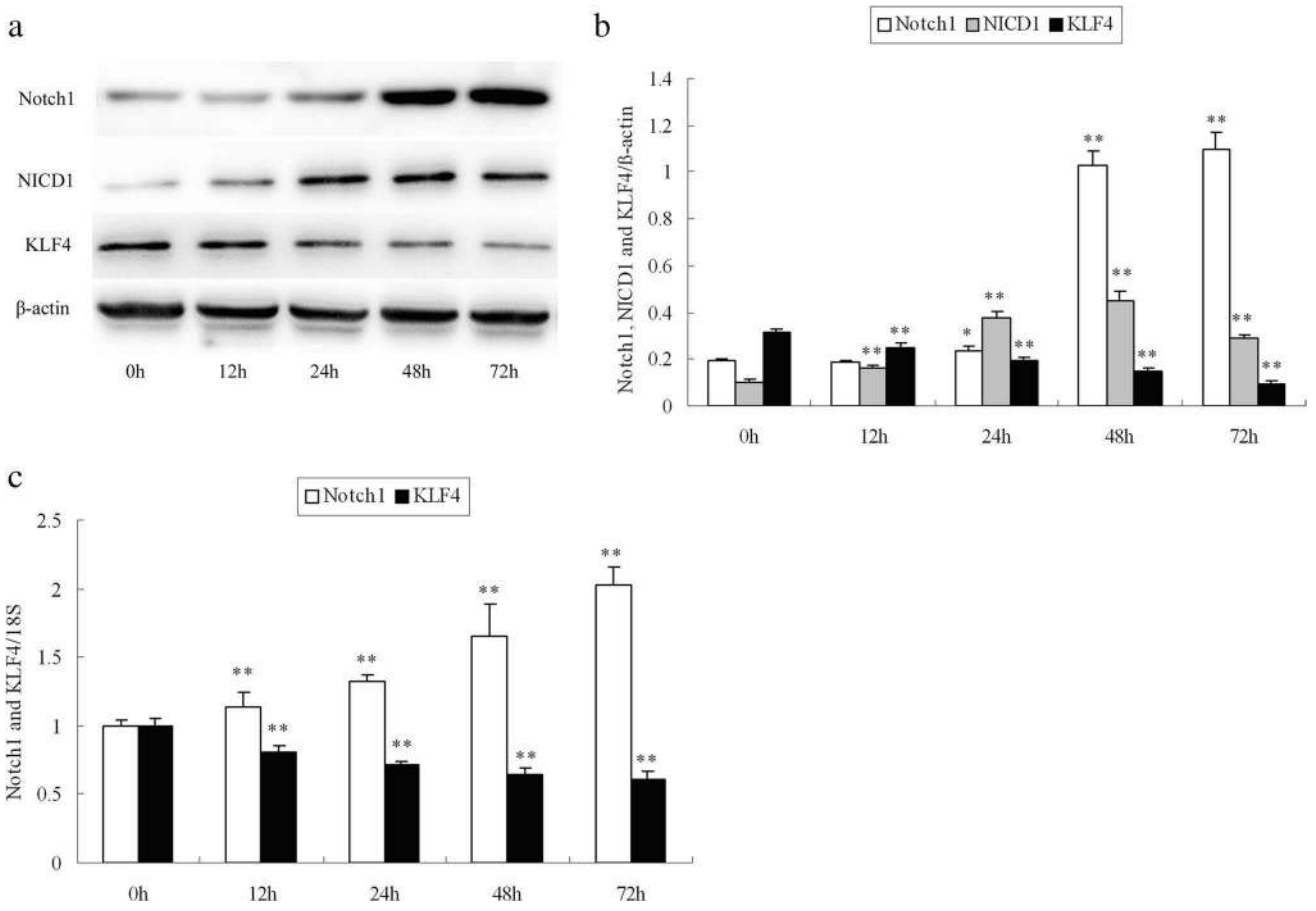

Fig. 1 Time course of the activation of the Notch pathway and KLF4 by HG in podocytes. Podocytes were incubated with HG at the indicated times (0–72 h). (**a**) The protein expression of Notch1, NICD1, and KLF4 was analyzed by Western blot. (**b**) The protein levels of Notch1, NICD1, and KLF4 were quantified by densitometry. (**c**) The mRNA expression of Notch1 and KLF4 was assessed by real-time PCR analysis. Values are expressed as means ± SD, $n = 6$. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control (0 h)

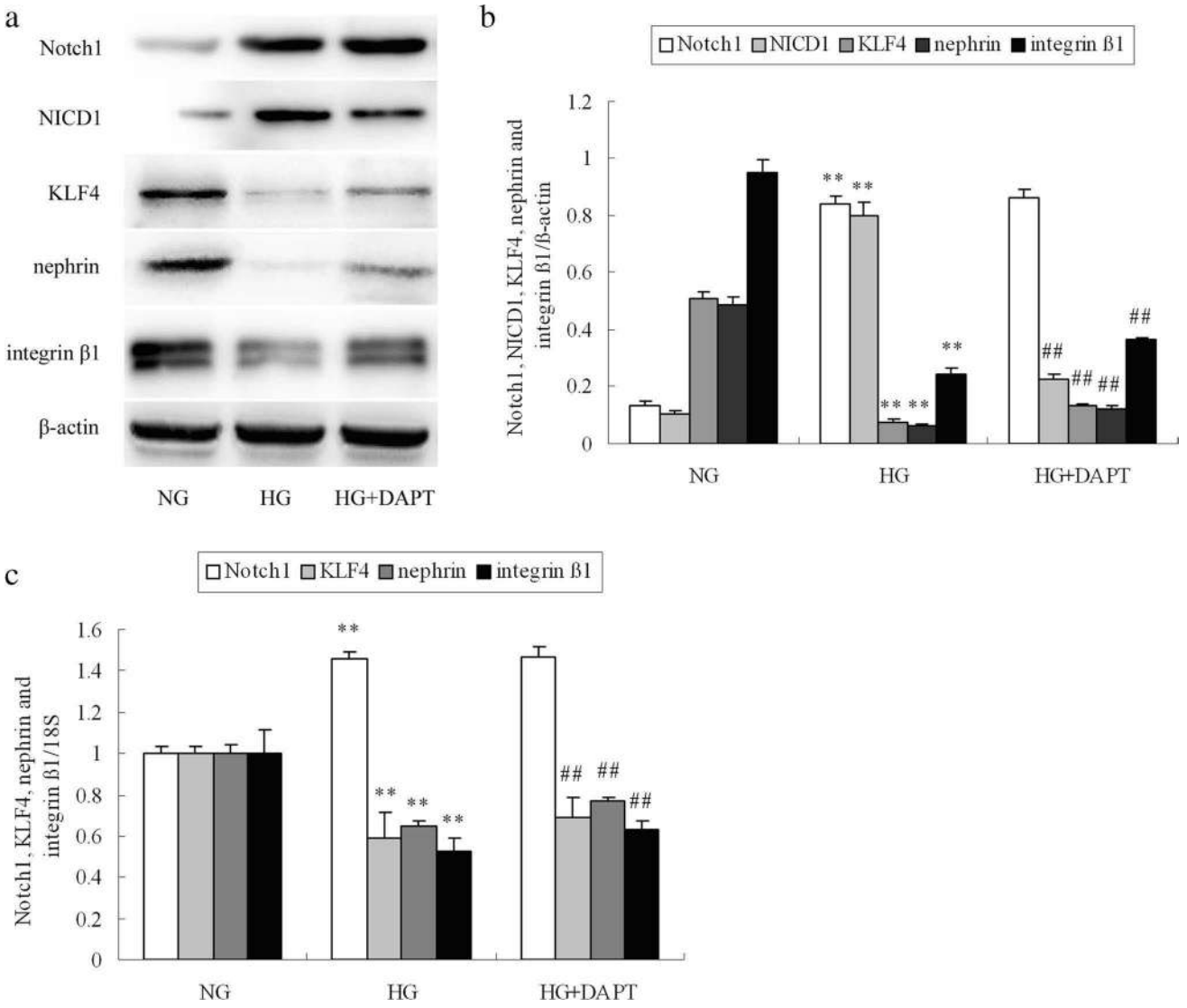

Fig. 2 Effect of DAPT on HG-induced expression of Notch1, NICD1, KLF4, nephrin, and integrin β1 in podocytes. (**a**) The protein expression of Notch1, NICD1, KLF4, nephrin, and integrin β1 was analyzed by Western blot. (**b**) The protein levels of Notch1, NICD1, KLF4, nephrin, and integrin β1 were quantified by densitometry. (**c**) The mRNA levels of Notch1, KLF4, nephrin, and integrin β1 were analyzed by real-time PCR. Values are expressed as means ± SD, $n = 6$. $^{**}p < 0.01$ vs. NG, $^{\#\#}p < 0.01$ vs. HG

were markedly increased ($p < 0.01$, Fig. 2a-c). DAPT upregulated the expression of KLF4, nephrin, and integrin β1 in HG-induced podocytes.

KLF4 silencing changed the expression of nephrin and integrin β1 in HG-induced podocytes

As shown in Fig. 3a-c, HG stimulation reduced KLF4 protein and mRNA expression compared with NG for 48 h ($p < 0.01$). KLF4-siRNA was added to further inhibit KLF4 expression in the HG group. The protein and mRNA levels of nephrin and integrin β1 in the HG group were significantly lower than those in the NG group. Nephrin and integrin β1 expression was further decreased after podocytes were transfected with KLF4-siRNA ($p < 0.01$). KLF4-siRNA downregulated nephrin and integrin β1 expression in HG-induced podocytes.

Discussion

The normal structure and function of podocytes play important roles in maintaining the integrity of GFB. When DN occurs, the cytoskeleton changes, and the structure of the slit diaphragm (SD) protein complex is destroyed [10]. Nephrin is a type 1 transmembrane protein that is the central molecule of the SD protein complex and plays a role in bridging adjacent pods through nephrin-nephrin or nephrin-Neph-1

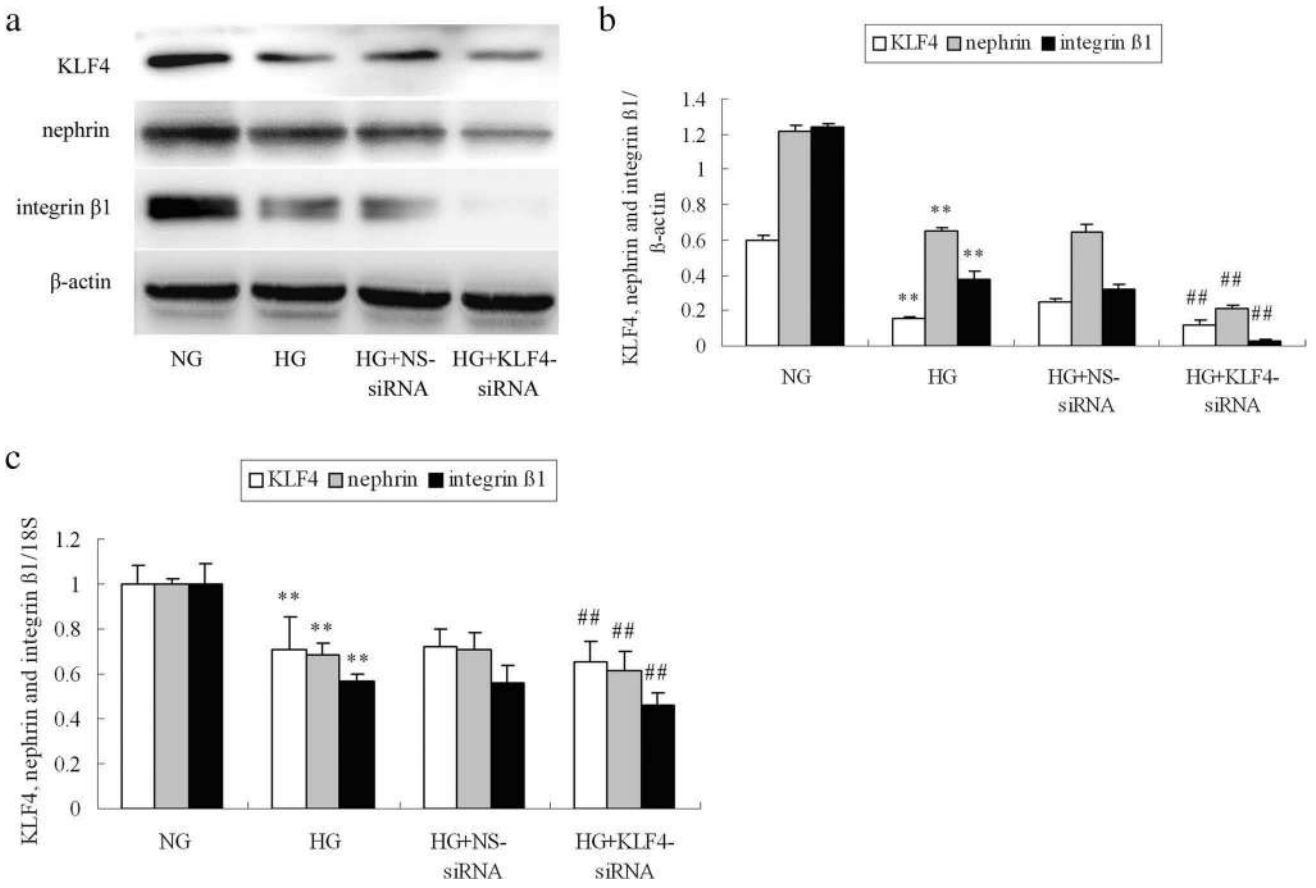

Fig. 3 Effect of KLF4 interference on HG-induced expression of KLF4, nephrin, and integrin β1 in podocytes. (**a**) The protein expression of KLF4, nephrin, and integrin β1 was analyzed by Western blot. (**b**) The protein levels of KLF4, nephrin, and integrin β1 were quantified by densitometry. (**c**) The mRNA levels of KLF4, nephrin, and integrin β1 were analyzed by real-time PCR. Values are expressed as means ± SD, $n = 6$. $^{**}p < 0.01$ vs. NG, $^{\#\#}p < 0.01$ vs. HG + NS-siRNA

interactions. A decrease in nephrin expression in DN causes foot process effacement, podocyte detachment from GBM, and the occurrence of proteinuria [11]. The heterodimer formed by integrin β1 and integrin α3 is the main component of podocyte adhesion, and integrin β1 and integrin α3 expression is abnormal in DN [12]. The results of this study also confirmed that nephrin and integrin β1 levels decreased in podocytes stimulated by HG. The loss of these podocyte-specific proteins can cause podocyte injury in DN.

The Notch pathway is a relatively conserved and widely existing signal transduction pathway between cells [13]. The Notch pathway is activated by the interaction between the receptor and the ligand. The ligand induces the conformational change in the Notch receptor, which is hydrolyzed under the action of extracellular metalloproteinase, and then releases NICD [3]. NICD represents an activated form of the Notch pathway, which enters the nucleus and affects cell differentiation, proliferation, and apoptosis. We found that the Notch pathway was activated in HG-induced podocytes and played a critical role in podocyte injury in DN. Elevated circulating growth hormone (GH) levels are associated with podocyte injury and proteinuria in diabetes, and induced TGF-β1-dependent Notch1 signaling contributes to the mitotic catastrophe of podocytes [14]. The Notch pathway has been shown to be activated in a diabetic mouse model and HG-induced podocytes, which decreases the expression of nephrin and podocin and results in proteinuria and accumulation of extracellular matrix [13]. In this study, DAPT inhibited the activation of the Notch pathway and increased the expression of nephrin and integrin β1 in HG-induced podocytes. It was proven that the Notch pathway, as an important signaling pathway in the progression of DN, may regulate the production of nephrin and integrin β1 in podocytes.

KLF4 is an important member of the KLF family. Hayashi et al. [15] found that the expression of KLF4 in glomerular podocytes is reduced in adriamycin (ADM)-induced nephrotic mice with increased proteinuria. KLF4 mRNA expression in patients with type 2 diabetes and diabetic nephropathy was found to be lower than that in the

control group [16]. We also found that KLF4 expression was decreased in HG-induced podocytes. With the decrease in KLF4, the nephrin and integrin β1 protein and mRNA levels were also obviously reduced. When KLF4-siRNA was used to inhibit the expression of KLF4 in podocytes, nephrin and integrin β1 expression was further reduced. These results suggested that KLF4 may regulate nephrin and integrin β1 expression in podocytes under HG conditions.

It has been confirmed that the Notch pathway inhibits KLF4 expression and promotes hepatic fibrosis. When the activation of the Notch pathway is inhibited by DAPT in primary hepatic stellate cells, the KLF4 levels generally increase [17]. After inhibition of Notch1 expression in human bladder cancer cells, the expression of KLF4 is increased, the growth and proliferation of cancer cells are inhibited, tumor cells are arrested in G0/G1 phase, and cell apoptosis is increased [18]. The Notch pathway promotes tumor-derived endothelial transdifferentiation by regulating the expression of KLF4, which changes the phenotype of tumor vessels and increases the probability of tumor metastasis [19]. In this study, we found that Notch pathway inhibition by DAPT in HG-induced podocytes alleviated the decrease in KLF4 expression caused by HG and then increased nephrin and integrin β1 levels. In HG-cultured podocytes, the Notch pathway may regulate the expression of nephrin and integrin β1 through KLF4. Since we only used DAPT to reduce NICD1 production and inhibit the activation of the Notch pathway, it may be possible that the Notch pathway has other ways to regulate KLF expression in podocyte injury in DN. To determine this will require further study. The Notch pathway and KLF4 are involved in regulating the differentiation of specific segments of nephrons and single cells during kidney development [20]. In the process of podocyte injury in DN, the Notch pathway and KLF4 jointly regulate the expression of podocyte-specific proteins.

This study suggests that the Notch pathway may regulate the expression of nephrin and integrin β1 through KLF4 in podocyte injury in DN.

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Declarations

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

Ethical approval Not required.

References

1. Zhong F, Chen H, Xie Y, Azeloglu EU, Wei C, Zhang W, et al. Protein S protects against podocyte injury in diabetic nephropathy. J Am Soc Nephrol. 2018;29(5):1397–410.
2. Chen J, Dong Y, Peng J, Zhang J, Gao X, Lu A, et al. Notch signaling mitigates chemotherapy toxicity by accelerating hematopoietic stem cells proliferation via c-Myc. Am J Transl Res. 2020;12(10):6723–39.
3. Yao M, Li G, Zhang T, Chi Y, Gao F. Notch pathway mediates podocyte injury and extracellular matrix synthesis in diabetic nephropathy. Int J Clin Exp Med. 2018;11(3):1873–81.
4. Niranjan T, Bielesz B, Gruenwald A, Ponda MP, Kopp JB, Thomas DB, et al. The Notch pathway in podocytes plays a role in the development of glomerular disease. Nat Med. 2008;14(3):290–8.
5. Gao F, Yao M, Shi Y, Hao J, Ren Y, Liu Q, et al. Notch pathway is involved in high glucose-induced apoptosis in podocytes via Bcl-2 and p53 pathways. J Cell Biochem. 2013;114(5):1029–38.
6. Ghaleb AM, Yang VW. Kruppel-like factor 4 (KLF4): what we currently know. Gene. 2017;611:27–37.
7. He M, Zheng B, Zhang Y, Zhang XH, Wang C, Yang Z, et al. KLF4 mediates the link between TGF-beta1-induced gene transcription and H3 acetylation in vascular smooth muscle cells. FASEB J. 2015;29(9):4059–70.
8. Gong J, Zhan H, Li Y, Zhang W, Jin J, He Q. Kruppel-like factor 4 ameliorates diabetic kidney disease by activating autophagy via the mTOR pathway. Mol Med Rep. 2019;20(4):3240–8.
9. Ghaleb AM, Aggarwal G, Bialkowska AB, Nandan MO, Yang VW. Notch inhibits expression of the Kruppel-like factor 4 tumor suppressor in the intestinal epithelium. Mol Cancer Res. 2008;6(12):1920–7.
10. New LA, Martin CE, Jones N. Advances in slit diaphragm signaling. Curr Opin Nephrol Hypertens. 2014;23(4):420–30.
11. Tung CW, Hsu YC, Shih YH, Chang PJ, Lin CL. Glomerular mesangial cell and podocyte injuries in diabetic nephropathy. Nephrology (Carlton). 2018;23(Suppl 4):32–7.
12. Sawada K, Toyoda M, Kaneyama N, Shiraiwa S, Moriya H, Miyatake H, et al. Upregulation of α3β1-integrin in podocytes in early-stage diabetic nephropathy. J Diabetes Res. 2016;2016:9265074.
13. Mertens PR, Raffetseder U, Rauen T. Notch receptors: a new target in glomerular diseases. Nephrol Dial Transplant. 2008;23(9):2743–5.
14. Nishad R, Mukhi D, Singh AK, Motrapu M, Chintala K, Tammineni P, et al. Growth hormone induces mitotic catastrophe of glomerular podocytes and contributes to proteinuria. Cell Death Dis. 2021;12(4):342.
15. Hayashi K, Sasamura H, Nakamura M, Azegami T, Oguchi H, Sakamaki Y, et al. KLF4-dependent epigenetic remodeling modulates podocyte phenotypes and attenuates proteinuria. J Clin Invest. 2014;124(6):2523–37.
16. Coskun ZM, Ersoz M, Adas M, Hancer VS, Boysan SN, Gonen MS, et al. Kruppel-like transcription factor-4 gene expression and DNA methylation status in type 2 diabetes and diabetic nephropathy patients. Arch Med Res. 2019;50(3):91–7.
17. Xue YK, Tan J, Dou DW, Chen D, Chen LJ, Ren HP, et al. Effect of Kruppel-like factor 4 on Notch pathway in hepatic stellate cells. J Huazhong Univ Sci Technol Med Sci. 2016;36(6):811–6.
18. Ai X, Jia Z, Liu S, Wang J, Zhang X. Notch-1 regulates proliferation and differentiation of human bladder cancer cell lines by inhibiting expression of Krüppel-like factor 4. Oncol Rep. 2014;32(4):1459–64.
19. Chen HF, Wu KJ. Endothelial transdifferentiation of tumor cells triggered by the Twist1-Jagged1-KLF4 axis: relationship between cancer stemness and angiogenesis. Stem Cells Int. 2016;2016:6439864.
20. El-Dahr SS, Aboudehen K, Saifudeen Z. Transcriptional control of terminal nephron differentiation. Am J Physiol Renal Physiol. 2008;294(6):F1273–8.

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ORIGINAL ARTICLE

Prevalent vascular complications in people with diabetes: a multicentre observational cohort study

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Abstract

Background Type 2 diabetes is a chronic metabolic disease characterized by vascular complications, the prevalence of which varies widely.

Aim Determine the prevalence of microvascular and macrovascular complications in type-2 diabetes in North India and their correlation with various risk factors.

Design Prospective observational study

Setting Tertiary referral centres

Methods A total of 6400 patients attending diabetes clinic were screened, out of which 6105 patients were enrolled during April 2018 to February 2021. Data were collected using a standardized electronic case record form. Prevalence estimates of microvascular and macrovascular complications were performed, and multivariate regression was applied for the analysis of correlation with known modifiable and non-modifiable risk factors. ROC analysis was done to find the best cut-offs for predictors of diabetic vascular complications.

Results The mean age of the participants was 58.3 ± 9.1 years (57% males). The mean duration of diabetes was 11.3 ± 7 years and HbA1c was 8.4 ± 4%. Prevalent diabetic neuropathy was observed in 30.1%, nephropathy in 18.8% and retinopathy in 13.7%. Coronary artery disease (CAD) was prevalent in 15.7%, cerebrovascular accident (CVA) in 3.6% and foot complications in 2.9%. In the multivariate regression model, all microvascular complications significantly correlated with the longer duration of diabetes but the same was not observed for macrovascular complications. Obesity [BMI cut-off>26.4 kg/m^2 (AUC 0.51, p=0.04) and waist circumference>94 cm (male) and >98 cm (female)] significantly predicted prevalent diabetic neuropathy and nephropathy. A BMI of >25.7 kg/m^2 had a sensitivity of 55.2% (AUC 0.57), p<0.001 for predicting foot complications.

Conclusion There is a strong association of obesity and longer diabetes duration with the prevalent microvascular and foot complications irrespective of the glycemic control but not for macrovascular complications.

Keywords Diabetic neuropathy · Nephropathy · Diabetic foot · Coronary artery disease · Microvascular complications · Macrovascular complications · Glycemia · HbA1c

Introduction

According to the diabetes atlas (9th edition), the global prevalence of diabetes is estimated at 463 million and is expected to reach 578 million by 2030 and 700 million by 2045 [1]. Of all the nations, India leads the world and has earned the distinction of 'Diabetes capital of the world'. This is possible due to the Asian-Indian phenotype characterized by higher insulin resistance, increased abdominal adiposity, lower adiponectin which predisposes Indians to a higher risk of diabetes and its vascular complications. In the Global burden of disease study, it was shown that among the major non-communicable diseases, diabetes had the highest increase in DALY rate in India from 1990 to 2016, with a crude increase of 80.0% and an age-standardized increase of 39.6% [2]. In 2016, the age-standardized DALY rate of diabetes in India was 1.3

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times the global average. Much of this increased morbidity and mortality has been attributed to delayed diagnosis and poor glycemic control leading to long-term damage and diabetic complications mainly affecting the eyes, nerves, kidneys and heart.

The increasing prevalence of diabetes and its vascular complications have burdened the national health systems which are now facing huge economic losses. A study in India in 2008–2009 found that total costs per hospitalization visit comprising consultation charges and medicines for patients without complications were Rs.4493 compared to Rs.14,691.75 (US$ 301.32) for patients with complications. Among the different types of complications investigated, foot complications incur the highest cost [3, 4]. Patients with foot complications spent four times more than patients without such complications along with higher mortality [5, 6]. Patients with renal disease, cardiovascular and retinal complications spent three times more than those without complications. Consultation and hospitalization costs were especially high for patients with complications. Hence, it is imperative to estimate the real burden of vascular complications and their risk factors for the early identification of individuals within a diabetic population and also to formulate preventive measures and realign the healthcare strategies for screening and management of these complications.

A linear relationship between microvascular complications and duration of disease has been established by the authors where they documented the presence of microvasculopathy across different age groups in 25–40% of diabetic patients aged >25 years with more than 5 years duration of diabetes [7].

Previous trials like UKPDS [8] and DCCT [9] have shown that intensive control of hyperglycemia decreases the incidence of microvascular complications, thus proving a cause-and-effect relationship between cumulative glycemic burden and microvascular damage. The Wisconsin Epidemiological Study of Diabetic Retinopathy showed a consistent exponential relationship between worsening glycemic control and prevalence of microvascular complications [10]. The Veterans Affairs (VA) Cooperative Study on Glycemic Control and Complications in Diabetes showed a significant reduction in proteinuria at the end of 2 years with more intensive glycemic control [11]. However, whether the same holds true for development of macrovascular complications like coronary artery disease, stroke or peripheral artery disease still remains controversial.

A favourable beneficial effect between the glycemic control and cardiovascular disease (CVD) is expected but a substantial evidence in the form of outcome studies is still lacking. Also, whether this macrovascular damage is sequential to microvascular complications, with the two representing a continuum or they are isolated events sharing some risk factors (common soil), remains debatable.

Hence, the aim of this study is to estimate the prevalence of microvascular and macrovascular complications in patients with established type 2 diabetes and to identify its relationship with the duration and severity of hyperglycemia.

Materials and methods

Patient enrollment

A total number of 6400 patients attending diabetes clinic of PGIMER, Chandigarh and PGIMS, Rohtak, were screened for the study, out of which 6105 patients were enrolled in duration of April 2018 to February 2021. Two hundred patients were excluded because of the secondary cause of diabetes (steroid-induced, pancreatogenous) or shorter duration of diabetes (<5years). Ninety-five patients were further excluded due to incomplete information about their vascular complications.

Data collection

The variables recorded at baseline included demographic and socioeconomic characteristics, physiological parameters (blood pressure, pulse rate, weight, height, body mass index [BMI] and waist circumference), change in glucose-lowering therapy and reason(s) for change, glycated hemoglobin (HbA_{1c}) level and other laboratory parameters (serum urea, creatinine and urine albumin), occurrence of major and minor hypoglycemic events, comorbidities (including microvascular and macrovascular complications), co-medications and patient-reported outcomes. Duration of diabetes was assessed as time since diagnosis of diabetes until data analysis. Hypertension was defined as blood pressure > 130/80 mm Hg or the history of use of anti-hypertensives. In line with the observational nature of the study, information with regard to glycemic control and other clinical variables was collected as measured in routine clinical practice at each site, according to the prevalent standard of care. The values of Hba1c, blood glucose (self-monitoring of blood glucose) [12] and renal function tests, within last available visit, were considered for analysis.

Definitions

Vascular complications

The presence of vascular complications was assessed by history as well as relevant investigations. Diagnosis and classification of complications were based on the best clinical judgement of the investigators.

Microvascular complications

- Retinopathy was diagnosed by history of laser therapy or history of anti-VEGF injection or a detailed fundus examination done as a part of routine screening.
- Nephropathy was diagnosed by presence of chronic kidney disease defined by eGFR < 60 ml/min/1.73 m^2 as estimated by MDRD (modification of diet in renal disease) equation. Measurement of urine albuminuria or proteinuria was not available for all participants, in line with the prospective nature of the study.
- Neuropathy: history of peripheral neuropathy (both positive and negative symptoms — numbness, cotton wool sensation, tingling, paresthesias or burning pain) was taken. Patients with a vibration perception threshold (VPT) test >25 and/or absence of monofilament perception at five standardized plantar sites were considered to have distal symmetric polyneuropathy (DSPN).

Macrovascular complications

- Coronary artery disease: history of stroke, angina, myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting and mortality from the same were obtained.
- Cerebrovascular complications: history of stroke, transient ischemic attack, carotid artery stenting or carotid endarterectomy.
- Peripheral artery disease: history of peripheral artery disease including revascularization procedures, diabetic foot and amputation was recorded. Ankle brachial index (ABI) was recorded and an ABI<0.9 was included for the diagnosis of PAD with or without symptoms.

Foot complications

- Presence or a history of prior diabetic foot ulcer or Charcot neuroarthropathy with or without amputation.

Additional information on mortality was obtained from hospital medical records in case of in-hospital death.

Statistical analysis

Data analysis was performed using the Statistical Package of Social Sciences (SPSS) version 21. Normality of the data was evaluated by Kolmogorov-Smirnov test and Shapiro-wilk test. In case of normal distribution, mean with SD was used for descriptive statistics. Prevalence of the complications in subgroups was compared by chi-square tests. The significance test was two-tailed and *p*-value of less than 0.05 was considered statistically significant. Multiple logistic regression analysis with stepwise additions of variables (age, duration of diabetes, Hba1c and hypertension) was performed to assess their association with each of the diabetic complication studied. To determine the relevance of predictors for vascular complications, a receiver operating characteristic curve (ROC) analysis was done to derive sensitivity, specificity and best cut-offs for parameters depicting cumulative glycemic burden, i.e. Hba1c, duration of diabetes and anthropometric parameters of obesity — BMI and waist circumference for early prediction of vascular complications. Youden index (J) was calculated. Waist circumference was analysed separately for males and females, given the gender-specific cut-offs for defining central obesity.

Results

A total of 6105 patients were included for analysis. The mean duration of diabetes was 11.3 ± 7 years and Hba1c was 8.4 ± 3.9 % as shown in Table 1. Majority of the participants (83%) were on at least two or three classes of oral hypoglycemic agents (OHA) and 43.5% were on both OHAs and insulin. The most common prevalent microvascular complication was diabetic neuropathy with estimated prevalence of 30.1% followed by diabetic nephropathy in 18.8% and retinopathy in 13.7% of the study population. Of the macrovascular disease, prevalent CAD was observed in 15.7% of the individuals. CVA was recorded in 3.6% and foot complications were present in 2.9% of the participants. Hypertension was present in 68.6% of the participants.

Correlation analysis for vascular complications with duration of diabetes and prevalent glycemic values

There was a significant correlation between the prevalence of all the microvascular complications (neuropathy, nephropathy and retinopathy) and the duration of the diabetes ($p <$ 0.01 for each) as shown in Table 2. Among macrovascular

Table 1 Baseline characteristics of study population.

Parameters	*N* = 6105
Age (years)	58.3 ± 9.1
HbA1c (%)	8.4 ± 4
Gender (*n*%)	Male = 3481 (57%)
	Female = 2623 (43%)
Duration of diabetes (years)	11.3 ± 7.0
On OAD (*n*%)	3851 (63.1%)
On OAD + Insulin (*n*%)	2651 (43.5%)

Data has been represented as mean values and percentage of population

OAD, oral antidiabetic drugs

Table 2 Correlation of microvascular and macrovascular complications with duration of diabetes.

Parameter	Duration of diabetes (years)				*p* value
	<6.0	6.0–10	10.01–15	>15	
Neuropathy (*n*%)	288 (15.7%)	471 (25.6%)	444 (24.2%)	635 (34.5%)	0.01*
Nephropathy (*n*%)	165 (14.4%)	261 (22.7%)	287 (25%)	435 (37.9%)	0.01*
Retinopathy (*n*%)	97 (11.6%)	190 (22.6%)	196 (23.4%)	356 (42.4%)	0.01*
CAD (*n*%)	176 (18.4%)	246 (25.7%)	226 (23.6%)	309 (32.3%)	0.01*
CVA (*n*%)	51 (23%)	69 (31.1%)	50 (22.5%)	52 (23.4%)	0.483
Foot complications (*n*%)	29 (16.6%)	48 (27.4%)	43 (24.6%)	55 (31.4%)	0.04*

*p value <0.05 is considered significant

CAD, coronary artery disease

CVA, cerebrovascular disease

complications, cardiovascular events increased statistically with increasing duration of diabetes (p<0.01). There was no correlation between the prevalence of stroke and diabetes duration (p=0.483).

With respect to severity of prevalent hyperglycemia, neuropathy ($p = 0.01$) and retinopathy (p=0.09) showed significant correlation with the increasing HbA1c but the same was not observed for nephropathy (p=0.41) as shown in Table 3. CAD had no correlation (p=0.57), but CVA had significant correlation with increasing HbA1c (p=0.02).

Regression models for predictors of vascular complications

In the multivariate regression model comprising the pertinent risk factors of age, duration of diabetes, HbA1c and hypertension, both the age of the patient and duration of diabetes independently predicted the occurrence of microvascular complications. For retinopathy and nephropathy, presence of hypertension was an equally important risk factor. Similarly, age and presence of hypertension were the most important risk factors for prevalent cardiovascular disease. The risk for CAD was found to be increased only with poorly controlled diabetes, i.e. at HbA1c more than 9.5% and duration of diabetes more than 10 years. The odds of having CAD was 1.43 (95% CI: 1.05–1.94) with diabetes duration of > 10 years and 1.35 times (95% CI: 1.15–2.07) if the HbA1c was > 9.5%. For CVA, none of the risk factors was found to be significantly associated except Hba1c > 7.9% after at which the odds of having stroke increased by twofold (Table 4).

ROC analysis for identifying anthropometric parameter and diabetes characteristics cut-offs to predict vascular complications

ROC analysis highlighted that Hba1c was not a significant predictor for any of the vascular complications whereas duration of diabetes was an important predictor for all complications — neuropathy (sensitivity: 65%, $p < 0.001$), nephropathy (sensitivity: 55.8 %, $p < 0.001$), retinopathy (sensitivity: 62.2%, $p < 0.001$), CAD (sensitivity: 55.9 %, $p < 0.001$), foot complications (sensitivity: 56%, $p = 0.01$), except CVA, as shown in Table 5. Among obesity-related parameters, BMI was significant factor for predicting neuropathy (sensitivity: 51.4%, p: 0.04) and foot complications

Table 3 Correlation of microvascular and macrovascular complications with prevalent HbA1c.

Parameters	Prevalent HbA1c (%)				*p* value
	<6.8	6.8–7.9	7.9–9.5	>9.5	
Neuropathy (*n*%)	235 (22.7%)	253 (24.5%)	259 (25%)	287 (27.8%)	0.01*
Nephropathy (*n*%)	192 (27%)	184 (25.8%)	177 (24.9%)	159 (22.3%)	0.41
Retinopathy (*n*%)	96 (24.1%)	115 (28.9%)	108 (27.1%)	79 (19.8%)	0.01*
HTN (*n*%)	652 (28.9%)	572 (25.4%)	525 (23.3%)	507 (22.5%)	0.01*
CAD (*n*%)	113 (24.8%)	114 (25.1%)	110 (24.2%)	118 (25.9%)	0.57
CVA (*n*%)	22 (17.6%)	27 (21.6%)	36 (28.8%)	40 (32%)	0.02*
Foot complications (*n*%)	15 (16.0%)	26 (27.7%)	24 (25.5%)	29 (30.9%)	0.076

*p value <0.05 is considered significant

HTN, hypertension

CAD, coronary artery disease

CVA, cerebrovascular disease

Table 4 Regression analysis of vascular complications of diabetes with hypertension, duration of diabetes, HbA1c and age of the patient.

		Neuropathy			Nephropathy			Retinopathy			CAD			CVA		
		OR	**CI**	***p***	**OR**	**CI**	***p***	**OR**	**CI**	***p***	**OR**	**CI**	***p***	**OR**	**CI**	***p***
HTN	**Present**	1.15	0.96–1.37	0.11	1.64	1.33–2.03	0.00*	1.87	1.40–2.48	0.00*	2.19	1.67–2.86	0.01*	1.38	0.96–1.37	0.13
	Absent															
Duration of diabetes (years)	**6–10**	1.57	1.25–1.98	0.00*	1.33	1.01–1.75	0.03*	1.57	1.06–3.52	0.00*	1.07	0.78–1.47	0.64	1.49	1.25–1.98	0.10
	10.05–15	1.95	1.55–2.46	0.00*	1.84	1.40–2.41	0.00*	2.43	1.67–3.52	0.00*	1.43	1.05–1.94	0.02*	1.00	1.55–2.46	0.98
	>15	2.01	1.61–2.51	0.00*	2.52	1.95–3.24	0.00*	3.87	2.73–5.48	0.00*	1.54	1.15–2.07	0.00*	0.81	1.61–2.51	0.47
	<6															
Hba1c (%)	**6.8–7.9**	1.04	1.02–1.58	0.72	1.04	0.82–1.32	0.72	1.30	0.97–1.75	0.07	1.13	0.78–1.47	0.37	1.44	1.02–1.58	0.20
	7.9–9.5	1.02	1.10–1.71	0.81	1.02	0.80–1.31	0.81	1.21	0.89–1.64	0.21	1.15	1.05–1.94	0.32	2.06	1.10–1.71	0.01*
	>9.5	0.97	1.39–2.15	0.85	0.97	0.76–1.25	0.85	0.91	0.66–1.26	0.58	1.35	1.15–2.07	0.00*	2.32	1.39–2.15	0.01*
	<6.8															
Age (years)	>58	1.86	1.59–2.19	0.00*	1.80	1.49–2.16	0.00*	1.3	1.03–1.64	0.02*	1.68	1.35–2.08	0.00*	1.38	1.59–2.19	0.09
	≤ 58															

*p value<0.05 is considered significant

95% confidence interval and odds ratio for the same have been mentioned

HTN, hypertension

Table 5 ROC analysis for Hba1c and duration of diabetes as predictors of vascular complications.

Predictors of complication	Cut-off	Sensitivity (%)	Specificity (%)	Youden index (J)	AUC	*p*
Neuropathy						
HbA1c (%)	8.7	47.3	58.2	0.05	0.50	0.91
Diabetes duration (years)	9.8	65.0	51.3	0.16	0.60	<0.001*
Nephropathy						
HbA1c (%)	8.0	57.4	53.9	0.11	0.58	0.06
Diabetes duration (years)	11.2	55.8	62.0	0.17	0.61	<0.001*
Retinopathy						
HbA1c (%)	8.1	47.4	48.8	−0.03	0.50	0.34
Diabetes duration (years)	10.5	62.2	59.8	0.22	0.65	<0.001*
CAD						
HbA1c (%)	7.9	46.7	54.0	0.00	0.53	0.34
Diabetes duration (years)	10.1	55.9	54.0	0.09	0.56	<0.001*
CVA						
HbA1c (%)	7.9	56.3	54.4	0.10	0.61	0.13
Diabetes duration (years)	9.5	53.6	46.4	0.00	0.50	0.94
Foot complications						
HbA1c (%)	8.4	60.0	52.0	0.12	0.51	0.86
Diabetes duration (years)	10.1	56.0	52.7	0.08	0.55	0.01*

*p value <0.05 is considered significant

CAD coronary artery disease, *CVA* cerebrovascular disease

(sensitivity: 55.2%, p: 0.002) while waist circumference was significant for predicting neuropathy (male, sensitivity: 58%, p: <0.001; female, sensitivity: 65.7%, p: <0.001), nephropathy (male, sensitivity: 58.8%, p: <0.001; female, sensitivity: 50 %, p: <0.001) and risk of CVA (male, sensitivity: 57.1%, p: 0.004; female, sensitivity: 54.4%, p: 0.013) as shown in Table 6.

Discussion

This is a large observational cohort study from North India suggesting a significant burden of diabetic complications in people with type 2 diabetes. The mean HbA1c was much higher than recommended by ADA guidelines [13] suggesting an overall poor glycemic control in the studied population. This observation was like that of Diabcare-Asia study in which Indian patients had mean HbA1c level of 8.9 ± 2.1%, with more than 83% of participants having HbA1c levels above 7%. Similarly, ICMR-INDIAB phase I study [14] reported mean HbA1c of 9.1% with 25.3% of patients having HbA1c > 10%. So was observed by Borgharkar et al. [15] in the TIGHT study on real-world evidence of glycemic control among patients with type 2 diabetes in India where out of 55639 participants, two-thirds (76.65%) had poor glycemic control defined by Hba1c > 7%. These studies including ours clearly reflect a gap between the recommended HbA1c target and the real-world observation which may be attributed to poor awareness of targets, ignorance about vascular complications, clinical inertia in timely initiation and intensification of treatment and poor drug adherence.

The prevalence of microvascular complications correlated significantly with duration of diabetes than the glycemic control. The microvascular and macrovascular complications that occur in diabetes contribute substantially to the increased morbidity and mortality. These vascular changes are an outcome of metabolic changes induced by chronic hyperglycemia. Of late, it has been shown that these metabolic changes are not only related to the disease duration but also the severity of hyperglycemia. The acute glycemic excursions also have a significant impact on these metabolic changes leading to cellular damage and metabolic memory that persists even when hyperglycemic crisis resolves.

Despite multiple observational studies on the prevalence of vascular complications in patients with type 2 diabetes, the estimates from these studies vary greatly. This is due to the lack of standardization in the assessment methods used which renders them difficult to compare. Also, this may be due to the heterogeneity of the populations being studied as we know that these vascular complications depend not only on the acquired risk factors but also genetic imprints. A study by Mohan et al. [16] had shown that at any degree of hyperglycemia, Indians have a higher predilection for development of metabolic complications suggesting ethnicity-specific outcomes as described by Neels thrifty gene [17] and thin fat Indians [18] hypothesis. Hence, a better

Table 6 ROC analysis for anthropometric parameters as predictors of vascular complications.

Predictors of complication	Cut-off	Sensitivity (%)	Specificity (%)	Youden index (J)	AUC	*p*
Neuropathy						
Waist(M) (cm)	93.5	58.0	48.3	0.06	0.54	<0.001*
Waist(F)(cm)	93.1	65.7	40.5	0.06	0.54	<0.001*
BMI (kg/m^2)	26.4	51.4	50.6	0.02	0.51	0.04*
Nephropathy						
Waist(M) (cm)	94.7	58.8	50.6	0.09	0.56	<0.001*
Waist(F) (cm)	98.3	50.0	59.6	0.09	0.57	<0.001*
BMI (kg/m^2)	26.4	50.5	50.0	0.00	0.50	0.90
Retinopathy						
Waist(M) (cm)	95.3	50.5	52.3	0.02	0.50	0.70
Waist(F) (cm)	95.9	52.2	45.6	−0.02	0.51	0.36
BMI (kg/m^2)	26.3	52.0	51.1	0.03	0.52	0.06
CAD						
Waist(M) (cm)	94.9	52.4	49.2	0.01	0.50	0.73
Waist(F) (cm)	97.9	55.9	45.0	0.00	0.50	0.64
BMI (kg/m^2)	26.4	50.4	50.0	0.00	0.50	0.74
CVA						
Waist(M) (cm)	95.2	57.1	52.1	0.09	0.58	<0.001*
Waist(F) (cm)	97.9	54.4	54.0	0.08	0.57	0.01*
BMI (kg/m^2)	26.3	51.6	49.3	0.00	0.51	0.06
Foot complication						
Waist(M) (cm)	94.9	51.8	48.9	0.00	0.56	0.22
Waist(F)(cm)	97.9	54.8	53.8	0.08	0.57	0.13
BMI (kg/m^2)	25.7	55.2	56.5	0.11	0.57	<0.001*

*p value <0.05 is considered significant

CAD coronary artery disease, *CVA* cerebrovascular disease

understanding of the burden of vascular complications in patients with type 2 diabetes across the globe is of considerable importance, particularly in countries like India where population characteristics are diverse and available data about diabetic complications is scarce. The Global burden of Disease study had stratified Indian states based on epidemiological transition level (ETL), defined on the basis of the ratio of DALYs from communicable diseases to those from non-communicable diseases and injuries combined and had shown that there was a 2.5 times variation in the prevalence of diabetes between the states in 2016 and hence the subsequent variation in vascular complications [2]. Owing to this considerable heterogeneity, a region-wise study on the prevalence of vascular complications is likely to provide critically important information for making decisions on health policy and strategies for prevention of diabetic complications.

In our study, we observed the prevalence of diabetic retinopathy to be 13.7% at median duration of diabetes of 11.3 years. Previously, retinopathy prevalence of 17.6% (confidence interval 95% CI: 15.8–19.5] is reported by the Chennai Urban Rural Epidemiology Study (CURES) study, which was a population-based large cross-sectional study [19]. The Sankara Nethralaya DR Epidemiology and Molecular Genetic Study had estimated an urban prevalence of 18.0% [20] (95% CI: 16.0–20.1) and a rural prevalence of 10.3% (95% CI: 8.53–11.97%) of DR in South India [21]; Aravind Comprehensive Eye Study reported 10.5% prevalence of DR (in self-reported subjects with diabetes) in the rural South Indian population [22] and Ramchandran et al. [23] showed retinopathy in 23.7% at a diabetes centre in Chennai. In Northern India, Chawla et al. [24] reported DR prevalence of 21.2% and Agrawala [25] reported a prevalence of 32.5%. The prevalence rate in our study was lower than that estimated by other studies because participants with other forms of diabetic retinopathy such as mild to moderate non-proliferative DR may not have experienced sight-threatening complications and hence may not have sought fundus evaluation and treatment. However, this prevalence rate in our study is much higher than the 0.9% prevalence of PDR in South-east Asian cohort of the DISCOVER programme [26], which aimed to estimate and compare the prevalence rates of vascular complications across the globe. In the DISCOVER study, retinopathy was diagnosed on the basis of history of

retinopathy or laser photocoagulation. This low prevalence in the DISCOVER study could be due to the shorter duration of diabetes (mean duration 4.6 years compared to 11 years in the present study), with a comparable Hba1c of 8.6% and participants age of 53 years. On multivariate regression analysis, age of the patient, duration of diabetes and hypertension were found to be independent risk factors for the presence of retinopathy. A diabetes duration of >10.1 years significantly predicted the occurrence of DR unlike the presenting Hba1c, suggesting point of care Hba1c may not correlate with the occurrence of retinopathy. This is plausible as the microvasculature damage occurring at the level of retinal capillaries would take time to set in and is observed in 11% of prediabetics as well. However, prevalent retinopathy was independent of anthropometric variable of obesity.

The prevalence of nephropathy in our study was 18.8% as compared to CURES 45 data that reported a prevalence of 2.2% for overt diabetic nephropathy and 26.9% for microalbuminuria [15]. Agrawala et al. [25] had reported prevalence of 30.2% (combined macroalbuminuria and overt nephropathy) from Northwest India. Ramchandran et al. [23] had reported 19.7% prevalence of proteinuria in South Indian diabetes subjects while Gupta et al. had reported microalbuminuria in 26%. The DISCOVER programme showed prevalence of CKD and albuminuria as 0.7% and 5.6%, respectively, in South-east Asian cohort [26]. This low prevalence in the DISCOVER study could be attributed to shorter diabetes duration or may be lesser prevalence of hypertension in their study population. In our study, we did not measure albuminuria and estimated overt nephropathy. Our study showed a fairly high prevalence of overt nephropathy due to higher prevalence of hypertension in our study which is a well-known established risk factor for renal parenchymal disease. Our study was limited by the fact that urine albumin estimation was not available for all participants, while it is well known that diabetic kidney disease may manifest as isolated proteinuria without affecting GFR. Like retinopathy, multivariable regression revealed that increasing age, duration of diabetes > 11.2 years and presence of hypertension significantly predicted the occurrence of nephropathy unlike prevalent Hba1c. Also, a waist circumference of >94.7 cm (male) and 98.3 cm (female) significantly predicted the occurrence of diabetic nephropathy in the present study.

Diabetic neuropathy was the most prevalent microvascular complication in our study, observed in one-third of the participants. This was similar to that reported by Agrawala et al. [25] (26.85%) and Ramachandran et al. [23] (27.5%) while much less than that reported by Shobhana et al. [27] (70%). The increased prevalence noted by Shobhana et al. may be related to referral bias or the modality of assessment. The prevalence of peripheral neuropathy may vary according to the modalities and expertise for assessment as monofilament sensation testing and vibration perception assessment are subjective methods and have significant inter-observer variability unlike objective measures of nerve conduction study (NCS) or electromyography (EMG). In the DISCOVER study, the prevalence of diabetic neuropathy was estimated at 9.6% [26]. On multivariate regression, age and duration of diabetes were the risk factors and hypertension and HbA1c did not predict neuropathy. This correlation estimate was different from the study by Agrawala et al. [25] which revealed a positive association for duration of diabetes, blood pressure, fasting blood sugar, serum cholesterol, serum LDL and serum VLDL. We also observed that diabetes duration of >11.2 years and obesity with waist circumference of >93.5 (male) and 93.1 cm (female) and BMI>26.4 kg/m^2 were significantly associated with prevalent neuropathy.

Among the macrovascular complications, clinical CAD was reported by 15.7% of the participants in our cohort. Agrawal et al. [25] found the prevalence of CAD in 25.8% while Ramachandran et al. [23] found the prevalence of coronary artery disease in 11.4%. The occurrence of CAD correlated with duration of diabetes while CVA correlated with Hba1c value at the time of participation in our study. On multivariate regression, age of the patient and duration of diabetes (>10.1 years) were the most important risk factors for CAD occurrence. The odds of having CAD were 1.4 times (95% CI: 1.05–1.94) with diabetes duration > 10 years and 1.3 times (95% CI: 1.15–2.07) for Hba1c > 9.5%. This can be explained by the fact that the causation of atherosclerosis is multifactorial that depends not only on the cumulative glycemic burden in diabetes but also other risk factors like dyslipidemia, hypertension, smoking and familial predisposition. Much of the higher prevalence of ASCVD in diabetes can be explained by the common soil hypothesis, [28] which refers to the amalgamation of risk factors that are similar for the development of diabetes and atherosclerosis. Matheus and Gomes reported also reported that cardiovascular events may not go hand in hand with microvascular complications [29]. For CVA, there was a twofold higher odd of having stroke at HbA1c above 7.9% and the risk was independent of hypertension. However, anthropometric variables of waist circumference and BMI did not corelate with the prevalent CAD in the present study unlike CVA, where a waist circumference >95.2 cm (male) and 97.9 cm (female) significantly predicted the occurrence of CVA.

An assessment of prevalent foot complications among people with diabetes is pertinent considering an increased morbidity (limb amputation) and mortality risk as compared to diabetic patients without foot abnormalities [5, 6]. We observed that only 3% of participants had manifest foot complications in the form of foot ulcers or Charcot neuroarthropathy after a mean diabetes duration of 8 years. This could be an underestimation of foot complications as minor foot abnormalities like claw toes, other deformities or subclinical

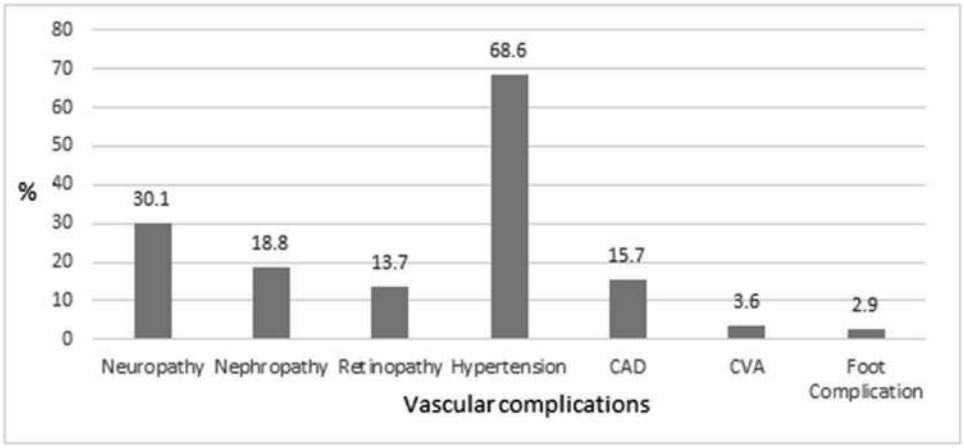

Fig. 1 Prevalence of microvascular and macrovascular complications in the study population. CAD, coronary artery disease; CVA, cardiovascular accident

peripheral arterial abnormalities might not be reported. A study from south India found that more than half of the studied patients had diabetic foot syndrome considering the IWGDF definition of foot at risk [30, 31]. We found that the duration of diabetes >10.1 years and obesity (BMI>25.7 kg/m^2) significantly predicted occurrence of foot complications.

Our study included a large number of participants from primarily north-west India. The study population was heterogenous and comprised of both urban and rural population. Since it was a prospective observational study, no specific intervention was planned, and this study represented the real-world scenario of vascular complications. However, there were several limitations. Patients with non-proliferative diabetic retinopathy were not included and a limited information about proteinuria or albuminuria was available which might have underestimated the prevalence of retinopathy and nephropathy. ASCVD was not further confirmed on ECG or 2D echocardiography in asymptomatic individuals and hence might not concur with the true prevalence of CVD. The present analysis is based on electronic registry database which is updated timely but a prospective time bound evaluation of participants was not ensured, limiting the estimation of incidence rate of vascular complications.

Conclusion

This real-world study of people with type 2 diabetes suggests poor glycemic control despite more than 10-year duration of diabetes and on multiple class of oral hypoglycemic agents. Diabetic neuropathy was the most prevalent vascular complication. Diabetic microvascular complications correlated with increasing duration of diabetes but not with the prevalent Hba1c. Obesity also significantly predicted the occurrence of diabetic neuropathy, nephropathy, cerebrovascular and foot complications independent of age and duration of diabetes Fig 1 .

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study was approved by Institute Ethics Committee vide letter No: PGI/IEC/2014/2341 and a written informed consent was obtained from all the participants. The patients were enrolled during their routine physical appointment in the clinic.

References

1. International Diabetes Federation.. IDF Diabetes Atlas. 9th ed. Brussels, Belgium: International Diabetes Federation; 2019.
2. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, et al. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. Lancet Glob Health. 2018;6(12):e1352-62.
3. Bansode B, Jungari DS. Economic burden of diabetic patients in India: a review. Diabetes Metab Syndr. 2019;13(4):2469–72.
4. Rastogi A, Bhansali A. Diabetic foot infection: an Indian scenario. J Foot Ankle Surg (Asia-Pacific). 2016;3(2):71–9.
5. Rastogi A, Goyal G, Kesavan R, Bal A, Kumar H, Mangalanadanam, et al. Long term outcomes after incident diabetic foot ulcer: multicenter large cohort prospective study (EDI-FOCUS investigators) epidemiology of diabetic foot complications study epidemiology of diabetic foot complications study. Diabetes Res Clin Pract. 2020;162:108113. https://doi.org/10.1016/j.diabres.2020.108113.
6. Chaudhary S, Bhansali A, Rastogi A. Mortality in Asian Indians with Charcot's neuroarthropathy: a nested cohort prospective study. Acta Diabetol. 2019;56:1259–64.
7. Chawla A, Chawla R, Bhasin GK, Soota K. Profile of adolescent diabetics in North Indian population. J Clin Diabetol. 2014;1:1–3.
8. UK Prospective Diabetes Study Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.

9. The DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977–86.
10. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. III. Prevalence and risk of diabetic retinopathy when age at diagnosis is 30 or more years. Arch Ophthalmol. 1984;102(4):527–32.
11. Levin SR, Coburn JW, Abraira C, Henderson WG, Colwell JA, Emanuele NV, et al. Effect of intensive glycemic control on microalbuminuria in type 2 diabetes. Veterans Affairs Cooperative Study on Glycemic Control and Complications in Type 2 Diabetes Feasibility Trial Investigators. Diabetes Care. 2000;23(10):1478–85.
12. Rao PV, Makkar BM, Kumar A, et al. RSSDI consensus on self-monitoring of blood glucose in types 1 and 2 diabetes mellitus in India. Int J Diabetes Dev Ctries. 2018;38:260–79.
13. American Diabetes Association. Standards of medical care in Diabetes—2018. Diabetes Care. 2018;41(Supplement 1):S13-27.
14. Unnikrishnan R, Anjana RM, Deepa M, Pradeepa R, Joshi SR, Bhansali A, et al. ICMR–INDIAB Collaborative Study Group. Glycemic control among individuals with self-reported diabetes in India–the ICMR-INDIAB Study. Diabetes Technol Ther. 2014;16(9):596–603.
15. Borgharkar SS, Das SS. Real-world evidence of glycemic control among patients with type 2 diabetes mellitus in India: the TIGHT study. BMJ Open Diab Res Care. 2019;7:e000654.
16. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res. 2007;125(3):217–30.
17. Neel JV. The "Thrifty Genotype" in 19981. Nutr Rev. 2009;57(5):2–9.
18. Misra A, Khurana L. Obesity-related non-communicable diseases: South Asians vs White Caucasians. Int J Obes. 2011;35(2):167–87.
19. Rema M, Premkumar S, Anitha B, Deepa R, Pradeepa R, Mohan V. Prevalence of diabetic retinopathy in urban India: The Chennai Urban Rural Epidemiology Study (CURES) eye study I. Invest Ophthalmol Vis Sci. 2005;46:2328–33.
20. Raman R, Rani PK, ReddiRachepalle S, Gnanamoorthy P, Uthra S, Kumaramanickavel G, et al. Prevalence of diabetic retinopathy in India: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study report 2. Ophthalmology. 2009;116:311–8.
21. Raman R, Ganesan S, Pal SS, Kulothungan V, Sharma T. Prevalence and risk factors for diabetic retinopathy in rural India. Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study III (SN-DREAMS III) report no 2. BMJ Open Diabetes Res Care. 2014;2:e000005.
22. Nirmalan PK, Tielsch JM, Katz J, Thulasiraj RD, Krishnadas R, Ramakrishnan R, et al. Relationship between vision impairment and eye disease to vision-specific quality of life and function in rural India: The Aravind Comprehensive Eye Survey. Invest Ophthalmol Vis Sci. 2005;46:2308–12.
23. Ramachandran A, Snehalatha C, Satyavani K, Latha E, Sasikala R, Vijay V. Prevalence of vascular complications and their risk factors in type 2 diabetes. J Assoc Physicians India. 1999;47(12):1152–6.
24. Chawla A, Chawla R, Chawla A. Correlation between retinopathy microalbuminuria and other modifiable risk factors. Presented on American Diabetes Association's 75th Scientific Session; June 5-9; Boston, Massachusetts; 2015.
25. Agrawal R, Ola V, Bishnoi P, Gothwal S, Sirohi P, Agrawal R. Prevalence of micro and macrovascular complications and their risk factors in type-2 diabetes mellitus. J Assoc Physicians India. 2014;62:6.
26. Kosiborod M, Gomes MB, Nicolucci A, Pocock S, Rathmann W, Shestakova M, et al. Vascular complications in patients with type 2 diabetes: prevalence and associated factors in 38 countries (the DISCOVER study program). Cardiovasc Diabetol. 2018;17(1):150.
27. Shobhana R, Ramarao P, Lavanya A, Vijay VV, Ramchandran A. Cost burden to diabetic patients with foot complications – a study from Southern India. JAPI. 2000;48:1147–50.
28. Stern MP. Diabetes and cardiovascular disease the "common soil" hypothesis. Diabetes Cardiovasc Dis. 1995;44:6.
29. Matheus AS, Gomes MB. Early aggressive macrovascular disease and type 1 diabetes mellitus without chronic complications: a case report. BMC Res Notes. 2013;6:22.
30. Bus SA, van Netten JJ, Lavery LA, Monteiro-Soares M, Rasmussen A, Jubiz Y, Price PE. International Working Group on the Diabetic Foot. IWGDF guidance on the prevention of foot ulcers in at-risk patients with diabetes. Diabetes Metab Res Rev. 2016;32(Suppl 1):16–24. https://doi.org/10.1002/dmrr.2696.
31. Vibha SP, Kulkarni MM, Kirthinath Ballala AB, Kamath A, Maiya GA. Community based study to assess the prevalence of diabetic foot syndrome and associated risk factors among people with diabetes mellitus. BMC Endocr Disord. 2018;18(1):43. https://doi.org/10.1186/s12902-018-0270-2.

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ORIGINAL ARTICLE

Utility of secondary screening in early detection of diabetic retinopathy and assessing diabetes management through rural referral mechanism

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Abstract

Background Diabetes mellitus is one of the major non-communicable diseases in India. Kamareddy district, with the support of Novartis under its Public Health Initiatives, has implemented secondary screening camps for subjects with higher risk of diabetes through screening under NPCDCS. In this analysis, we aim to identify the utility of the camps in monitoring diabetes management and early detection complications like diabetic retinopathy (DR).

Methods Seventeen camps were conducted in the rural Kamareddy district during Jun 2019 to Mar 2020. Subjects with RBS ≥ 140 mg/dl in the field screening were counselled and referred to the camps conducted as per schedule for further assessment through HbA1c, fundoscopy, ECG and lipid profile in an algorithm-based screening protocol.

Results Twelve percent (24,965/208,685) of screened subjects had abnormal RBS and were referred to the camps. Of them, 9.2% ($n = 2287$; male = 1291; female = 996) could avail HbA_1c testing in the camps, which consisted of 45% (1022) known diabetics. Forty-nine percent of the subjects belonged to age group 40–59 years (range: 6–88). Nearly one-third (34%) had HbA1c levels ≥ 7%. More than 63% of the known Diabetic subjects had HbA1c ≥ 7% indicating the need for strengthening treatment adherence ($p < 0.001$). Among subjects with HbA1c ≥ 7%, ~ 14% (75/547) had abnormal fundoscopy results indicating need for regular screening for DR.

Conclusion Our cross-sectional study observations suggest that there is high probability of undetected diabetes cases with or without DR in the field. Results indicate the need for regular active referral of known diabetics for necessary treatment adherence counselling for prevention and early detection of DR.

Keywords Diabetes · Diabetic retinopathy · Fundoscopy · Early detection · Complications · Rural referral mechanism · Active screening

Introduction

In India, the leading cause of death, accounting to nearly 60% of all deaths, is due to non-communicable diseases (NCDs) such as cardiovascular diseases (CVD), cancer, chronic respiratory diseases and diabetes mellitus (DM) [1]. This cause of pre-mature mortality due to NCDs is projected to increase over the years. Hence, the Government of India (GoI), in 2010, launched the National Program for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS) in 100 districts across 21 States, in order to prevent and control the major NCDs. The main focus of the program is on health promotion, early diagnosis, management and referral of cases, besides strengthening the infrastructure and capacity building [1].

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According to the National Multi-sectoral Action Plan (NMAP) for Prevention and Control of Common NCDs (2017–2022), revised in October 2017, [2] health promotion mechanisms is one of the envisaged roles to be played by the private sector in achieving the goals of NPCDCS. The framework also enlists the desired achievement of reduction in premature mortality due to NCDs by 10% by 2020 and by 25% by 2025. In the larger context of achieving the above-mentioned goal, the Department of Public Health and Family Welfare (DPHFW) of Telangana State has made a non-financial agreement with Novartis Healthcare Private Limited (Novartis) to utilize the support from Novartis Public Health Initiatives in 5 identified districts in the state. As part of the agreement, Novartis supported the District Medical and Health Office (DMHO) of Kamareddy district through arranging an algorithm based-screening camps [3, 4] in identified locations at stipulated frequency.

Kamareddy district is the 15th largest district in Telangana State in India with its spread over an area of 3,652.00 km^2 (1,410.05 sq mi). Kamareddy is bounded by Nizamabad district on North, Sircilla district and Siddipet district on East and South East respectively; it is bounded on South by Sangareddy district and Medak district and on the West and South West by Nanded district of Maharashtra and Bidar district of Karnataka state. According to 2011 Census of India, the district has a population of 972,625, making it the 15th most populous district in the state. Majority (92%, $n = 893{,}912$) of the population in the district is rural with its males and females are 439,532 (49.2%) and 454,380 (50.8%) respectively. In rural areas of Kamareddy district, sex ratio is 1036 females per 1000 males [5].

Diabetic subjects require life-long treatment and regular follow-up after diagnosis. Though there are concerns on the accuracy of HbA1c, the benefits such as ease of use, no need of fasting, being relatively cheap and high accessibility have made it as a go-to-test during follow-up. Among subjects with diabetes, prevalence of site-threatening diabetic retinopathy (STDR) is 10.2% globally [6]. There are several factors, which influence DR such as duration and type of DM, blood pressure and serum lipid. After onset of non-insulin-dependent DM, the DR develops generally in 4–7 years for which Ophthalmologists advise a comprehensive eye examination which includes dilated fundus examination for all subjects with diabetes on regular basis [7]. DR is the leading cause of visual disability in subjects with diabetes. The reported prevalence of DR in India ranges from 17.6 to 28.2% [8–11].

In this analysis, we are presenting the observations from this intervention, which can add value to the existing information on role of strengthening community-based screening and early detection of diabetic complications especially DR towards achieving the goals of NPCDCS.

Materials and methods

Our cross-sectional study was conducted in Kamareddy district of Telangana State in India during Jun 2019 to Mar 2020. Kamareddy district is one of the 5 districts identified for implementation of the non-financial collaboration between DPHFW, GoTS and Novartis under its public health initiatives. DMHO of Kamareddy, as per NPCDCS strategy, implemented the field level screening for identifying potential diabetes cases through house to house survey and using rapid test kits for Random Blood Sugar (RBS). Accredited Social Health Activist (ASHAs), Auxiliary Nurse Midwifery (ANMs) and other field health staff of DMHO, Kamareddy, conducted house-to-house survey in their respective villages to identify potential diabetes cases. These field staff also conducted RBS test on all the people above 40 years and those who are known diabetics irrespective of age. The known diabetics and the new suspects for DM if RBS $\geq$ 140 mg/dl were referred to the NCD screening camps arranged at predefined locations.

Fortnightly NCD screening camps with higher testing have been arranged based on the information generated from field-level screening camps and in accordance with the non-financial agreement. In the screening camps arranged at Primary Health Centres (PHCs) or Community Health Centres (CHCs) on a prefixed date as per the camp calendar, the Novartis team along with necessary test kits provided the additional screening tests as per the attached screening algorithm (Fig. 1). The study-specific algorithm-based screening has given options to cover all the eligible subjects with necessary tests while utilizing the resources effectively. According to the algorithm, known diabetics were provided with HbA1c right away and similarly subjects with markedly high hyperglycemia (HbA1c $\geq$ 10%) were provided with screening tests for complications irrespective of the age of subject and duration of diabetes (Fig. 1). Diabetes and its complications related to the retina and heart were covered through these camps.

HbA1c results were generated using rapid test kit (SD Multicare), which generates results within 3 min, and the same were shared with the subjects attending the camps on the spot. Guidelines by the American Diabetes Association (ADA) and the National Institute for Health and Care Excellence (NICE) of the United Kingdom suggest 6-monthly follow-up using HbA1c with a goal to maintain it < 7.% (52 mmol/mol) [12, 13]. A community-based study from Chandigarh demonstrated that HbA1c level cut-off point of 7.0% had 92% sensitivity for diagnosis of diabetes while cut-off point of 6.5% has 88% sensitivity [14]. In general, in Indian settings, though the HbA1c goal of < 6.5% is considered as decent (good) glycemic control, there is a shift towards the cut-off value of 7%

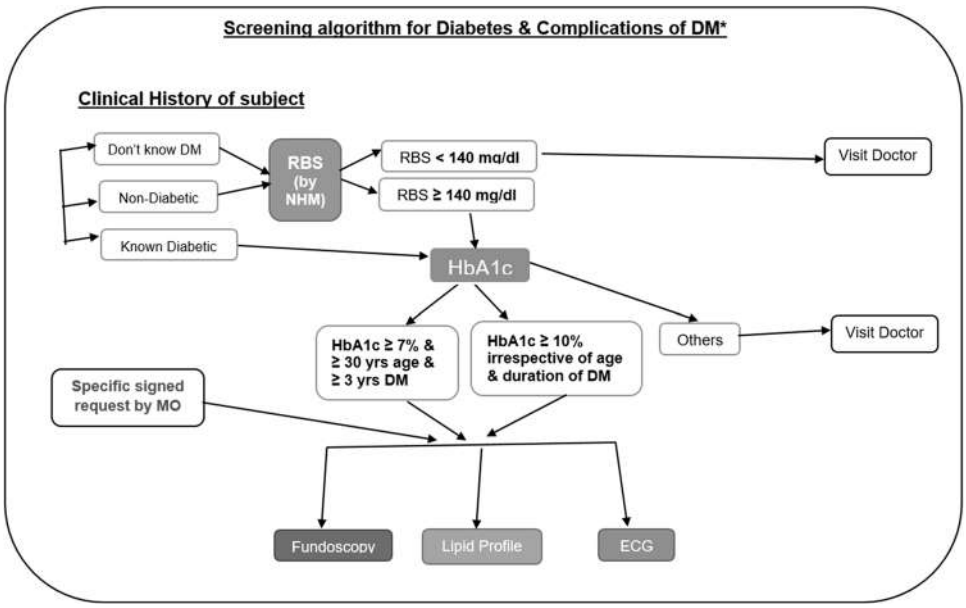

Fig. 1 Screening algorithm for diabetes and complications of DM. RBS, random blood sugar; HbA_1c, glycosylated haemoglobin; ECG, electrocardiogram; *DM*, diabetes mellitus

in recent times [15]. Hence, we have made the HbA1c levels into four categories such as 'Normal (< 5.7%), Good Control/Pre-diabetic (5.7–6.9%), Hyperglycemia (7–8.5%) and Marked Hyperglycemia (> 8.5%)'. A special category of 'Marked Hyperglycemia' was used to tag the severity level among follow-up subjects. If the HbA1c was ≥ 7% and based on other criteria of algorithm, the Medical Officer (MO) in the PHC/CHC recommended further testing with fundoscopy, ECG and lipid profile (Fig. 1).

Fundoscopy was performed using Bosch fundus camera and the image was shared immediately with the MO in the camp for an immediate counselling to the subject. However, a printed copy of the image with reporting by a qualified ophthalmologist was shared in courier to the health facility within 7 days for data updation in the NCD registers and further distribution to the subjects. As the focus of the study was limited to complications of diabetes, in our study, we labelled all the fundus abnormalities representing diabetic retinopathy as 'DR' and all other observations were labelled as 'Normal Fundus/Other than DR'. Electrocardiogram (ECG) was performed using a portable ECG machine and the printed report of ECG was handed over to the subject immediately after MO's review. Whole blood was collected for performing lipid profile. The sample was centrifuged using a portable centrifuge equipment brought by Novartis technicians and the sample was taken to the main lab for further processing. Results of lipid profile were shared in hard copy through courier to the health facility for updation in the NCD registers and further distribution to the subjects.

Results

A total of 24 secondary screening camps were conducted in the district during Jan 2019 to Mar 2020 through Novartis collaboration. Of them, 7 were in Urban and 17 were conducted in rural settings between Jun 2019 and Mar 2020. The 7 urban camps conducted at District Hospital, Kamareddy, were not considered for further analysis, as the subjects attended these camps were not through the referral mechanism and they were incidental outpatients visiting the hospital on the day of the camp.

Through the 17 rural camps, approximately 208,685 people were screened in the field through government health system and 24,965 (12.0%) subjects were found to have abnormal RBS and were referred to the camps. Camp-wise data is reflected in Table 1 which reflects that in 4 locations, the camps were conducted twice and in 9 locations, camp was conducted once only in the given period. One-tenth (9.9%; 2469/24965) of the referred could reach the camp on specified date and location. Among them, 92.6% (2287/2469) could actually utilize the HbA1c testing. Number of HbA1c tests conducted in one camp ranged from 50 to 295 (Table 1).

Among those utilizing the screening camps, 56.4% (1291/2287) were males and 43.6% (996/2287) were females. Of them, 1022 were known Diabetic subjects and 1265 did not know their DM status or were known non-diabetic. Age of the subjects ranged from 6 to 88 years, while majority (49%) of the subjects belonged to 40–59-year age group (Table 2). As the age increased, there was

Table 1 Camp-wise details of subjects utilizing the secondary screening camps

S. No	Camp date	Hospital	Population	No. of screened in the field	Abnormal RBS	Reached NCD camps	HbA_1c	HbA_1c (≥7%)	Fundo scopy	Abnormal fundoscopy	Lipid profile	ECG
1	6-Jun-2019	CHC Pitlam	36,575	5002	512	250	249	104	13	2	85	50
2	20-Jun-2019	PHC Bhiknoor	35,936	3679	580	315	295	118	12	0	160	90
3	27-Jun-2019	PHC Yellareddy	44,217	5252	551	90	90	44	50	7	65	36
4	11-Jul-2019	PHC Machareddy	35,952	5328	462	179	130	73	18	0	100	80
5	1-Aug-2019	PHC Sadashivnagar	27,220	10,167	1128	69	50	19	27	7	69	0
6	8-Aug-2019	PHC Bichkunda	65,625	11,375	2624	125	107	38	35	1	88	55
7	12-Sep-2019	PHC Dongli	61,977	21,575	2128	153	153	47	31	3	72	65
8	26-Sep-2019	PHC Birkur	50,112	19,264	1665	80	80	7	17	3	43	16
9	22-Oct-2019	PHC Ramareddy	28,250	12,554	764	92	80	23	7	1	62	48
10	5-Nov-2019	PHC Bhiknoor*	-	12,008	1783	125	118	17	53	1	102	90
11	19-Nov-2019	CHC Gandhari	62,084	10,428	2092	145	137	5	37	0	124	99
12	19-Dec-2019	PHC Jukkal	53,630	20,540	1459	172	156	23	50	3	106	80
13	17-Dec-2019	PHC Machareddy*	-	8968	1392	116	108	15	23	10	77	53
14	27-Jan-2020	PHC Bibipet	62,578	21,194	2025	180	174	65	36	10	137	77
15	4-Feb-2020	CHC Gandhari*	-	13,163	2092	137	137	63	37	8	126	76
16	18-Feb-2020	PHC Mathmal	44,217	16,285	1084	111	93	52	46	8	83	59
17	3-Mar-2020	CHC Bichkunda*	-	11,903	2624	130	130	76	53	11	72	68
		Total	608,373	208,685	24,965	2,469	2287	789	545	75	1571	1042

*Screening camps repeated in these locations. Hence, population catered by the health facility for second camp was not considered in total population covered

Table 2 Demographics of study subjects and the screening tests offered

Variable	Gender		Total	Pearson X^2 statistic and p-value
	Female	Male		
	n (row %)	*n* (row %)	*n*	
Total	996 (43.6%)	1291 (56.4%)	2287	
Age group (years)				
0–19	3 (50.0%)	3 (50.0%)	6	40.679
20–39	131 (53.0%)	116 (47.0%)	247	$p < 0.001$
40–59	533 (47.9%)	580 (52.1%)	1113	
60–79	321 (35.8%)	576 (64.2%)	897	
≥ 80	8 (33.3%)	16 (66.7%)	24	
Diabetes status prior to camp				
Known diabetic	395 (38.6%)	627 (61.4%)	1022	18.051
Unknown status/non-diabetic	601 (47.5%)	664 (52.5%)	1265	$p < 0.001$
HbA_1c results				
Normal (< 5.7%)	311 (48.4%)	331 (51.6%)	642	9.297
Good control (5.7–6.9%)	364 (42.5%)	492 (57.5%)	856	$p = 0.026$
Hyperglycemia (7–8.5%)	240 (40.5%)	353 (59.5%)	593	
Marked hyperglycemia (> 8.5%)	81 (41.3%)	115 (58.7%)	196	
Lipid profile	686 (43.7%)	885 (56.3%)	1571	NA
ECG	461 (44.1%)	583 (55.9%)	1044	NA
Fundoscopy	250 (45.7%)	297 (54.3%)	547	NA

ECG Electrocardiogram

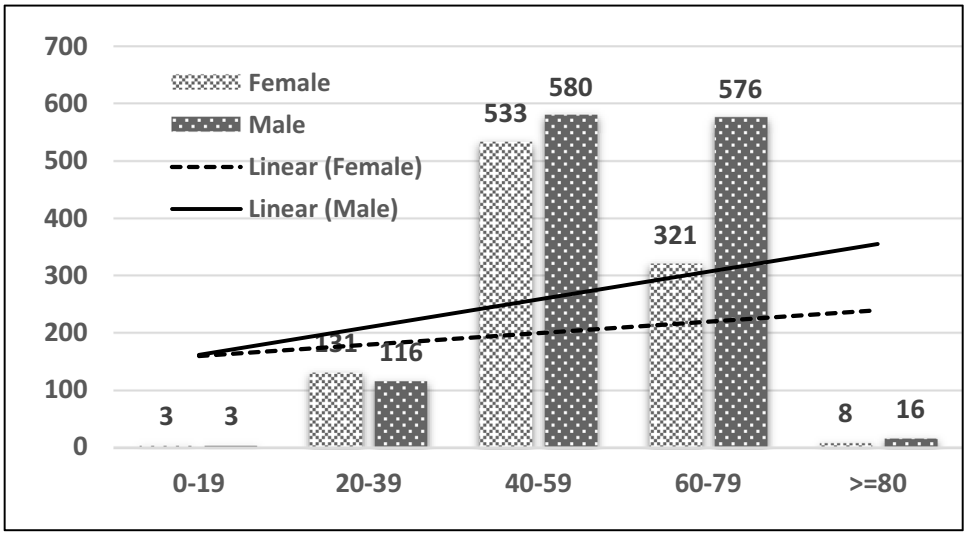

Fig. 2 Age distribution of the subjects by gender

significant higher utilization of screening camps by men compared to similar age group of women ($p < 0.001$) (Fig. 2 and Table 2).

The mean and standard deviation of HbA1c levels of the study population were 6.53% and 1.41%, respectively. The abnormal HbA1c results were significantly higher among men compared to women ($p < 0.026$). More than 63% of the known Diabetic subjects had HbA1c ≥ 7% that indicates the treatment adherence needs to be strengthened ($p < 0.001$). Nearly one-third (34%) had HbA1c levels ≥ 7% (Fig. 3).

Higher proportion of subjects with high HbA1c level were found among known Diabetics compared to the subjects without prior diabetes or unknown status ($p < 0.001$) (Table 3 and Fig. 4).

Of 2287 total cases, ECG was provided to 1042 (45.6%) and lipid profile to 1571 (68.7%) subjects as per screening algorithm (Table 2). However, data related to lipid profile

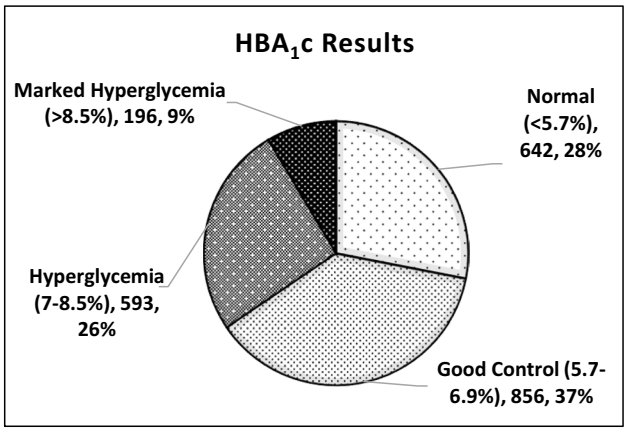

Fig. 3 HbA_1c results

and ECG could not be assessed further due to gaps in data recording as the primary focus was on complications of diabetes and the reports were not immediately available. Among 956 subjects with HbA1c ≥ 7%, 547 (58%) could have fundoscopy test during the camps of which 75 (13.7%) had fundoscopy results reflecting DR indicating need for regular screening for DR (Table 4). There was no significant difference in fundus abnormalities across gender. Fundus abnormalities did not differ significantly across known or unknown status of diabetes. However, abnormal fundus status has significantly increased with the increase in age of the subject between 20 and 80 years age (Table 4).

Discussion

Diabetes is one the major health concerns in India contributing to preventable blindness in India. It is estimated that the number of people with DM is likely to reach 79.4 million in numbers by 2030 while those with DR to reach 22.4 million [16]. Rural India has seen less prevalence of DM (10%) and DR (12%) compared to urban India, but never the less to be ignored as majority of Indian population (70%) resides in rural India [17, 18]. A population-based study from rural India demonstrated an abysmally low confirmation of DM in the study population due to low 'risk perception'. The study also suggested for community-focused risk communication interventions to increase the uptake of DM confirmatory tests [19]. A study on population-based study from India reported < 1% uptake of confirmatory tests by the DM high-risk patients identified through screening

Table 3 HbA_1c results versus DM status before testing in the camp

	Normal (< 5.7%)	Good control/pre-diabetic (5.7–6.9%)	Hyperglycemia (7–8.5%)	Marked hyperglycemia (> 8.5%)	Total	Pearson X^2 statistic and p-value
DM status not known	586	536	128	15	1265	807.45
Known diabetic	56	320	465	181	1022	$p < 0.0001$
Total	642	856	593	196	2287	

DM Diabetes mellitus

Fig. 4 HbA_1c results versus DM status before testing in the camp

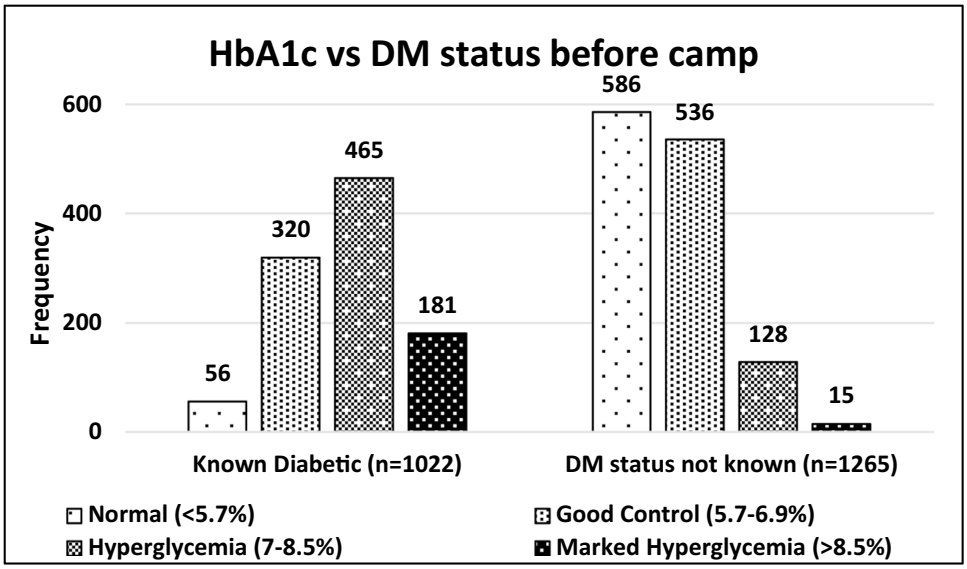

Table 4 Fundoscopy observations across age, gender and DM Status

	Diabetic retinopathy ($n=75$)	Normal fundus/other than DR ($n=472$)	Total ($n=547$)	Pearson X^2 statistic and p-value
Age group (years)				21.729
0–19	0	2	2	$\boldsymbol{p<0.001^{\#}}$
20–39	4	57	61	
40–59	21	234	255	
60–79	49	175	224	
≥ 80	1	4	5	
Gender				1.139
Female	30	220	250	$\boldsymbol{p=0.286}$
Male	45	252	297	
DM Status				1.797
Known diabetic	38	278	316	$\boldsymbol{p=0.180}$
DM status unknown	37	194	231	

DR, Diabetic retinopathy; *DM*, diabetes mellitus; $^{\#}X^2$ test performed only for 3 age groups, 20–39, 40–59 and 60–79 years, as both the extreme age groups have very minimal frequency of study subjects

[20]. However, few other studies reported varied uptake of confirmatory tests ranging from 28 to 50% [21–23]. In our study, we observed the uptake of HbA1c as confirmatory was at 9.2% (2287/24965).

Our analysis of 2287 rural population of Kamareddy district of Telangana State through 17 focused screening camps had observed that 34.5% of the subjects screened (789/2287) had HbA1c ≥ 7%. Higher proportion of these cases can be attributed to specific referral mechanism where the rural population with higher RBS value (> 140 mg/dl) was mobilized to these screening camps. However, it is interesting to find that of those with HbA1c ≥ 7%, only 18% (143/789) were either not aware of their DM status or they were found to be non-diabetic earlier, while 82% (646/789) were known Diabetic subjects. It implies that the blood sugar maintenance in the known diabetic needs a drastic improvement in those who are prone for all complications of DM unless the treatment adherence is achieved through proper counselling and management.

Diabetic retinopathy is a preventable complication if detected early among the patients with diabetes. According to a study on knowledge about DM and DR in rural India, 49.9% subjects had knowledge of DM and only 37.1% about DR. Knowledge and belief about controlling blood sugar that can avoid DR were limited to only 36.5% [24]. A study from Myanmar observed that 34% patients decided to visit an ophthalmologist for eye check-up when they had any problems with eye sight [25]. Lack of awareness about DR was the prime reason for the delay besides other factors such as poor doctor-patient communication, cultural beliefs, poor literacy and misconceptions about treatment [25, 26].

Our analysis also observed that around 14% (75/547) had abnormal retina in fundoscopy testing. Interestingly the abnormalities in fundus were seen in higher proportions in those without prior DM diagnosis 16% (37/231) compared to those in known DM cases 12% (38/316). This result implies that there is higher proportion of abnormal fundus cases seen even before the subjects are identified with diabetes. In line with general expectations, with age, the abnormal fundus results have increased from 0% (0/2) in 0–19 years age group, 6.6% (4/61) in 20–39 years, 8.2% (21/255) in 40–59 years, 22% (49/224) in 60–79 years and 20% (1/5) in subjects with > 80 years age group. However, interestingly, there was no gender difference in fundus results with 12% (30/250) abnormality in females and 15% (45/297) in males.

Some of the strengths of our study are that, firstly, for the first time in Telangana State, the secondary screening support provided through a non-financial collaboration was effectively integrated into the population-based screening program of DM as part of NPCDCS effectively. Secondly, a structured planning of schedule of the camps was done and the same was informed to population-based screening teams so that the referrals to the confirmatory tests on scheduled dates and locations are successfully completed. Thirdly, the report of confirmatory test for DM was instantaneous which helped the MO in the facility to take immediate necessary actions in terms of counselling and treatment modifications besides identifying candidates for further testing related to complications of DM. Results of fundoscopy were also instantaneous through images which helped the MO for providing further counselling and referral to higher centres for further management.

Limitations

One major limitation in our study is that only 58% of subjects with HbA1c ≥ 7% could utilize fundoscopy due to algorithm-based screening. Secondly, data on results of lipid profile and ECG could not be used effectively in identifying the other complications as the primary focus on complications of diabetes. Thirdly, only a tenth of the referred population have actually reached the camps on specified location on specified date, which reflects the need of regularizing the tests provided through camps at these peripheral locations.

Conclusions

The observations suggest that there is high probability of undetected diabetes cases with or without complications in the field, which might be missed during routine population-based screening, as the uptake of confirmatory test after referral from field is only 9.2%. Our results indicate the abnormality in fundus due to DM is higher (13.7%) than generally expected which emphasizes the need for structured counselling on treatment adherence to those who are already diagnosed with DM towards prevention and early detection of diabetic retinopathy.

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Declarations

Ethics approval Since all the provided services were part of implementation of the national program, NPCDCS, and no therapeutic or clinical interventions were made in the study, there was no breach of ethical issues of the subjects who were part of the analysis.

Conflict of interest There is no conflict of interest declared as the screening camps arranged by Novartis were as per the non-financial MoU between NHM, GoTS and Novartis and as per mutually agreed screening camps schedule and algorithm. Novartis has not collected/gathered any individual/personalized data from the screening camps. Novartis support limited to the arrangement of camps only. Data collection, analysis and further intervention based on the screening camps results are solely are owned by government, DM&HO Kamareddy, GoTS.

References

1. National Program on Prevention and Control of non-communicable diseases. Available on https://dghs.gov.in/content/1363_3_NationalProgrammePreventionControl.aspx. Accessed 15 Jul 2020.
2. National Multisectoral Action Plan (NMAP) for prevention and control of common NCDs (2017–22), revised in October 2017, supported by WHO Country Office for India
3. Training module for medical officers for prevention, control and population level screening of Hypertension, Diabetes and common cancers (Oral, Breast & Cervical) developed by national program for prevention and control of cancer, diabetes, cardiovascular diseases and stroke, Government of India. 2017
4. Operational guidelines for non-communicable diseases (NCDs), Pilot Project in Siricilla district of Telangana State developed by Department of Public Health and Family Welfare in collaboration with Novartis Healthcare Private Limited, October 2017
5. Demographic profile of Kamareddy district. Available on https://kamareddy.telangana.gov.in/demography/. Accessed 15 Jul 2020.
6. Yau JW, Rogers SL, Kawasaki R, et al. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012;35:556–64.
7. Harris MI, Klein R, Welborn TA, Knuiman MW. Onset of NIDDM occurs at least 4–7 yr before clinical diagnosis. Diabetes Care. 1992;15:815–9.
8. Dandona L, Dandona R, Naduvilath TJ, McCarty CA, Rao GN. Population based assessment of diabetic retinopathy in an urban population in Southern India. Br J Ophthalmol. 1999;83:937–40.
9. Rema M, Premkumar S, Anitha B, Deepa R, Pradeepa R, Mohan V. Prevalence of diabetic retinopathy in urban India: the Chennai urban rural epidemiology study (CURES) eye study. I Invest Ophthalmol Vis Sci. 2005;46:2328–33.
10. Narendran V, John RK, Raghuram A, Ravindran RD, Nirmalan PK, Thulasiraj RD. Diabetic retinopathy among self reported diabetics in Southern India: a population based assessment. Br J Ophthalmol. 2002;86:1014–8.
11. Agarwal RP, Ranka M, Beniwal R, et al. Prevalence of diabetic retinopathy in type 2 diabetics in relation to risk factors: hospital based study. Int J Diabetes Dev Countries. 2003;23:16–9.
12. American Diabetes Association. Abridged standards of care-2021: Clinical diabetes journals.org. Volume 39, Number 1, winter 2021. Page no. 23, 39
13. NICE. Type 2 diabetes in adults: management; 2016. https://www.nice.org.uk/guidance/ng28/chapter/1- recommendations. [Accessed 9 Feb 2017]
14. Kumar PR, Bhansali A, Ravikiran M, Bhansali S, Dutta P, Thakur JS, et al. Utility of glycated hemoglobin in diagnosing type 2 diabetes mellitus: a community-based study. J Clin Endocrinol Metab. 2010;95:2832–5 [PubMed] [Google Scholar].
15. Chawla R, Madhu SV, Makkar BM, Ghosh S, Saboo B, Kalra S, On behalf of the RSSDI-ESI Consensus Group*. RSSDI-ESI clinical practice recommendations for the management of type 2 diabetes mellitus 2020. Indian J Endocrinol Metab. 2020;24(1):1–122. https://doi.org/10.4103/ijem.IJEM_225_20.
16. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87:4–14.
17. Namperumalsamy P, Kim R, Vignesh TP, et al. Prevalence and risk factors for diabetic retinopathy: a population-based assessment from Theni district. South India Br J Ophthalmol. 2009;93:429–34.
18 Raman R, Ganesan S, Pal SS, Kulothungan V, Sharma T. Prevalence and risk factors for diabetic retinopathy in rural India. Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study III (SN-DREAMS III), report no 2. BMJ Open Diabetes Res Care. 2014;2:e000005.
19. Venkateshmurthy NS, Soundappan K, Gummidi B, et al. Are people at high risk for diabetes visiting health facility for confirmation of diagnosis? A population-based study from rural India. Glob Health Action. 2018;11:1416744.

20. Dyavarishetty P, Kowli S. Population based screening for diabetes: experience in Mumbai slums, Maharashtra. India Int J Res Med Sci. 2016;4:2766–9.
21. Vivekanand RV, Chintakayala G, Kumar P, Chatla C. Early detection of Diabetic Retinopathy through active screening of out-patients at ESI facility in Hyderabad. Natl J Res Community Med. 2020;9(2):090–5.
22. Venugopal V, Selvaraj K, Majumdar A, et al. Opportunistic screening for diabetes mellitus among adults attending a primary health center in Puducherry. Int J Med Sci Public Health. 2015;4:1206.
23 Shewade H, Palanivel C, Balamurugesan K, et al. Feasibility of opportunistic screening for type 2 diabetes mellitus: need for interventions to improve follow up. J Soc Health Diabetes. 2015;3:43.
24. Rani PK, Raman R, Subramani S, Perumal G, Kumaramanickavel G, Sharma T. Knowledge of diabetes and diabetic retinopathy among rural populations in India, and the influence of knowledge of diabetic retinopathy on attitude and practice. Rural and Remote Health. 2008;8(3):838. https://doi.org/10.22605/RRH838.
25. Huang OS, Zheng Y, Tay WT, Chiang PP, Lamoureux EL, Wong TY. Lack of awareness of common eye conditions in the community. Ophthalmic Epidemiol. 2013;20(1):52–60.
26 Lewis K, Patel D, Yorston D, Charteris D. A qualitative study in the United Kingdom of factors influencing attendance by patients with diabetes at ophthalmic outpatient clinics. Ophthalmic Epidemiol. 2007;14(6):375–80.

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ORIGINAL ARTICLE

Haptoglobin genotypes and risk of vascular complications in a northern Chinese Han population with type 2 diabetes mellitus

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Abstract

Background Vasculopathy is an important complication of type 2 diabetes mellitus (T2DM) and the leading cause of morbidity and mortality in type 2 diabetes mellitus (T2DM), which is closely related to oxidative stress and inflammation. Haptoglobin (Hp), an acute-phase protein, binds to hemoglobin (Hb) and prevents haeme-iron oxidative damage.

Objective To investigate the distribution of the frequencies of Hp genotypes and to analyse the association between Hp gene polymorphisms and vascular complications in diabetes patients.

Methods The study included 171 patients with T2DM without vascular complications and 797 patients with T2DM with vascular complications. The clinical characteristics of the studied population were recorded. Hp genotyping was performed using polymerase chain reaction–sequence specific primers (PCR-SSP).

Results Our results showed that the frequencies of Hp genotypes in all 968 T2DM patients were as follows: Hp1-1, 4.98%; Hp2-1, 38.07%; and Hp2-2, 56.95%. Although there was no significant difference in the Hp allele or genotype frequencies among individuals with vascular complications in the T2DM groups and controls regardless of gender, we found that the distribution of the Hp2-2 genotype and allele frequency was higher in males with diabetic retinopathy than in the control group ($p < 0.05$), and Hp2-2 had higher odds ratio (OR) for DR in univariate analysis (OR 3.760, [95% confidence interval [CI] (1.374–10.286)], $p = 0.010$). In multivariate logistic regression analysis, Hp2-2 genotype was used as a predictor of increased DR risk; after adjusting for major risk factors, such as DC, TG, and TC, the increased risk of DR predicted by the Hp2-2 genotype could not be estimated.

Conclusions The Hp2-2 genotype may be associated with an increased risk of male diabetic retinopathy with T2DM in the northern Chinese Han population.

Keywords Haptoglobin · Haptoglobin genotypes · Type 2 diabetes mellitus · Diabetic retinopathy

Introduction

Diabetes mellitus is a common chronic clinical syndrome that is characterized by a disorder in blood glucose metabolism, resulting from a complex inheritance-environment interaction [1]. The eighth edition of the global diabetes map released by International Diabetes Federation in 2017 showed that there were 451 million adults with diabetes worldwide, and the figure may reach 700 million by 2045 [2]. According to its pathogenesis, diabetes mellitus can be divided into four categories: type 1 diabetes (T1DM), type 2 diabetes (T2DM), gestational diabetes (GDM), and special type diabetes [3]. China has the largest diabetes population and one of the most dramatic rises in diabetes prevalence in the world. The main type of diabetes in China is T2DM, accounting for more than 90.0% [4]. Diabetic vascular complications are divided into micro- and macrovascular complications. Microvascular complications include diabetic retinopathy (DR) and diabetic nephropathy (DN), and macrovascular complications include cardiovascular disease (CVD)

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[5, 6]. The pathogenesis of T2DM and diabetic vascular complications have been associated with inflammation and oxidative stress [7, 8].

Haptoglobin (Hp) is an acute phase protein produced and released by the liver. The human Hp gene is located on chromosome 16q22 and is characterized by two alleles: Hp1 and Hp2 [9]. The Hp1 allele is conserved among species. The Hp2 allele is the result of a small intragenic duplication within the fourth and second introns of two Hp 1 genes [10]. The two common alleles for Hp1 and Hp2 result in three common phenotypes (Hp1-1, Hp2-2, and Hp2-1) in human populations and a rare allele deletion genotype, Hpdel. Hp binds specifically to hemoglobin (Hb), which is released into the circulation by hemolysis or normal red blood cell turnover, and then the Hp-Hb complexes are degraded by endocytosis mediated by the scavenger receptor CD163 on macrophages [11]. Therefore, Hp possesses antioxidative and anti-inflammatory properties. The distributions of Hp alleles and genotypes are ethnically and regionally different. In European Americans, the distribution of Hp allele frequencies is approximately 40% Hp1 and 60% Hp2 and for Hp genotypes is approximately 16% Hp1-Hp1, 48% Hp2-Hp1, and 36% Hp2-Hp2 [12]. In contrast, in Singapore, compared with a prevalence of 67% in Chinese individuals, the prevalence of the Hp2 allele is 83% in Indians [13]. Three common genotypes can be amplified by polymerase chain reaction–sequence specific primers (PC-SSP), followed by agarose electrophoresis for Hp phenotyping [14].

The development of vascular diseases accounts for the majority of morbidity and mortality in diabetes patients. Clinical and experimental data have demonstrated that Hp genotyping is an independent risk factor for diabetic vascular disease. Hp genotypes have been more consistent with cardiovascular complications in T1DM [13]. Kathryna et al. [11]found that the Hp1-1 genotype was shown to be associated to T2DM. Another study also showed that the Hp1-1 can serve as a genetic marker to regulate the possibility of developing diabetic complications in patients with T2DM [15]. Some previous studies found that the Hp2-2 was a genotypic risk factor for CVD in T2DM [16, 17]. The Hp2-2 was associated with increased risk of T2DM in the northern Chinese Han population [18]. However, another reported that Hp1-1 phenotype was associated with increased risk of incident AMI in Chinese T2DM patients [19]. Although the relationship between vascular complications and Hp has been reported in different populations, the results remain unclear. We describe our analysis of the association between Hp gene polymorphism and clinical parameters in a group of northern Chinese Han populations with T2DM.

Materials and methods

Patients

A total of 968 patients with T2DM, including 491 males and 477 females, were randomly selected from The Second Hospital of Shijiazhuang from August 2016 to June 2019. The average age of patients was 63.95 ± 8.76 years, and the average body mass index (BMI) was 25.54 ± 4.34. All patients met the 1999 WHO diagnostic criteria for diabetes: FPG $\geq$ 7.0 mmol/L, 2 hPG $\geq$ 11.1 mmol, or HbA1c $\geq$ 47.5 mmol/mol (6.5%). Diagnoses of diabetic retinopathy, diabetic nephropathy, and cardiovascular disease were performed by the relevant physicians and specialists according to Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (2013 Edition). The inclusion criteria were as follows: individuals with T2DM; 50 years of age or older. The exclusion criteria were as follows: immune vasculitis; acute cardiovascular and cerebrovascular events in the past 3 months; acute or chronic infection; and acute complications of diabetes.

Blood biochemical assessments

The basic demographic information, including age, sex, course of diabetes, height, and weight, were recorded for all 968 patients with T2DM. All participants fasted for 10 h, and peripheral blood was collected the next morning to detect the clinical biochemical indexes and for DNA extraction. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automated sphygmomanometer (HEM-1000, OMRON, Japan) after 5 min of rest in the sitting position. Fasting venous blood sample was analysed for estimation of fasting blood-glucose (FPG) by glucose oxidase peroxidase method. Serum was collected from remaining blood sample and was subjected to biochemical analyses by an automatic biochemical analyser (AU5800, Beckman Coulter, USA) and instrument-based reagents, including triglycerides (TG), total cholesterol (TC), α1 microglobulin (α1-M),β2 microglobulin (β2-M), blood urea nitrogen (BUN), serum creatinine (Cr), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). TC, TG, and BUN were measured using enzymatic techniques. α1-M andβ2-M were measured using immunoturbidimetry. Cr was measured using picric acid method. LDL-C and HDL-C were measured using direct method. C-peptide (CP) and fasting insulin (FIns) were measured using an electrochemiluminescence analyser (Cobas e 601, Roche, Swiss). Glycosylated hemoglobin (HbA1c) was measured

using high-performance liquid chromatography by an automated glycohemoglobin analyser (HLC-723G8, Tosoh Corporation, Japan). Hemoglobin (Hb) was measured using cyanide methemoglobin colorimetric by a blood cell analyser (DxH800, Beckman Coulter, USA). Urine microalbumin (mAlb) was measured using immunoturbidimetry (DCA Vantage2000, Siemens, Germany).

Hp genotyping

Genomic DNA isolation was performed in an automated nucleic acid extraction instrument (GeneRotex96, TIANLONG, China) using a nucleic acid extraction kit (ZTLYQY40, TIANLONG, China). Hp genotyping was performed using polymerase chain reaction–sequence specific primers (PCR-SSP) according to a report by Koch et al. [10]. The 20 μL reactions contained 20 ng of DNA, 1 μL of upstream primer and downstream primer (10 μM/L), 2X Rapid Taq Master Mix as suggested by the supplier (Vazyme, China), and ddH_2O to 20 μL. Primers A (5′-GAGGGGAGCTTGCCTTTCCATTG-3′) and B (5′-GAGATTTTTGAGCCCTGGCTGGT-3′) and primers C (5-CCTGCCTCGTATTAACTGCACCAT-3) and D (5-CCGAGTGCTCCACATAGCCATGT-3) were synthesized by Sangon Biotech. Primers A and B were used for amplification of the 1757-bp Hp1 allele–specific sequence and the 3481-bp Hp2 allele–specific sequence. Then, only the 349 bp Hp2 allele–specific sequence was amplified using primers C and D. The PCR procedure was as follows: initial denaturing at 95 °C for 2 min, followed by 33 cycles of 95 °C for 1 min, 69 °C for 2 min (primer A and B) or 1 min (primer C and D), followed by 7 min at 72 °C for a final extension. PCR products were observed and photographed in the imaging system after 1.5% agarose electrophoresis.

Sanger sequencing

After Hp phenotyping, the electrophoresis strips were cut off and the DNA was recovered by a recovery kit (Tiangen, China), and sent to Sangon Biotech (China) for first-generation sequencing. Forward sequencing was used, and then the gene sequences were compared with standard sequences for further verification.

Statistical analysis

All statistical analyses were performed using SPSS software (version 21.0, IBM, USA). The allele frequencies or genotype frequencies were compared using either the x^2 test or Fisher's exact test. For comparison of clinical and biochemical parameters among groups, Student's *t*-test was used for continuous variables, and Mann–Whitney test was used for non-parametric variables. All results are expressed as *n* or the mean ± standard deviation (SD) or median (range). A two-sided p value < 0.05 was considered significant. Initial diabetes risk factors including Hp genotyping variables were assessed in univariate analysis, and then multivariate logistic regression was used to analyse the major risk factors for DR.

Results

Hp genotyping based on PCR

We determined the Hp genotypes of 968 T2DM samples by PCR-SSP. The three phenotypes (Hp1-1, Hp2-1, and Hp2-2) were easily distinguished by representative bands (Fig. 1). Then, we confirmed that the target fragments obtained by PCR were Hp1 and Hp2 through first-generation sequencing. The obviously redundant bands of approximately 1 kbp and 2 kbp obtained by primer A and B amplification of genomic DNA were confirmed to be the product of separate amplification of primer B and haptoglobin-related protein precursor (HPR), respectively, through Primer-BLAST (PubMed) and first-generation sequencing.

Association between Hp genotypes and vascular complications in patients with T2DM

We examined the association between Hp genotypes and vascular complications in patients with T2DM. The genotypic

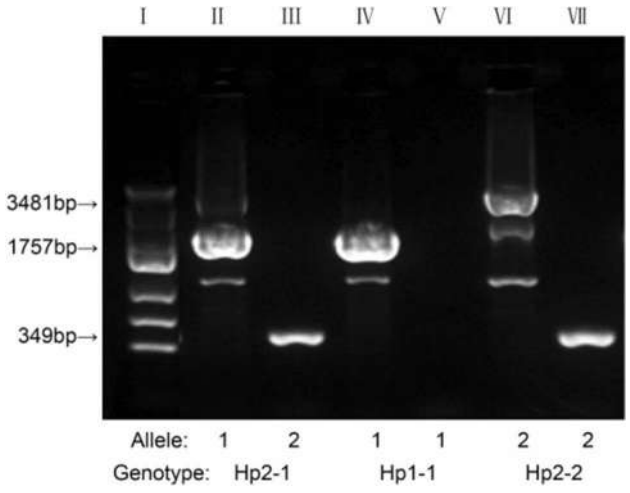

Fig. 1 Haptoglobin genotyping. The agarose gel indicated the genotypes of three individuals representing Hp1-1, Hp2-1, and Hp2-2, respectively, Lane I, DNA marker III (Biosharp); the reactions in lanes II, IV, VI contain DNA products from the synthesis of primers A and B of three individuals, respectively; the reactions in lanes III, V, and VII contain DNA products from the synthesis of primers C and D of three individuals, respectively; lanes II and III, genotype Hp2-1; lanes IV and V, genotype HP1-1; lanes VI and VII, genotype Hp2-2

distributions were consistent with Hardy–Weinberg proportions in each group. We divided 968 patients into six groups: T2DM without vascular complications group (control); type 2 diabetes mellitus with diabetic nephropathy group (DN); type 2 diabetes mellitus with diabetic retinopathy group (DR); type 2 diabetes mellitus with cardiovascular diseases group (CVD); type 2 diabetes mellitus with diabetic nephropathy and retinopathy (DN + DR) group; and type 2 diabetes mellitus with diabetic nephropathy, retinopathy, and cardiovascular diseases group (DN + DR + CVD). The Hp allele frequencies or genotype frequencies among each group and control group were not significantly different ($p > 0.05$) (Table 1). In our study, microvascular complications included DR and DN, and macrovascular complications included CVD. Hpdels were detected in only very few cases in several groups.

Clinical characteristics of all the patients by Hp genotypes

Table 2 includes the clinical characteristics for the samples and for each HP genotype group. For all participants, 964 individuals carried at least one Hp1 or Hp2 genotype, and 4 individuals carried the Hpdel allele. Among the patients without the Hpdel allele, the common Hp genotypes obtained in 964 individuals with their distribution were as follows: Hp1-1 48 (4.98%), Hp2-1 367 (38.07%), and Hp2-2 549 (56.95%). The common Hp genotypes were in Hardy–Weinberg equilibrium. There were significant differences among the different common Hp genotypes: the mean age was 63.95 ± 8.76 years (Hp1-1: 64.00 ± 9.58 years, Hp2-1: 63.51 ± 12.61 years, and Hp2-2: 61.56 ± 13.56 years; $p < 0.035$), the mean TG was 21.2 ± 2.43 (Hp1-1: 3.11 ± 6.48 mmol/L, Hp2-1: 2.02 ± 2.44 mmol/L, and Hp2-2: 2.10 ± 1.63 mmol/L; $p < 0.034$), and the mean LDL-C was 2.83 ± 1.82 (Hp1-1: 3.02 ± 1.04 μg/mL, Hp2-1: 2.82 ± 2.70 μg/mL, and Hp2-2: 2.82 ± 0.96 μg/mL; $p < 0.045$), with the trend that Hp2-2 was significantly lower than Hp1-1; the mean BMI was 25.45 ± 3.58 (Hp1-1: 23.80 ± 2.90 kg/m^2, Hp2-1: 25.00 ± 3.32 kg/m^2, and Hp2-2: 25.88 ± 3.68 kg/m^2; $p < 0.007$), the mean Hb was 132.13 ± 22.86 (Hp1-1: 128.89 ± 25.33 g/L, Hp2-1: 131.56 ± 18.59 g/L, and Hp2-2: 132.72 ± 25.16 g/L; $p < 0.009$), the mean α1-M was 4.52 ± 7.09 (Hp1-1: 4.12 ± 4.99 μg/mL, Hp2-1: 4.16 ± 7.51 μg/mL, and Hp2-2: 4.83 ± 6.96 μg/mL; $p < 0.044$), and the mean β2-M was 0.59 ± 2.19 (Hp1-1: 0.25 ± 0.51 μg/mL, Hp2-1: 0.50 ± 1.46 μg/mL, and Hp2-2: 0.69 ± 2.66 μg/mL; $p < 0.036$), with the trend that Hp2-2 was significantly greater than Hp1-1. The groups of different Hp genotype carriers showed no significant difference in gender, course of diabetes, blood pressure, FBP, HbA1c, FIns, C-peptide, TC, HDL-C, mAlb, Cr, or BUN ($p > 0.05$).

Association between common Hp genotypes and vascular complications in male patients with T2DM

We further investigated the correlation between Hp genotypes and vascular complications in different genders with T2DM (Table 3). The genotypic distribution was consistent with Hardy–Weinberg equilibrium in each group. We divided all participants into six groups as shown in Table 1 for males and females. Only the Hp genotype and allele frequencies among male DR and male control were significantly different ($p < 0.05$). The frequencies of Hp genotypes in the group of male DR and male control were as follows: Hp1-1, 3.12% and 0.00%; Hp2-1, 36.46% and 14.71%; Hp2-2, 59.38% and 85.29%, respectively.

Comparison of clinical characteristics between male T2DM patients with DR and T2DM non-DR controls

We included 96 male T2DM patients without DR and 34 male T2DM patients with DR for this analysis. The

Table 1 Distribution of Hp genotype and allele frequency in T2DM patients among each group

Group	*n*	Allele [*n*(%)]			Genotype [*n*(%)]				*p* value	
		Hp1	Hp2	Hpdel	Hp1-1	Hp2-1	Hp2-2	Hpdel	Allele	Genotype
Control	171	78 (22.81)	260 (76.02)	4 (1.17)	9 (5.26)	60 (35.09)	100 (58.48)	2 (1.17)		
DN	148	70 (23.65)	226 (76.35)		8 (5.40)	54 (36.49)	86 (58.11)		0.925	0.981
DR	74	27 (18.24)	121 (81.76)		2 (2.70)	23 (31.08)	49 (66.22)		0.281	0.536
DN + DR	143	75 (26.22)	211 (73.78)		8 (5.59)	59 (41.26)	76 (53.15)		0.401	0.560
CVD	117	58 (24.79)	172 (73.50)	4 (1.71)	7 (5.98)	44 (37.61)	64 (54.70)	2 (1.71)	0.617	0.828
DN + DR + CVD	315	155 (24.60)	475 (75.40)		14 (4.44)	127 (40.32)	174 (55.24)		0.636	0.545

Control, type 2 diabetes mellitus without vascular complications group; *DN*, type 2 diabetes mellitus with diabetic nephropathy group; *DR*, type 2 diabetes mellitus with diabetic retinopathy group; *CVD*, type 2 diabetes mellitus with cardiovascular disease group. *p* values indicate the significance of differences among each group and Control. $p < 0.05$ are shown in bold

Table 2 Clinical and biochemical data among groups of Hp genotype in T2DM patients ($n=964$)

Variable	Total cohort	Hp genotype			*p* value
		Hp1-1 ($n=48$)	Hp2-1 ($n=367$)	Hp2-2 ($n=549$)	
Male/female	491/475	22/26	170/197	297/252	0.055
Age (years)	63.95 ± 8.76	64.00 ± 9.58	63.51 ± 12.61	61.56 ± 13.56	0.035
BMI (kg/m^2)	25.45 ± 3.58	23.80 ± 2.91	25.00 ± 3.32	25.88 ± 3.68	0.007
DC (years)	10 (0–40)	10 (1–23)	10 (0–40)	10 (1–40)	0.584
SBP (mmHg)	130 (75–210)	130 (100–180)	130 (88–210)	130 (75–180)	0.138
DBP (mmHg)	80 (55–120)	80 (60–112)	80 (55–110)	80 (60–120)	0.913
FPG (mmol/L)	8.83 ± 3.51	9.48 ± 4.60	8.54 ± 3.41	8.97 ± 3.47	0.091
HbA1c (%)	8.52 ± 3.43	8.73 ± 2.20	8.30 ± 1.96	8.64 ± 4.20	0.250
FIns (mU/L)	9.00 (0.20–813.90)	9.50 (2.52–163.60)	9.05 (1.36–813.90)	8.95 (0.20–783.40)	0.876
Hb (g/L)	132.13 ± 22.86	128.89 ± 25.33	131.56 ± 18.59	132.72 ± 25.16	0.009
CP (ng/mL)	2.37 ± 1.31	2.69 ± 1.35	2.36 ± 1.27	2.35 ± 1.34	0.214
TG (mmol/L)	2.12 ± 2.43	3.11 ± 6.48	2.02 ± 2.44	2.10 ± 1.63	0.034
TC (mmol/L)	4.60 ± 1.49	4.93 ± 1.89	4.46 ± 1.45	4.66 ± 1.48	0.112
α1-M (μg/mL)	4.52 ± 7.09	4.12 ± 4.99	4.16 ± 7.51	4.83 ± 6.96	0.044
β2-M (μg/mL)	0.59 ± 2.19	0.25 ± 0.51	0.50 ± 1.46	0.69 ± 2.66	0.036
HDL-C (mmol/L)	1.23 ± 0.33	1.22 ± 0.34	1.23 ± 0.35	1.23 ± 0.31	0.581
LDL-C (mmol/L)	2.83 ± 1.82	3.02 ± 1.04	2.82 ± 2.70	2.82 ± 0.96	0.045
mAIb (μg/mL)	11.04(0.03–506.00)	16.60(0.50–506.00)	10.00(0.20–330.40)	11.04(0.03–481.60)	0.319
Cr (μmol/L)	82.05 ± 31.82	87.07 ± 25.79	82.96 ± 33.73	81.09 ± 31.08	0.098
BUN (mmol/L)	5.82 ± 2.62	6.26 ± 2.57	5.83 ± 2.90	5.78 ± 2.43	0.181

Continuous data are presented as mean ± standard deviation or median (range),categorical data are presented as numbers (percentages). $p<0.05$ are shown in bold. *Hp,* haptoglobin; *BMI*, body mass index; *DC*, course of the disease; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *FPG*, fasting blood-glucose; *HBA1c*, glycosylated haemoglobin; *FIns*, fasting insulin; *Hb*, haemoglobin; *CP*, C-peptide; *TG*, triglycerides; *TC*, total cholesterol, *α1-M*, α1 microglobulin; *β2-M*, β2 microglobulin; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *mAlb*, urine microalbumin; *Cr*, serum creatinine; *BUN*, blood urea nitrogen. *p* values indicate the significance of differences among common Hp genotypes and clinical traits

clinical and anthropometric characteristics of all patients are described in Table 4. There was no significant difference in age, BMI, SBP, DBP, FPG, HbA1c, FIns, Hb, CP, α1-M, β2-M, HDL-C, LDL-C, mAlb, Cr, or BUN between male T2DM patients with or without DR. Patients with DR presented higher TC (4.89 ± 2.48, $p<0.0001$) levels and lower TG (1.55 ± 0.80, $p<0.0001$) levels, and a longer duration of diabetes (10 (3–30), $p<0.005$), than those without DR.

Risk factor analysis of DR

As determined by univariate logistic regression, patients with Hp2-2 were more likely to have DR than those with other genotypes (odds ratio (OR) 3.760, (95% confidence interval (CI) (1.374–10.286)), $p=0.010$). Among the established biochemical and clinical characteristics, DC, TG, and TC were the only independent predictors of DR. In multivariate regression analyses prepared for the Hp2-2 genotype as a predictor of an increased risk of DR, the significant association between Hp2-2 and DR identified in univariate testing did not exist in multivariate testing (OR 2.445 (95%CI (0.577–10.360)), $p=0.225$), as shown in Table 5.

Discussion

To our knowledge, there are few trials investigating the association between the Hp phenotypes and the risk of vascular complications with T2DM in the northern Chinese Han population. To date, the majority of studies have focused on investigating the association of the Hp level or Hp genotypes with diabetic macrovascular complications. Our study showed that the Hp2-2 genotype may be associated with an increased risk of diabetic retinopathy (DR) in male patients with T2DM.

First, we presented the genotype and allele distribution of the Hp gene (Hp2-2 56.95%, Hp2-1 38.07%, Hp1-1 4.98%) in participants, which is similar to the results of previous studies that there were significant differences in the genotypic and allelic distributions between the T2DM patient group (Hp2-2 51.7%, Hp2-1 39.7%, and Hp1-1 8.6%) and the nondiabetic control group (Hp2-2 44.1%, Hp2-1 45.1%, and Hp1-1 10.9%) in the northern Chinese Han population [18]. One study reported that individuals with the Hp2-2 phenotype are under greater oxidative stress [16]. Another study found that compared to controls, Hp2 allele carriers

Table 3 Distribution of Hp genotype and allele frequency in T2DM Patients among each group

Gender	Group	*n*	Allele [*n* (%)]			Genotype [*n* (%)]				*p* value	
			Hp1	Hp2	Hpdel	Hp1-1	Hp2-1	Hp2-2	Hpdel	Allele	Genotype
Male	Control	96	41 (21.36)	149 (77.60)	2 (1.04)	3 (3.12)	35 (36.46)	57 (59.38)	1 (1.04)		
	DN	76	34 (22.37)	118 (77.63)		5 (6.58)	24 (31.58)	47 (61.84)		0.896	0.499
	DR	34	5 (7.35)	63 (92.65)		0 (0.00)	5 (14.71)	29 (85.29)		**0.009**	**0.026**
	DN + DR	82	43 (26.22)	121 (73.78)		4 (4.88)	35 (42.68)	43 (52.44)		0.319	0.593
	CVD	51	17 (16.67)	83 (81.37)	2 (1.96)	2 (3.92)	13 (25.49)	35 (68.63)	1 (1.96)	0.440	0.415
	DN + DR + CVD	152	74 (24.34)	230 (75.66)		8 (5.26)	58 (38.16)	86 (56.58)		0.513	0.744
Female	Control	75	37 (24.67)	111 (74.00)	2 (1.33)	6 (8.00)	25 (33.33)	43 (57.34)	1 (1.33)		
	DN	72	36 (25.00)	108 (75.00)		3 (4.17)	30 (41.67)	39 (54.16)		1.000	0.439
	DR	40	22 (27.50)	58 (72.5)		2 (5.00)	18 (45.00)	20 (50.00)		0.752	0.502
	DN + DR	61	32 (26.23)	90 (73.77)		4 (6.56)	24 (39.34)	33 (54.10)		0.889	0.796
	CVD	66	41 (31.06)	89 (67.43)	2 (1.51)	5 (7.58)	31 (46.97)	29 (43.94)	1 (1.51)	0.232	0.243
	DN + DR + CVD	163	81 (24.85)	245 (75.15)		6 (3.68)	69 (42.33)	88 (53.99)		1.000	0.217

Control, type 2 diabetes mellitus without vascular complications group; *DN*, type 2 diabetes mellitus with diabetic nephropathy group; *DR*, type 2 diabetes mellitus with diabetic retinopathy group; *CVD*, type 2 diabetes mellitus with cardiovascular disease group. *p* values indicate the significance of differences among each group and control. $p < 0.05$ are shown in bold

contributed to higher Hp levels in T2DM [11]. Additionally, some studies have also reported an association between the Hp2-2 genotype and T2DM [16, 18, 19]. Moreover, the Hp2-2 phenotype is a genotypic risk factor for vascular complications in T2DM patients [20]. However, some studies failed to find any association between the Hp genotype and

Table 4 Clinical and biochemical data of male T2DM patients with and without retinopathy ($n = 130$)

Variable	Total cohort	Non-DR ($n = 96$)	DR ($n = 34$)	*p* value
Age (years)	60.70 ± 8.18	60.89 ± 8.28	60.18 ± 7.97	0.506
BMI (kg/m^2)	25.91 ± 2.77	25.85 ± 3.06	26.01 ± 2.27	0.501
DC (years)	8 (1–30)	7 (1–20)	10 (3–30)	0.003
SBP (mmHg)	130 (100–180)	130 (100–180)	130 (102–175)	0.680
DBP (mmHg)	80 (60–120)	80 (60–120)	80 (60–100)	0.211
FPG (mmol/L)	8.15 ± 3.32	8.01 ± 3.32	8.57 ± 3.34	0.157
HbA1c (%)	8.11 ± 2.16	8.00 ± 2.12	8.41 ± 2.27	0.264
FIns (mU/L)	8.64 (1.66–53.35)	8.14 (1.66–22.87)	9.29 (3.84–53.35)	0.198
Hb (g/L)	144.51 ± 15.07	144.77 ± 12.94	143.76 ± 20.21	0.987
CP (ng/mL)	2.35 ± 1.31	2.43 ± 1.44	2.10 ± 0.72	0.522
TG (mmol/L)	2.77 ± 1.84	3.21 ± 1.92	1.55 ± 0.80	0.000
TC (mmol/L)	3.45 ± 2.27	2.94 ± 1.97	4.89 ± 2.48	0.000
α1-M (μg/mL)	3.50 ± 3.60	3.63 ± 3.77	3.16 ± 3.13	0.311
β2-M (μg/mL)	0.54 ± 1.45	0.64 ± 1.65	0.25 ± 0.62	0.102
HDL-C (mmol/L)	1.13 ± 0.25	1.13 ± 0.22	1.12 ± 0.30	0.814
LDL-C (mmol/L)	2.74 ± 0.92	2.79 ± 0.91	2.68 ± 0.97	0.837
mAIb (μg/mL)	8.31 (0.5–35.16)	8.53 (0.50–35.16)	8.29 (0.50–24.90)	0.598
Cr (μmol/L)	83.28 ± 22.03	81.96 ± 12.90	87.05 ± 37.52	0.794
BUN (mmol/L)	5.55 ± 1.56	5.44 ± 1.45	5.87 ± 1.82	0.482

non-DR, male without vascular complications in type 2 diabetes mellitus; *DR*, male with diabetic retinopathy in type 2 diabetes mellitus. Data are presented as mean ± standard deviation, median (range) or percentages. *Hp*, haptoglobin; *BMI*, body mass index; *DC*, course of the disease; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *FPG*, fasting blood-glucose; *HBA1c*, glycosylated haemoglobin; *FIns*, fasting insulin; *Hb*, haemoglobin; *CP*, C-peptide; *TG*, triglycerides; *TC*, total cholesterol, *α1-M*, α1 microglobulin; *β2-M*, β2 microglobulin; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *mAlb*, urine microalbumin; *Cr*, serum creatinine; *BUN*, blood urea nitrogen. *p* values indicate the significance of differences among male with diabetic retinopathy in type 2 diabetes mellitus and male without vascular complications in type 2 diabetes mellitus. $p < 0.05$ are shown in bold

Table 5 Logistic regression analysis of relative factors for retinopathy

Variable	B	SE	Wald	OR	95C%I	*p* value
Hp genotype	0.894	0.737	1.472	2.445	0.577–10.360	0.225
DC	0.450	0.116	14.952	1.569	1.249–1.971	0.000
TG	− 1.187	0.349	11.580	0.305	0.154–0.604	0.001
TC	1.170	0.332	12.425	3.222	1.681–6.175	0.000

DC, course of the disease; *TG*, triglycerides; *TC*, total cholesterol

vascular complications in T2DM [21, 22]. In summary, the current data show conflicting results, and the correlation of the Hp genotypes and diabetes mellitus remains undetermined. Then, we subdivided the T2DM participants into control, DN, DR, DN + DR, CVD, and DN + DR + CVD. However, the Hp allele frequencies or genotype frequencies among each group and control still had no significant difference. Although our result was not expected, it shows the Hp genetic characteristics of the northern Chinese Han population with T2DM.

Then, we compared the clinical characteristics of all the patients among each Hp genotype group. Regardless of their younger age, those in the Hp2-2 genotype group had higher BMI, Hb, α1-M, and β2-M levels ($p < 0.05$). In addition, we found that the Hp2-2 genotype was associated with the levels of TG and LDL-C ($p < 0.05$), which are also risk factors for T2DM. To our knowledge, obesity, especially abdominal obesity, negatively affects glucose metabolism and is an independent risk factor for T2DM development [23]. Similarly, our study found that Hp2-2 were more obese than Hp1-1, which may be a risk for developmental complications. The Hp expressed by the Hp2 allele presents a lower ability to bind Hb and the Hp-Hb complex to the CD163 receptor in T2DM, and Alshiek et al. [14] have also shown that the clearance efficiency of the Hp2-2-Hb complex is lower than that of the Hp1-1-Hb complex, especially in DM, explaining our results that the Hp2-2 genotype is associated with higher levels of Hb. The increased amounts of Hp2-2-Hb complexes can specifically bind to ApoA1 on HDL particles, leading to oxidative modification and HDL dysfunction. HDL-C can mediate cholesterol outflow from macrophages, prevent the accumulation of cholesterol in the arterial wall, and transport cholesterol to the liver for metabolism [24]. Although our study did not find an association between the Hp genotype and cardiovascular events in type 2 diabetes, oxidative damage to HDL due to high levels of Hb and Hp2-2-Hb may explain the high risk of developing vascular complications in diabetic patients with Hp2-2 phenotype. The important risk factors for vascular diseases are high levels of TC, LDL-C, and TG; interestingly, our results show that the Hp2-2 genotype has lower LDL-C and TG levels than the Hp1-1 genotype, which means that the Hp1-1 genotype may also contribute to lipid metabolism in T2DM. Our results show increasing levels of α1-M and β2-M, suggesting that early Hp-susceptible cases of nephropathy may have been included. Taken together, these results emphasize that Hp1-1 and Hp2-2 may have their respective contributions in T2DM.

Bilkan et al. [25] believe that, in general, males are at a higher risk for diabetic microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) than females, while the consequences of macrovascular complications may be greater in females. Mogarekar et al. [26] reported that Hp2-2genotype is a risk factor of retinopathy in T2DM. Therefore, we further studied the correlation between the Hp genotype and vascular complications in male and female T2DM patients, respectively. We found that there was a significant difference in allele frequency and genotype distribution between male DR with T2DM and male T2DM controls, and the Hp2 allele frequency and Hp2-2 genotype were higher in the male DR group with T2DM ($p < 0.05$). In Scotland and the UK, male sex has been reported to be an independent risk factor for advanced DR in T2DM for progression of the disease [27, 28]. There is a growing body of evidence that oestrogens are generally considered to be cardioprotective and androgens are detrimental to cardiovascular health [25]. The principle steroidal androgens are testosterone and its metabolite 5α-dihydrotestosterone (DHT), while testosterone increases mitochondrial ROS generation in vascular smooth muscle cells [29]. Oxidative stress is implicated in the development and progression of diabetic retinopathy [30]. Therefore, the Hp2-2 genotype may increase the risk of DR by affecting androgens in males.

We further analysed the clinical and anthropometric characteristics and found that the male DR group had a longer course of disease ($p < 0.01$), higher TC ($p < 0.001$), and lower TG ($p < 0.001$) levels than the male control group. According to univariate logistic regression analysis, the male diabetics with Hp2-2 genotype had higher OR for the development of DR than those with Hp2-1or Hp1-1 genotype. In multivariate logistic regression analysis, Hp2-2 genotype was used as a predictor of increased DR risk; after adjusting for major risk factors, such as DC, TG, and TC, the increased risk of DR predicted by the Hp2-2 genotype could not be estimated. Therefore, Hp2-2 genotype is an additional risk factor of retinopathy in type 2 diabetes mellitus, and there may be other unconsidered factors that influence the role of Hp2-2 genotype in the development of male DR. Nevertheless, some limitations of this study must be acknowledged.

First, we did not analyse the effect of the serum Hp and sex hormone levels as a direct indicator in the case and control groups. Second, this study requires larger prospective studies for further investigation.

Conclusion

Considering the global prevalence of diabetes and vascular complications, few studies have examined or reported sex differences in vascular complications in diabetes. Our study is the first to report that the Hp2-2 genotype may be associated with an increased risk of male DR with T2DM in the northern Chinese Han population, suggesting that determination of Hp genotype may be helpful in the risk assessment and management of male DR. Furthermore, population-based prospective studies in a larger cohort are necessary to validate our findings in different ethnic groups.

Author contribution ZCL, SL, and QSH conceived and designed the research; PPZ, YXZ, HYG, MD, and WLZ conducted the research and wrote the manuscript and had primary responsibility for the final content of the manuscript; YCS, YS, JZ, SZN, and RL provided materials and performed statistical analyses. All authors read and approved the final manuscript.

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Declarations

Ethical approval The study was approved by The Second Hospital of Shijiazhuang Ethics Committee according to the Declaration of Helsinki. All participants signed an informed consent form.

Conflict of interest The authors declare no competing interests.

References

1. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014;11:1185–200.
2. Carracher AM, Marathe PH, Close KL. International Diabetes Federation 2017. J Diabetes. 2018;10:353–6.
3. Khodaeian M, Enayati S, Tabatabaei-Malazy O, Amoli MM. Association between genetic variants and diabetes mellitus in Iranian populations: a systematic review of observational studies. J Diabetes Res. 2015;2015:585917
4. Ma R. Epidemiology of diabetes and diabetic complications in China. Diabetologia. 2018;61:1249–60.
5. Hirakawa Y, Arima H, Zoungas S, Ninomiya T, Cooper M, Hamet P, et al. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: the ADVANCE trial. Diabet Care. 2014;37:2359–65.
6. Hata J, Arima H, Rothwell PM, Woodward M, Zoungas S, Anderson C, et al. Effects of visit-to-visit variability in systolic blood pressure on macrovascular and microvascular complications in patients with type 2 diabetes mellitus: the ADVANCE trial. Circulation. 2013;128:1325–34.
7. Grandl G, Wolfrum C. Hemostasis, endothelial stress, inflammation, and the metabolic syndrome. Semin Immunopathol. 2018;40:215–24.
8. Jha JC, Ho F, Dan C, Jandeleit-Dahm K. A causal link between oxidative stress and inflammation in cardiovascular and renal complications of diabetes. Clin Sci (Lond). 2018;132:1811–36.
9. Suarez-Sanchez F, Vazquez-Moreno M, Herrera-Lopez E, Gomez-Zamudio JH, Peralta-Romero JJ, Castelan-Martinez OD, et al. Association of rs2000999 in the haptoglobin gene with total cholesterol, HDL-C, and LDL-C levels in Mexican type 2 diabetes patients. Medicine (Baltimore). 2019;98:e17298
10. Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schomig A, et al. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clin Chem. 2002;48:1377–82.
11. Rodrigues KF, Pietrani NT, Carvalho L, Bosco AA, Sandrim VC, Ferreira CN, et al. Haptoglobin levels are influenced by Hp1-Hp2 polymorphism, obesity, inflammation, and hypertension in type 2 diabetes mellitus. Endocrinol Diabetes Nutr. 2019;66:99–107.
12. Carter K, Worwood M. Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases. Int J Lab Hematol. 2007;29:92–110.
13. Dalan R, Liuh LG. The protean role of haptoglobin and haptoglobin genotypes on vascular complications in diabetes mellitus. Eur J Prev Cardiol. 2018;25:1502–19.
14. Alshiek JA, Dayan L, Asleh R, Blum S, Levy AP, Jacob G. Antioxidative treatment with vitamin E improves peripheral vascular function in patients with diabetes mellitus and Haptoglobin 2–2 genotype: a double-blinded cross-over study. Diabetes Res Clin Pract. 2017;131:200–7.
15. Stempkowska A, Walicka M, Franek E, Naruszewicz M, Panczyk M, Sanchak Y, et al. Hp1–1 as a genetic marker regulating inflammation and the possibility of developing diabetic complications in patients with type 2 diabetes-cohort studies. Genes (Basel). 2020;11
16. Awadallah S, Madkour M, Hamidi RA, Alwafa EA, Hattab M, Zakkour B, et al. Plasma levels of Apolipoprotein A1 and Lecithin: Cholesterol Acyltransferase in type 2 diabetes mellitus: correlations with haptoglobin phenotypes. Diabetes Metab Syndr. 2017;11(Suppl 2):S543–6.
17. Adams JN, Cox AJ, Freedman BI, Langefeld CD, Carr JJ, Bowden DW. Genetic analysis of haptoglobin polymorphisms with cardiovascular disease and type 2 diabetes in the Diabetes Heart Study. Cardiovasc Diabetol. 2013;12: 31
18. Shi X, Sun L, Wang L, Jin F, Sun J, Zhu X, et al. Haptoglobin 2–2 genotype is associated with increased risk of type 2 diabetes mellitus in northern Chinese. Genet Test Mol Biomarkers. 2012;16:563–8.
19. Al-Safar H, Kamal W, Hassoun A, Almahmeed W, Rais N. Combined association analysis of interleukin 1-receptor antagonist (IL-1RN) variable number of tandem repeat (VNTR) and Haptoglobin 1/2 polymorphisms with type 2 diabetes mellitus risk. J Diabetes Metab Disord. 2015;15: 10
20. Dalan R, Goh LL, Lim CJ, Seneviratna A, Liew H, Seow CJ, et al. Impact of vitamin E supplementation on vascular function in haptoglobin genotype stratified diabetes patients (EVAS Trial): a randomised controlled trial. Nutr Diabetes. 2020;10: 13
21. Koda Y, Soejima M, Yamagishi S, Amano S, Okamoto T, Inagaki Y, et al. Haptoglobin genotype and diabetic microangiopathies in Japanese diabetic patients. Diabetologia. 2002;45:1039–40.

22. Costacou T, Ferrell RE, Ellis D, Orchard TJ. Haptoglobin genotype and renal function decline in type 1 diabetes. Diabetes. 2009;58:2904–9.
23. Standards of medical care in diabetes--2012. Diabet Care. 2012;35 Suppl 1:S11–S63
24. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. 2011;364:127–35.
25. Maric-Bilkan C. Sex differences in micro- and macro-vascular complications of diabetes mellitus. Clin Sci (Lond). 2017;131:833–46.
26. Mogarekar MR, Hampe MH. Haptoglobin2-2 phenotype is an additional risk factor of retinopathy in type 2 diabetes mellitus. Indian J Hum Genet. 2013;19:154–8.
27. Looker HC, Nyangoma SO, Cromie D, Olson JA, Leese GP, Black M, et al. Diabetic retinopathy at diagnosis of type 2 diabetes in Scotland. Diabetologia. 2012;55:2335–42.
28. Kostev K, Rathmann W. Diabetic retinopathy at diagnosis of type 2 diabetes in the UK: a database analysis. Diabetologia. 2013;56:109–11.
29. Lucas-Herald AK, Alves-Lopes R, Montezano AC, Ahmed SF, Touyz RM. Genomic and non-genomic effects of androgens in the cardiovascular system: clinical implications. Clin Sci (Lond). 2017;131:1405–18.
30. Nuzzi R, Scalabrin S, Becco A, Panzica G. Gonadal hormones and retinal disorders: a review. Front Endocrinol (Lausanne). 2018;9: 66

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ORIGINAL ARTICLE

Neutrophil lymphocyte ratio: a reliable biomarker for diabetic nephropathy?

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Abstract

Background Inflammation plays a central role in pathogenesis of diabetic nephropathy (DN), a major cause of morbidity and mortality in type 2 diabetes mellitus (T2DM). Neutrophil lymphocyte ratio (NLR) is a novel and easily available inflammatory marker that can be used to predict DN.

Objective The objective was to evaluate NLR as a predictive and prognostic marker for DN.

Material and methods It was an observational cross-sectional study. A total of 324 T2DM patients and 212 healthy controls (HC) were selected by consecutive sampling between June 2019 and June 2020. Complete blood count, erythrocyte sedimentation rate (ESR), renal function parameters, 24-h urinary protein, and fundoscopy were done. Appropriate statistical analysis was applied using SPSS software.

Results Of 324 T2DM patients, 146 (45%) had DN and 178 (55%) did not. Mean NLR (± SD) for T2DM without DN, T2DM, with DN and HC was 2.73 ± 0.91, 4.85 ± 1.37, and 2.05 ± 0.73, respectively (p-value < 0.05). Positive correlation between NLR vs ESR ($r = +0.335$), creatinine ($r = +0.282$), and 24-h urinary protein ($r = +0.508$) (p-value < 0.001) and negative correlation with hemoglobin ($r = -0.335$) and estimated glomerular filtration rate ($r = -0.163$) (p-value = 0.001) was observed. Receiver operating characteristic curve for NLR was highest (0.882) (Std. error − 0.019 and p-value < 0.000), and best cut-off value was 3.28 (sensitivity = 89.7% and specificity = 69.7%).

Conclusion NLR is a better and reliable inflammatory marker compared to a frequently assayed inflammatory parameter like ESR. Thus, it can be considered as a predictive and prognostic marker for DN.

Keywords Biomarker · Diabetes · Diabetic nephropathy · Neutrophil–lymphocyte ratio

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Introduction

Diabetic nephropathy (DN) is a major microvascular complication occurring in approximately 30% and 40% of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), respectively [1]. DN is a major cause of morbidity and mortality in diabetes mellitus (DM) patients as it not only leads to end-stage renal disease (ESRD) but also is a major contributor to cardiovascular adverse events. DN is clinically detected by the presence of albuminuria or proteinuria with diabetic retinopathy (DR) in DM patients without evidence of other non-diabetic kidney diseases [2]. Currently, 463 million people worldwide are affected by diabetes and it is expected to increase to 700 million by 2045. After China, India has the world's largest population living with diabetes approximately 82 million as of 2017, and estimated to be 151 million by 2045 [3]. The prevalence of

DN is increasing parallelly with a dramatic worldwide rise in the prevalence of diabetes [4].

Though the exact molecular mechanism of pathogenesis of DN is unknown, various mechanisms have been proposed such as kidney injury by activating several cellular pathways including diacylglycerol (DAG)-protein kinase C (PKC) pathway, advanced glycation end-products (AGE), polyol pathway, hexosamine pathway, and oxidative stress due to direct glucotoxicity. As inflammation is the final common outcome of these cellular pathways, thus, it plays a critical role in developing DN [5]. Moreover, there is growing evidence that chronic inflammation and inflammatory cytokines such as TNF-α, IL-1β, and IL-6 play a central role in endothelial dysfunction, and atherosclerosis contributing to both developments as well as the acceleration of microangiopathy and macroangiopathy in DM patients [6, 7].

Neutrophil–lymphocyte ratio (NLR) stands out as a novel marker of chronic inflammation because it reflects a counterbalance between two complementary components of the immune system; neutrophils being the active nonspecific mediator of inflammation, whereas lymphocytes act as the protective or regulatory component of inflammation [8].

NLR has been demonstrated to be a greater risk factor and better prognostic marker than total leucocyte count (TLC) in the prediction of adverse outcomes in various medical conditions like cancer and cardiovascular diseases [9, 10]. Though microalbuminuria is considered as the earliest marker for DN, a substantial percentage of patients with microalbuminuria may remain microalbuminuric or revert to normoalbuminuria over a period of time [11]. Hence, a reliable biomarker is lacking in these subsets of patients. Thus, there is a quest to find a novel biomarker for the detection of individuals at risk to develop DN.

However, after doing an extensive search using various search engines, only one study was found evaluating the predictive and prognostic value of NLR in our country [12]. Probably, due to this reason, NLR is not being used frequently in clinical practice, hoping that our study would add to the literature that would give more confidence to the clinicians to rely on this parameter especially in a resource-poor setting. Thus, considering these aspects and the dearth of literature present in context to the Indian population, we conducted this study aiming to evaluate NLR as a novel biomarker for DN.

Materials and methods

This was a hospital-based observational cross-sectional study conducted from June 2019 to June 2020. All diagnosed T2DM patients aged > 13 years with a minimum duration of 5 years from the time of diagnosis, attending out-patient, or admitted in medicine department were screened. Age and sex-matched healthy controls (HC) were also taken from the same population.

Among those excluded from the study were diagnosed cases of T1DM; gestational diabetes; secondary diabetes; non-diabetic organic kidney disease; patients with active infections, for example, urinary tract infection (UTI), respiratory tract infections (RTI), gastrointestinal infection, genital infection, skin infection, otitis media, viral hepatitis, pyrexia of unknown origin, tuberculosis, and AIDS; patients with systemic diseases such as cardiovascular disease, obesity, chronic liver disease, blood disorders, autoimmune disorders such as systemic lupus erythematosus; cancer patients, known and unknown poisoning; patients on non-steroidal anti-inflammatory drugs, systemic or topical steroids, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers; alcoholics; smokers; patients with uncontrolled hypertension; and patients having diseases affecting urinary protein excretion as nephritic syndrome, urolithiasis, renal artery stenosis, pregnancy, and dehydration states. At the time of sample collection, patients who performed excessive exercise within 24 h or had fever were excluded. Furthermore, on urine sample, processing those with significant bacteriuria or hematuria were also excluded.

All the participants were subjected to a comprehensive history and clinical examination. Relevant information such as age, sex, duration of diabetes, drug history, family history, alcohol, smoking, and any other medical or surgical illness, height, weight, body mass index (BMI), waist circumference, waist to hip ratio, blood pressure, pulse rate, and the temperature was documented.

Routine blood investigations such as complete blood count (CBC), liver function tests (LFTs), kidney function tests (KFTs), fasting lipid profile, thyroid profile, fasting blood sugar (FBS)/postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c), chest X-ray, and ultrasonography of the abdomen were done. Fundoscopy was done to assess diabetic retinopathy. Absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and NLR were calculated by analyzing differential leukocyte count.

Also, a mid-stream morning urine sample was taken for routine urine analysis as well as for urine albumin to creatinine ratio (UACR) estimation, and 24 h urine was collected for 24-h urinary protein excretion estimation. The estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease-epidemiology collaboration (CKD-EPI) formula. Diabetes was diagnosed as per American Diabetes Association (ADA) 2019 criteria [13]. DN was diagnosed as 24-h urinary albumin excretion > 300 mg supported by the co-existence of DR after excluding other non-diabetic organic kidney diseases [2].

All the data collected were analyzed using Social Sciences (SPSS for Windows, version 23.0 Chicago, SPSS Inc.) software. For the analysis of continuous quantitative data,

Student *t*-test and ANOVA were applied whereas for qualitative data, Chi-square test was used. The reciever operating characteristic (ROC) curve analysis was done to test the ability of various parameters to predict DN risk, while the Spearman correlation coefficient (*r*) was used for correlation analysis between NLR and other relevant parameters. A *p*-value of <0.05 was considered statistically significant.

Results

This study included 324 T2DM cases with a mean age ($\pm$ standard deviation (SD)) 56.21 ± 10.37 years, categorized into 2 groups: 178 (55%) T2DM without DN (DM-DN), and 146 (45%) T2DM with DN (DM + DN), and 212 HC with mean age 55.85 ± 10.52 years. The baseline parameters were compared and projected in Table 1 with their respective *p*-value. There was a significant difference between the groups with respect to renal parameters, triglyceride, HDL, and LDL-cholesterol (*p*-value <0.05). The inflammatory parameters like erythrocyte sedimentation rate (ESR), TLC, ANC, ALC, and NLR were compared among the DM-DN group (group 1), DM + DN group (group 2), and HC group (group 3) and projected in Table 2. The analysis was also done between group 1 vs group 2, group 1 vs group 3, and group 2 vs group 3. Though on comparing all the groups together, we got the *p*-value <0.05 in each section but on comparing group 1 vs group 3, TLC was not found to have a significant difference (*p*-value >0.05). Similarly, there was no significant difference in ALC when group 1 and group 3 were compared ($p=0.82$). The mean value ($\pm$ SD with 95% confidence interval) of NLR for DM-DN, DM + DN, and HC group was 2.73 ± 0.91, 4.85 ± 1.37, and 2.05 ± 0.73, respectively (Fig. 1). Correlations between NLR and other studied parameters are shown in Table 3. There was a significant positive correlation between NLR vs ESR ($r=+0.335$), creatinine ($r=+0.282$) and 24-h urinary protein ($r=+0.508$) (*p*-value <0.001). Significant

Table 2 Comparison of various inflammatory parameters between DM-DN group DM + DN group and Healthy Control (HC) group

Parameters	DM-DN 178 (mean ± SD)	DM + DN 146 (mean ± SD)	HC 212 (mean ± SD)	*p*-value
ESR	38.37 ± 19.08	63.09 ± 25.87	21.64 ± 15.5	**< 0.001**
*Significance between groups: p1– **0.0001**, p2– **0.003**, p3- < **0.001**				
TLC	6985 ± 1420	7440 ± 1764	5736 ± 1327	**0.0004**
*Significance between groups: p1– **0.005**, p2– 0.34, p3- **0.0001**				
ANC	4731 ± 1126	5741 ± 1522	3869 ± 912	**< 0.001**
*Significance between groups: p1– **0.0001**, p2– **0.003**, p3- < **0.001**				
ALC	1708 ± 573	1209 ± 440	1917 ± 564	**0.0017**
*Significance between groups: p1– **0.001**, p2– 0.82, p3- **0.001**				
NLR	2.731 ± 0.91	4.85 ± 1.37	2.05 ± 0.73	**< 0.001**
*Significance between groups: p1- < **0.001**, p2-**0.002**, p3- < **0.001**				

ESR erythrocytic sedimentation rate, *TLC* total leucocyte count, *ANC* absolute neutrophil count, *ALC* absolute lymphocyte count, *NLR* neutrophil lymphocyte ratio; mean ± standard deviation includes the 95% confidence interval

*p1, *p*-value for comparing between DM-DN group and DM + DN group; p2, *p*-value for comparing between DM-DN group and HC group; p3, *p*-value for comparing between DM + DN group and HC group

Table 1 Comparison of baseline parameters of diabetic patients

Biochemical parameters	DM–DN *(mean ± S.D.)*	DM + DN *(mean ± S.D.)*	*p-value*
Numbers	178 (55%)	146 (45%)	
Gender (male)	109 (61.2%)	86 (58.9%)	0.66
Age(years)	55.59 ± 10.64	56.66 ± 10.23	0.62
Hb *(gm%)*	11.00 ± 2.49	9.03 ± 2.32	0.37
HbA1c *(%)*	7.99 ± 2.32	8.36 ± 2.52	0.29
Duration of diabetes	12.83 ± 3.83	13.34 ± 3.57	0.37
Serum Creatinine*(mg/dl)*	0.91 ± 0.23	2.74 ± 1.80	**< 0.001**
eGFR (ml/min/1.73m^2)	98.78 ± 11.88	35.27 ± 29.79	**< 0.001**
24-h urinary protein (*gm/24 h*)	18.91 ± 5.60	1241.14 ± 814.85	**< 0.001**
UACR *(mg/gm)*	15.37 ± 4.76	1133.38 ± 775.29	**< 0.001**
Total cholesterol *(mg/dl)*	143.07 ± 47.40	152.08 ± 53.86	0.10
Triglycerides *(mg/dl)*	122.22 ± 52.16	148.99 ± 78.43	**< 0.001**
HDL *(mg/dl)*	40.67 ± 13.01	37.05 ± 10.90	**0.02**
LDL–C *(mg/dl)*	79.87 ± 27.53	83.47 ± 36.86	**< 0.001**

Mean ± standard deviation includes the 95% confidence interval

Abbreviatons: *Hb* hemoglobin, *HbA1c* glycated hemoglobin, *eGFR* estimated glomerular filtration rate, *UACR* urinary albumin creatinine ratio, *HDL* high density lipoprotein, *LDL-C* low-density lipoprotein cholesterol

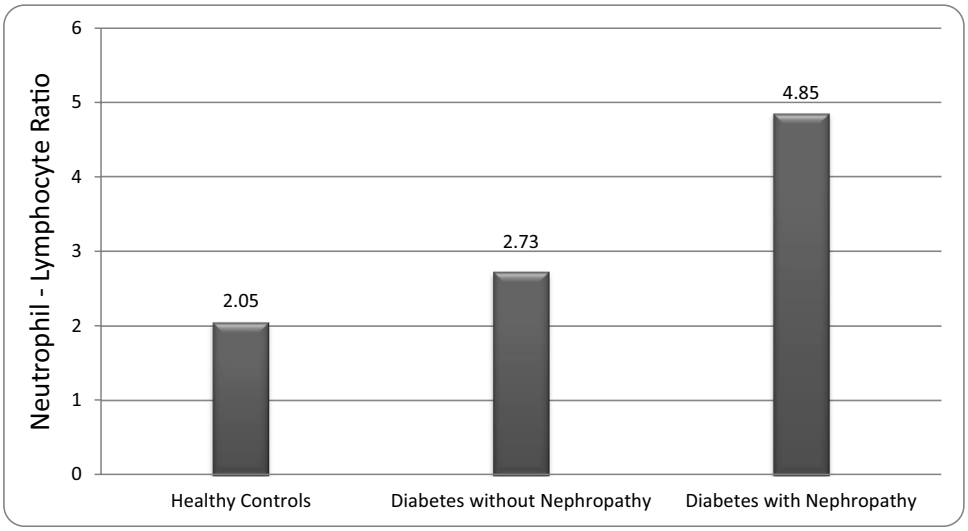

Fig. 1 Mean Neutrophil–Lymphocyte Ratio of various groups

Table 3 Correlation between Neutrophil–Lymphocyte Ratio and other studied variable

Study parameters	Correlation coefficient (*r*)	*p*-value
ESR (mmHr)	+0.335	**< 0.001**
Hemoglobin (gm/dl)	−0.335	**< 0.001**
HbA1c	+0.063	0.254
Duration of diabetes (years)	+0.095	0.295
Creatinine (mg/dl)	+0.282	**< 0.001**
eGFR (ml/min/1.73m^2)	−0.163	**0.003**
24 h urinary protein (gm/dl)	+0.508	**< 0.001**

ESR erythrocytic sedimentation rate, *HbA1c* glycosylated hemoglobin, *eGFR* estimated GFR

The ones which are in bold are of significance

negative correlation was seen between NLR vs hemoglobin ($r = -0.335$) and eGFR ($r = -0.163$) (p-value = 0.001). A ROC curve was plotted for NLR, ESR, creatinine, total WBC count (TC), ANC, and ALC (Fig. 2). The area under the curve (AUC) for NLR was 0.882 (Std. error – 0.019 and p 0.000). Based on the graph, 3.28 was found to be the best cut-off value (sensitivity = 89.7% and specificity = 69.7%) which predicts the presence of DN in T2DM patients (CI 95% 0.846–0.919). The AUC for ESR and creatinine was 0.765 (Std. error – 0.027 and p-value = 0.000) and 0.773 (Std. error – 0.026 and p-value = 0.000), respectively. The AUC values for TC, ANC, and ALC were 0.606 (Std. error – 0.032 and p-value = 0.001), 0.751 (Std. error – 0.028 and p-value = 0.000), 0.225 (Std. error 0.026 and p-value = 0.000), respectively.

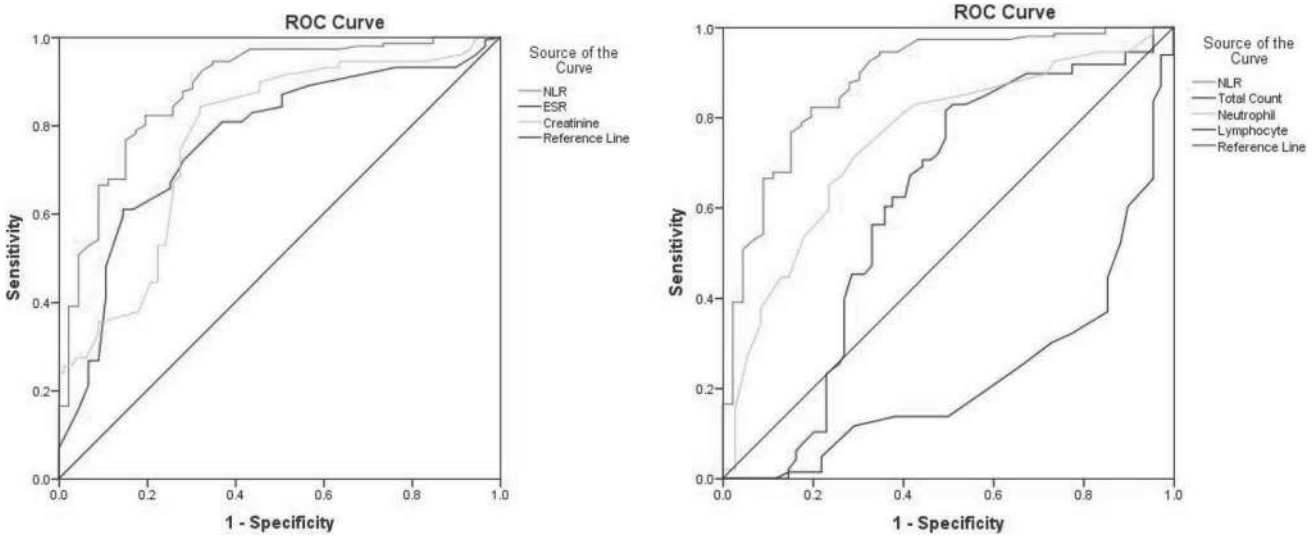

Fig. 2 ROC analysis of the predictive accuracy of NLR and other markers

Discussion

In this study, high NLR levels were found to be significantly associated with T2DM with DN as compared to T2DM without DN, as well as HC. DN being the most common and dreaded complication of DM needs to be detected at the earliest to decrease mortality and morbidity. A major role of inflammation and endothelial dysfunction in diabetes and progression to DN has been well established in various studies [14]. In several studies, higher TLC was related to increased urinary albumin excretion rates [15, 16]. The exact biological mechanisms by which leukocytes and their subtypes play a role in mediating increased protein and albumin excretion are not completely understood. The increased spontaneous adherence of neutrophils to endothelial cells was described as a possible mechanism of DN and proteinuria [17].

NLR is considered superior to other leukocyte parameters such as TLC, ALC, and ANC as its stability is less influenced by physiological, pathological, and physical factors [18]. Moreover, it is a dynamic marker of inflammation representing a combination of two parameters of uncontrolled chronic inflammatory condition (i.e., high neutrophil and low lymphocyte) [19]. Estimation of NLR is simple, easy, and relatively cheap compared to other inflammatory markers, e.g., C-reactive protein (CRP), cytokines, such as IL-6, IL-1b, and TNF-α. In our study, the mean NLR among T2DM with DN patients was significantly higher as compared to T2DM without DN (p-value = 0.001) and healthy controls (p-value < 0.001). In concordance with our study, Huwang et al. also reported significantly higher NLR values in diabetic patients with evidence of nephropathy (2.48 ± 0.59) than in diabetic patients without nephropathy (2.20 ± 0.62) and HC subjects (1.80 ± 0.64) [20]. Similarly, Khandare et al. found the mean NLR among Indian diabetic patients with proteinuria (2.83 ± 0.85) to be significantly higher than those without proteinuria (1.94 ± 0.65) [12]. Asfar et al. reported that NLR could be associated with DN as increased NLR was independently associated with both 24-h urinary protein (p-value < 0.001) and urinary albumin excretion (p-value < 0.001) in newly diagnosed Turkish patients with type 2 diabetes [21]. Recently, Onalan et al. reported NLR to be a predictive hematological parameter for microvascular complications in T2DM [22].

Ashar et al. found NLR values to be higher in patients with DN though it did not reach the significant level (p-value > 0.05). However, a significant association was observed with retinopathy and peripheral neuropathy (p-value < 0.0001) which upholds the evidence that NLR has got an association with microvascular complications in diabetics. The difference can be explained by the fact the author had excluded ESRD patients who might have severe inflammation with higher NLR [23].

We found that NLR had a positive correlation with ESR, serum creatinine, and 24-h urinary protein excretion and a negative correlation with hemoglobin and eGFR. These findings strongly advocate NLR to be considered as a novel biomarker for DN. In agreement with the above finding, a positive correlation between NLR and ESR was reported by Moursy et al. [24]. Similarly, Kahraman et al. reported that NLR was significantly negatively correlated with eGFR, and positively with albuminuria and CRP [25].

As we excluded the potential causes of inflammation and active infection, results of our study reflect the inflammation associated with diabetic nephropathy. In our study, NLR showed better predictive value for DN with AUC of 0.882 as compared to other inflammatory parameters such as TLC, ANC, ALC, and ESR as evident from the ROC curve. Moreover, NLR showed a better predictive value for renal dysfunction than creatinine. Similar findings were reported in a study by Huang et al. where the performance of NLR was higher (AUC 87.2%) than TLC, ANC, ALC, and creatinine [26].

There were several limitations to our study. As it was a single-centered cross-sectional study comprising a relatively small sample size, it did not provide any direct evidence for a cause-effect association between NLR and DN. Multicenter, prospective studies with larger sample size are required for establishing the direct association between NLR and DN. Furthermore, the analyses in this study were based on a single measurement of TLC and NLR, the results of this study might not reflect the relationship between NLR and DN over time. It would be fruitful to measure the serial changes of TLC and NLR to further clarify the prognostic role of NLR in DN. We were unable to correlate the prognostic value of NLR with other inflammatory markers such as IL-6, TNF-α, CRP, and fibrinogen as they were costly and not routinely done.

Conclusion

We conclude that NLR is a reliable marker of inflammation and can be considered as a novel predictive as well as a prognostic marker for diabetic nephropathy. Further studies are required to support the findings of this study.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval Ethical approval was granted by the Instructional Ethics Committee (ref no- PMC/EC/2019/05–015) dated 13/05/2019.

References

1. Park C. Diabetic kidney disease: from epidemiology to clinical perspectives. Diabetes Metab J. 2014;38(4):252.
2. Rossing P, Fioretto P, Feldt-Rasmussen B, Parving H-H. Brenner & Rectors the kidney. In: Skorecki K, Chertow G, Marsden P, Brenner B, Rector F, editors. 10th ed. Philadelphia: Elsevier, 2016. p. 1283 1320.
3. International Diabetes Federation Eighth Edition. 2017. Available from: https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html. Accessed 17 Jul 2020.
4. Pálsson R, Patel UD. Cardiovascular complications of diabetic kidney disease. Adv Chronic Kidney Dis. 2014;21(3):273–80.
5. Lin Y-C, Chang Y-H, Yang S-Y, et al. Update of pathophysiology and management of diabetic kidney disease. J Formos Med Assoc. 2018;117(8):662–75.
6. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19(3):433–42.
7. Rivero A, Mora C, Muros M, Garcia J, Herrera H, Navarro- Gonzalez JF. Pathogenic perspectives for the role of inflammation in diabetic nephropathy. Clin Sci (Lond). 2009;116(6):479–92.
8. Bhutta H, Agha R, Wong J, Tang TY, Wilson YG, Walsh SR. Neutrophil lymphocyte ratio predicts medium-term survival following elective major vascular surgery: a cross-sectional study. Vasc Endovascular Surg. 2011;45:227–31.
9. Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med. 2012;5:2.
10. Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Kubota K. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer. Br J Cancer. 2013;109:401–7.
11. Gaede P, Tarnow L, Vedel P, et al. Remission to normoalbuminuria during multifactorial treatment preserves kidney function in patients with type 2 diabetes and microalbuminuria. Nephrol Dial Transplant. 2004;19(11):2784–8.
12. Khandare SA, Chittawar S, Nahar N, Dubey TN, Qureshi Z. Study of neutrophil-lymphocyte ratio as a novel marker for diabetic nephropathy in type 2 diabetes. Indian J Endocrinol Metab. 2017;21(3):387–92.
13 American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in Diabetes-2019. Diabetes Care. 2019;42(Suppl. 1):S13–28.
14. Pitsavos C, Tampourlou M, Panagiotakos DB, et al. Association between low-grade systemic inflammation and type 2 diabetes mellitus among men and women from the ATTICA study. Rev Diabet Stud. 2007;4:98–104.
15. Tong PC, Lee KF, So WY, et al. White blood cell count is associated with macro- and microvascular complications in Chinese patients with type 2 diabetes. Diabetes Care. 2004;27:216–22.
16. Cavalot F, Massucco P, Perna P, Traversa M, Anfossi G, Trovati M. White blood cell count is positively correlated with albumin excretion rate in subjects with type 2 diabetes. Diabetes Care. 2002;25:2354–5.
17. Takahashi T, Hato F, Yamane T, et al. Increased spontaneous adherence of neutrophils from type 2 diabetic patients with overt proteinuria: possible role of the progression of diabetic nephropathy. Diabetes Care. 2000;23(3):417–8.
18. Lou M, Luo P, Tang R, et al. Relationship between neutrophil-lymphocyte ratio and insulin resistance in newly diagnosed type 2 diabetes mellitus patients. BMC Endocr Disord. 2015;15(1):4–9.
19. Woo SJ, Ahn SJ, Ahn J, Park KH, Lee K. Elevated systemic neutrophil count in diabetic retinopathy and diabetes: a hospital-based cross-sectional study of 30,793 Korean subjects. Invest Ophthalmol Vis Sci. 2011;52:7697–703.
20. Huang W, Huang J, Liu Q, et al. Neutrophil-lymphocyte ratio is a reliable predictive marker for early-stage diabetic nephropathy. Clin Endocrinol (Oxf). 2015;82(2):229–33.
21. Asfar B. The relationship between neutrophil-lymphocyte ratio with urinary protein and albumin excretion in newly diagnosed patients with Type 2 Diabetes. Am J Med Sci. 2013;347(3):217–20.
22 Onalan E, Gozel N, Doder E. Can hematological parameters in type 2 diabetes predict microvascular complication development? Pak J Med Sci. 2019;35(6):1511–5.
23. Fawwad A, Butt AM, Siddiqui IA, Khalid M, Sabir R, Basit A. Neutrophil-to-lymphocyte ratio and microvascular complications in subjects with type 2 diabetes: Pakistan's perspective. Turk J Med Sci. 2018;48(1):157–61.
24. Youssef ME. Relationship between neutrophil-lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. Am J Intern Med. 2015;3(6):250.
25. Kahraman C, Kahraman NK, Aras B, Coşgun S, Gülcan E. The relationship between neutrophil-to- lymphocyte ratio and albuminuria in type 2 diabetic patients: a pilot study. Arch Med Sci. 2016;12(3):571–5.
26. Huang L, Xie Y, Dai S, Zheng H. Neutrophil-to-lymphocyte ratio in diabetic microangiopathy. Int J Clin Exp Pathol. 2017;10(2):1223–32.

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ORIGINAL ARTICLE

Isolation and characterization of bacteria from diabetic foot ulcer: amputation, antibiotic resistance and mortality rate

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Abstract

Background Diabetic foot ulcer (DFU) is one of the most serious complications of diabetes mellitus with devastating outcomes. Poorly treated DFU leads to osteomyelitis, gangrene and limb amputation. There is an increased risk of mortality for the amputees and increased number of bacterial resistance in survived patients. Struggle on choice of the best antibiotic(s) for DFU is escalating.

Objectives To determine risk factors associated with mortality in patients with DFU. To investigate bacterial drug resistance in survived or deceased patients around amputation.

Methodology This is a retrospective cohort study that involved all diabetic patients who had DFU or minor or major amputation at Hebron Governmental Hospital from 2013 to 2020. Antibiotic use and bacterial isolates along with culture and sensitivity test results were retrieved from patients' profiles and laboratory records. Major outcome of study was survival rate around amputation. Patients who missed test results for FBS or HbAc1, or who had no wound culture were excluded. SPSS version 22 was used to analyze data.

Results Eighty four subjects were included in this study, 64.8 ± 12.58 years old, 63.1% males who had diabetic foot ulcer, minor or major limb amputation between 2013 and 2020 at Hebron Governmental Hospital. Forty tow patients (50%) had diabetic foot ulcer, 28 patients (33.3%) had major limb amputation, and 14 patients (16.7%), succumbed to minor amputation. Average FBS was 292.8 ± 136.33 mg/dl and average HbA1C was 8.55 ± 1.89%. Mortality rate was 9.5%. Using the Chi square test, we found a significant relationship between mortality and type of isolated bacteria, $p = 0.033$ and between diabetic complications (nephropathy) and mortality, $p = 0.033$. There was a significant relationship between antibiotic use and mortality, $p = 0.04$, especially with metronidazole and colistin, if they were used around limb amputation.

Conclusions Mortality of diabetic patients with DFU was associated with nephropathy and Acinetobacter or *E. coli* infections.

Keywords DFU · Amputation · Mortality rate · Antibiotic-resistance · Bacterial isolates

Introduction

Diabetes mellitus (DM) is a chronic multifactorial condition. It occurs when the pancreas is unable to produce enough insulin or the human body is incapable of using effectively the secreted insulin. Patients with DM suffer a variety of complications due to micro- and macro-vascular abnormalities. Complications such as neuropathy, nephropathy, obesity, hypertension, and increased susceptibility to a variety of infectious diseases are most common. However, poor control over blood sugar leads to life-threatening complications such as cardiovascular disease, foot damage, hearing impairment, fungal and bacterial infection, Alzheimer disease, memory deficits and depression [1].

Type I DM affects 5–10% of patients and commonly occurs in young ages which have autoimmune destruction of beta-cell. This type is treated mainly by insulin. Patients in this type are non-obese [2].

Type II DM affects 90% of patients and commonly occurs in older people which have low sensitivity to insulin. This type of DM is treated by diet. If not well-controlled by diet alone, oral hypoglycemic agent is added to the regimen, then, insulin is added as per case. Nutritional

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medical treatment is another option. Patients in this type are obese and highly affected by family history of DM [3].

Patient education is crucial for all diabetic patients especially those with high risk of DM. High risk patients should be advised to make dietary adjustments, lose excess weight, do regular light exercise, check their blood sugar periodically, smoking cessation, and foot care.

A Palestinian study performed in 2019 demonstrated that smoking, sensory loss to vibration, sensory loss to monofilament, loss of pedal pulse, presence of calluses, nephropathy, retinopathy, neuropathy, and poor self-care behaviors were associated with DFU [4].

The same study above recommended that: knowing risk factors and predictors of amputation will help nurses and physicians design a suitable program to minimize the incidence of DFU development such as integrating audio-visual teaching strategies. Also, increasing public awareness and knowledge about risk factors and importance of self-care practices by the healthcare team can decrease the incidence of diabetic foot ulcers in Palestine.

One of the most serious complications of DM is diabetic foot ulcer (DFU). It occurs more commonly in males than females as suggested by a recent study in Saudi Arabia. Up to 2–3% of patients with DM are thought to have an active foot ulcer, it is also increasingly recognized that latter stages of complications from foot ulcers are associated with serious morbidity and overall reduction in quality of life. Managing tissue damage and enhancing wound healing and repair in DFU might be quite challenging. Wound dressings that might form a barrier against contamination and debridement by removal of necrotic and hyperkeratotic tissue are widely used. Improving vascularization through Percutaneous Trans luminal angioplasty (PCTA) is the gold standard for placement of narrow blood vessels with or without stents. This procedure can also improve blood flow and promote healing in a timely manner. It is estimated that more than two-thirds of non-traumatic lower limb amputations are preceded by an ulcer [5, 6].

Foot ulcer commonly affects people with type II DM. It can lead to infection and amputation of lower extremities. The risk of developing an ulcer increases with peripheral vascular disease, neuropathy, diabetes duration ≥ 10 years, insulin use, retinopathy, nephropathy, age 45 years, cerebral vascular disease, and poor glycemic control. Increasing cumulative glycemic burden, coronary artery disease, male gender, smoking, and hypertension are all present in these patients [7–10].

Patients with poorly treated DFU may develop diabetic foot osteomyelitis leading to gangrene and amputation. Diabetic foot infections (DFI) are predominantly polymicrobial and multidrug resistant (MDR) with the ability to form biofilm [11].

Our study aims at determining main risk factors associated with lower limb amputation among diabetic patients in Palestine. We focused on survival rate and quality of life of patients after amputation. This issue was poorly studied in the literature.

We aim at tracing antibiotic resistance among non-hospitalized patients with recurrent infection and re-hospitalization at time of severe infection and amputation.

Methods

We decided to review all diabetic patients' profiles with DFU who went through foot, part of foot, or below knee amputation at the orthopedic department at Hebron Governmental Hospital between 2013 and 2020. We defined 126 patients who complied with our inclusion criteria. Unfortunately, we had to exclude some of them due to missing information such as FBS or HbAc1, bacterial culture and antibiotic sensitivity test results around amputations. Sensitivity test was done using fully automated Mcfarland antibiotic sensitivity test using VITEX 2 compact device, without Mueller Hinton agar. Bacterial swabs were taken from all DFU patients and bacterial identification by culture was done. SPSS version 22 was used for analysis of data.

Results

A total of 84 patients with diabetic foot infection were included in the present study whose sociodemographic and clinical data are shown in Table 1 below. The mean age of patients was 64.8 ± 12.58 with predominantly male patients 53 (63.1%). Majority of subjects (95.24%) had Type 2 diabetes mellitus. Mean HbA1c of the patients was $8.55 \pm 1.89\%$ and mean FBS was 292.8 ± 136.33 mg/dl (Table 1).

Patients were then categorized according to main outcome of study, the survival rate. Bacterial isolates from survived or dead patient's foot were identified as shown in Table 2.

Chi square test showed a significant relation between death and microorganism isolated from diabetic foot, $p = 0.033$. The results also indicated a significant relation between diabetic complications and death, $p = 0.033$, Table 2.

We also depicted percentage of bacteria species isolated from diabetic foot as related to main outcomes of the study as shown in Fig. 1.

However, when we stratified bacterial species with diabetic complications, we found that all dead patients suffered from some kind of diabetic complications. For example, 100% of dead patients who had *Proteus*, *Enterococcus* or *Enterobater* species isolates, had nephropathy.

Table 1 Sociodemographic, diabetic complications, microorganism isolated and antibiotic use per diagnosis

Diagnosis			
Variables	Diabetic foot ulcer N (%)	Major amputation N (%)	Minor amputation N (%)
Microorganism			
Proteus spp.	9 (21.4)	3 (21.4)	5 (17.9)
Klebsiella pneumoniae	5 (11.9)	1 (7.1)	9 (32.1)
Stappylococcus aureas	5 (11.9)	2 (14.3)	3 (10.7)
Pseudomonas spp.	3 (7.1)	1 (7.1)	2 (7.1)
MRSA	8 (19)	2 (14.3)	3 (10.7)
Acinetobacter spp.	4 (9.5)	3 (21.4)	1 (3.6)
‡*E. coli*	4 (9.5)	1 (7.1)	4 (14.3)
Enterococcus spp.	1 (2.4)	1 (7.1)	1 (3.6)
Enterobacter spp.	2 (4.8)	0 (0)	0 (0)
Staphylococcus epidermidis	1 (2.4)	0 (0)	0 (0)
Total	42 (100)	14 (100)	28 (100)
Complication			
Nephropathy	3 (7.1)	3 (21.4)	3 (10.7)
Retinopathy	2 (4.8)	1 (7.1)	3 (10.7)
Cardiovascular problem	2 (4.8)	1 (7.1)	1 (3.6)
Peripheral vascular disease	4 (9.5)	1 (7.1)	8 (28.6)
Neuropathy	31 (73.8)	8 (57.1)	13 (46.4)
Total	42 (100)	14 (100)	28 (100)
D.M*			
Type 1	2 (4.8)	0 (0)	2 (7.1)
Type 2	40 (95.2)	14 (100)	26 (92.9)
Total	42 (100)	14 (100)	28 (100)
Antibiotic			
Ceftriaxon	16 (38.1)	2 (14.3)	8 (28.6)
Ceftazidime	3 (7.1)	0 (0)	2 (7.1)
Meropenem	6 (14.3)	3 (21.4)	3 (10.7)
Colistin	4 (9.5)	3 (21.4)	1 (3.6)
Teicoplanin	1 (2.4)	3 (21.4)	2 (7.1)
Gentamicine	2 (4.8)	0 (0)	0 (0)
Cefuroxime	(9.5)	3 (21.4)	3 (10.7)
Amoxicillin/clavulanic acid	1 (2.4)	0 (0)	2 (7.1)
Metronidazole	4 (9.5)	0 (0)	3 (10.7)
Vancomycin	1 (2.4)	0 (0)	1 (3.6)
Ciprofloxacin	0 (0)	0 (0)	3 (10.7)
Total	42 (100)	14 (100)	28 (100)
FBG§			
70–110	0 (0)	2 (15.4)	0 (0)
111–126	3 (8.1)	0 (0)	2 (8.7)
127–199	9 (24.3)	3 (23.1)	4 (17.4)
More than 200	25 (67.6)	8 (61.5)	17 (73.9)
Total	42 (100)	14 (100)	28 (100)
Age			
30–45	1 (2.4)	0 (0)	0 (0)
46–61	15 (35.7)	5 (35.7)	11 (39.3)
62–77	20 (47.6)	5 (35.7)	13 (46.4)
78–93	6 (14.3)	4 (28.6)	4 (14.3)
Total	42 (100)	14 (100)	28 (100)

Table 1 (continued)

Diagnosis			
Variables	Diabetic foot ulcer N (%)	Major amputation N (%)	Minor amputation N (%)
Mortality			
Survived	36 (85.7)	14 (100)	26 (29.9)
Deceased	6 (14.3)	0 (0)	2 (7.1)
Total	42 (100)	14 (100)	28 (100)

‡ *E. coli*, Escherichia coli; * *D.M*, Diabetes Mellitus; § *FBG*, Fasting Blood Glucose, mg/dl

Table 2 Percentage survival rates per microorganisms isolated or diabetes mellitus complications at time of amputation

	Survived N (%)	Dead N (%)	Chi-square statistic	Sig
Microorganism				
Proteus spp.	16 (21.1)	1 (12.5)	14.016	0.033
Klebsiella pneumoniae	15 (19.7)	0 (0)		
Staphylococcus aureas	10 (13.2)	0 (0)		
Pseudomonas spp.**	5 (6.6)	1 (12.5)		
MRSA‡	13 (17.1)	0 (0)		
Acinetobacter spp.	6 (7.9)	2 (25)		
E. coli	7 (9.2)	2 (25)		
Enterococcus spp.	2 (2.6)	1 (12.5)		
Enterobacter spp.	1 (1.3)	1 (12.5)		
Staphylococcus epidermidis	1 (1.3)	0 (0)		
Total	76 (100)	8 (100)		
Complications				
Nephropathy	5 (6.6)	4 (50)	14.016	0.033
Retinopathy	6 (7.9)	0 (0)		
Cardiovascular problem	4 (5.3)	0 (0)		
Peripheral vascular disease	11 (14.5)	2 (25)		
Neuropathy	50 (65.8)	2 (25)		
Total	76 (100)	8 (100)		

‡ *MRS*, Methicillin Resistant *Staphylococcus aureas*; ** *spp*., species

On the other hand, *Pseudomonas* species isolates along with neuropathy lead to death for all patients, as shown in Fig. 2.

We also sought antibiotic percentage use among dead and survived subjects to provide a clear picture of antibiotic contribution to the main outcome. As shown in Fig. 3, high percentage usage of certain antibiotic was found in dead subjects around amputation, such as metronidazole.

We followed the trends of antibiotic use and bacterial resistance over the past 7 years (2013–2020). There was inconsistent use of antibiotics through these years for many reasons such as; antibiotic availability, appearance of specific antibiotic-resistant bacteria, and protocol and clinical decision.

We could find sufficient data for the past 5 years only about trends of antibiotic use and bacterial isolate, see Fig. 4. Empty year columns mean no use of that antibiotic at that year due to emergence of resistance as was confirmed by bacterial culture and sensitivity test. (Only consistently used antibiotics with confirmed cultures and sensitivity tests during these years were shown here. You can follow by color any antibiotic through all years or any bacteria per antibiotic per year in this figure.)

Discussion

Many studies focused on microorganism species isolated from diabetic foot without linking these findings to limb amputation or survival rates among afflicted patients. Here are some of these studies. Kaimkhani et al. found that the most common organisms isolated from DFU are *Staphylococcus aureus*, *Proteus*, *Pseudomonas*, and *Escherichia coli* [12].

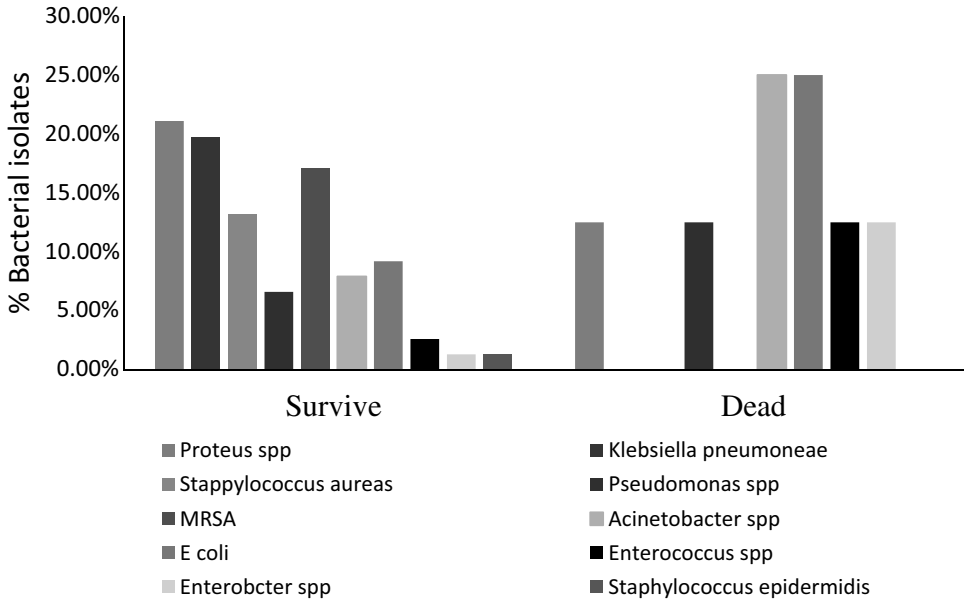

Fig. 1 Percentage of bacterial isolates from subjects before death or in survived patients around amputation

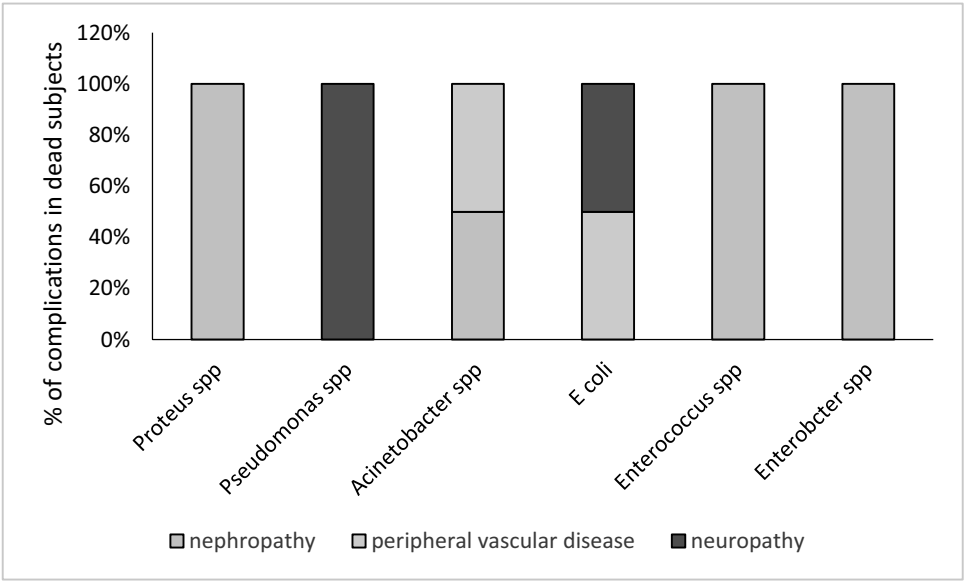

Fig. 2 Percentage of diabetic complications and type of bacteria isolated from foot of dead subjects at time of amputation

Carro et al. (2019) found that gram negative bacteria were the most frequent microorganisms isolated from DFU and the study recommended an empiric therapy of antibiotics with amoxicillin/clavulanate plus ciprofloxacin should be given as a regimen of choice for management [13].

In Lebanon, a study found that *Pseudomonas* spp. was the most common Gram-negative organism isolated from DFU in some Middle Eastern countries. The study also showed that a combination of amoxicillin/clavulanate and ciprofloxacin was the most appropriate empirical oral antibiotic for outpatient. Piperacillin/tazobactam would then be the treatment of choice for hospitalized a patient if oral treatment has failed [14].

In another study that was conducted in Kenya, the majority of organisms isolated were gram negative bacteria. Most common were *S. aureus*, *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These bacteria showed resistance to commonly used antibiotics like ampicillin, amoxicillin, ceftazidime and pipracillin with tazobactam [15].

In our study, gram negative bacteria were also the most common bacteria isolated from diabetic foot, such as *Proteus* species (17 patients), *Klebsiella* (15 patients), and MRSA (gram positive) (13 patients), among others such as *S. aureus*, *E. coli*, and *Acinobacter* species. This comes in align with some studies as above. However, subjects in our

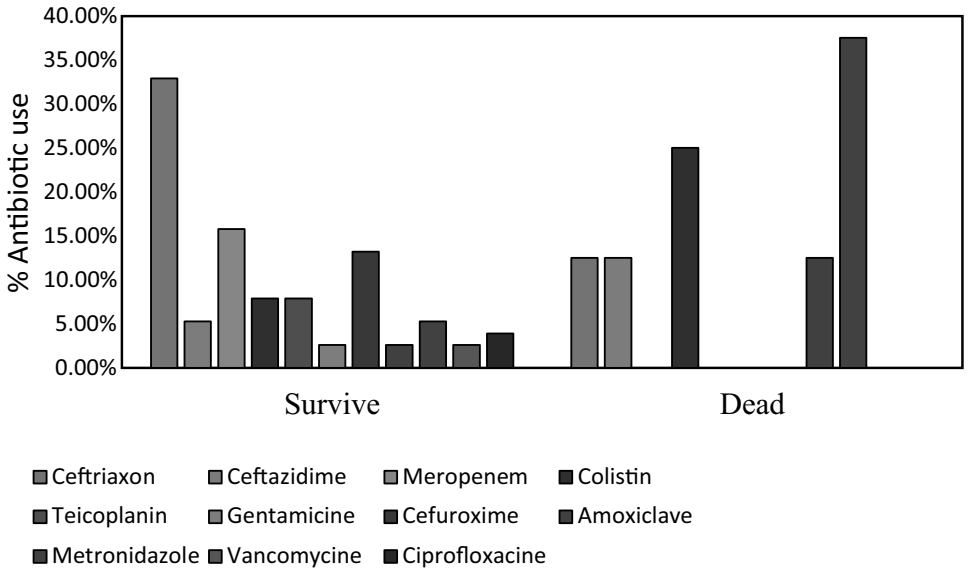

Fig. 3 Percentage of antibiotics use among patients at time of amputation as related to main outcome of the study

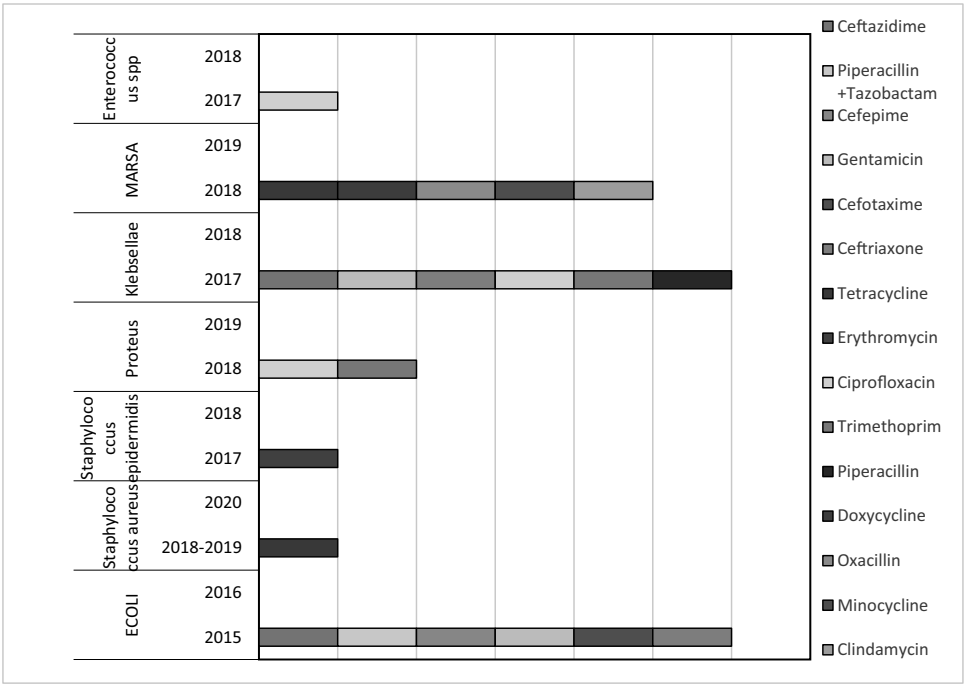

Fig. 4 Bacterial isolates form diabetic foot and antibiotic-resistance among all subjects of the study during the past 5 years

study have higher percentage of MRSA which is usually hospital-acquired infection that is hard to eradicate.

Our study showed an extensive use of strong antibiotics by parental route (IV or IM) in hospital setting. As shown in Figs. 3 and 4 above, 2nd, 3rd, and 4th generation cephalosporins are extensively used and still showing activity. Almost 34% of survived patients were on cefuroxime IM around amputation and 37% of dead subjects were on metronidazole (Fig. 3). Colistin, metronidazole, and amoxicillin/clavulanic acid are facing higher rates of bacterial resistance and their use is increasingly associated with death. This is similar to the results of Kenya and Lebanon studies above.

Mixed infections (bacterial with fungal) are rare but also found in DFU patients. *Candida* species (albicans and parapsilosis) and *Aspergillus flavus* were the most common fungal isolates from DFU. They were successfully treated with oral fluconazol. Seemingly, another study showed that candida was resistant to fluconazol and *Aspergillus* was resistant to itraconazole. Fungal infection if not recognized and treated can impede wound healing [16–18]. Minority of cases in our

study had mixed infection and were treated successfully but were not included in the study.

A plethora of studies addressed the risk factors associated with DF amputation whether it was major amputation (above the ankle) and minor amputation (below the ankle). Major amputations were associated with short recovery time and greater mortality rate. The rate of amputation was directly proportional to HbA1c values more or equal to 8%. Risk factors for major amputation include increase in WBC count and ulcer history. Minor amputation risk factors include; increased duration of diabetes, increased WBC count, infection, revascularization history, and decreased postprandial blood sugar. Foot deformity and serum urea were also associated with diabetic lower extremity amputations [19–22].

In this study, almost all patients had uncontrolled diabetes as shown by average HbAC1 around 8.55% and average FBS around 292 mg/dl. However, we could not find a significant relation between some of the studied factors including cumulative blood sugar, FBS, and other factors in Table 1 and amputation risk. This could be attributed to 2 factors; first, FBS and HbAC1 were not different among subjects of our study whether they had minor or major amputation or DFU. All patients (dead or survived) had uncontrolled diabetes with complications at varying degrees upon admission to orthopedic department.

Second, we didn't study severity of DFU infection prospectively, as per University of Texas scale [9], which plays major role in progression of infection into major or minor amputation. However, we found a significant relation between diabetic complications and survival rate of patients after amputation. Diabetic complications are an inevitable end result of uncontrolled diabetes. All dead patients (8 patients) had diabetic complications, Table 2.

Nephropathy (10% of patients) was associated with death of all patients who had this complication along with *Proteus* species isolates, Fig. 2. Noteworthy, *Proteus* itself, as independent factor, was not associated with highest number of deaths as shown in Table 2. This proves valid the conclusion that nephropathy plays a major role in death in patients with DFU around amputation due to direct effect on infection complications and intervention with antibiotic efficacy and/or excretion rate. This comes in align with a study by Shojaiefard et al. (2008) that found higher incidence of nephropathy(as independent factor) in patients who needed amputation [23].

Neuropathy (62%) of patients and peripheral vascular disease (15%) were independently associated with death of half patients with *E. coli* isolates, Fig. 2. Other studies related neuropathy and peripheral vascular disease to amputation in DFU but not to death [24, 25].

One might expect that MRSA, a multidrug resistant bacteria, should lead to death in rates more than *E. coli* or other species. This was not the case in our study since patients who had MRSA infection didn't have diabetic complications as shown in Fig. 2 above.

However, *Acinobacter* species and *E. coli* were associated with highest percentage of death in our patients around amputation. Antibiotic-resistance might be one of the contributing factors and the deterioration of patient condition might be the main reason. This is an epidemiologic evidence of increasing death rate with these bacterial species. We can't really confirm causative relationship with death, Table 2. We didn't confirm direct cause of death among these patients neither did we calculate time elapsed between amputation and death. This might be one of the limitations of this study.

A study by Martins-Mendes, et al., found that complication count and previous DFU were related with death [26]. They also concluded that previous DFU was associated with DFU, amputation and death. Actually, many of our patients had recurrent DFU infections and went through serial amputations before final major amputation with or without death.

Our study doesn't present a solution for antibiotic resistance neither gives suggestions for antibiotic of choice for use, since there isn`t one antibiotic or a combination of antibiotics that might fit all patents at all times. This came along with other studies conclusions where it was found that no single antibiotic used empirically offers adequate coverage for all potential bacteria found in diabetic foot ulcers [27]. In addition to that, poor penetration of antibiotics into the lower limb tissue due to peripheral arterial disease makes therapy less effective [28].

Thirteen of our patients (15%) (11 survived after amputation and 2 dead) had peripheral vascular disease as explained above.

It was so frustrating to see the extensive use of parenteral antibiotics in these patients to the degree they are no longer effective and patients are losing their choices. Actually patients and their families or care-providers share responsibility in that. We have noticed that subjects in this study came to hospital at end stage of infection with very poor control of blood sugar. Both factors lead to devastating end of amputation.

To make sense of all what we have done, we followed trends of antibiotic use along with bacterial resistance as was confirmed by bacterial culture and sensitivity tests during the past years in this hospital. It was shown that *E. coli*, which happens to be sensitive to many agents in the year 2015, was no longer sensitive to any of them in 2016, as shown in Fig. 4 above. On the other hand, *Klebsiella* species that were sensitive to many of the listed agents in 2017, were no longer sensitive to any of them in 2018. *S. aureus* became resistant to almost all listed drugs in 2020 including cefotaxime that was effective in 2018–2019. MRSA was found to be resistant to most listed drugs since 2019.

Management of DFU firstly can be achieved by good glycemic control. Pharmacological therapy using suitable antibiotics depending on wound culture result comes next. Quite few studies suggested that intake of some vitamins improves wound healing. These vitamins include; magnesium, zinc and vitamin D. Significant improvement in DFU was observed when these vitamins were taken for 12 weeks [29–31].

Patients with DFUs and COVID-19 infection who require surgical intervention should be treated with considerable care. The surgery should be performed under protective conditions in a negative-pressure operating room. General anesthesia should be avoided in these patients [32].

We highly recommend early diagnosis and treatment of DFU, routine foot inspection, training and employment of Podiatricians in all diabetic clinics of the ministry. Also early use of pressure off-loading devices might protect patients' foot and prevent amputation or worsening of bacterial infection.

We have noticed that amoxicillin/clavulanate and/or ciprofloxacin were the drugs of choice for empirical treatment in most patients in this hospital as part of the general protocol of management by the ministry. However, as we found in our study later, most bacterial species are resistant now to both agents and to combination of both. We recommend highly the personal approach of management of DFU and that there is no single antibiotic that fits all patients. On the light of that, we refused to accept the use of antibiotics upon availability or as per the hospital formulary or best list. The use of IV, or IM antibiotics should be limited to severe cases and for few days only, then a patient has to step down to oral treatment in order to minimize kidney failure and antibiotic resistance. In addition to that, we recommend exploring the possibility of Ozone clinics in the country.

Study limitations

We didn't determine the survival rate of patients per infection severity neither did we classify bacterial infection into aerobic or anaerobic bacteria, which could shed light on important aspects of resistance and severity of infection.

We focused on sensitivity of bacteria to antibiotic(s) used around amputation without linking it to severity of DFI according to Wagnar or other universal systems due to the retrospective nature of the study and availability of information in the profiles.

Year of survival after amputations was not determined, since it wasn't one of our end results to determine survival rate, rather than death or amputation as the main outcomes.

Small sample size makes it hard to globally generalize conclusions from this study; however, results might be valid in the Middle East.

Conclusions

Mortality rate is alarmingly increasing in diabetic patients who suffer from diabetic foot ulcer with or without amputation. Mortality was associated with nephropathy and *Acinetobacter* species or *E. coli* infections. Wise use of antibiotics and personalized treatment of DFU should be adopted.

Acknowledgements We would like to thank the staff at Hebron Governmental Hospital (Formerly Queen Alia Hospital) especially the laboratory department, attending physicians in the orthopaedic department, and pharmacy department for their cooperation with our team in order to get this work done.

Declarations

Ethics approval and consent to participate First, we took the permission to run this study from the ethics committee at Hebron University (IRB). Then we applied to the Ministry of Health, department of research and continuous learning to get their permission in order to get access to patients` profiles at the intended hospital. After we got the approval from the minister of health, we approached the hospital administration board and president in order to start the work.

Conflict of interest We declare no conflict of interest for this research.

References

1. Kim H-G. Cognitive dysfunctions in individuals with diabetes mellitus. Yeungnam Univ J Med. 2019;36(3):183.
2. Mellitus D. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2005;28(S37):S5–10.
3. Serván PR. Diet recomendations in diabetes and obesity. Nutr Hosp. 2018;35(Spec No4):109–15.
4. Salameh BS, Abdallah J, Naerat EO. Case-Control study of risk factors and self-care behaviors of foot ulceration in diabetic patients attending primary healthcare services in Palestine. J Diabetes Res. 2020;2020.
5. Lim JZM, Ng NSL, Thomas C. Prevention and treatment of diabetic foot ulcers. J R Soc Med. 2017;110(3):104–9.
6. Qari FA, Akbar D. Presentation and treatment. Saudi Med J. 2000;21(5):443–6.
7. Wouter B, Abu-Hanna A, Bus SA. Development of a multivariable prediction model for plantar foot ulcer recurrence in high-risk people with diabetes. BMJ Open Diabetes Res Care. 2020;8(1):e001207.
8. Tolossa T, Mengist B, Mulisa D, Fetensa G, Turi E, Abajobir A. Prevalence and associated factors of foot ulcer among diabetic patients in Ethiopia: a systematic review and meta-analysis. BMC Public Health. 2020;20(1):1–14.
9. Smith-Strøm H, Iversen MM, Igland J, Østbye T, Graue M, Skeie S, et al. Severity and duration of diabetic foot ulcer (DFU) before seeking care as predictors of healing time: a retrospective cohort study. PLoS One. 2017;12(5):e0177176.
10. Dekker RG, Qin C, Ho BS, Kadakia AR. The effect of cumulative glycemic burden on the incidence of diabetic foot disease. J Orthop Surg Res. 2016;11(1):1–8.
11. Banu A, Hassan MMN, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: a prospective study. Australas Med J. 2015;8(9):280.

12. Kaimkhani GM, Siddiqui AA, Rasheed N, Rajput MI, Kumar J, Khan MH, et al. Pattern of infecting microorganisms and their susceptibility to antimicrobial drugs in patients with diabetic foot infections in a tertiary care hospital in Karachi, Pakistan. Cureus. 2018;10(6).
13. Carro GV, Carlucci E, Priore G, Gette F, Llanos MDLÁ, Losada D, et al. Infecciones en pie diabético. Elección del tratamiento antibiótico empírico. Medicina (Buenos Aires). 2019;79(3).
14. Obeid M, Moughames E, Aboulhosn P, Madi R, Farah M, Feghali J, et al. Epidemiology and susceptibility profiles of diabetic foot infections in five hospitals in Lebanon. J Infect Dev Ctries. 2018;12(05):347–51.
15. Mutonga DM, Mureithi MW, Ngugi NN, Otieno FC. Bacterial isolation and antibiotic susceptibility from diabetic foot ulcers in Kenya using microbiological tests and comparison with RT-PCR in detection of S. aureus and MRSA. BMC Res Notes. 2019;12(1):1–6.
16. Torrence GM, Schmidt BM. Fungal osteomyelitis in diabetic foot infections: a case series and comparative analysis. Int J Low Extrem Wounds. 2018;17(3):184–9.
17. Raiesi O, Siavash M, Mohammadi F, Chabavizadeh J, Mahaki B, Maherolnaghsh M, et al. Frequency of cutaneous fungal infections and azole resistance of the isolates in patients with diabetes mellitus. Adv Biomed Res. 2017;6.
18. Missoni EM, Kalenić S, Vukelić M, De Syo D, Belicza M, Kern J, et al. Role of yeasts in diabetic foot ulcer infection. Acta Med Croat . 2006;60(1):43–50.
19. Atosona A, Larbie C. Prevalence and determinants of diabetic foot ulcers and lower extremity amputations in three selected tertiary hospitals in Ghana. J Diabetes Res. 2019;2019.
20. Jiang Y, Ran X, Jia L, Yang C, Wang P, Ma J, et al. Epidemiology of type 2 diabetic foot problems and predictive factors for amputation in China. Int J Low Extrem Wounds. 2015;14(1):19–27.
21. Armstrong DG, Lipsky BA. Diabetic foot infections: stepwise medical and surgical management. Int Wound J. 2004;1(2):123–32.
22. Jbour A, Jarrah NS, Radaideh AM, Shegem NS, Bader IM, Batieha AM, et al. Prevalence and predictors of diabetic foot syndrome in type 2 diabetes mellitus in Jordan. Saudi Med J. 2003;24(7):761–4.
23. Shojaiefard A, Khorgami Z, Larijani B. Independent risk factors for amputation in diabetic foot. Int J Diabetes Dev Ctries. 2008;28(2):32.
24. Shin JY, Roh S-G, Sharaf B, Lee N-H. Risk of major limb amputation in diabetic foot ulcer and accompanying disease: a meta-analysis. J Plast Reconstr Aesthet Surg. 2017;70(12):1681–8.
25. Li X, Xiao T, Wang Y, Gu H, Liu Z, Jiang Y, et al. Incidence, risk factors for amputation among patients with diabetic foot ulcer in a Chinese tertiary hospital. Diabetes Res Clin Pract. 2011;93(1):26–30.
26. Martins-Mendes D, Monteiro-Soares M, Boyko EJ, Ribeiro M, Barata P, Lima J, et al. The independent contribution of diabetic foot ulcer on lower extremity amputation and mortality risk. J Diabetes Complicat. 2014;28(5):632–8.
27. Rahim F, Ullah F, Ishfaq M, Afridi AK, ur Rahman S, Rahman H. Frequency of common bacteria and their antibiotic sensitivity pattern in diabetics presenting with foot ulcer. J Ayub Med Coll Abbottabad. 2016;28(3):528–33.
28. Datta P, Chander J, Gupta V, Mohi GK, Attri AK. Evaluation of various risk factors associated with multidrug-resistant organisms isolated from diabetic foot ulcer patients. J Lab Phys. 2019;11(1):58.
29. Razzaghi R, Pourbagheri H, Momen-Heravi M, Bahmani F, Shadi J, Soleimani Z, et al. The effects of vitamin D supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. J Diabetes Complicat. 2017;31(4):766–72.
30. Razzaghi R, Pidar F, Momen-Heravi M, Bahmani F, Akbari H, Asemi Z. Magnesium supplementation and the effects on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. Biol Trace Elem Res. 2018;181(2):207–15.
31. Momen-Heravi M, Barahimi E, Razzaghi R, Bahmani F, Gilasi HR, Asemi Z. The effects of zinc supplementation on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. Wound Repair Regener. 2017;25(3):512–20.
32. Tao F, Tang X, Tao H, Luo Y, Cao H, Xiang W, et al. Surgical treatment of diabetic foot ulcers during the COVID-19 pandemic in China. J Diabetes Complicat. 2020;34(9):107622.

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ORIGINAL ARTICLE

Comparison of Mini-Mental State Examination and Addenbrooke's Cognitive Examination III in detection of cognitive impairment in patients with type 2 diabetes

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Abstract

Introduction There is an increased prevalence of cognitive impairment (CI) in patients with type 2 diabetes; thus, there is a need for a sensitive and a convenient screening tool for its detection. While Mini-Mental State Examination (MMSE) is a widely used tool for the assessment of cognition, it is not available for free for research. Hindi version of Addenbrooke's Cognitive Examination III is a freely available cognition assessment tool that has been widely used on diverse group of diseases including diabetes.

Aims and objectives To assess cognitive function in patients with type 2 diabetes using Hindi version of ACE-III and compare its performance with MMSE.

Materials and methods Cognition was assessed using validated Hindi version of ACE-III and MMSE among 54 participants with type 2 diabetes and similar number of age, sex, and educational status matched healthy controls. The cut-off for CI was taken at 82/100 for ACE-III, and for MMSE, education level–based cut-offs were used: 21 for the illiterate group, 22 for the low education group, 23 for the middle education group, and 24 for the high education group.

Results Mean age of the study population was 64.5 ± 5.3 years. The average duration of diabetes was 8.5 ± 6.0 years with mean HbA1C of $8.8 \pm 2.5\%$. Both the tools showed high prevalence of CI among cases, but ACE-III was more sensitive in detection of CI when compared to MMSE (64.8% vs. 55.6%)**.** There was a good agreement between ACE-III and MMSE outcomes (Cohen's kappa = 0.732).

Conclusion Type 2 diabetes mellitus is an independent risk factor for cognitive decline in elderly patients. ACE-III is an easy to use, sensitive, and freely available screening tool for cognition assessment in diabetes population.

Keywords Diabetes · Elderly · MMSE · ACE-III

Introduction

Diabetes is strongly associated with cognitive decline, and it also increases risk for vascular dementia and Alzheimer's disease by about 100–160% and 45–90%, respectively [1]. Diabetes is also associated with faster progression from mild cognitive impairment (MCI) to frank dementia, and patients with type 2 diabetes and cognitive impairment (CI) are less likely to receive adequate diabetes care compared to those who have diabetes alone [2]. On the other hand, CI in diabetes also increases risk of both hyperglycemia and hypoglycemia [3]. Neuroimaging studies have shown that there is increased prevalence and faster progression of brain atrophy in diabetes affecting both gray and white matter which is related to poor cognitive function [4, 5].

The prevalence of CI in diabetes is variable and depends on the underlying population and the assessment tool. For instance, one study had shown a 96.31% prevalence of CI among diabetic patients [6] but the cohort was from neurology clinic and hence had more chances of underlying CI irrespective of diabetes. Other studies have shown prevalence to vary from 48 to 63%, but the assessment tools were different in these studies [7–9]. The CI may produce difficulty in managing diabetes particularly for patients on

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multiple oral drugs and insulin as it can affect correct insulin administration, timing, and dose adjustments. Thus, it is necessary to detect CI in patients with diabetes at an early stage to plan for better overall care. Moreover, there is some evidence that a strict glycemic control in midlife prevent CI [10] but a caution to be exercised in older patients as intensive glycemic control may not be beneficial to retard progression of CI [11].

The Mini-Mental State Examination (MMSE) has been the most widely used test for assessment of cognitive function in diabetes [7, 12, 13], while some studies have used Montreal Cognitive Assessment (MoCA) [9]. There has not been any standard tool for cognitive assessment in diabetes. The Addenbrooke's Cognitive Examination III (ACE-III) is a cognitive screening tool in patients above 50 years of age [14]. The ACE-III had been used in Chinese cohort of type 2 diabetes subjects with good reliability, and it was able to detect impairment in global and individual domains of cognition [15]. ACE-III is also available in Hindi version [16] and is free from copyright with the facility of online training (https://www.mvls.gla.ac.uk/aceIIItrainer/). However, there have been very few studies in India using ACE in diabetes [6]. The present case–control study was thus designed to assess the cognition among patients with diabetes using ACE-III and MMSE tools and compare their utility in detection of CI.

Materials and methods

Study design and setting

We conducted a case control study over a period of 2 years (May 2018 to April 2020). The study was conducted in the Department of Internal Medicine, GSVM Medical College, Kanpur, and subjects were recruited from the outpatient department.

Participants

Patients of age ≥ 60 years with type 2 diabetes of duration ≥ 2 years were considered for the study. The diagnosis of diabetes was based on previous biochemical investigations (fasting plasma glucose and/or HbA1c) or those diagnosed elsewhere but requiring treatment for glycemic control. Those who were willing to participate were evaluated for exclusion criteria. Patients with known depression, epilepsy, or patients on antiepileptics, psychiatric illness, acutely ill patients, or patients with chronic systemic illness, substance abuse, stroke, and encephalopathy were excluded from the study. Patients who were unable to read, prior stroke with impaired higher mental function, and those who had significant hypoglycemia over past 4 weeks (a documented glucose of ≤ 54 mg/dl and/or loss of consciousness secondary to hypoglycemia) were not considered for the study. A total of 180 type 2 diabetes patients were surveyed and 118 subjects were excluded. Remaining 62 subjects were interviewed for Beck's Depression Inventory – Hindi version (H-BDI) [17] to exclude patients with underlying undiagnosed depression (subject with a score of ≥ 30 was excluded from the study). Finally, we had 54 type 2 diabetes subjects as study participants. We took 54 age-, sex-, and educational status–matched healthy subjects as controls for comparison. The control participants were recruited from the relatives of non-diabetic patients presenting in out-patient department.

Study tools

Subjects from both the groups were assessed for cognitive dysfunction by using paid Hindi version of the Mini-Metal State Examination (MMSE) (https://www.parinc.com/Products/Pkey/243) as well as validated Hindi version of Addenbrooke's Cognitive Function Test or ACE-III (https://www.sydney.edu.au/content/dam/corporate/documents/brain-and-mind-centre/ace-diagnostic-tests/ace-iii---standard/ACE-III-Administration-Hindi.pdf). MMSE includes tests of orientation, attention, memory, language, and visuospatial skills. The maximum score for MMSE is 30. As the subjects had heterogeneous education level, we used education level–based cut-offs based on a previous study [18] and following cut-offs were used: 21 for the illiterate group, 22 for the low education group, 23 for the middle education group, and 24 for the high education group. ACE-III tool assesses 5 cognitive sub-domains: attention, memory, fluency, language, and visuospatial. It has a maximum score of 100 and recommended cut-off for CI is 82 with sensitivity of 93% and specificity of 100% [14]. The two investigators SA and ST had a formal training for ACE-III assessment from university of Glasgow, UK (online). We used illiterate and literate versions of ACE-III for illiterate and literate (primary education and above) individuals, respectively, for assessment.

Sample size

For sample size calculation, the prevalence of cognitive impairment in elderly population was assumed to be 25% and among diabetic population was taken to be 54% based on two previous Indian studies on elderly general population and elderly diabetes population, respectively [19, 20]. The required sample in each group was 45; we recruited 54 subjects in each group.

Statistical analysis

The data obtained was transferred to Microsoft Excel (version 2016). Analysis was done using SPSS software version 22.0. The descriptive analysis was done in terms of range, frequency, percentages, mean, and standard deviation. The significance of difference for cognition assessment by ACE-III and MMSE was done by chi square test using recommended cut-offs and odds ratio was also calculated. "*p*" value < 0.05 denoted significant difference. We also investigated the agreement between ACE-III and MMSE scores using Bland Altman plot and calculated the Cohen's kappa agreement between these two tools. Controls were matched for age and education status; however, hypertension and dyslipidemia were possible confounding factors between the two groups.

Results

Table 1 describes the baseline characteristics of the cases and controls. The diabetic subjects were matched with controls in terms of age, gender, and educational status. Mean age of the study population was 64.5 ± 5.3 years with an average duration of diabetes of 8.5 ± 6 years. The two groups had similar prevalence of smoking and alcohol intake. However, there was a significant difference between the cases and controls in prevalence of hypertension and HbA1c which is expected in the former. Mean MMSE and ACE-III scores were significantly lower among cases compared to controls (Table 2). Thus, both the tools depicted a higher incidence of CI in diabetes; however, CI detection was better with ACE-III compared to MMSE in both cases and controls. There was also good agreement between the two tools (Fig. 1) with Cohen's kappa value of 0.732 which suggests that as MMSE score decreases, ACE-III score is also expected to decrease.

Discussion

Our cohort of diabetic participants had an advanced age, long duration of diabetes, and poor glycemic control. Earlier studies have found an increased prevalence of CI in diabetes [7–9]. Thus, it is required to have formal cognitive assessment in this population. In this study, both the tools (MMSE and ACE-III) detected a high prevalence of CI among patients with diabetes when compared to controls (Table 2). A screening tool which is more sensitive

Table 1 Demographic profile of cases and controls

Variable		Cases (mean ± SD)	Controls (mean ± SD)	*p* value
Age (years)		64.5 ± 5.3	64.9 ± 4.8	0.356 (t test)
Gender (%)	Males	50	50	$p = 0.15$ (χ^2)
	Females	43	57	
Education (%)	Illiterate	14.8	18.5	$p = 0.86$ (χ^2)
	Primary school	38.9	46.3	
	High school	25.9	13	
	Graduate and above	20.3	22.2	
Hypertension (%)		46.3	14.8	0.004 (χ^2)
Duration of diabetes (years)		8.5 ± 6	NA	
HbA1C (%)		8.8 ± 2.5	5.2 ± 0.2	0.003 (t test)
Smokers*(%)		9.3	11.1	0.507 (χ^2)
Alcoholic# (%)		8.9	14.8	0.732 (χ^2)

*Tobacco smoking (including cigarettes and bidi) for several years

#Daily alcohol intake of ≥ 2 drinks for men and ≥ 1 drink for women for several years

Table 2 Comparison of ACE-III and MMSE scores

	Subjects with diabetes	Subjects without diabetes	Odds ratio	95% CI	Z statistic	*p* value
Mean score (SD) on ACE-III	74.98 ± 11.2	86.92 ± 5.3				< 0.001
Mean score (SD) on MMSE	23.15 ± 4.4	25.63 ± 3.0				< 0.001
Subjects with low score on ACE-III (%)	34 (64.8)	11 (20.3)	6.6	2.8—15.7	4.305	< 0.0001
Subjects with low score on MMSE (%)	30 (55.6)	8 (14.9)	7.2	2.9 -18.1	4.189	< 0.0001

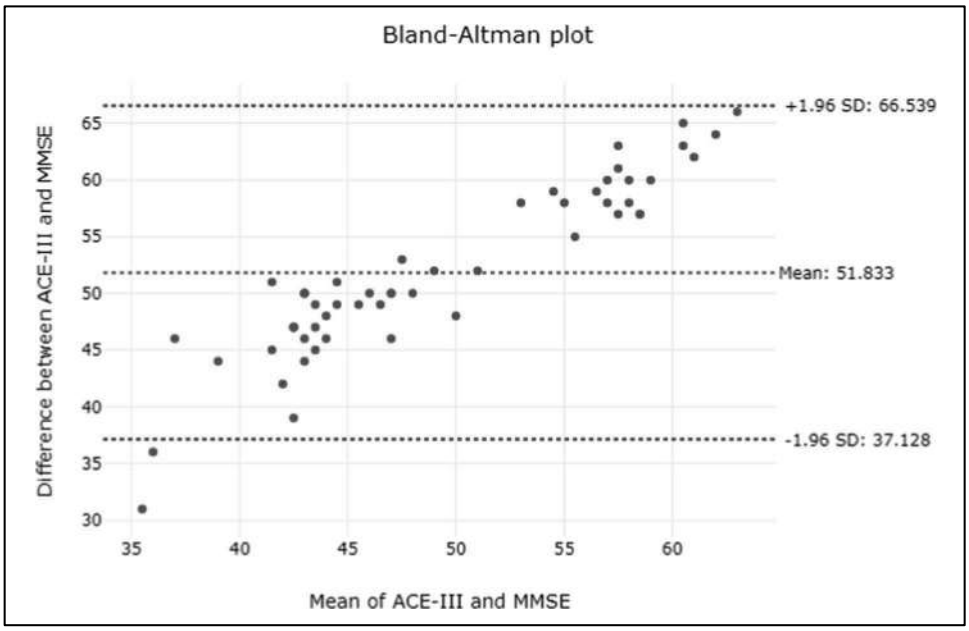

Fig. 1 Agreement between ACE-III and MMSE using Bland Altman plot

may be more helpful, and ACE-III was a more sensitive tool compared to MMSE for detection of CI in our study as it picked up more cases with probable CI (64.8% vs. 55.6%). ACE-III picked up 4 more cases of CI among diabetes compared to MMSE. Moreover, ACE-III also picked up 3 additional cases of CI among control participants compared to MMSE. The agreement between the two tools on Bland Altman analysis was also good (Fig. 1), suggesting that ACE-III has comparable diagnostic accuracy when compared to MMSE, and thus, ACE-III can also work as a screening tool for cognition assessment. This tool has also been validated in a previous study on type 2 diabetes [15]. Moreover, as MMSE has issues of copyright after 2001 [21], ACE-III can be used for research studies and as it had also shown comparable results to other screening tools like Montreal Cognitive Assessment (MoCA) and Rowland Universal Dementia Assessment Scale for discriminating healthy individuals from CI [22, 23]. Another important advantage with ACE-III over MMSE is its ability to detect mild cognitive impairment (MCI) [24]. Moreover, the subdomains of ACE-III determine the day-to-day functional impairments better than MMSE and MoCA [25]. One study also suggested that it can detect moderate to severe dementia at a cut-off of 61 [25]. However, caution is to be exercised as specificity of both the scores and optimal cut-offs in diabetic population is still unknown and any patient diagnosed to have low scores in the absence of obvious clinical history and/or examination suggestive of CI should undergo formal neuropsychological assessment before making a final diagnosis.

Conclusion

Considering an exceedingly high prevalence of CI among elderly diabetic patients, cognitive assessment must be included in evaluation of diabetic patients. ACE-III is an easy to use, sensitive, reliable, and freely available screening tool for cognitive assessment of patients with diabetes and has similar diagnostic utility as MMSE. However, abnormal ACE-III scores should be supplemented with appropriate neuropsychological tests.

Strengths of the study

The study used most updated ACE-III tool for assessment of cognitive function in diabetes. The population studied was carefully selected and after exclusion of possible confounding factors and excluding subjects with underlying undiagnosed depression by use of H-BDI tool.

Limitations

This is not a validation study and hence does not define optimal cut-offs for defining CI in diabetes. We used cut-offs for CI which were defined from different set of population. Moreover, unlike MMSE, we did not have education level–based cut-offs for ACE-III (although we used

illiterate and literate versions of ACE-III) as there are limited studies in this regard.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study was approved by the Ethics Committee of the institute (EC/09/ethics/2018, dated 21/03/2018).

References

1. Kravitz E, Schmeidler J, Schnaider BM. Type 2 diabetes and cognitive compromise: potential roles of diabetes-related therapies. Endocrinol Metab Clin North Am. 2013;42(3):489–501.
2. Handley M, Bunn F, Goodman C. Dementia-friendly interventions to improve the care of people living with dementia admitted to hospitals: a realist review. BMJ Open. 2017;7(7):e015257.
3. Punthakee Z, Miller ME, Launer LJ, Williamson JD, Lazar RM, Cukierman-Yaffee T, et al. Poor cognitive function and risk of severe hypoglycemia in type 2 diabetes: post hoc epidemiologic analysis of the ACCORD trial. Diabetes Care. 2012;35(4):787–93.
4. Moran C, Phan TG, Chen J, Blizzard L, Beare R, Venn A, et al. Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. Diabetes Care. 2013;36(12):4036–42.
5. van Elderen SGC, de Roos A, de Craen AJM, Westendorp RGJ, Blauw GJ, Jukema JW, et al. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. Neurology. 2010;75(11):997–1002.
6. Kant S, Poddar K, Kamle M, Patil C. Assessment of cognitive impairment by using Addenbrooke's Cognitive Examination (ACE) and Montreal Cognitive Assessment (MoCA) amongst type 2 diabetes mellitus patients in Eastern Uttar Pradesh. India IP Indian J Neurosci. 2020;4(4):185–90.
7. N S, Karan. Assessment of the Cognitive Status in Diabetes Mellitus. J Clin Diagn Res JCDR. 2012;6(10):1658–62.
8. Solanki RK, Dubey V, Munshi D. Neurocognitive impairment and comorbid depression in patients of diabetes mellitus. Int J Diabetes Dev Ctries. 2009;29(3):133–8.
9. Lalithambika CV, Arun CS, Saraswathy LA, Bhaskaran R. Cognitive impairment and its association with glycemic control in type 2 diabetes mellitus patients. Indian J Endocrinol Metab. 2019;23(3):353.
10. Rawlings AM, Sharrett AR, Schneider ALC, Coresh J, Albert M, Couper D, et al. Diabetes in midlife and cognitive change over 20 years: a cohort study. Ann Intern Med. 2014;161(11):785–93.
11. Launer LJ, Miller ME, Williamson JD, Lazar RM, Gerstein HC, Murray AM, et al. Effects of intensive glucose lowering on brain structure and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. Lancet Neurol. 2011;10(11):969–77.
12. Shanmugapriya S, Dhandapani N, Saravanan T. A study comparing cognitive function assessment in type-2 diabetes mellitus using Rowland Universal Dementia Assessment Scale and Mini Mental State Examination. Int J Health Allied Sci. 2018;7(3):184.
13. Wilson RS, Barnes LL, Krueger KR, Hoganson G, Bienias JL, Bennett DA. Early and late life cognitive activity and cognitive systems in old age. J Int Neuropsychol Soc JINS. 2005;11(4):400–7.
14. Bruno D, Schurmann VS. Addenbrooke's cognitive examination III in the diagnosis of dementia: a critical review. Neuropsychiatr Dis Treat. 2019;15:441–7.
15. Xin J, Xiao X, Chen X, Pan X. Application of Chinese version of ACE-III in type 2 diabetes mellitus patients with mild cognitive impairment. Zhonghua Yi Xue Za Zhi. 2017;97:3455–9.
16. Bajpai S, Upadhyay A, Sati H, Pandey RM, Chaterjee P, Dey AB. Hindi Version of Addenbrooke's Cognitive Examination III: Distinguishing Cognitive Impairment Among Older Indians at the Lower Cut-Offs. Clin Interv Aging. 2020;15:329–39.
17. Kumar K J. 1. Kushwaha, Jitendra Kumar (2016): Beck Depression Inventory: Hindi Translation and Psychometric Properties for the students of Higher Education. Journal of Research in Humanities and Social Sciences (Quest Journals), Vol. 4, No. 9, Pp 39–49. ISSN: 2321–9467 (Online Access). J Res Humanit Soc Sci. 2016; 4:39–49.
18. Kochhann R, Varela JS, Lisboa CS de M, Chaves MLF. The Mini Mental State Examination: Review of cutoff points adjusted for schooling in a large Southern Brazilian sample. Dement Neuropsychol. 2010;4(1):35–41.
19. Patel RM, Singh US. Prevalence study of cognitive impairment and its associated sociodemographic variables using mini-mental status examination among elderly population residing in field practice areas of a medical college. Indian J Community Med. 2018 Apr 1;43(2):113.
20. Goswami A, Reddaiah VP, Kapoor SK, Singh B, Dey AB, Dwivedi SN, et al. Prevalence and determinants of cognitive impairment in rural elderly population in India. Help Age India Res Dev J. 2006;12(1):8–15.
21. Seshadri M, Mazi-Kotwal N. A copyright-free alternative to the mini-mental state examination is needed. BMJ. 2012;345:e8589.
22. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695–9.
23. Rowland JT, Basic D, Storey JE, Conforti DA. The Rowland Universal Dementia Assessment Scale (RUDAS) and the Folstein MMSE in a multicultural cohort of elderly persons. Int Psychogeriatr. 2006;18(1):111–20.
24. Slachevsky A, Villalpando JM, Sarazin M, Hahn-Barma V, Pillon B, Dubois B. Frontal assessment battery and differential diagnosis of frontotemporal dementia and Alzheimer disease. Arch Neurol. 2004;61(7):1104–7.
25. Giebel CM, Challis D. Sensitivity of the Mini-Mental State Examination, Montreal Cognitive Assessment and the Addenbrooke's Cognitive Examination III to everyday activity impairments in dementia: an exploratory study. Int J Geriatr Psychiatry. 2017;32(10):1085–93.

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ORIGINAL ARTICLE

Periodontitis and diabetes mellitus—an awareness and perception study among endocrinologists and diabetologists

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Abstract

Background of the study Periodontitis is a chronic inflammatory disease caused by pathogenic dental plaque which causes microbial dysbiosis leading to destruction of supporting structures of teeth with a consequent tooth loss. Diabetes mellitus and periodontitis are non-communicable diseases with a bidirectional relation. The awareness of the medical community about potential effect of periodontal infections on systemic health is important. The aim of this study was to understand the physician's knowledge, practice behavior, and awareness about periodontal disease and its association with diabetes.

Methods A cross-sectional study was undertaken among 210 endocrinologists and diabetologists based on the selection criteria. A validated questionnaire was sent to the participants wherein the respondent demographic characteristics, awareness about the periodontal disease, and information about their practice was collected. Descriptive analysis was done for the collected data and was presented as frequency counts and percentages. The association between categorical variables was done by Pearson's chi-square test.

Results The response rate for the study was 90.3% ($p < 0.001$). Seventy-nine percent of the respondents were males with a fair knowledge about oral hygiene aids. Oral health awareness regarding frequency of dental examination (80%) and oral prophylaxis (69%) was significant. Only 11% respondents referred their patients for dental examination. Eighty-five percent of the participants agreed that oral health is necessary for overall health and 65% were in favor of bidirectional referrals among the dentist and endocrinologists. Significant response was also collected on inclusion of patient's oral health records in diabetic health history assessments (58%). Eighty percent of the respondents strongly agreed on the collaborative training for medical and dental students in their educational curriculum.

Conclusion Our study concluded that in the awareness about periodontal disease, its bidirectional relation was high among the study participants but did not significantly relate to their referral rate to dental office. Integrated collaborative approaches by physicians and periodontists should be a part of the management protocols diabetes and oral/periodontal health. Such collaborations would aim for an early detection of the diabetic patients at periodontal risk (as a pre-diabetic screening parameter) and vice versa.

Keywords Periodontitis · Endocrinologists · Periodontal medicine · Diabetes · Periodiabetes · HbAIc · Inter professional education

Introduction

Periodontitis is an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation and recession or both [1]. Periodontal pathogens and their toxins enter the bloodstream leading to systemic production of inflammatory mediators causing and/or contributing to the development of systemic diseases/conditions. These include cardiovascular diseases, diabetes mellitus,

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preterm delivery of low-birth-weight baby, and respiratory infections. There has been a growing interest with respect to association between periodontal disease and systemic diseases/conditions. The scientific basis of evidence for independent associations between type 2 diabetes mellitus (T2DM) and periodontitis is its bidirectional influence [2]. The underlying mechanisms that link these two diseases involve impaired immune function, neutrophil activity, and cytokine biology [3, 4]. Epidemiological data confirm that diabetes is a major risk factor for periodontitis as susceptibility to periodontitis is increased threefold in diabetics [5]. Incidence of macroalbuminuria and end-stage renal disease is increased in diabetics with severe periodontitis versus diabetics with a healthy mouth [6]. One of the first published study investigating the progression of HbA1c among diabetes-free individuals concluded that the patients with the severe periodontitis demonstrate fivefold increase in HbA1c over the 5 years as compared with those with no periodontitis at baseline [7]. Uncontrolled HbA1c levels and elevated cardiovascular risk factors significantly increase the severity of periodontitis in type 2 diabetes mellitus [8].

Oral health problems despite affecting overall quality of life remains a neglected and under-recognized issue. The aim of this study was to understand the current knowledge and practices of endocrinologists and diabetologist on the periodontal disease and diabetes continuum.

Material and methods

This study was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. Ethics approval was obtained from the Institutional Ethical Committee at Indraprasatha Apollo Hospital, New Delhi, India. Two hundred ten board-registered endocrinologists were screened and invited to participate in the study. Written informed consent was obtained from all participants included in the study. The inclusion criteria for this study was specialists practicing/teaching (full time or part time) in a public, private, or government practice. Retired doctors were excluded from the study. A closed-ended survey questionnaire was designed with variables: 2 point scale response (yes/no) or 5-point scale response (strongly agree/agree/don't know/disagree/strongly disagree). The validity of the questionnaire was assessed by the faculties and revealed a high degree of agreement during test–retest of questionnaire. The reliability of the questionnaire was determined by Cronbach's α coefficients. The final questionnaire was prepared that comprised of thirty-four questions divided into three sections: (1) awareness about periodontal disease and its relation to diabetes, (2) demographics and oral health, (3) practice setting. In order to maintain the confidentiality of the records, coded questionnaire form was sent along with the cover note via email to all the study participants. The participants emailed back their completed forms to the investigators.

Data analysis

For statistical analysis, data were entered into a Microsoft Excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Participant characteristics were presented as means and standard deviations (SD) for continuous variables and as percentages for categorical variables. *Z*-test (Standard Normal Deviate) was used to test the significant difference of proportions. Chi-square test (χ^2) was performed to detect the significant associations between the unpaired proportions. Statistical significance was set at $p \leq 0.05$.

Results

Analysis of demographic data

Maximum respondents were in the age group of 41–50 years (64.8%) and least in the age group less than 30 years (1%). 28.6% respondents followed the correct frequency of brushing but a good level of awareness is evitable by the fact that 44.8% used interdental and supplemental aids. Sixty-one percent practiced tongue brushing (Table 1).

Analysis of oral health awareness

80.5% had their dental examination in the last 6–12 months out of which 61.9% had their last prophylaxis within this time frame. High response rate reveals a good oral health awareness level. 56.2% were informed about their oral hygiene status and 69% knew that prophylaxis was required every 6–12 months (Table 2).

Analysis of practice setting

43.8% reported inquiring patients about their dental problems. Although 72.9% respondents were aware of a dental facility in the vicinity, periodontal referral was only 11%. 70.5% of the respondents were aware of the fact that the dental specialization dealing with treatment of gingival and periodontal disease is periodontology and the trained specialist doctor is called a periodontist. Sixty-five percent were in favor of a bidirectional referral. 74.8% of the specialists had a hospital-based practice and only 20% were

Table 1 Survey demographic data by number and percentage of respondents and non-respondents

Variable	Respondents		Non-respondents		*Z* score
	N	%	*N*	%	
Age in years			5	2.4	1.8999
< 30	2	1			
31–40	15	7.5	5		
41–50	136	64.8			
Gender			10	2.1	11.906
Male	166	79	5		
Female	44	21	5		
Frequency of brushing			2	1%	8.5883
Once	148	70.5			
Twice	60	28.6			
Use of dental floss/interdental aid			–	–	7.8576
Yes	94	44.8			
No	94	44.8			
Sometimes	22	10.5			
Tongue brushing			5	2.4	7.702
Yes	128	61			
No	50	23.8			
Sometimes	27	12.9			

$p < 0.05$ = level of significance

N number, % percentage

into academics. Majority of the clinicians (57.6%) had been practicing for 10–20 years (Table 3).

Analysis of awareness about periodontology as a specialty

85.2% of the specialists agreed that oral health is necessary for overall health, and 64% were aware about periodontal disease and its symptoms. 49.5% strongly agreed that inflammation is the key component between these two diseases. Forty-eight percent responded in affirmation on the effect of periodontal treatment on diabetes management. Periodontitis as a complicating factor in diabetes management presents a 35% agreement. A significant response for diabetics being more prone to periodontal disease was received (55%). The level of awareness about the research studies coincided with the level of agreement about bidirectional relationship between the two diseases. Inclusion of oral/periodontal health report in diabetic health review received a high level of agreement (58%). Dose-dependent relationship between severity of periodontitis and complications of diabetes, e.g., macro albuminuria and end-stage renal disease, was not quite well known with the respondents (28%) (Table 4).

Analysis of dental care recommendations

Inclusion of oral health examination as a part of diabetic screening was found significant in relation to type of practice ($p = 0.0001$, hospital based) and age group ($p = 0.003$,

Table 2 Survey respondents oral health practice and information

Variable	Respondents		Non-respondents		*Z* score
	N	%	*N*	%	
Last dental examination visit			6	2.9	2.4876
6 months	72	34.3			
12 months	97	46.2			
Never done	18	8.6			
Don't remember	17	8.1			
Last oral prophylaxis			9	4.3	1.8999
6 months	56	26.7			
12 months	74	35.2			
Never done	50	23.8			
Don't remember	21	10			
Informed about periodontal health status?			10	4.8	5.114
Yes	118	56.2			
No	66	31.4			
Maybe	16	7.6			
Awareness about prophylaxis (6–12 months)?			7	3.3	8.4951
Yes	145	69			
No	58	27.6			

$p < 0.05$ = level of significance

N number, % percentage

Table 3 Survey respondents: inquiring patients about dental problems, referral to dental professional, awareness about periodontology as speciality, concept of collaborative practices, years and type of practice

Variable	Respondents		Non-respondents		*Z* score
	N	%	*N*	%	
Do you ask about dental or oral problems from patients with diabetes?			22	10.5	0.3925
Yes	92	43.8			
No	96	45.7			
Any dental college/dental department in practice vicinity			8	3.8	11.788
Yes	153	72.9			
No	33	15.7			
Not aware	16	7.6			
Periodontal referral for diabetics			4	19	0.3217
Yes	23	11			
No	60	28.6			
Occasionally	60	28.6			
No, but a good idea	63	30			
Collaborative practice between endocrinologist and periodontist			6	2.9	0.2932
Yes, bidirectional referral is good	136	65			
No	50	24			
I don't know	24	11.6			
Are you aware about the specialist who treat periodontal diseases is known as a periodontist?			6	2.9	8.9819
Yes	148	70.5			
No	56	26.7			
Type of practice			4	1.9	11.2383
Hospital-based practice	157	74.8			
Academics	42	20			
Solo practice	7	3.3			
Years in practice			3	1.4	7.9103
< 5	13	6.2			
5–10	31	6.2			
10–20	121	57.6			
> 20	42	20			

$p < 0.05$ = level of significance

N number, % percentage

41–50 years and experience ($p = 0.021, > 20$ years). Dental referral for diabetics showed significant relation with experience ($p = 0.0198$, 11–20 years) and type of practice ($p = 0.024$, hospital based). The question on collaborative training of medical and dental students received a significant response with respect to participants years of practice ($p = 0.0129$, 11–20 years). Bidirectional collaborative practice between endocrinologist and dentist had a significant response with age of participant ($p = 0.021$, 41–50 years) and experience ($p = 0.030$, 11–20 years) (Table 5).

Analyses of participant's awareness on periodontal health and its association to diabetes

Effect of periodontal inflammation on diabetes and its role in increasing diabetic complications had a significant response in relation to age ($p = 0.0005$, $p = 0.028$, 41–50 years) and

Table 4 Survey respondents: awareness on importance of periodontal health in diabetics, scientific evidence between periodontitis and diabetes, importance of oral health screening and intervention in diabetic management protocol

Variable	Respondents *N* (%)					Non-respondents *N* (%)	*Z* score
	Strongly agree	Agree	Don't know	Disagree	Strongly disagree		15.9479
Good oral health is important for overall health	179 (85.2)	16 (7.6)	–	–	–	15 (7.1)	
Aware about periodontal diseases?						10 (4.8)	
Yes	136 (64.8)						
No	64 (30.5)						
Do you know the symptoms of periodontal diseases?						29 (13.8)	8.3995
Yes	131 (62.4)						
No	46 (21.9)						
Inflammation is a key component between periodontal disease and diabetes	104 (49.5)	52 (24.8)	28 (13.3)	14 (6.7)	12 (5.7)	23(4.8)	5.2513
Control of periodontal infection is important in management of diabetes	54 (25.7)	101 (48.1)	17 (8.1)	20(9.5)	1 (0.5)	17(8.1)	4.7526
Periodontitis increases risk for complications of diabetes	56 (26.7)	73 (34.8)	44 (21)	19 (9)	5 (2.4)	13 (6.2)	4.4965
Diabetics are more likely to be suffering from periodontitis	24 (11.4)	94 (44.8)	38 (18.1)	22 (10.5)	19 (9)	13(6.2)	5.8861
Should there be a collaborative training for medical and dental students	168 (80.4)	36 (17.2)	3 (1.4)	–	–	2(1)	12.8871
Periodontitis is sixth complication of diabetes—is awareness important among endocrinologist?	76 (36.2)	122 (58.1)	–	–	–	12(5.7)	4.4965
Severe periodontitis increases the incidence of macro albuminuria and end-stage renal disease in diabetics	2 (1)	59 (28.1)	86 (41)	26 (12.4)	9 (4.3)	28(13.3	2.771
Should endocrinologists include an oral and periodontal report in their review?	56 (26.7)	121 (57.6)	13 (6.2)	2 (1)	–	20 (9.5)	6.4232
There is a bidirectional relationship between periodontal diseases and diabetes	83 (39.5)	73 (34.8)	35 (16.7)	12 (5.7)	–	7 (3.3)	1.0099
Are you aware about the studies linking periodontal disease and diabetes?	78 (37.1)	88 (41.9)	26 (12.4)	–	–	18 (8.6)	0.9981

$P < 0.05$ = level of significance

N number, % percentage

experience ($p = 0.017$, 11–20 years). Effective control of periodontitis in diabetes management was also significantly associated to participant's age ($p = 0.052$, 41–50 years), experience ($p = 0.018$, 11–20 years), and type of practice ($p = 0.018$, hospital based). Participants' years of experience (11–20 years) and type of practice (hospital based) showed a significant response in relation to (a) their awareness for symptoms of periodontal diseases ($p = 0.004$), (b) increased risk of periodontitis among diabetics ($p = 0.02$), and (c) bidirectional relation between periodontitis and diabetes ($p = 0.0001$) (Table 6).

Table 5 Comparative outcomes of participant's age, experience and type of practice on dental care recommendations

Recommendation	Age of the participant *p* value, *n* (%), response category	Type of practice *p* value, *n* (%), response category	Years in practice *p* value, *N* (%), response category
Dental problems should be inquired from diabetic patients	0.046*, 18 (26.5) (41–50 years)	0.003*, 48 (70.6) (hospital based	0.0812
Oral and periodontal health examination should be included in diabetic screening protocols	0.003*, 53 (73.5) (41–50 years)	0.0001*, 31 (68.9) (hospital based)	0.021*, 27 (51.2) (> 20 years)
Diabetics patients should be referred for dental check up	0.1905	0.024*, 14 (60.9) (hospital based)	0.019*, 26 (47.2) (11–20 years)
Collaborative training for medical and dental students should be a part of the curriculum	0.6494	0.2596	0.012*, 22 (13) (11–20 years)
Collaborative practice should be there between endocrinologist and dentist	0.02*, 45(65.2) (41–50 years: bidirectional referral)	0.172	0.030*, 37 (71.2) (11–20 years)

$p < 0.05$

* = significant, *N* (%) = number (percentage), ** = survey questionnaire response

Analyses of participant's personal dental health awareness

Significant association was observed in relation to participant age and experience with regard to his/her last dental examination ($p = 0.0001$, 41–50 years, last dental visit was 12 months) and use of supplemental oral hygiene aids ($p = 0.0019$, 11–20 years) respectively (Table 7).

Discussion

EFP/AAP 2012 workshop on periodontitis and systemic diseases concluded with recommendation for drafting guidelines for periodontal care in diabetes both for professionals and for patients [9]. Research indicates that collaboration between dental and medical community on co-management for diabetics is sub-optimal and may be lagging due to paucity of knowledge resulting in non-utilization of scientific research into practice [10]. European Federation of Periodontology (EFP) and International Diabetes Federation (IDF) 2017 updated the evidence from the EFP/AAP 2012 workshop and released a dual set of guidelines: (1) for the patients with diabetes, (2) for the physicians/other medical health professionals treating diabetes and periodontitis. The guidelines emphasized need for the (a) oral healthcare personnel to identify the undiagnosed diabetes and pre-diabetic conditions and (b) need for the physicians to be aware of periodontal disease and its implications on glycemic control

Table 6 Comparative outcomes of participant's age, experience and type of practice on awareness of diabetes and periodontitis

Periodontitis and diabetes mellitus	Age of the participant *p* value, *n* (%), response category	Type of practice *p* value, *n* (%), response category	Years in practice *p* value, *n* (%), response category
Inflammation is a key component between periodontal disease and diabetes	0.0005*, 22 (27.5) 41–50 years	0.4998	0.7822
Control of periodontal infection important in management of diabetes	0.042*, 9 (26.5) 41–50 years	0.018*, 22 (64.7) hospital based	0.018*, 6 (17.6) 11–20 years
Periodontitis increases risk of diabetic complications	0.028*, 12 (25.5) 41–50 years	0.0668	0.017*, 8 (17) 11–20 years
Do you know the symptoms of periodontal diseases?	0.473	0.004*, 65 (66.3) hospital based	0.044*, 14 (14.3) 11–20 years
There is a bidirectional relationship between periodontal diseases and diabetes	0.1988	0.0001*, 47 (62.7) hospital based	0.018*, 12 (16) 11–20 years
Diabetics have increased risk of poor periodontal health	0.3512	0.0278*, 38 (73.1) hospital based	0.021*, 11 (21.2) 11–20 years

$p < 0.05$

* = significant, *n* (%) = number (percentage), ** = survey questionnaire response

Table 7 Comparative outcomes of participant's age, experience, and type of practice on dental health awareness

Characteristic	Age of the participant *p* value, *n* (%), response category	Type of practice *p* value, *n* (%), response category	Years in practice *p* value, *n* (%), response, category
When was your last dental examination visit?	0.0001*, 25 (36.2) 41–50: 12 months	0.4754	0.2665
Do you floss your teeth/use any interdental aid?	0.2843	0.1880	0.001*, 6 (11.3) 11–20 years

*$p < 0.05$

* = significant, *n* (%) = number (percentage), ** = survey questionnaire response

[11]. It is prudent for the medical physicians to have a fair understanding about the oral health and systemic health [12].

A Turkish study done on medical doctors demonstrated that 90.8% respondents believed that there is a relationship between periodontal disease and systemic health and the most frequent systemic disease (66.8%) known to be related to periodontal disease was diabetes. [13]. However, this awareness was not supported by precise knowledge and did not translate into appropriate clinical practice. Medical doctors got the information regarding the relationship between periodontal disease and its association with systemic health, primarily from lessons/workshops, dental practitioners, medical journals, and magazines [14].An effort has been made in our study to highlight the knowledge of bidirectional link and not mere reporting effect of diabetes on periodontal health. To the best of our knowledge, this is the first Indian study undertaken exclusively among the super specialists. The Indian studies published so far have enrolled either only patients/interns/medical practitioners [12, 15, 16]. A study from University of North Carolina (UNC) conducted among medical interns (85%) and endocrinologists (15%) demonstrated a fairly high knowledge about periodontal disease and diabetes. However, only 21% were aware about the bidirectional link [17]. This is in contrast to a relatively high level of awareness (73%) about this link in our study and is comparable to a study from Hong Kong (76%) [18]. The UNC study revealed 50% of the subjects did not know or disagreed that treatment of periodontal disease might improve glycemic control which proves lack of knowledge about evidence-based studies [15]. In our study, 80% of the respondents strongly agreed that collaborative training for medical and dental students is important in their educational curriculum. The participants concurred that control of periodontal infection is important in management of diabetes. Total referral rate in the North Carolina study was 28.7%, wherein endocrinologist's referral rate was more (48.7%) as compared to internists (25.1%).This translates to the fact that super specialists are better versed with the association between two diseases [15]. Referral rate in our study was 11% and is similar to the study from Hong Kong (12%) [19], higher than a study from Iran (4.4%) [20], and lower than the published studies from Cyprus (42%) [21] and China (26%) [18].

A study involving 510 general practitioners (232 physicians and 278 dentists) concluded that dentists were significantly more aware (52%) on the bidirectional association than the physicians (31%) [14]. Awareness on bidirectional link between diabetes and periodontitis in our study was (73%) and is comparable to study from Hong Kong (76%) [19]. Awareness on diabetics more prone to periodontitis was reported higher (90%) in the study from Hong Kong [19] as compared to our study (55%). 56.5% of the medical doctors refer their patients to a periodontist for different reasons out of which gingival bleeding was most common (44%) [13]. A qualitative study exploring the perception and barriers of GPs (general practitioners) in Australia revealed that despite the current recommendations, GPs' oral health care practices among diabetic patients is limited. Development of appropriate oral health training programs and risk assessment tools along with accessible referral pathways are required to address the current barriers [22].

A recent study demonstrated a predictive model (age, gender, ethnicity, HbA1c, and smoking habit) that could be used as a reliable screening tool for periodontitis in primary medical care settings to facilitate referral of patients at risk for oral examination. This study further reinforced the need for guidelines to screen for periodontitis, pre-diabetes, and diabetes in dental and medical care settings. Measures of modifiable risk factors for periodontitis and diabetes, such as glycemic control and adiposity, were reported to be universally applicable screening parameters [23]. With a response rate of 90.3% and on comparison of the outcomes, our study revealed a significant association of dental care recommendations with type of practice and experience years of participants ($p < 0.05$). This suggests the role of the participant's own experience in reaching out to conclusions pertaining to the relationship between diabetes and periodontitis and its effect on the dental care recommendations they would provide. It is important to understand here that the type of practice, suggestive of conducive environment, is also a

Table 8 Future recommendations [24–27]

- ☐ Formulation of clinical practice guidelines would be fruitful only if we promote collaboration between dental and medical fraternity in clinical practice
- ☐ Cross-training and inter-professional education: Medical Council of India and Dental Council of India (governing bodies for medical and dental education in India) should introduce chapters in the undergraduate curriculums about the importance of diabetes and periodontal/oral health
- ☐ At the national level, the policy makers in India should emphasize the need for public health strategies to integrate oral health within the existing non-communicable disease control programs and its importance on systemic health
- ☐ Frequent scientific interactions between the medical and dental fraternity via continuing medical/dental sessions/conferences/symposium and other scientific exchange forums
- ☐ Development of public awareness modules/social connect programs on the digital platforms
- ☐ Incorporation of dental health screening in diabetes practice as a part of their regular consultation and diabetes management protocols: this can be disseminated by educating and creating awareness among the nurses, nurse practitioner/diabetes educator
- ☐ Basic oral health information and patient counseling/education tools must be a part of a diabetic and dental practice
- ☐ Cross referrals among the diabetologists and periodontists/dentist for early detection and diagnosis of diabetes and periodontitis
- ☐ Glucometers should be a part of diagnostic armamentarium at dental office for prediabetic/diabetic chairside screening

significant factor in including oral health examination and subsequent referral. Similarly, the awareness on relation between diabetes and periodontitis had a significant association with years of experience and type of practice. These results hint towards a lacuna in the training imparted during medical education due to which there is a seemingly more dependence on experience and possibly less utilization of scientific research into practice. Hitherto, the main thrust comes from clinicians' own experience, a favorable environment, and his/her own interest and endeavor for continuous learning through published research studies and scientific conferences.

Conclusion

Our study concluded that the participants had a high level of awareness about periodontal disease and its bidirectional relation with diabetes. However, it did not significantly translate to the referral rate to dental office. Interdisciplinary collaborative approaches should be a part of the management protocol for diabetes and periodontitis. Such team approach would aim at detecting the diabetic patients at periodontal risk (as a pre-diabetic screening at dental office) and vice versa. Table 8 proposes few future recommendations that can be incorporated in the realm of clinical practice by the dentist and physician to bridge the lacuna between documented evidence and clinical practice in India.

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Declarations

Ethical approval This study was in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by institutional ethics review committee of Indraprastha Apollo Hospital, New Delhi India.

Patient consent Written informed consent was obtained from each participant.

Consent for publication Written informed consent was received from the study participants for publication of study results.

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

References

1. John Novak M. Classification of diseases and conditions affecting the periodontium. Carranza's' Clinical periodontology 10 ed. Sauders Elsevier; 2006.
2. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. Ann Periodontol. 2001;6:99–112.
3. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care. 1993;6:329–34.
4. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012;55:21–31.
5. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. Periodontol. 2000;2007(44):127–53.
6. Shultis WA, Weil EJ, Looker HC, Curtis JM, Shlossman M, Genco RJ, et al. Effect of periodontitis on overt nephropathy

and end-stage renal disease in type 2 diabetes. Diabetes Care. 2007;30:306–11.
7. Demmer RT, Desvarieux M, Holtfreter B, Jacobs DR, Wallaschofski H, Nauck M, et al. Periodontal status and A1C change: longitudinal results from the study of health in Pomerania (SHIP). Diabetes Care. 2010;33:1037–43.
8. Dhir S, Wangnoo S, Kumar V. Impact of glycemic levels in type 2 diabetes on periodontitis. Indian J EndocrinolMetab. 2018;22:672–7.
9. Chapple ILC, Genco R, on behalf of working group 2 of the joint EFP/AAP. Diabetes and periodontal diseases: consensus report of the joint EFP/AAP workshop on periodontitis and systemic diseases. J Periodontol. 2013;84:S106–12.
10. Ahdi M, Teeuw WJ, Meeuwissen HG, Hoekstra JB, Gerdes VE, Loos BG, et al. Oral health information from the dentist to the diabetologist. Eur J Intern Med. 2015;26(7):498–503.
11. Sanz M, Ceriello A, Buysschaert M, Chapple I, Ryan TD, Graziani F, et al. Scientific evidence on the links between periodontal diseases and diabetes: consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. J Clin Periodontal. 2018;45:138–49.
12. Jaiswal R, Shenoy N, Biju T. Extent of awareness regarding periodontal disease in diabetic patients among medical interns. Nitteuniv J Health Sci. 2015;5:17–21.
13. Tasdemir Z, Alkan BA. Knowledge of medical doctors in Turkey about the relationship between periodontal disease and systemic health. Braz Oral Res. 2015;29:1–8.
14. Al-Khabbaz AK, Al-Shammari KF, Al-Saleh NA. Knowledge about the association between periodontal diseases and diabetes mellitus: contrasting dentists and physicians. J Periodontol. 2011;82(3):360–6.
15. Kaur S, Khurana P, Kaur H. A survey on acquaintance, orientation and behavior of general medical practitioners toward periodontal diseases. J IndSoc Perio. 2015;19:322–6.
16. Obulareddy VT, Nagarkanti S, Chava VK. Knowledge, attitudes and practice behaviours of medical specialists for the relationship between diabetes and periodontal disease. J Family Med Prim Care. 2018;7(1):175–8.
17. Owens BJ, Wilder RS, Southerland JS, Janet HS, Buse JB, Malone RM. North Carolina internists and endocrinologists' knowledge, opinions, and behaviors regarding periodontal disease and diabetes: need and opportunity for interprofessional education. J Dent Edu. 2011;75:329–38.
18. Lin H, Zhang H, Yan Y, et al. Knowledge, awareness, and behaviors of endocrinologists and dentists for the relationship between diabetes and periodontitis. Diabetes Res ClinPrac. 2014;106:428–34.
19. Tse SY. Diabetes mellitus and periodontal disease: awareness and practice among doctors working in public general out-patient clinics in Kowloon West Cluster of Hong Kong. BMC Fam Pract. 2018;19:1–9.
20. Moghadam FA, Haerian A, Salami MS, Karbasi MHA, Tabatabayi RF, Vaziri F. Evaluation of knowledge, attitude and practice between periodontal disease and diabetes. J Comm Health Res. 2013;2:124–30.
21. Galazi AA, Siskou O, Karagkaoni I, Giannaki C, Charalampous C, Konstantakopoulou O, et al. Investigating physicians' and patients' oral health knowledge: a field needed interdisciplinary policy making approach. Int J Health Prom & Edu. 2019;57:1–12.
22. Poudel P, Griffiths R, Wong VW, et al. Perceptions and practices of general practitioners on providing oral health care to people with diabetes - a qualitative study. BMC Fam Pract. 2020;21:1–11.
23. Montero E, Herrera D, Sanz M, Dhir S, Van Dyke T, Sima C. Development and validation of a predictive model for periodontitis using NHANES 2011–2012 data. J Clin Periodontal. 2019;46:420–9.
24. Herman W, Taylor GW, Jacobson JJ, Burke R, Brown MB. Screening for prediabetes and type 2 diabetes in dental offices. J Public Health Dent. 2015;75(3):175–82.
25. Bansal S, Dhir S, Wangnoo SK. Diabetes mellitus and periodontitis: relevance of the diabolic duo in India. Apollo Med. 2020;17(4):267–71.
26. Dhir S, Lalwani R, Sharma JK, Kolte A, Bansal S, Gupta A. "The Perio-Diabetes Symposium": consensus report of the Indian Society of Periodontology and Research Society for the Study of Diabetes in India - A joint event on Periodontitis and Diabetes. J Indian Soc Periodontol. 2019;23:593–4.
27. Fisher CSD, Kanjirath PP, Peters M, Borgnakke W. Oral health: an untapped resource in managing glycemic control in diabetes and promoting overall health. The J Nurs Pract. 2015;11:889–96.

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ORIGINAL ARTICLE

Cardiac autonomic recovery in response to aerobic versus resistance exercise in type 2 diabetes mellitus patients

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Abstract

Purpose To investigate the effect of aerobic exercise (AE) versus resistance exercise (RE) on outcomes of cardiac autonomic recovery in type 2 diabetes mellitus (T2DM) patients with cardiac autonomic neuropathy (CAN).

Methods Fifty T2DM patients with CAN (age: 52.2 ± 6.8 years) were recruited for the present study. They were randomly allocated into two groups: AE ($n = 25$) and RE ($n = 25$). The AE group performed graded maximal exercise test using Balke protocol while the RE group performed 5 sets of leg press exercise at 10 repetition maximum (RM) intensity. Heart rate variability (HRV) was assessed for 10 min after both exercise bouts while heart rate recovery (HRR) was recorded for the first 3 min after completion of the exercise bouts. Key outcome measures for the study were standard time and frequency domain parameters of HRV and HRR_{1min}, HRR_{2min}, and HRR_{3min}. Standard statistical analysis which included independent *t*-test and repeated measures analysis of variance was performed in order to test the study hypothesis.

Results Significant impairment was observed in both HRV and HRR after both AE and RE ($p < 0.05$). However, alterations in both HRV and HRR responses were found to be more after AE as compared to RE ($p < 0.05$) in T2DM patients with CAN.

Conclusion Findings of the present study suggest significant blunting of autonomic responses after both AE and RE in T2DM patients with CAN. However, deterioration in them was found to be more after AE as compared to the RE indicating that AE may pose greater stress on the cardiovascular system of T2DM patients with CAN.

Keywords Autonomic nervous system · Diabetes · Exercise · Recovery

Abbreviations

30:15 ratio	Ratio of the longest R-R interval during 30 s and the shortest R-R interval during 15th second of the head-up tilt test
AE	Aerobic exercise
ANCOVA	Analysis of covariance
ANS	Autonomic nervous system
BP	Blood pressure
BRS	Baroreflex sensitivity
CAN	Cardiac autonomic neuropathy
CARTs	Cardiovascular autonomic reflex tests
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DBT	Deep breathing test
E:I ratio	Ratio of the average of longest R-R interval during expiration and the shortest R-R interval during inspiration of the deep breathing test
ECG	Electrocardiogram
HF	High frequency
HGT	Hand grip test
HR	Heart rate
HRR	Heart rate recovery
HRV	Heart rate variability
HUT	Head-up tilt
LF/HF ratio	Ratio of low- and high-frequency power
LF	Low frequency
Mean NN	Average of N–N intervals
MHR	Maximal heart rate
pNN50	Proportion of differences in consecutive N–N intervals that are longer than 50 ms
PNS	Parasympathetic nervous system
RE	Resistance exercise

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RM	Repetition maximum
RMSSD	Root mean square of successive differences between adjacent R-R intervals
SBP	Systolic blood pressure
SDNN	Standard deviation of N–N intervals
SNS	Sympathetic nervous system
T2DM	Type 2 diabetes mellitus
TP	Total power
VM	Valsalva maneuver
VR	Valsalva ratio
ΔHR	Change in R-R intervals during six consecutive cycles of deep inspiration and expiration

Introduction

Type 2 diabetes mellitus (T2DM) is a global epidemic linked to various macro- and micro-vascular complications of which cardiovascular complications are quite common [1]. Cardiac autonomic neuropathy (CAN) manifested in the form of cardiac autonomic dysfunction is one of the commonest cardiovascular complication in T2DM patients which is strongly associated with the occurrence of ventricular arrhythmias and myocardial infarction [2].

The heart has been suggested to be vulnerable to cardiovascular events during exercise and the recovery phase after completion of exercise due to the predominant sympathetic activity [3]. CAN in T2DM not only impairs resting cardiac autonomic tone but also negatively influences cardiac autonomic recovery. During exercise, a continuous and reciprocal functioning of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) regulates heart rate (HR) [3]; however, this interaction between the two arms of autonomic nervous system (ANS) is disrupted during recovery phase after exercise due to the differences in firing patterns between these systems [4]. There is parasympathetic reactivation followed by sympathetic withdrawal in the later stages which protects the heart by a timely and sequential reduction in HR. However, this autonomic recovery pattern is largely disrupted in T2DM patients with CAN, with prolonged SNS activity and delayed PNS reactivation during the recovery phase [4, 5]. Post-exercise heart rate variability (HRV) and heart rate recovery (HRR) are often utilized to measure cardiac autonomic recovery after exercise [5, 6]. Reduction in HRV parameters and blunting of HRR response during the recovery period are hallmarks of impaired cardiac autonomic recovery and is often observed in T2DM patients [4–6].

International guidelines strongly recommend exercise as part of the comprehensive rehabilitation of T2DM [7]. Exercise can be performed in a variety of modes; however, most common forms are still aerobic (AE) and resistance exercises (RE) which are strongly recommended for T2DM patients as well [7]. Since acute exercise has been shown to disrupt the cardiac autonomic activity [4], understanding the autonomic recovery responses to these exercise forms is imperative in order to comment on the cardiovascular safety during these exercises. Moreover, this research question becomes even more important and relevant for patients with CAN in which there is quite evident disruption of autonomic responses [8]. Therefore, the present study intended to compare the cardiac autonomic recovery after AE versus RE in T2DM patients with CAN. We hypothesized that there will be a significant difference in the autonomic recovery patterns after AE versus RE in T2DM patients with CAN.

Materials and methods

Participants

Sample size for the present study was calculated using the Software G. Power (3.1.9.7) with the effect size obtained in a previous study [9] ($d = 1.06$) between high-frequency (HF) power of HRV after aerobic versus resistance exercise. A sample size of 50 T2DM patients ($n = 25$ in each group) with 0.98 power and 0.05 α was found to be necessary to test the study hypothesis. Patients with T2DM (HbA1c > 6.5%) [10] which were found to be positive for CAN as per the Ewing's criteria [11] with a duration of T2DM ≥ 1 year were recruited into the study. Patients with any severe cardiovascular disease [12] or morbid obesity (> 40 kg/m^2) or those who suffered from uncontrolled hypertension (systolic blood pressure/diastolic blood pressure (SBP/DBP) > 165/95 mmHg) or any musculoskeletal problem that could limit his/her ability to participate in exercise testing without inducing pain, those involved in any structured exercise training for last 6 months, or those who were unable to follow directions and recognize symptoms were excluded. A random sample of 50 T2DM patients with CAN was selected from the population. Fifty T2DM patients with CAN were randomly allocated into either AE or RE group using the lottery method wherein a researcher randomly picks numbers, with each number corresponding to a subject or item, in order to create the sample. The present study constituted comparative cross-sectional design wherein comparison of autonomic recovery was made between the AE and RE groups. The present study was approved by the Institutional Ethics Committee of Jamia Millia Islamia (Proposal No 16/9/146/JMI/IEC/2017), and the study procedures were executed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Experimental procedures

The present investigation was conducted at Jamia Millia Islamia, New Delhi, India, after taking approval from the Institutional Ethics Committee between April, 2018, and January, 2019. Eligible participants were evaluated for demographic and clinical parameters followed by standard cardiovascular autonomic reflex tests (CARTs) for diagnosing CAN. Thereafter, 50 T2DM patients with CAN were randomly divided into AE ($n=25$) and RE ($n=25$) group. Both groups underwent HRV assessment at rest followed by AE and RE in the AE and RE groups respectively. Post-exercise HRV and HRR were recorded in both groups after completion of respective exercise bouts.

Aerobic and resistance exercise

For the AE, a maximal graded exercise test was conducted on a motorized treadmill in accordance with the Balke protocol [13]. The test was terminated when either attainment of 90% of age-predicted maximal HR (MHR) or volitional fatigue or VO_{2max} was achieved (defined as VO_2 that increased < 1 mL/kg/min for ≥ 30 s despite increment in workload) [14].

RE consisted of 5 sets of 10 repetitions (at 10 repetition maximum (RM) intensity) each on a leg press machine (8 station multi-gym, Fitness World, India). Prior to the actual test, 10 RM for each participant was calculated using a ramp protocol. Ten RM was defined as the load at which eleventh repetition could not be performed. Two minutes of rest period was provided between each set and the pace of repetitions was controlled by a metronome.

Autonomic function assessment

Cardiovascular autonomic reflex tests

CARTs proposed by Ewing et al. [11] evaluate CAN on the basis of 5 tests of autonomic reactivity. The CART battery comprises of three HR tests (deep breathing test (DBT), Valsalva maneuver (VM), and head-up tilt (HUT) test) and two blood pressure (BP) tests (HUT and hand-grip test (HGT)) which provides objective information on parasympathetic and sympathetic reactivity respectively. CARTs were performed following the standard procedures and variables such as E:I ratio, ΔHR, VR, 30:15 ratio, ΔSBP, and ΔDBP were calculated [11].

Heart rate variability and heart rate recovery assessment

HRV at rest was evaluated under controlled conditions in a quiet, temperature-controlled room. A rest period of 15 min was provided in supine position prior to the actual testing. After the rest period, the actual test included electrocardiogram (ECG) recording for 10 min with a standard lead II configuration. Of the 10-min ECG record, the last 5 min was utilized for HRV analysis. For post-exercise HRV, participants were asked to assume supine position on a nearby bed immediately after completion of the exercise bouts. Again, ECG was recorded (standard lead II configuration) for the next 10 min which included a 3-min transitional phase. This transitional phase was provided so as to provide the participant adequate time to dismount from the treadmill/leg press machine, assume supine position on the bed, and safeguard the position of electrodes. Post-exercise HRV analysis was performed on the R-R intervals between 3 and 8 min of the post-exercise ECG record [15].

HR was derived from a continuous ECG record obtained via lead II ECG configuration during the recovery phase. HRR was calculated as the absolute difference between HR at peak exercise and HR recorded at 1 (HRR_{1min}), 2 min (HRR_{2min}), and 3 min (HRR_{3min}) after the AE and RE [16].

Heart rate variability analysis

We recorded HRV data at the sampling rate of 1000 Hz using a low-pass filter (at 40-Hz cutoff frequency). The beat classifier module of the software was utilized to visually and automatically inspect the ectopic beats which were then interpolated as per standard guidelines [17]. Fast Fourier transform was utilized in order to decompose the data into its frequency components for frequency domain HRV analysis. Total power (TP) and the power density in areas of low-frequency (LF) (0.04 to 0.15 Hz) and HF (0.15 to 0.4 Hz) bands were calculated at rest and after exercise along with the ratio of LF and HF power, i.e., LF/HF ratio. Considering the possible impact of respiratory oscillations on HRV during the post-exercise period, the range of HF band was extended (HF, 0.15–0.5 Hz) for post-exercise HRV analysis [18]. Standard time domain indices such as average of N–N intervals (Mean NN), standard deviation of N–N intervals (SDNN), root mean square of successive differences between adjacent R-R intervals (RMSSD), and proportion of differences in consecutive N–N intervals that are longer than 50 ms (pNN50) were also calculated. Mean NN, SDNN, and TP denote overall variability in R-R intervals while RMSSD, pNN50, and HF power are indicators of parasympathetic cardiac control. LF power represents sympathetic cardiac control whereas LF/HF ratio is an indicator of sympatho-vagal balance [19]. HRV recording and analysis were performed in accordance with the guidelines proposed by the Taskforce of European Society of Cardiology and North American Society of Pacing and Electrophysiology [19] using the AD instruments Lab Chart version 7.3.7 with HRV module version 1.4.2 (Power Lab 8 SP, AD Instruments, Australia).

Statistical analysis

Data was analyzed using SPSS software version 21. Data are presented in the form of mean ± standard deviation, median (interquartile range), and frequencies/percentages wherever required in the tables. Normality of the data was examined using the Shapiro–Wilk test. Non-normal data was log transformed prior to any further analysis. Demographic and clinical variables were compared between the groups (AE versus RE) using independent *t*-test/Mann–Whitney *U* test. Data recorded in frequencies/percentages such as co-morbidities, complications, drugs, and gender was compared between the groups using chi-square test. CARTs and HRV parameters at rest were also compared between the groups (AE versus RE) using independent *t*-test/Mann–Whitney *U* test. A 2 × 2 repeated measures analysis of variance was employed to examine main effects of time (rest versus post-exercise), group (AE versus RE), and group × time interaction. If any outcome variable was found to be significantly different between the groups at rest, analysis of covariance (ANCOVA) was applied to compute group differences by considering the baseline value as a covariate. Statistical significance was set at $p < 0.05$.

Results

Demographic and clinical characteristics were found to be statistically similar between the groups (Table 1). CARTs and resting HRV parameters were also found to be statistically similar between the groups except SDNN and HF power (Table 2) which were analyzed using ANCOVA.

All time domain HRV parameters showed significant time effect (Mean NN, $p < 0.001$; RMSSD, $p < 0.001$; pNN50, $p < 0.001$) indicating significant impairment in these parameters from rest to post-exercise period in both AE and RE groups. Moreover, a significant group and interaction effect was observed for Mean NN (Group, $p = 0.04$; Interaction, $p = 0.007$), and a significant group effect was observed for SDNN ($p < 0.001$), and RMSSD ($p = 0.008$) showing significantly greater impairment in these parameters after AE as compared to RE (Table 3).

Among frequency domain parameters, TP ($p < 0.001$) and LF power ($p = 0.01$) showed significant time effect signifying significant impairment in these parameters from rest to post-exercise period in both groups. Moreover, a significant group and interaction effect was observed for LF power (Group, $p = 0.005$; Interaction, $p = 0.006$) and a significant group effect was observed for HF power ($p = 0.001$) demonstrating significant impairment in these parameters after AE as compared to RE (Table 3).

Table 1 Comparison of demographic and clinical characteristics between the groups

Variables	RE ($n = 25$) Mean ± SD	AE ($n = 25$) Mean ± SD	*p*-value
Demographic variables			
Age (years)	53.4 ± 8.01	51.1 ± 5.59	0.24
Gender, M/F (*n*, *n*%)	16/9	17/8	0.76
Height (m)	1.6 ± 0.07	1.6 ± 0.09	0.87
Weight (kg)	73.5 ± 9.42	74.8 ± 11.72	0.66
BMI (kg/m^2)	28.0 ± 3.84	28.4 ± 4.73	0.80
DM duration (years)	7.7 ± 5.37	8.6 ± 6.26	0.51
HR_{rest} (beats/min)	81.8 ± 12.98	81.1 ± 12.60	0.84
SBP (mmHg)	124.8 ± 13.56	131.5 ± 15.13	0.10
DBP (mmHg)	78.4 ± 6.42	80.5 ± 9.47	0.39
FBG (mg/dl)	171.8 ± 69.21	181.9 ± 66.91	0.48
PPBG (mg/dl)	234.8 ± 94.89	244.1 ± 86.26	0.63
HbA1c (%)	8.3 ± 1.81	9.0 ± 1.78	0.11
TC (mg/dl)	178.2 ± 33.03	174.7 ± 41.61	0.73
TG (mg/dl)	140.9 ± 36.95	158.3 ± 44.84	0.14
HDL (mg/dl)	45.0 ± 8.38	42.0 ± 6.80	0.27
LDL (mg/dl)	103.9 ± 31.10	104.4 ± 38.98	0.79
VLDL (mg/dl)	26.9 ± 5.98	27.4 ± 9.53	0.84
Co-morbidities (*n*, *n*%)			
HTN	13 (52)	13 (52)	0.77
Dyslipidemia	15 (60)	17 (68)	0.55
Thyroid dysfunction	5 (20)	3 (12)	0.44
Musculoskeletal issues	4 (16)	5 (20)	0.71
DM complications (*n*, *n*%)			
Peripheral neuropathy	6 (24)	10 (40)	0.22
Retinopathy	2 (8)	3 (12)	0.63
Nephropathy	0 (0)	1 (4)	0.31
Drugs (*n*, *n*%)			
Metformin	11 (44)	12 (48)	0.77
Insulin	2 (8)	4 (16)	0.38
Sulfonylureas	3 (12)	5 (20)	0.44
ACE inhibitors	1 (4)	1 (4)	1.00
Statins	4 (16)	2 (8)	0.38
Calcium channel blockers	0 (0)	3 (12)	0.07
DPP4 inhibitors	1 (4)	2 (8)	0.55
Thiazolidnedione	1 (4)	0 (4)	0.31
α Glucosidase inhibitors	0 (4)	2 (8)	0.14
Dapaglifzone	1 (4)	1 (4)	1.00

SD standard deviation, *RE* resistance exercise, *AE* aerobic exercise, *CI* confidence intervals, *M* male, *F* female, *DM* diabetes mellitus, *BMI* body mass index, HR_{rest} resting heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *PPBG* post-prandial blood glucose, *HbA1c* glycosylated hemoglobin, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very-low-density lipoprotein, *HTN* hypertension, *ACE* angiotensin-converting enzyme, *DPP4* dipeptidyl peptidase 4; independent t-test/Mann–Whitney *U* test applied; to compare frequencies, chi-square test applied

Table 2 Comparison of cardiovascular autonomic reflex tests and heart rate variability parameters at rest between the groups at baseline

Variables	RE ($n=25$)	AE ($n=25$)	SMD (95% CI)	p-value
CARTs, n (n%)				
Early	18	14	-	0.13
Definite	04	07	-	0.30
Severe	02	04	-	0.38
	Mean ± SD	Mean ± SD		
E:I ratio	1.16 ± 0.11	1.14 ± 0.14	0.00 (-0.55, 0.56)	0.52
ΔHR (beats/min)	9.9 ± 5.26	11.4 ± 9.01	-0.40 (-0.96, 0.16)	0.70
VR	1.02 ± 0.66	1.21 ± 0.56	-0.03 (-0.58, 0.53)	0.20
ΔDBP (mmHg)	17.6 ± 5.39	16.1 ± 5.80	0.26 (-0.29, 0.82)	0.45
30:15 ratio	1.06 ± 0.09	1.12 ± 0.14	-0.50 (-1.07, 0.06)	0.07
#ΔSBP (mmHg)	-3.00 (-14.00, 14.00)	-2.00 (-16.00, 22.00)		0.91
HRV (rest)				
Mean NN (ms)	746.5 ± 114.73	723.3 ± 80.48	0.23 (−0.33, 0.79)	0.41
SDNN (ms)	28.5 ± 10.89	36.8 ± 15.61	−0.14 (−0.70, 0.41)	0.01*
RMSSD (ms)	27.5 ± 8.35	24.5 ± 11.66	0.24 (−0.32, 0.79)	0.13
pNN50 (%)	1.59 ± 1.32	1.59 ± 1.22	0.00 (−0.55, 0.55)	0.45
TP (ms^2)	757.5 ± 372.11	711.9 ± 284.74	0.14 (−0.42, 0.69)	0.62
LF power (ms^2)	247.0 ± 172.98	198.9 ± 109.80	0.20 (−0.35, 0.76)	0.54
HF power (ms^2)	320.2 ± 187.61	165.7 ± 82.88	0.99 (0.40, 1.58)	0.005*
LF/HF ratio	1.20 ± 0.01	1.28 ± 0.51	−0.00 (−0.56, 0.55)	0.22

SD standard deviation, *SMD* standardized mean difference, *CARTs* cardiovascular reflex tests, *HRV* heart rate variability, *RE* resistance exercise, *AE* aerobic exercise, *CI* confidence intervals, *E:I ratio* ratio of the average of longest R-R interval during expiration and the shortest R-R interval during inspiration of the deep breathing test, *ΔHR* change in R-R intervals during six consecutive cycles of deep inspiration and expiration, *30:15 ratio* ratio of the longest R-R interval during 30 s and the shortest R-R interval during 15th s of the head-up tilt test, *VR* valsalva ratio, *ΔSBP* change in systolic blood pressure during head-up tilt test, *ΔDBP* change in diastolic blood pressure during hand grip test, *HR* heart rate, *Mean NN* average of N–N intervals, *SDNN* standard deviation of N–N intervals, *RMSSD* root mean square of successive differences between adjacent N–N intervals, *pNN50* Proportion of differences in consecutive N–N intervals that are longer than 50 ms, *TP* total power, *LF* low frequency, *HF* high frequency, *LF/HF ratio* ratio of low and high frequency power; #median (interquartile range) reported and non-parametric tests applied for ΔSBP; *significant difference between the groups; chi-square test applied to compare frequencies in the different stages of CAN and independent t-test applied to compare other variables

Peak HR showed a significant time effect ($p<0.001$) indicating significantly greater rise in HR at peak exercise from the resting conditions. HRR_{1min}, HRR_{2min}, and HRR_{3min} also demonstrated significant time effect ($p<0.001$) indicating a significant decline in post-exercise HR from the peak HR. A significant group and interaction effect was observed for HRR_{1min} (Group, $p=0.03$; Interaction, $p<0.001$) and HRR_{2min} (Group, $p=0.04$; Interaction, $p<0.001$) while a significant interaction effect was seen for HRR_{3min} ($p<0.001$) indicating a significantly greater impaired HRR after AE as compared to RE (Table 3).

Discussion

Purpose of the present study was to investigate and compare the autonomic recovery patterns after AE versus RE in T2DM patients with CAN. Significantly greater impairment was observed in autonomic recovery pattern after AE bout than the RE bout suggesting that AE was more strenuous than RE for T2DM patients with CAN.

Attenuated HRR after exercise has shown to be linked with reduced parasympathetic activity [20] and is also found to increase the risk for cardiovascular events and associated mortality in asymptomatic patients with T2DM [21]. It has been proposed that HRR represents the coordinated interaction between sympathetic and parasympathetic arms of ANS in order to safeguard the heart with parasympathetic reactivation initially (HRR_{1min}) followed by sympathetic withdrawal in later parts of recovery (HRR_{2min} and HRR_{3min}) [22]. Our findings demonstrated that HRR from 1st to 3rd minute post-exercise remained significantly greater after the RE bout than the AE bout which points towards greater cardiovascular stress posed by AE in the present sample. Findings of the present study suggested that both parasympathetic reactivation and sympathetic withdrawal (denoted by altered $HRR_{1\text{-}3\ min}$) were significantly impaired in both AE and RE groups; however, greater impairments were

Table 3 Comparison of post-exercise heart rate variability and heart rate recovery parameters between the groups

Variables	RE ($n=25$) Mean ± SD	AE ($n=25$) Mean ± SD	SMD (95% CI)	*p*-values Group	Time	Group × time
Post-exercise HRV						
Mean NN (ms)	680.8 ± 93.67	604.6 ± 65.67	0.93 (0.34, 1.51)	0.04*	< 0.001*	0.007*
#SDNN (ms)	24.0 ± 4.77	18.8 ± 7.93	0.78 (0.21, 1.36)	< 0.001*		
RMSSD (ms)	15.2 ± 5.80	10.7 ± 4.29	-0.87 (0.29, 1.45)	0.008*	< 0.001*	0.12
pNN50 (%)	1.18 ± 1.07	0.45 ± 0.35	0.90 (0.32, 1.49)	0.16	< 0.001*	0.15
TP (ms^2)	498.7 ± 93.21	351.9 ± 224.19	0.84 (0.26, 1.42)	0.12	< 0.001*	0.24
LF power (ms^2)	208.9 ± 54.27	128.4 ± 90.61	1.06 (0.47, 1.66)	0.005*	0.01*	0.006*
#HF power (ms^2)	156.4 ± 36.09	98.3 ± 86.81	0.86 (0.28, 1.44)	0.001*		
LF/HF ratio	1.24 ± 0.33	1.41 ± 0.58	0.35 (−0.91, 0.20)	0.12	0.08	0.57
Post-exercise HRR						
HR_{peak} (beats/min)	152.3 ± 10.16	166.6 ± 12.69	−1.22 (−1.83, −0.62)	0.005*	< 0.001*	< 0.001*
HRR_{1min} (beats/min)	17.5 ± 14.14	14.3 ± 5.55	0.29 (−0.26, 0.85)	0.03*	< 0.001*	< 0.001*
HRR_{2min} (beats/min)	27.6 ± 12.06	25.7 ± 6.22	0.19 (−0.36, 0.75)	0.04*	< 0.001*	< 0.001*
HRR_{3min} (beats/min)	59.4 ± 15.8	43.1 ± 12.40	1.13 (0.53, 1.73)	0.72	< 0.001*	< 0.001*

SD standard deviation, *SMD* standardized mean difference, *HRV* heart rate variability, *HRR* heart rate recovery, *RE* resistance exercise, *AE* aerobic exercise, *CI* confidence intervals, *Mean NN* average of N–N intervals, *SDNN* standard deviation of N–N intervals, *RMSSD* root mean square of successive differences between adjacent N–N intervals, *pNN50* Proportion of differences in consecutive N–N intervals that are longer than 50 ms, *TP* total power, *LF* low frequency, *HF* high frequency, *LF/HF ratio* ratio of low and high frequency power; #analysis of covariance applied; *significant difference; 2 × 2 repeated measures analysis of variance applied to calculate the main effects of group (AE versus RE), time (rest versus post-exercise) and group × time interaction

observed post-AE. Blunted HRR has been observed after acute exercise in T2DM patients; however, to the best of our knowledge, no study so far exists which compares HRR after AE versus RE in T2DM patients. Vianna et al. [23] have demonstrated significant impairment in HR kinetics after lower limb and upper limb resistance exercise with greater deterioration following lower limb exercise. Moreover, HR kinetics was also found to be impaired in healthy men following AE [24]. Regarding the comparison of autonomic responses between control, AE, and RE, Niemela et al. [25] also observed significant impairment in recovery pattern of baroreflex sensitivity (BRS) after both exercise forms in comparison to the control condition. The BRS response after high-intensity RE was blunted for a duration of 60 min as compared to 30 min in the AE group [25].

The variables of HRV provide direct information on the parasympathetic cardiac control and inferred information on the sympathetic cardiac control [19]. Findings suggested that most of the HRV parameters demonstrated significant impairment after both AE and RE bouts in T2DM patients with CAN. Previous studies [5, 26] have demonstrated the same and have shown that short-term exercise significantly blunts autonomic responses after exercise in patients with T2DM. A recent study [5] demonstrated slower autonomic recovery after maximal aerobic exercise in patients with diabetes than non-diabetic controls which was further slowed down by the presence of hypertension. Similar to aerobic exercise, research [26] has shown altered HRV kinetics as compared to resting conditions in patients with hypertension. The present study revealed that most of the HRV measures showed significant deterioration from rest to post-exercise conditions in both exercise groups while greater impairment was observed in the AE group. In contrary to our findings, studies by Heffernan et al. [27, 28] have shown significantly greater alteration in the autonomic recovery pattern after RE bout than AE in healthy male participants. BRS was reduced after both bouts of exercise, but significantly greater reductions were seen following RE [27]. Heffernan et al. [28] in another experiment reported a significant increase in LF/HF ratio after both AE and RE, and a decrease in TP, LF, and HF power was also observed which was more pronounced after RE than AE indicating that RE was more strenuous than AE in the healthy male participants. However, it is to be noted that they [27, 28] investigated autonomic responses to exercise in healthy participants in contrary to our diseased sample and that physiological responses are highly dependent on the presence or absence of pathology [29]. Also, in their studies [27, 28], BRS and HRV, i.e., autonomic recovery was recorded 20 min and 30 min after completion of the exercise bouts in contrary to our experimental procedures which involved assessment of immediate post-exercise autonomic recovery (up to 10 min after exercise). Differences in the findings of these studies [27, 28] and the present study could be accounted by differences in exercise volume because these previous investigations [27, 28] involved whole-body RE protocols in contrast to our protocol which involved only

leg press exercise and indicates that the amount of muscle mass involved during exercise is also relevant. Another important point which should be taken into consideration is that resistance exercise protocol utilized in the present study was intermittent which may explain the differences observed by us and the studies of Heffernan et al. [27, 28].

Though there exists plenty of literature regarding the effects of exercise training on autonomic modulation in T2DM patients, however, the literature remains to be scarce regarding the acute effects of these exercise forms on cardiac autonomic recovery. Moreover, it was important to understand the acute effects of these exercise forms in order to quantify autonomic stress posed by them. AE has direct effects on the cardiovascular system while RE has inferred effects. Therefore, it may be speculated that due to its direct actions on the cardiovascular system [30], AE exerted greater cardiovascular stress in T2DM patients in the present study. RE on the other hand may be considered to pose lesser stress on the cardiovascular system since it primarily works upon the peripheral muscles in order to direct its cardiovascular effects [30]. Of notice is the fact that peak HR achieved during RE was significantly less than the peak HR achieved during AE (Table 3) which might be responsible for exaggerated blunting of autonomic recovery after AE in the present investigation.

The present study had a few limitations. RE bout was performed at 10 RM intensity which is equivalent to 75% of 1 RM whereas AE bout utilized a maximal graded exercise test. Therefore, differences in exercise intensity might have influenced the results. Also, we did not measure energy expenditure during the two exercises which might have some influence on the observed findings. Moreover, these patients were on anti-diabetic medications too which could have modified autonomic function; however, the intake of medicines was prohibited 24 h prior to the autonomic testing. Focus of the present study was immediate autonomic recovery and we did not assess long-term autonomic recovery which is also relevant from a physiological point of view and therefore, future research should focus on long-term recovery from exercise along with immediate autonomic recovery. Also, it would be interesting to investigate and compare autonomic recovery after different intensities of AE and RE. Furthermore, we did not include a control with no exercise in this study, inclusion of which could have further strengthened the design of the present study.

Conclusion

Findings of the present study suggest that cardiac autonomic function parameters showed significant impairment after both AE and RE; however, deterioration in them was found to be more after AE in comparison to RE in T2DM patients with CAN. These findings are relevant since AE and RE are routinely performed in these patients and recovery methods should be practiced keeping in mind the amount of autonomic stress posed by two exercise forms.

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Declarations

Ethics approval and consent to participate The present trial was ethically approved by the Institutional Ethics Committee, Jamia Millia Islamia (A Central University), New Delhi, India. A written informed consent was obtained from each participant for their participation in the study.

Consent for publication Not applicable.

Competing interest The authors declare no competing interests.

References

1. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020. Diabetes Care. 2020;43:S14-31. https://doi.org/10.2337/dc20-S002.
2. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: risk factors, diagnosis and treatment. World J Diabetes. 2018;9:1–24. https://doi.org/10.4239/wjd.v9.i1.1.
3. Michael S, Graham KS, Davis GM. Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals—a review. Front Physiol. 2017;8:301. https://doi.org/10.3389/fphys.2017.00301.
4. Banthia S, Bergner DW, Chicos AB, Ng J, Pelchovitz DJ, Subacius H, et al. Detection of cardiovascular autonomic neuropathy using exercise testing in patients with type 2 diabetes mellitus. J Diabetes Complications. 2013;27:64–9. https://doi.org/10.1016/j.jdiacomp.2012.09.002.
5. Verma S, Bhati P, Ahmad I, Masroor S, Ali K, Singla D, et al. Co-existence of hypertension worsens post-exercise cardiac autonomic recovery in type 2 diabetes. Indian Heart J. 2018;70:S82–9. https://doi.org/10.1016/j.ihj.2018.06.007.
6. Sacre JW, Jellis CL, Coombes JS, Marwick TH. Diagnostic accuracy of heart-rate recovery after exercise in the assessment of diabetic cardiac autonomic neuropathy. Diabet Med. 2012;29:e312–20. https://doi.org/10.1111/j.1464-5491.2012.03719.x.
7. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. Diabetes Care. 2010;33:2692–6. https://doi.org/10.2337/dc10-9990.
8. Bissinger A. Cardiac autonomic neuropathy: why should cardiologists care about that? J Diabetes Res. 2017;2017:1–9. https://doi.org/10.1155/2017/5374176.
9. de Paula T, Neves MF, da Silva IA, Monteiro W, Farinatti P, Cunha FA. Acute effect of aerobic and strength exercise on heart rate variability and baroreflex sensitivity in men with autonomic dysfunction. J Strength Cond Res. 2019;33:2743–52. https://doi.org/10.1519/JSC.0000000000002372.

10 American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2016;39:S13–22. https://doi.org/10.2337/dc16-S005.
11. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care. 1985;8:491–8. https://doi.org/10.2337/diacare.8.5.491.
12. Criteria Committee of the New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston: Little, Brown & Company; 1994. p. 253–6.
13. Beltz NM, Gibson AL, Janot JM, Kravitz L, Mermier CM, Dalleck LC. Graded exercise testing protocols for the determination of VO_{2max}: historical perspectives, progress, and future considerations. J Sports Med. 2016. https://doi.org/10.1155/2016/3968393.
14. Bhati P, Moiz JA, Naqvi IH, Hussain ME. Diagnostic performance of resting and post-exercise heart rate variability for detecting cardiac autonomic neuropathy in type 2 diabetes mellitus. Auton Neurosci. 2019;219:53–65. https://doi.org/10.1016/j.autneu.2019.04.003.
15. Neves VR, Kiviniemi AM, Hautala AJ, Karjalainen J, Piira OP, Catai AM, et al. Heart rate dynamics after exercise in cardiac patients with and without type 2 diabetes. Front Physiol. 2011;2:57. https://doi.org/10.3389/fphys.2011.00057.
16. Cheng YJ, Lauer MS, Earnest CP, Church TS, Kampert JB, Gibbons LW, et al. Heart rate recovery following maximal exercise testing as a predictor of cardiovascular disease and all-cause mortality in men with diabetes. Diabetes Care. 2003;26:2052–7. https://doi.org/10.2337/diacare.26.7.2052.
17. Choi A, Shin H. Quantitative analysis of the effect of an ectopic beat on the heart rate variability in the resting condition. Front Physiol. 2018;9:922. https://doi.org/10.3389/fphys.2018.00922.
18. Gąsior JS, Sacha J, Jeleń PJ, Zieliński J, Przybylski J. Heart rate and respiratory rate influence on heart rate variability repeatability: effects of the correction for the prevailing heart rate. Front Physiol. 2016;7:356. https://doi.org/10.3389/fphys.2016.00356.
19. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93:1043–65. https://doi.org/10.1161/01.CIR.93.5.1043.
20. Storniolo JL, Esposti R, Cavallari P. Heart rate kinetics and sympatho-vagal balance accompanying a maximal sprint test. Front Psychol. 2020;10:2950. https://doi.org/10.3389/fpsyg.2019.02950.
21. Chacko KM, Bauer TA, Dale RA, Dixon JA, Schrier RW, Estacio RO. Heart rate recovery predicts mortality and cardiovascular events in patients with type 2 diabetes. Med Sci Sports Exerc. 2008;40:288–95. https://doi.org/10.1249/mss.0b013e31815c4844.
22. Enciu E, Stanciu S, Matei D, Roul G. Autonomic nervous system dysfunction and abnormal heart rate recovery. Arch Cardiovasc Dis Suppl. 2018;10:116. https://doi.org/10.1016/j.acvdsp.2017.11.198.
23. Vianna JM, Werneck FZ, Coelho EF, Damasceno VO, Reis VM. Oxygen uptake and heart rate kinetics after different types of resistance exercise. J Hum Kinet. 2014;42:235–44. https://doi.org/10.2478/hukin-2014-0077.
24. Schamne JC, Ferreira Junior A, Araújo AC, Lima-Silva AE, Bertuzzi RC, Okuno NM. Cardiac autonomic responses during and after a single session of aerobic exercise with and without blood flow restriction. Motriz Rev Educ Fís. 2019;25. https://doi.org/10.1590/s1980-6574201900030015.
25. Niemela TH, Kiviniemi AM, Hautala AJ, Salmi JA, Linnamo V, Tulppo MP. Recovery patternof baroreflex sensitivity after exercise. Med Sci Sports Exerc. 2008;40:864–70. https://doi.org/10.1249/MSS.0b013e3181666f08.
26. Trevizani GA, Peçanha T, Nasario-Junior O, Vianna JM, Silva LP, Nadal J. Cardiac autonomic responses after resistance exercise in treated hypertensive subjects. Front Physiol. 2015;6:258. https://doi.org/10.3389/fphys.2015.00258.
27. Heffernan KS, Kelly EE, Collier SR, Fernhall B. Cardiac autonomic modulation during recovery from acute endurance versus resistance exercise. Eur J Cardiovasc Prev Rehabil. 2006;13:80–6. https://doi.org/10.1097/01.hjr.0000197470.74070.46.
28. Heffernan KS, Collier SR, Kelly EE, Jae SY, Fernhall B. Arterial stiffness and baroreflex sensitivity following bouts of aerobic and resistance exercise. Int J Sports Med. 2007;28:197–203. https://doi.org/10.1055/s-2006-924290.
29. Bhati P, Moiz JA, Menon GR, Hussain ME. Does resistance training modulate cardiac autonomic control? A systematic review and meta-analysis. Clin Auton Res. 2019;29:75–103. https://doi.org/10.1007/s10286-018-0558-3.
30. Patel H, Alkhawam H, Madanieh R, Shah N, Kosmas CE, Vittorio TJ. Aerobic vs anaerobic exercise training effects on the cardiovascular system. World J Cardiol. 2017;9:134–8. https://doi.org/10.4330/wjc.v9.i2.134.

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ORIGINAL ARTICLE

Evaluation of the prevalence of inappropriate hba1c examination requests at the General Hospital of Dokter Saiful Anwar Malang

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Abstract

Background/purpose of the study The HbA1c test was officially introduced by the American Diabetes Association (ADA) as a diagnostic and monitoring tool for diabetes mellitus in 2010.The following year, the World Health Organization (WHO) decided to accept the use of this test for similar reasons. The HbA1c test reflects average plasma glucose over the proceeding 8 to 12 weeks. Therefore, this test should ideally be performed every 2 to 3 months. The HbA1c value can be inaccurate with certain comorbidities such as hemoglobinopathies, high turnover of erythrocytes, and following blood transfusion. In developing countries, HbA1c tests are quite expensive so its demand must be appropriate. This study aims to evaluate the early HbA1c test, which was performed in less than 3 months, and its association with the incidence of hemoglobinopathies in the Dr. Saiful Anwar Hospital.

Methods This is a retrospective study using historical data from the Laboratory Information System (LIS) in the period of 2012 until 2017.

Results Over 5 years, there were 32,000 HbA1c tests. About 18% were repeated more than two times in less than 3 months. Of this number, 6.4% was performed in less than a month, 5.9% in 1 to 2 months, and 5.7% in 2 to 3 months.

Conclusion This may not only be caused by the lack of communication between clinicians and laboratory personnel, but also the presence of hemoglobinopathies, causing unreliable results. Requesting HbA1c test too early can result in inaccurate measurements. As a consequence, there is a need for better communication between clinicians and laboratory personnel.

Keywords Repeated examinations · Diabetes mellitus · Hemoglobinopathy · Laboratory management · HbA1c

Introduction

Glycated hemoglobin (HbA1c) is a form of hemoglobin, glycated non-enzymatically through the condensation of glucose. HbA1c is used by clinicians to diagnose and monitor hyperglycemia in type 2 diabetes patients. It also serves as a predictor of diabetic complications [1–6]. The HbA1c level reflects the average value of plasma glucose over 8 to 12 weeks and is not meaningfully affected by glycemic instability after adjusting for mean blood glucose [2, 7, 8]. Therefore, it is a more accurate tool to evaluate diabetic patients, compared to blood glucose tests [7]. The World Health Fund has made efforts to reduce costs while maintaining quality. As a consequence, this will affect the type of laboratory examinations, of which 70 to 80% are for diagnostic or therapeutic decisions, with costs covered by government insurance [9].

One of the things that are urgent is the increase in inappropriate test orders resulting in overutilization (over-ordering) of tests. Many studies suggest that about 25% of clinical pathology examinations are unnecessary; other studies have even found higher numbers. The UK Department of Health stated that a similar number may occur against requests for clinical pathology that should have been done but were not (underutilization or under-ordering) [9]. Driskell et al. stated that inappropriate HbA1c test orders, that do not comply with the guidelines, may significantly affect diabetes control [10]. However, only a few studies with evidence in this

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regard have been published, probably because of the difficulty in collecting such data [9].

The large variability in the average test orders between general and hospital practice supports the notion that inappropriate test orders are widely prevalent. It is also observed in diabetes mellitus, a disease in which guidelines for tests frequency had been established by the American Diabetes Association (ADA), the UK National Institute for Health and Clinical Excellence (NICE), and the Canadian Diabetes Association. Identifying the variations, prevalence, and consequence of inappropriate test orders done too soon or too late has major implications, mainly excessive healthcare costs. This study was conducted to determine the prevalence of inappropriate HbA1c test orders [9].

The UK NICE guideline for HbA1c testing on uncontrolled type 1 and 2 diabetes recommends a 2- to 6-month gap, with measurement taken every 3 months. Meanwhile, patients on routine medication with controlled diabetes are advised to have HbA1c testing in 6 to 12 monthly intervals. ADA recommends HbA1c testing at least twice a year in patients who have achieved therapeutic goals and have stable glycemic control, and once every 3 months in patients whose treatment regimens have changed or have not reached glycemic targets. Based on ADA and NICE recommendations, HbA1c levels less than 7.0% indicate stable glycemic control. This cut-off point can be used as a guide to determine whether test orders are too soon (defined as sooner than 6 months for patients with initial HbA1c < 7% and less than 3 months for patients with 7% or over) or too late (defined as more than 12 months for patients with initial HbA1c < 7.0% and more than 6 months for patients with 7.0% or over). The latest HbA1c results are required to precisely determine the next test interval. In this case, the optimum interval for each patient may change with fluctuations in glycemic control over time [9].

Despite being favored as one of the diagnostic parameters in diabetes mellitus, HbA1c testing possesses some limitations. This test cannot be used in pediatric patients, pregnant women, women in the early postpartum period (< 2 months), patients with suspected type 1diabetes, short duration of diabetic symptoms (< 2 months), and with high risk of diabetes in severe illness, patients on medication that raise blood glucose levels such as corticosteroids or antipsychotic drugs, patients with history of acute pancreas damage or post-pancreatic surgery, chronic kidney disease, chronic liver disease, and patients on antiretroviral therapy. Careful use and interpretation of HbA1c is needed in patients with hemoglobinopathies, anemia, changes in erythrocytes age (i.e. post-splenectomy patients), and recent blood transfusion [11–14]. Vitamin C and E can inhibit non-enzymatic glycation of hemoglobin, resulting in false low HbA1c values [15]. Furthermore, there are several markers that correlate with HbA1c in patients with type 2 diabetes mellitus. These include frailty score [16], neuregulin [17], uric acid/HDL cholesterol ratio [18], neutrophil/lymphocyte ratio [19], platelet/lymphocyte ratio [20], red cell distribution width [21], and serum vitamin D [22].

Considering the high prevalence of diabetes, inappropriate overuse of HbA1c test is very likely to occur. Futility in repeated laboratory tests is considered important. Especially in developing countries with limited financial resources, laboratory tests utilization should be accurate and efficient [1]. Given the lack of local data, it is necessary to study the accuracy of HbA1c tests utilization in Indonesia, which is a developing country with a high prevalence of diabetes. In addition, the cost of HbA1c testing is also quite expensive.

In this retrospective study, the goal is to identify the prevalence of inappropriate HbA1c test orders in the Dr. Saiful Anwar General Hospital in Malang, Indonesia. The results can later become the basis for finding ways to reduce unnecessary tests, which in turn would ease some of the burden on health care costs without affecting the quality of patient care.

Materials and methods

The study was conducted in the central laboratory of the Dr. Saiful Anwar General Hospital from October 2017 to March 2018. Data on HbA1c test orders were obtained from the Laboratory Information System (LIS). This study was approved by ethics committee with ethical clearance number 400/028/K.3/302/2019.

The study sample included all patients tested for HbA1c levels in the Dr. Saiful Anwar General Hospital, Malang from August 27, 2012 until September 25, 2017 whose data were recorded in the LIS. Within this timeframe, there were 32,000 HbA1c test orders from 16,439 patients in the hospital. Patients with incomplete data were excluded from the analysis.

Inappropriate HbA1c test orders can be defined as either over-ordered or under-ordered. In this case, over-ordering means the test was carried out in less than 3 months for patients with initial HbA1c > 7.0% and less than 6 months for patients with less than 7.0%. Meanwhile, the term under-ordering means the test was carried out in more than 6 months for patients with initial HbA1c ≥ 7.0% and more than 12 for patients with less than 7.0%. Analysis was performed using Microsoft Excel to calculate the percentage of the under-requests, over-requests, or appropriate requests.

Results

HbA1c examination data were obtained retrospectively through data in LIS; the HbA1c data was taken from August 27, 2012 to September 25, 2017. Within this timeframe,

Table 1 HbA1c tests ordered more than once in less than 3 months in the Central Laboratory

Interval of repeated tests	Frequency	Percentage	Percentage of all tests
Same-day	108	1.88%	0.34%
1–10 days	823	14.30%	2.57%
10–20 days	365	6.34%	1.14%
20–30 days	743	12.91%	2.32%
1–2 days	1.887	32.79%	5.90%
2–3 days	1.829	31.78%	5.72%
Total	**5.755**	**100%**	**17.98%**

Table 2 Reasons for repeating HbA1c tests on the same day

Reason	Frequency	Percentage	Percentage of all tests
Didn't know prior result	76	70.37%	0.24%
Inappropriate sample	27	15.74%	0.08%
Inappropriate result	4	3.70%	0.01%
N/A	1	0.93%	0.003%
Total	**108**	**100%**	**0.34%**

Table 3 Reasons for repeating HbA1c tests in within 1 to 10 days

Reason	Frequency	Percentage	Percentage of all tests
Didn't know prior result	809	98.3%	2.53%
Inappropriate sample	4	0.49%	0.01%
Inappropriate result	10	1.22%	0.03%
N/A	0	0%	0%
Total	**823**	**100%**	**2.57%**

there were 32,000 HbA1c examinations from 16,439 patients in the Dr. Saiful Anwar General Hospital.

As many as 13,364 tests were carried out in less than 6 months. Of this number, 10,144 (31.7% of all tests) tests were carried out in less than 3 months (over-ordering). About more than half of it, 5,755 tests (17.98% of all tests) were ordered more than once in less than 3 months. For a more in-depth analysis, these 5,755 tests were further divided into several groups based on inter-demand intervals described in Table 1.

From the table above, 108 tests were repeated on the same day and 823 tests were repeated within 1 to 10 days. The data was then analyzed to find the reasons for repeated tests, as shown in Tables 2 and 3. The reasons for repeated tests were listed in the LIS. Based on the HbA1c results, the data was further grouped into time intervals as shown in Tables 4 and 5. From this data, 11,034 (34.48%) tests were considered too soon, 3,616 (11.3%) were done in a timely manner, and 17,350 (54.22%) were considered too late.

Table 4 HbA1c tests performed within a 3- to 6-month interval

HbA1c result	Frequency	Note
< 7.0%	890	Over request
≥ 7.0%	2,330	Appropriate

Table 5 HbA1c tests performed within a 6- to 12-month interval

HbA1c result	Frequency	Note
< 7.0%	1,286	Appropriate
≥ 7.0%	2,162	Under request

Discussion

Of all HbA1c tests, very low HbA1c levels of less than 5% were found in 505 tests; < 4% in 51 tests; and 0% in as much as 36%. We use the cut-off value of < 5% for low HbA1c based on a study by Aggarwal et al. [23] and < 4% based on studies by Carson et al. and Christman et al. [24, 25]. The 51 tests with HbA1c < 4% levels were further divided into two groups, one with high HbA2 values (44 tests in 20 patients) and the other with unknown HbA2 data (seven tests in six patients). In the first group, there were 32 repeated tests from eight patients, while the other 12 were not repeated. In the second group, two tests originated from one patient and five tests from five patients were not repeated. Patients with high HbA2 who had undergone repeated tests turned out to be tested for two to five times with the shortest interval of one day.

HbA1c results supplemented with HbA2 levels were found in 320 tests from 141 patients. Based on these tests, nine tests from nine patients were not repeated and the other 311 from 132 patients were repeated. A total of 110 patients were retested one time (220 tests in total), 20 patients were retested three times (80 tests in total), one patient was retested four times (five tests in total), and the other one was retested five times (six tests in total).

Based on these data, it can be concluded that there were many instances of over-ordering of HbA1c tests. The proportion of tests that were considered too soon reached 35.48% of all tests in the 5-year period. In fact, 56.73% of the tests were repeated more than once. It was found that unknown results of previous HbA1c level (within the last 3 months) were the most common reason for repeating the test, particularly those performed more than once. This might occur due to lack of communication between the clinician and the patient. It could be that the clinicians might have forgotten

to explain the monitoring plan or the patients failed to understand the clinician's explanation. A similar thing could also happen between the clinician and the laboratory personnel.

Another frequent cause is the presence of untrusted results or hemoglobinopathies. In the case of hemoglobinopathies, HbA1c results may be inaccurate. These hemoglobinopathies may either falsely raise or lower the results. Poor communication would cause the clinician to doubt the test result which is not in line with the patient's condition. There were 311 (1%) repeated HbA1c tests from 132 patients with hemoglobinopathies. This should raise the concern that simply improving communication can significantly lower the costs of unnecessary repetition of tests. In fact, the central laboratory of the Dr. Saiful Anwar General Hospital has made a policy that every HbA1c test in patients with hemoglobinopathies should be accompanied with a note stating that inaccurate result. However, lack of communication between both parties caused frequent premature HbA1c tests.

In the UK, Driskell et al. found that from 2001 to 2011 17.7% of the tests were a result of over-ordering of HbA1c tests [9] while other study in Turkey, by Akan et al., showed that from 2002 to 2004 35.5% of the tests were results of over-ordering of HbA1c tests [2]. Another study in the US conducted from2001 to 2013 by McCoy et al. found that there were 60.3% of the tests resulted from the over-ordering of HbA1c tests [26].

On the other hand, this study found that 54% of all HbA1c tests were considered too late. This was likely to occur when the patient had previous normal HbA1c results or the patient had not complied. In this case, better communication between clinicians and patients is needed. In developing countries like Indonesia, HbA1c test is costly. Therefore, repetition of ultimately futile tests must be avoided to reduce unnecessary health care expenditure.

In conclusion, over-ordering of HbA1c tests in the Dr. Saiful Anwar General Hospital was still prevalent. The test was useless because the HbA1c value is constant for 3 months. Lack of communication between the clinician, the laboratory personnel, and the patient could be the root of the problem. These futile tests must be avoided to reduce unnecessary health expenses. HbA1c test is costly, so reducing laboratory test orders will certainly lower the government's expenditure.

This study has some limitations, particularly because it was conducted only at one health care center. Studies from other health care centers are needed to support the results. Another limitation is that the reasons for repeating HbA1c tests were more of a hypothesis rather than being obtained from a questionnaire.

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Author contribution All authors have contributed to this study.

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Declarations

Ethics approval The study was conducted according to the Declaration of Helsinki and was approved by the ethics committee of our institution.

Conflict of interest The authors declare no competing interests.

References

1. Salinas M, López-Garrigós M, Flores E, Leiva-Salinas C, Tests PGotAUoL, Valero VP, et al. Glycated hemoglobin: a powerful tool not used enough in primary care. J Clin Lab Anal. 2018;32(3):e22310.
2. Akan P, Cimrin D, Ormen M, Kume T, Ozkaya A, Ergor G, et al. The inappropriate use of HbA1c testing to monitor glycemia: is there evidence in laboratory data? J Eval Clin Pract. 2007;13(1):21–4.
3. Hanna FW, Geen J, Issa BG, Tahrani AA, Fryer AA. Limitations of glycosylated haemoglobin (HbA1c) in diabetes screening. Pract Diabetes. 2012;29(1):29–31.
4. Owora AH. Diagnostic validity and clinical utility of HbA1c tests for type 2 diabetes mellitus. Curr Diabetes Rev. 2018;14(2):196.
5. Kim H-J, Choi EY, Park EW, Cheong YS, Lee H-Y, Kim JH. The utility of HbA1c as a diagnostic criterion of diabetes. Kor J Fam Med. 2011;32(7):383.
6. Nadkarni P, Weinstock RS. Chapter 16: Carbohydrates. In: McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 23rd Edition. St. Louis, Missouri. Elsevier Health Sciences; 2017. p 209.
7. Khan MAH, Rabeya MR, Saiedullah M. Measurements of HbA1c by high performance liquid chromatography in D-10 analyzer and immunological method by Beckman Coulter AU480 System: a comparative study. J Enam Med Coll. 2012;2(2):62–6.
8. Drugs CAf, Health Ti. HbA1c testing frequency: a review of the clinical evidence and guidelines: Canadian Agency for Drugs and Technology in Health; 2015.
9. Driskell OJ, Holland D, Hanna FW, Jones PW, Pemberton RJ, Tran M, et al. Inappropriate requests for glycated hemoglobin (HbA1c) are widespread: assessment of prevalence, impact of national guidance, and practice-to-practice variability. Clin Chem. 2012:clinchem. 2011.176487.
10. Driskell OJ, Holland D, Waldron JL, Ford C, Scargill JJ, Heald A, et al. Reduced testing frequency for glycated hemoglobin, HbA1c, is associated with deteriorating diabetes control. Diabetes Care. 2014;37(10):2731–7.
11. Kilpatrick ES, Atkin SL. Using haemoglobin A1c to diagnose type 2 diabetes or to identify people at high risk of diabetes. Bmj. 2014;348:g2867.
12. Sehrawat T, Jindal A, Kohli P, Thour A, Kaur J, Sachdev A, et al. Utility and limitations of glycated hemoglobin (HbA1c) in patients with liver cirrhosis as compared with oral glucose tolerance test for diagnosis of diabetes. Diabetes Ther. 2018;9(1):243–51.

13. Hellman R. When are HBA1C values misleading? AACE Clin Case Rep. 2016;2(4):e377–9.
14. Amin Mohsin Z, Paul A, Devendra S. Pitfalls of using HbA1c in the diagnosis and monitoring of diabetes. London J Prim Care. 2015;7(4):66–9.
15. Thevarajah M, Nadzimah M, Chew Y. Interference of hemoglobinA1c (HbA1c) detection using ion-exchange high performance liquid chromatography (HPLC) method by clinically silent hemoglobin variant in University Malaya Medical Centre (UMMC)—a case report. Clin Biochem. 2009;42(4–5):430–4.
16. Bilgin S, Aktas G, Kurtkulagi O, Atak BM, Duman TT. Edmonton frail score is associated with diabetic control in elderly type 2 diabetic subjects. J Diabetes Metab Disord. 2020;19:511–4.
17. Kocak MZ, Aktas G, Atak BM, Duman TT, Yis OM, Erkus E, et al. Is neuregulin-4 a predictive marker of microvascular complications in type 2 diabetes mellitus? Eur J Clin Investig. 2020;50(3):e13206.
18. Aktas G, Kocak MZ, Bilgin S, Atak BM, Duman TT, Kurtkulagi O. Uric acid to HDL cholesterol ratio is a strong predictor of diabetic control in men with type 2 diabetes mellitus. Aging Male. 2019;1–5.
19. Duman TT, Aktas G, Atak BM, Kocak MZ, Erkus E, Savli H. Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. Afr Health Sci. 2019;19(1):1602–6.
20. Atak B, Aktas G, Duman TT, Erkus E, Kocak MZ, Savli H. Diabetes control could through platelet-to-lymphocyte ratio in hemograms. Rev Assoc Med Bras. 2019;65(1):38–42.
21. Bilgin S, Aktas G, Zahid Kocak M, Atak BM, Kurtkulagi O, Duman TT, et al. Association between novel inflammatory markers derived from hemogram indices and metabolic parameters in type 2 diabetic men. Aging Male. 2019;1–5.
22. Erkus E, Aktas G, Kocak MZ, Duman TT, Atak BM, Savli H. Diabetic regulation of subjects with type 2 diabetes mellitus is associated with serum vitamin D levels. Rev Assoc Med Bras. 2019;65(1):51–5.
23. Aggarwal V, Schneider AL, Selvin E. Low hemoglobin A1c in nondiabetic adults: an elevated risk state? Diabetes Care. 2012;35(10):2055–60.
24. Carson AP, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM, et al. Low hemoglobin A1c and risk of all-cause mortality among US adults without diabetes. Circ: Cardiovasc Qual Outcomes. 2010;3(6):661–7.
25. Christman AL, Lazo M, Clark JM, Selvin E. Low glycated hemoglobin and liver disease in the US population. Diabetes Care. 2011;34(12):2548–50.
26. McCoy RG, Van Houten HK, Ross JS, Montori VM, Shah ND. HbA1c overtesting and overtreatment among US adults with controlled type 2 diabetes, 2001–13: observational population based study. Bmj. 2015;351:h6138.

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SHORT ARTICLE

Diabetes prevalence is associated with obesity, hypertension, dyslipidemia, and sociodemographic factors in adults living in Casablanca-Settat and Rabat-Sale-Kenitra regions, Morocco

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Abstract

Background Diabetes is increasing at an alarming rate worldwide, but little is known about its risk factors in Morocco.
Objective This study aimed to investigate the prevalence and risk factors of diabetes.
Methods Data from the national survey on risk factors for non-communicable diseases conducted in 2017–2018 was used. Data collection was based on the WHO STEPwise approach to surveillance (STEPS). A total of 1522 adults aged 18 years and older were included in our analysis.
Results The overall prevalence of prediabetes and diabetes was 12.8% and 17.9%, respectively. Women had a significantly higher likelihood of diabetes than men ($p = 0.049$). Adults aged 45 years or older were more likely than the 18–29-year-old group to have prediabetes ($p < 0.033$) and diabetes ($p < 0.001$). Similarly, urban individuals were more likely to develop diabetes than rural individuals (odds ratio (OR): 4.58; 95% confidence interval (95%CI): 3.06–6.84). Overweight/obesity, abdominal obesity, hypertension, and dyslipidemia were associated with a significantly higher risk for prediabetes and diabetes. Compared with never smokers, former smokers were found to be at increased risk of diabetes, while current smokers had a reduced risk of both prediabetes and diabetes. Alcohol drinkers also had a slightly lower risk of prediabetes and diabetes than non-drinkers.
Conclusions More than 30% of adults had prediabetes or diabetes. Increased age (≥ 45 years), female gender, living in urban areas, overweight/obesity, hypertension, and dyslipidemia were associated with a greater risk for prediabetes and diabetes. Public health interventions are urgently needed to prevent and control diabetes and therefore avoid associated morbidity and mortality.

Keywords Alcohol consumption · Diabetes · Dyslipidemia · Education level · Hypertension · Obesity · Overweight · Place of residence · Prediabetes · Smoking

Introduction

Worldwide, diabetes prevalence is increasing at an alarming rate [1]. This increase is likely due to the aging of the world's population, economic development, and rapid urbanization that have resulted in increasingly sedentary lifestyles and greater consumption of unhealthy foods associated with rising prevalence rates of overweight and obesity [2]. Even though genetic predisposition partly determines individual susceptibility to diabetes, this disease may be associated with multiple risk factors including ethnicity, family history, level of education, and socioeconomic status, as well as increased body weight, metabolic syndrome, and its components [3, 4].

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There is evidence that diabetes can lead to serious lifelong complications such as blindness, kidney failure, cardiovascular diseases, and lower limb amputation [5]. The burden of diabetes and related complications has been rising more rapidly in developing countries than in developed countries. African, Middle Eastern and Northern African, and Southeast Asian regions are expected to experience the highest increase in diabetes incidence during the next three decades [6].

Although diabetes has been the fourth cause of death in Morocco and remains a major public health problem [7], little is known about its risk factors particularly in some densely populated regions such as Casablanca-Settat and Rabat-Sale-Kenitra regions. Identifying individuals living with diabetes or prediabetes and addressing the upstream determinants of diabetes are important not only for making projections of the probable health burden in the country but also for designing effective strategies that aim to prevent and control this disease.

The main purpose of our study was to estimate the prevalence of prediabetes and diabetes and to investigate their association with gender, age, place of residence, education level, general and abdominal obesity, hypertension, dyslipidemia, smoking history, and alcohol consumption among Moroccan adults.

Methods

Study design

This study reports data collected as part of a recent national survey on risk factors for non-communicable diseases. The survey was conducted during the years 2017–2018 among adults aged 18 years and older who were randomly selected using a multistage stratified and geographically clustered sampling design.

Study setting

In this paper, only diabetes data collected in Casablanca-Settat and Rabat-Sale-Kenitra regions are presented and analyzed. These regions are highly populated and account for 32% of the total Moroccan population (36 million in 2018) of which 69% live in urban areas [8]. It should be noted that Rabat city is the national political capital, and Casablanca is the largest city in Morocco and is considered the economic and business center of the country.

Data collection

Data were collected by trained investigators based on the World Health Organization (WHO) STEPwise approach to surveillance (STEPS) methodology, including three standardized "steps" [9].

Step 1 consisted of a face-to-face interview using a structured questionnaire to collect information on sociodemographic parameters (age, education level, and place of residence, among others), smoking history, alcohol consumption, and medical history.

Step 2 entailed physical measurements of survey participants using standardized methods and equipment. Height was measured to the nearest 0.1 cm using an adult portable stadiometer (Seca, Germany; 200 ± 0.1 cm). Body weight was measured to the nearest 0.1 kg using a digital weighing scale (Seca, Germany; 150 ± 0.1 kg). Waist circumference (WC) was horizontally measured using a non-stretchable measuring tape to the nearest 0.1 cm. Body mass index (BMI) was calculated as a ratio of weight (kg) to height squared (m^2). Body weight status was categorized into four groups: underweight ($BMI < 18$ kg/m^2), normal weight (18 kg/$m^2 \leq BMI < 25$ kg/m^2), overweight (25 kg/$m^2 \leq BMI < 30$ kg/m^2), and obese ($BMI \geq 30$ kg/m^2) according to WHO criteria [10]. Abdominal obesity was defined based on criteria from the NCEP ATP III ($WC > 102$ cm for men and > 88 cm for women) [11].

Hypertension was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg or current treatment with antihypertensive drugs [12].

Step 3 dealt with biochemical measurements, including fasting plasma glucose (FPG) and total cholesterol. Venous blood samples were collected for biochemical analyses after a 12-h fasting. All blood samples were centrifuged immediately to separate serum, and transferred under cold chain condition to the Reference Laboratory of the Joint Research Unit in Nutrition and Food, Regional Designed Center of Nutrition (AFRA/IAEA), Ibn Tofail University-CNESTEN, Rabat, Morocco. FPG values for the diagnosis of prediabetes and diabetes were set according to the WHO criteria [13]. Individuals who self-reported history of diagnosed diabetes with a physician prescription of diabetes medication, and/or diagnosed diabetes, and/or had recorded survey-measured FPG levels ≥ 7 mmol/L (126 mg/dL) were considered diabetic. Those who had recorded survey-measured FPG levels of 6.1–6.9 mmol/L (110–125 mg/dL) were considered prediabetic. There was no distinction between type 1 diabetes and type 2 diabetes. Dyslipidemia was defined as total cholesterol ≥ 5 mmol/L ($\sim$ 190 mg/dL) [14].

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software (version 20.0; IBM Corp, Armonk, NY, USA). The Kolmogorov–Smirnov test was initially used to investigate the normality of each variable. Results are expressed as medians and interquartile

ranges (IQR: 25th–75th percentile) or as proportions and 95% confidence interval using descriptive statistics. Differences between proportions and medians of the three diabetes status groups were assessed by the Chi-square test and the Kruskal–Wallis test, respectively. Multinomial logistic regression analysis was applied to investigate the association of prediabetes and diabetes with weight status and sociodemographic factors. A two-tailed p-value of < 0.05 was considered to be statistically significant.

Results

A total of 1522 adults were included in our analyses. The estimated prevalence of prediabetes and diabetes in the present study was 12.8% and 17.9%, respectively (Fig. 1). The median age was 46.0 years (IQR: 35.0–59.0), and the proportion of men was 36.7%. Subjects with diabetes or prediabetes had significantly higher age, BMI, WC, and FPG than those with normal blood glucose levels ($p < 0.001$). Similarly, overweight and both general and abdominal obesity were more common among individuals with diabetes or prediabetes compared to those without diabetes or prediabetes ($p < 0.001$). The prevalence of hypertension and dyslipidemia was associated with diabetes status, with statistically significant positive trends across the three diabetes status groups ($p < 0.001$ and $p = 0.001$, respectively). However, there were no significant differences in smoking and alcohol consumption between diabetes status groups (Table 1).

Logistic regression analysis showed that women had a significantly higher likelihood of diabetes as compared with men (odds ratio (OR): 1.37; 95% confidence interval (95%CI): 1.00–1.88). Adults aged 45 years or older were significantly more likely than the 18–29-year-old group to have prediabetes ($p < 0.033$) and diabetes ($p < 0.001$). Similarly, urban individuals were more than twice as likely to have prediabetes and diabetes as rural individuals (OR: 2.81; 95%CI: 1.87–4.22; and OR: 4.58; 95%CI: 3.06–6.84, respectively). However, the likelihood of having prediabetes and diabetes did not differ substantially between groups with different levels of education. Overweight/obese subjects were more likely to have diabetes than their non-overweight peers (21.8% vs. 10.7%), with an OR of 2.51 (95%CI: 1.83–3.44). Abdominally obese individuals also were 3 times more likely to have diabetes compared to non-abdominally obese with an OR of 3.06 (95%CI: 2.30–4.07). Both hypertension and dyslipidemia were associated with a significantly higher risk for prediabetes and diabetes. Compared with never smokers, former smokers were found to be at increased risk of diabetes (OR:1.45; 95%CI: 0.82–2.56), while current smokers had a reduced risk of prediabetes (OR: 0.52; 95%CI: 0.24–1.13) and diabetes (OR: 0.92; 95%CI: 0.44–1.92). Alcohol drinkers also had a slightly lower risk of prediabetes and diabetes than non-drinkers (OR: 0.88; 95%CI: 0.50–1.56; and OR: 0.66; 95%CI: 0.38–1.15, respectively) (Table 2).

Discussion

The overall prevalence of diabetes and prediabetes was 17.9% and 12.8%, respectively. Our estimate of diabetes prevalence is in line with that reported in 2019 in some MENA countries such as Saudi Arabia (18.3%), Sudan (17.9%), Pakistan (17.1%), United Arab Emirates (15.4%), and Egypt (15.2%), but higher than national, regional (MENA), and global estimates (7.4%, 12.8%, and 9.3%,

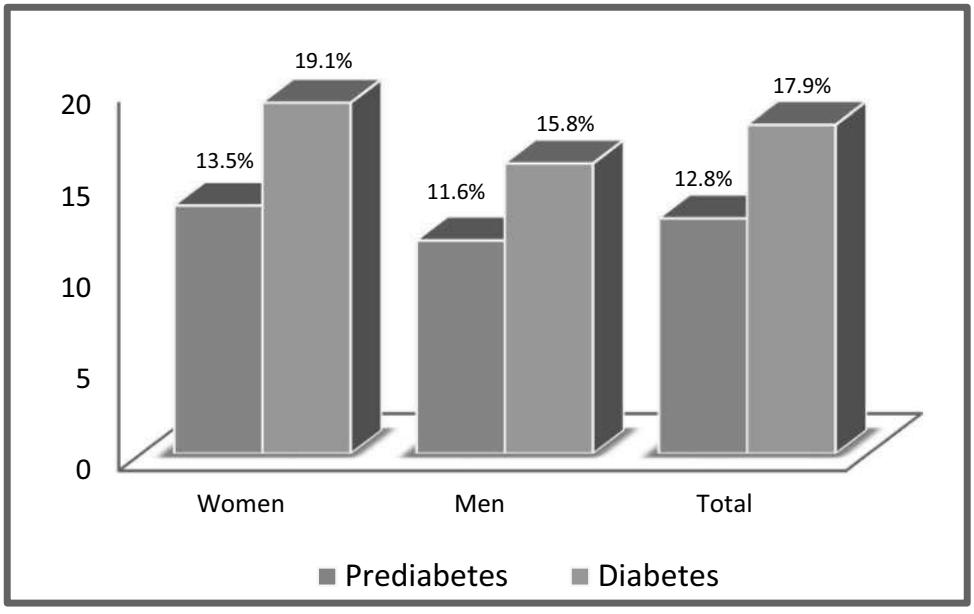

Fig. 1 Prevalence of prediabetes and diabetes

Table 1 Characteristics of the study population according to diabetes status

Characteristics	All ($N=1522$)		Diabetes status						
			Normal glycemia ($n=1055$)		Prediabetes ($n=195$)		Diabetes ($n=272$)		p-value *
	Median/%	IQR/95%CI	Median/%	IQR/95%CI	Median/%	IQR/95%CI	Median/%	IQR/95%CI	
Age (years)	46.0	35.0–59.0	43.0	32.0–54.0	52.0	39.0–64.0	56.0	44.3–65.0	<0.001
Sex (% men)	36.7	34.2–39.2	38.4	35.7–41.4	33.3	27.7–40.0	32.4	26.8–37.9	0.108
BMI (Kg/m^2)	26.6	23.4–30.1	25.9	22.7–29.3	27.7	24.2–32.5	28.4	25.3–32.0	<0.001
WC (cm)	93.0	84.0–101.0	90.0	82.0–98.0	97.0	88.0–105.0	100.0	92.0–107.0	<0.001
FPG (mg/dL)	96.0	85.0–113.0	90.0	81.0–97.0	116.0	112.0–121.0	145.0	130.0–185.8	<0.001
Abdominal obesity(%)	50.3	47.8–52.6	43.1	40.1–46.1	61.5	54.4–68.2	69.9	64.3–75.4	<0.001
Weight status									
Underweight (%)	3.1	2.3–64.1	2.9	1.9–4.1	6.2	3.1–9.7	1.5	0.4–2.9	<0.001
Normal weight(%)	32.5	30.2–34.8	37.5	34.6–40.5	23.1	16.9–29.7	19.9	15.1–24.6	
Overweight(%)	39.2	36.7–41.6	39.2	36.3–42.2	33.8	27.2–41.0	43.0	36.8–49.6	
Obese(%)	25.2	23.2–27.4	20.3	17.7–22.7	36.9	30.3–43.6	35.7	29.8–41.5	
Hypertension									
With	27.5	24.7–30.1	20.6	17.3–23.6	36.6	29.1–44.8	43.2	36.6–49.8	<0.001
Without	72.5	69.9–75.3	79.4	76.4–82.7	63.4	55.2–70.9	56.8	50.2–63.4	
Dyslipidemia									
With	14.7	12.9–16.6	12.6	10.7–14.7	18.5	12.8–23.6	20.2	15.8–25.0	0.001
Without	85.3	83.4–87.1	87.4	85.3–89.3	81.5	76.4–87.2	79.8	75.0–84.2	
Smoking status									
Never	7.4	6.0–8.7	7.5	6.1–9.2	9.2	5.1–13.3	5.5	2.9–8.4	0.371
Former	83.4	81.5–85.3	82.2	79.6–84.5	84.1	79.0–89.2	87.5	83.5–91.2	
Current	9.3	7.9–10.7	10.3	8.5–12.3	6.7	3.6–10.3	7.0	4.0–10.3	
Ever alcohol consumption									
No	92.0	90.6–93.3	91.4	89.5–93.2	92.3	88.2–95.9	94.1	91.2–96.7	0.138
Yes	8.0	6.7–9.4	8.6	6.8–10.5	7.7	4.1–11.8	5.9	3.3–8.8	

Data are presented as median and interquartile range 25th–75th percentile (IQR) for continuous variables and as proportion and 95% confidence interval (95%CI) based on 1000 bootstrap samples for categorical variables. *BMI* body mass index; *WC* waist circumference; *FPG* fasting plasma glucose

*Differences between diabetes status groups were assessed using the Chi-square test for proportions and the Kruskal–Wallis test for medians

respectively) [6]. It is also higher than estimates of diabetes prevalence observed in some Maghreb countries including Algeria (7.2%) and Tunisia (10.2%) [6]. Similarly, the proportion of individuals with prediabetes (12.8%) is higher than that observed at both regional and global levels (8.3% and 7.5%, respectively) [6]. These results indicate that diabetes is an emerging public health problem in Morocco.

Although it is difficult to compare our findings with those of previous studies, due to disparities in terms of ethnicity, sex, age, socioeconomic status, geographic location, and criteria for diagnosing diabetes, our estimated prevalence of diabetes is not markedly different from that reported in some earlier studies in Morocco and elsewhere. For instance, one of these studies found among the general Moroccan population that 13.4% of participants were diabetic [15]. In another study among a large sample of women aged 20–69 years from Meknes region, Morocco, the prevalence of diabetes was 19% [16]. Also, there was no noticeable difference between our results and those of available STEPwise surveys carried out in some MENA countries such as Kuwait (18.8%) [17] and Algeria (14.7%), as well as in some African countries including Niger (22.5%), Liberia (19.2%), and Swaziland (14.5) [18]. Furthermore, our results are consistent with trends in diabetes in the MENA region suggested by Azizi et al., indicating that diabetes prevalence in Morocco is expected to rise from 12.5% in 2010 to 21.8% in 2025 [19].

The high prevalence rates of prediabetes and diabetes found in our study sample are likely to be due to the rapid growth of urban agglomerations, uncontrolled population increase, and significant socioeconomic development that Casablanca-Settat and Rabat-Sale-Kenitra regions have experienced over the last few decades. Such changes were found to be associated with an increased prevalence of

Table 2 Association of prediabetes and diabetes with gender, age, place of residence, education level, weight status, abdominal obesity, hypertension, dyslipidemia, smoking status, and alcohol consumption among Moroccan adults

	Sub-group (*n*)	Prediabetes				Diabetes			
		%	OR	95%CI	*p*-value	%	OR	95%CI	*p*-value
Gender									
Men	558	11.6	Ref			15.8	Ref		
Women	964	13.5	1.24	0.88–1.75	0.221	19.1	1.37	1.00–1.88	0.049
Age group (years)									
18–29	234	7.7	Ref			7.3	Ref		
30–44	473	11.6	1.57	0.89–2.78	0.123	10.8	1.58	0.87–2.84	0.130
45–59	459	12.4	1.89	1.05–3.41	0.033	20.3	3.41	1.91–6.06	0.000
60–69	214	19.6	4.52	2.38–8.56	0.000	31.8	8.42	4.51–15.70	0.000
≥ 70	142	16.2	3.25	1.57–6.73	0.002	30.3	7.16	3.61–14.19	0.000
Place of residence									
Rural	454	8.1	Ref			7.7	Ref		
Urban	1068	14.8	2.81	1.87–4.22	0.000	22.2	4.58	3.06–6.84	0.000
Education level									
None (illiterate)	708	14.1	Ref			20.6	Ref		
Primary education	340	11.8	0.81	0.53–1.26	0.355	15.0	0.85	0.57–1.28	0.441
Secondary education	351	11.4	0.77	0.49–1.21	0.259	15.4	0.88	0.58–1.33	0.538
Higher education	123	12.2	0.82	0.43–1.56	0.546	17.1	0.91	0.51–1.62	0.751
Weight status									
Non-overweight	542	10.5	Ref			10.7	Ref		
Overweight/obese	980	14.1	1.65	1.18–2.30	0.003	21.8	2.51	1.83–3.44	< 0.001
Abdominal obesity									
Without	757	9.9	Ref			10.8	Ref		
With	765	15.7	2.11	1.54–2.89	< 0.001	24.8	3.06	2.30–4.07	< 0.001
Hypertension									
Without	737	11.5	Ref			16.4	Ref		
With	279	17.6	2.22	1.49–3.30	< 0.001	33.0	2.93	2.10–4.07	< 0.001
Dyslipidemia									
Without	1298	12.2	Ref			16.7	Ref		
With	224	16.1	1.57	1.05–2.35	0.029	24.6	1.76	1.2-–2.49	0.001
Smoking status									
Never	112	16.1	Ref			13.4	Ref		
Former	1269	12.9	0.83	0.49–1.42	0.498	18.8	1.45	0.82–2.56	0.205
Current	141	9.2	0.52	0.24–1.13	0.099	13.5	0.92	0.44–1.92	0.820
Ever alcohol consumption									
No	1400	12.9	Ref			18.3	Ref		
Yes	122	12.3	0.88	0.50–1.56	0.668	13.1	0.66	0.38–1.15	0.141

Odds ratio (OR) and 95%confidence interval (95%CI) using multinomial logistic regression analysis

diabetes worldwide [20]. This may be attributed to improvement in the economic situation, nutrition transition, reduced physical activity, and increased prevalence of overweight and obesity [4, 20–22]. It should be noted that the combined prevalence of overweight and obesity in our study population (Table 1) is higher than previously reported in a national survey (64.4% vs. 50.8%) [23]. An increase in diabetes diagnosis and reduced mortality among people affected by diabetes may also have contributed to its rising prevalence in the target regions, particularly in middle- and high-socioeconomic neighborhoods [3].

Although the worldwide prevalence of diabetes in 2019 was estimated to be slightly lower in women than in men (9.0% vs. 9.6%) [6], among our study sample, women showed a significantly higher risk of diabetes compared to men. Several factors can explain this sex difference such as multiple pregnancies, lack of motivation or skills in sport, and environmental and cultural barriers to outdoor physical activity for girls and women in Arab countries, including Morocco [21]. Additionally, a previous national survey revealed that obesity, known as a diabetes risk

factor, was more prevalent among women compared to men in Morocco (26.8% vs. 8.2%) [23].

The prevalence of prediabetes and diabetes increased steadily with age group, being lowest in the youngest group (18–29 years) and highest in the 60–69-year-old group. These findings are consistent with other recent studies [24, 25]. Another finding of concern was that diabetes prevalence was not only high among the elderly, but a significant proportion of young adults were diabetic. Many factors may explain this high prevalence of diabetes among our study population such as increasing incidence of type 1 diabetes in children, increasing incidence of type 2 diabetes in all age groups due to reduced physical activity and high-energy dietary intakes, intergenerational effects of hyperglycemia in pregnancy, and general aging of the population. Another possible explanation could be that early diagnosis and better management of all types of diabetes would lead to greater life expectancy [1, 3].

The risk for prediabetes and diabetes was significantly higher among adults from urban versus rural areas. These findings are aligned with earlier studies that reported a positive association between urbanization and diabetes [6, 20, 21]. Such association may be mediated, though not entirely explained, by an increasing prevalence of some diabetes risk factors including unhealthy dietary habits, sedentary lifestyle, physical inactivity, obesity [22], and stress in the built urban environment [26].

As expected, increased education levels tended to have a protective effect against prediabetes and diabetes. Our results are consistent with those of previous studies that found a higher prevalence of diabetes among illiterate people compared to educated people [22, 27, 28]. Although literacy levels had little effect, our findings suggest that it may be useful for public health policymakers to consider the role of education in interventions aimed to prevent and control diabetes.

In our study, individuals with overweight/obesity were significantly more likely to develop prediabetes and diabetes when compared to non-overweight individuals. Similarly, abdominally obese individuals were 3 times more likely to have diabetes compared to non-abdominally obese. These findings are consistent with previous studies indicating that increased body mass index and waist circumference are good predictors of diabetes risk [10, 29, 30].

Furthermore, our data confirm that both general and abdominal obesity are strong risk factors for diabetes [10]. It has been demonstrated that excess adiposity is closely associated with the inflammatory process characterized by increased circulating levels of pro-inflammatory cytokines that can progressively lead to various metabolic disorders, including insulin resistance [31]. Elevated blood glucose levels are also associated with metabolic syndrome components, including central obesity [6]. In addition, genetic polymorphisms, as well as genetic predisposition to obesity or excess body weight, are significant risk factors for diabetes [32]. Thus, public health interventions to promote the maintenance of healthy body weight, through regular physical activity and appropriate energy intake, may be effective in preventing or delaying the onset of diabetes.

The current study revealed that hypertensive and dyslipidemic subjects had a significantly higher likelihood of prediabetes and diabetes compared to healthy subjects. This is aligned with previous studies suggesting that increased blood glucose levels may be associated with hypertension and dyslipidemia [4–6]. Our findings highlight the importance of preventing and controlling hypertension and dyslipidemia as risk factors for diabetes, particularly among vulnerable groups such as obese and elderly persons.

Our results showed that former smoking was positively associated with diabetes risk, while current smoking was inversely associated with both prediabetes and diabetes risk. In contrast, epidemiological studies have demonstrated that cigarette smoking is one of the key risk factors implicated in the pathogenesis of type 2 diabetes mellitus [4]. Clinical data also suggest an effect of smoking and nicotine on body composition, insulin sensitivity, and pancreatic β cell function [33]. More studies are needed to delineate the basis of the observed associations between smoking and diabetes risk among our study sample. In addition, smoking cessation and weight control post-cessation should be promoted as essential public health interventions for diabetes prevention [33].

We found that individuals who reported that they drink or drank alcohol at some point in their lifetime had a slightly lower likelihood of prediabetes and diabetes. Although this finding is consistent with some previous studies suggesting that consumption of alcohol is associated with a reduced risk for diabetes [34], it should be interpreted with caution, because all of our study participants were Muslims who do not drink alcohol or might not report it reliably. Moreover, alcohol consumption remains one of the major risk factors for diabetes [4].

Strengths of the present study include the use of standardized methods of the WHO STEPS survey and the large sample size which allowed us to have reasonably accurate estimates of prediabetes and diabetes prevalence. However, this work has some limitations to be considered. First, the cross-sectional study design does not allow drawing causal relationships between obesity, hypertension, dyslipidemia, and sociodemographic factors and diabetes. Second, the proportion of men was lower than women among our study population. This could be due to a lower response rate among men than women. Third, the definition of prediabetes and diabetes was based on FPG levels and did not include HbA1c and a 2-h oral glucose tolerance test. Therefore, our blood glucose measurements could lead to either an underestimation or overestimation of the true prevalence of prediabetes

and diabetes. Finally, although the aforementioned diabetes risk factors pose a worldwide health problem, it should be acknowledged that not all of our findings can be extrapolated and used to describe the association between each one of these risk factors and diabetes within the specific context of other regions.

In conclusion, more than 30% of our study population had prediabetes or diabetes. Increased age (≥ 45 years), female gender, living in urban areas, low educational attainment, general and abdominal obesity, hypertension, and dyslipidemia were associated with a greater risk for prediabetes and diabetes. Our findings underscore the urgent need for diabetes awareness-raising interventions, effective education, and follow-up of patients diagnosed as diabetic or prediabetic to reduce morbidity and mortality from diabetes and related complications. Further studies are warranted to address other risk factors for diabetes such as unhealthy dietary patterns, lifestyle behaviors, consanguineous marriages, and family antecedents of diabetes.

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Author contribution All authors have contributed to the study design, data collection, and manuscript write-up. All authors read and approved the final version of the manuscript.

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Data availability Data and materials are available upon request to the corresponding author.

Declarations

Ethics approval The survey was conducted according to the ethical principles of the World Medical Association Declaration of Helsinki and was approved by the Biomedical Research Ethics Committee of the Faculty of Medicine and Pharmacy in Rabat, Morocco. All participants provided written informed consent before data collection.

Conflict of interest The authors declare no competing interests.

References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843.
2. Basu S, Yoffe P, Hills N, Lustig RH. The relationship of sugar to population-level diabetes prevalence: an econometric analysis of repeated cross-sectional data. PLoS ONE. 2013;8(2):e57873. https://doi.org/10.1371/journal.pone.0057873.
3. Weisman A, Fazli GS, Johns A, Booth GL. Evolving trends in the epidemiology, risk factors, and prevention of type 2 diabetes: a review. Can J Cardiol. 2018;34(5):552–64.
4. Kyrou I, Tsigos C, Mavrogianni C, Cardon G, Van Stappen V, Latomme J, et al. Sociodemographic and lifestyle-related risk factors for identifying vulnerable groups for type 2 diabetes: a narrative review with emphasis on data from Europe. BMC Endocr Disord. 2020;20(1):1–3.
5. The International Diabetes Federation. Diabetes complications. 2020: https://www.idf.org/aboutdiabetes/complications.html (Accessed: September 20, 2020).
6. International Diabetes Federation. IDF Diabetes Atlas, 9th edition. Brussels; 2019.
7. World Health Organization. Noncommunicable diseases (NCD) country profiles, 2018: https://www.who.int/nmh/publications/ncd-profiles-2018/en/ (Accessed: August 22, 2020).
8. World Bank. Perspective monde de la Banque Mondiale : Pyramide des âges au Maroc en 2018. http://perspective.usherbrooke.ca/bilan/servlet/BMPagePyramide?codePays=MAR&annee=2018 (Accessed 23 April 2020).
9. Riley L, Guthold R, Cowan M, Savin S, Bhatti L, Armstrong T, et al. The World Health Organization STEPwise approach to noncommunicable disease risk-factor surveillance: methods, challenges, and opportunities. Am J Public Health. 2016;106:74–78. https://ajph.aphapublications.org/doi/10.2105/AJPH.2015.302962. Accessed 20 Sept 2020.
10. World Health Organization. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight; 2018. (Accessed 12 September 2021).
11. Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
12. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension. 2003;42(6):1206–52.
13. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. 2006: https://apps.who.int/iris/bitstream/handle/10665/43588/9241594934_eng.pdf (Accessed 20 Aug 2020).
14. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2007;28(19):2375–414.
15. Gharbi MB, Elseviers M, Zamd M, Alaoui AB, Benahadi N, Trabelssi EH, et al. Chronic kidney disease, hypertension, diabetes, and obesity in the adult population of Morocco: how to avoid "over"-and "under"-diagnosis of CKD. Kidney Int. 2016;89(6):1363–71.
16. El Boukhrissi F, Bamou Y, Ouleghzal H, Safi S, Balouch L. Prévalence des facteurs de risque des maladies cardiovasculaires et du syndrome métabolique chez les femmes de la région de Meknès, Maroc. *Med. des Mal. Metab.* 2017;11(2):188-94.
17. Alkandari A, Longenecker JC, Barengo NC, Alkhatib A, Weiderpass E, Al-Wotayan R, et al. The prevalence of pre-diabetes and diabetes in the Kuwaiti adult population in 2014. Diabetes Res Clin Pract. 2018;144:213–23.
18. World Health Organization. Report on the status of major health risk factors for noncommunicable diseases. Geneva: WHO African Region, 2015 (2016). https://apps.who.int/iris/bitstream/handle/10665/253568/Contact?sequence=1. Accessed 15 Aug 2020.

19. Azizi F, Hadaegh F, Hosseinpanah F, Mirmiran P, Amouzegar A, Abdi H, et al. Metabolic health in the Middle East and north Africa. The lancet Diabetes endocrinol. 2019;7(11):866–79.
20. Gassasse Z, Smith D, Finer S, Gallo V. Association between urbanisation and type 2 diabetes: an ecological study. BMJ Glob Health. 2017;2:e000473. https://doi.org/10.1136/bmjgh-2017-000473.
21. Abuyassin B, Laher I. Diabetes epidemic sweeping the Arab world. World J Diabetes. 2016;7:165–74. https://doi.org/10.4239/wjd.v7.i8.165.
22. Badran M, Laher I. Type II diabetes mellitus in Arabic-speaking countries. Int J Endocrinol. 2012;2012:902873. https://doi.org/10.1155/2012/902873.
23. High Commission of Planning (HCP). Enquête Nationale Anthropométrique 2011. *Cahiers du Plan.* 2011;43(1):9–19. https://www.hcp.ma/Les-Cahiers-du-Plan-N-43-Mars-Avril-2013_a1248.html (Accessed 26 April 2020).
24. Cho N, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81. https://doi.org/10.1016/j.diabres.2018.02.023.
25. Orces CH, Lorenzo C. Prevalence of prediabetes and diabetes among older adults in Ecuador: analysis of the SABE survey. Diabetes Metab Syndr. 2018;12(2): 147–153. https://www.sciencedirect.com/science/article/abs/pii/S1871402117303776?via%3Dihub. Accessed 20 Sept 2020.
26. Pasala SK, Rao AA, Sridhar GR. Built environment and diabetes. Int J Diabetes Dev Ctries. 2010;30:63–68. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2878692/. Accessed 20 Sept 2020.
27. Azimi-Nezhad M, Ghayour-Mobarhan MP, Parizadeh MR, Safarian M, Esmaeili H, Parizadeh SM, et al. Prevalence of type 2 diabetes mellitus in Iran and its relationship with gender, urbanization, education, marital status and occupation. Singap Med J. 2008;49(7):571. http://www.smj.org.sg/sites/default/files/4907/4907a9.pdf. Accessed 20 Sept 2020.
28. Al-Moosa S, Allin S, Jemiai N, Al-Lawati J, Mossialos E. Diabetes and urbanization in the Omani population: an analysis of national survey data. Popul Health Metr. 2006;4(1):5. https://link.springer.com/article/10.1186/1478-7954-4-5. Accessed 20 Sept 2020.
29. Venkatrao M, Nagarathna R, Patil SS, Singh A, Rajesh SK, Nagendra H. A composite of BMI and waist circumference may be a better obesity metric in Indians with high risk for type 2 diabetes: an analysis of NMB-2017, a nationwide cross-sectional study. Diabetes Res Clin Pract. 2020;161:108037.
30. Luo J, Hodge A, Hendryx M, Byles JE. BMI trajectory and subsequent risk of type 2 diabetes among middle age women. Nutr Metab Cardiovasc Dis. 2020;31(4):1063–70.
31. Pirola L, Ferraz JC. Role of pro-and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity. World J Biol Chem. 2017;8(2):120.
32. Sarkar P, Chatterjee D, Bandyopadhyay AR. Association of CAPN10 (SNP-19) genetic polymorphism and obesity with T2DM: a study on Bengali Hindu caste population. Int. J. Diabetes Dev. Ctries. 2020:1–6.
33. Maddatu J, Anderson-Baucum E, Evans-Molina C. Smoking and the risk of type 2 diabetes. Transl Res. 2017;184:101–7.
34. Holst C, Becker U, Jørgensen ME, Grønbæk M, Tolstrup JS. Alcohol drinking patterns and risk of diabetes: a cohort study of 70,551 men and women from the general Danish population. Diabetologia. 2017;60(10):1941–50.

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COMMEMORATING GOLDEN JUBILEE OF RESEARCH SOCIETY FOR STUDY OF DIABETES IN INDIA (RSSDI)

Origin and concept

Dr. Vinod Kumar
Founder Secretary, RSSDI (July 1972-December 1980) and currently the RSSDI Patron

The Beginning

Research Society for Study of Diabetes in India (RSSDI) was founded by Prof. MMS Ahuja. He sought the cooperation of Dr. BB Tripathy and both requested Dr. OP Gupta of Ahmedabad and originally a distinguished alumni from Agra to be President of the Society. Accordingly, Dr. Vinod Kumar registered RSSDI on July 29th, 1972 and located the Society's office in the Department of Medicine at All India Institute of Medical Sciences (AIIMS) in New Delhi.

RSSDI was established with following aims and objectives:

- To encourage and support clinical and laboratory research in the field of diabetes.
- To hold workshops and scientific meetings within the country; to gather scientists involved in the field for discussions or demonstration of laboratory procedures and seminars
- To encourage collaborative studies at national level and help training workers in various research techniques in the field.

As per Records of Minutes, First RSSDI Executive Committee (EC) met on September 25, 1972 with OP Gupta in chair and attended by BB Tripathy, Vinod Kumar, M Bhaskar Rao, MMS Ahuja and GS Mutalik.

Pictures of Members who attended the First EC Meeting on September 25, 1972 and their affiliations are illustrated below.

MMS Ahuja, Dept. of Medicine
AIIMS, New Delhi
(RSSDI Founder 1972)

OP Gupta, Dept. of Medicine
BJ Medical College, Ahmedabad
(RSSDI President 1972 and 1973)

BB Tripathy, Dept. of Medicine
SCB Medical College, Cuttack
(RSSDI President 1974)

GS Mutalik, Dept. Of Medicine
BJ Medical College, Pune
(RSSDI EC Member 1972)

Vinod Kumar, Dept. of Medicine
AIIMS, New Delhi
(RSSDI Founder Secretary 1972-80)

MB Rao, Dept. of Medicine
MA Med College, New Delhi
(RSSDI Treasure 1972-77)

Executive and General Body Meetings (ECs & AGMs)

Ably steered by MMS Ahuja and various Presidents, Secretaries, Treasurers and Members of Executive Committees, 20 EC meetings and 9 AGMs were held during the decade. This journey witnessed many stalwarts of the day becoming the attendees at ECs/AGMs for the first time like OP Gupta and BB Tripathy at the 1st EC (25th Sept, 1972), SGP Moses and GS Sainani at the 2nd AGM (25th Jan, 1974), RS Hoon at the 5th EC (2nd Sept, 1975) and M Vishwanathan at the 12th EC (29th Oct, 1976) and all of these stalwarts later becoming the RSSDI Presidents. Successive RSSDI Secretaries during the 1st decade were Vinod Kumar (1972-1980) followed by B Krishna Ram from 1981 onwards and successive RSSDI Treasurers were M Bhaskar Rao (1972-1977) and B Krishna Ram (1978-1980) followed by MG Karmarkar from 1981 onwards.

Some of the deliberations of ECs and AGMs included:

1. Extensive discussion happened on a request received in 1973 from Diabetic Association of India (DAI), a well-established organization of the time with emphasis on public awareness of diabetes for joining hands with RSSDI, an organization which was recently established to foster research and training. Negotiations in this direction, however failed due to lack of consensus. There were two more such communications between DAI and RSSDI in 1976 and 1978 but each time efforts to forge a linkage failed again.
2. Other linkages discussed in RSSDI meetings pertained to establishing an Asian Pacific Association for the Study of Diabetes (APASOD) and participation of RSSDI which actually happened in conferences as a part of Federation of Societies of Endocrinology and Reproduction in October, 1976 at Delhi and in November, 1977 at Lucknow.
3. Other notable events were grant of 80 G tax relief under IT Act informed to EC in 1973, approval of RSSDI Insignia by EC in 1977, establishing 1st RSSDI Oration in 1978 (1st & 2nd Orations given by Moses & Kumar) and discussion on publishing a RSSDI Bulletin every 3 months. EC meetings with quorum continued to happen and it was only after

a decade in 1982 that the then Secretary of RSSDI raised the issue of starting adjourned EC meetings to tide over the problem of quorum, should it ever arise.

4. Several scientific initiatives in relation to diabetes came up for discussion in various RSSDI EC and AGM meetings from time to time. Some of these were Diet calendars, Health calendars, Diet exhibits, Diabetic recipes competition, Cyclamates, Yoga and diabetes, Postgraduate course in diabetes, Transparencies designing for post graduate lectures in diabetes etc. and Madras being established as first RSSDI Branch by Moses.
5. Some of the research proposals that were floated and discussed in EC meetings included Islet cell antibodies in juvenile diabetes, Pancreatic histology in diabetes, Food toxins affecting the pancreas, Home monitoring of blood glucose, Prospective study of therapy of maturity onset diabetes, Hypertension in diabetes, Microvascular disease of nephropathy, Genetic markers of diabetes, Cardiovascular morbidity in impaired glucose intolerance, Effect of nutrition on biochemical profile of diabetes etc.

Know your Founder and Presidents of RSSDI

Pictures of RSSDI Founder and Presidents of RSSDI during this time and glimpses of their distinguished professional career given below. **Few Presidents served for more than a year.**

RSSDI Founder, MMS Ahuja**,** (1929-1998), MBBS 1952 (Madras), Joined AIIMS in 1958, rose to Prof & Head of Medicine and then of Endocrinology and Dean and was Jawahar Lal Nehru Fellow in 1989. Founder Editor of International Journal of Diabetes in Developing Countries and chief Editor of Progress in Medicine Book Series. On the left is one of Dr. Ahuja's oldest pictures (seen on right in the photo) at Nalsarovar Lake near Ahmedabad (1967).

RSSDI President, OP Gupta (1929-2021), MBBS 1954 (Agra), Prof & Head of Medicine (1961-74), Dean (1971-74) at BJ Medical College, Ahmedabad, Director Medical Education (1974-80), Director Health Services (1981-88), Govt of Gujrat. Expertise in Diabetes Prevalence. Awardee of Govt Fellowship for training in London, WHO Fellowship to work in USA, BC Roy Awardee. Chairman of ICMR Expert Committee in Endocrinology.

RSSDI President, BB Tripathy (1923-2010), MBBS (Patna), Became Professor of Medicine in 1964 and then Head of Medicine and Medical Superintendent at SCB Medical College, Cuttack. Well known expert in diabetes epidemiology and recognised the association of diabetes with malnutrition in India, the J type of diabetes later rechristened as Protein Deficient Diabetes Mellitus (PDDM). He was Editor in Chief of RSSDI Textbook of Diabetes Mellitus-1st & 2nd Editions.

RSSDI President, Sam GP Moses born in 1927, obtained his MBBS in 1949 and was the Honorary Physician in Medicine at Madras Medical College for more than 30 years till his retirement in 1983. He was the youngest person to be promoted as Professor of Medicine at age of 30 years and was the first Honorary Professor of Diabetology at this college. He had started the 1st Diabetic Clinic at Madras Medical College in 1953. Prof. Moses was awarded the Lifetime Achievement Award in Endocrinology from Harvard University in USA.

RSSDI President, Lt. Gen. RS Hoon, PVSM, born in 1917, was Brigadier Professor and Head of Medicine Department at AFMC, Pune in 1960s and rose to become Lt General in the Indian Army and then became Director General, Armed Forces Medical Services (DGAFMS) in 1976. Served in the Middle East, Palestine, Korea & headed a medical team to investigate jaundice epidemic in Congo. Was a Dr. BC Roy National Awardee from MCI in recognition of being good and capable teacher in Medicine.

RSSDI President, M Vishwanathan completed his medical degree from Stanley Medical College, Madras in 1946. He established the first Centre for Diabetes in 1954, MV Hospital for Diabetes in 1971 and a Diabetes Research Centre in 1972. Diabetes Research Centre was the first Institute to award Diploma in Diabetology and got recognition from ICMR and DST. MV Hospital for Diabetes was the first of its kind as an institution solely devoted to the care of Diabetes.

RSSDI President, GS Sainani, born in 1931, graduate from Medical College, Nagpur, Prof & HOD there at age 32. He is Emeritus Director at Jaslok Hospital, Mumbai, Emeritus Professor of National Academy of Medical Sciences, Co-ordinator for Global Research in Geriatrics, formerly Chairman of International Congress in Geriatrics & Director of All India Heart Foundation. Awardee of 8 Lifetime Achievements, he is the Hon. Brigadier, Past Presidents of many scientific organizations including Association of Physicians of India and has served as Hon. Physician to President of India.

Financial Matters

Compared to modern India of today, period of 1970s had limited resources. Instead of emails and social media, communications depended on time consuming postal correspondence, in the absence of photocopiers, distributions of notices and records depended on cyclostyling and instead of computers and word processors, typing depended on skilled shorthand stenos and typists. Office bearers travelling on scooters & trains was common.

Membership fee for life, ordinary and associate member was decided right in the beginning as Rs. 100/-, 50/- and 10/- respectively and it remained the same during the whole first decade. Even at the end of this decade, any ordinary member could become life member by paying the same amount of Rs. 100/-. Further, any life member of Endocrine Society of India could become life member of RSSDI by paying just the half amount (Rs. 50/- only/-).

RSSDI Scientific Conferences with Administrative/Organizing Support from Secretariat at AIIMS (1976* and 1977* meetings were held as a part of Federation of Endocrine Societies)

YEAR	DATES	THEME SUBJECT	VENUE	PUBLICATION	EDITOR
1972	December 19-23	Etiogenesis of Diabetes and Panel discussion on biological characterization of insulin	AIIMS, Delhi	Abstracts Book (Only 30 delegate/22 abstracts)	
1974	January 24-25	Disorders of Carbohydrate Metabolism	MAMC, Delhi	Monograph	Vinod Kumar
1975	January 17-19	Complications of Diabetes	MC, Madras	Abstracts Book	
1976	January 21	Chemical Pathology of Diabetes and Workshop on laboratory techniques	AIIMS, Delhi	Abstracts Book	
1976*	October 30	Challenges of Therapy of Diabetes in Developing Countries	AIIMS, Delhi	Collection of cyclostyled articles	DK Hazra
1977*	November	Diabetes Mellitus	Lucknow	Monograph	MB Rao
1978	January 19	Health Care Delivery of Diabetes	Bhopal		
1979	January 17	Diabetes in Young	MC, Madras	Abstracts Book	
1980	January	Diabetes Mellitus	MC, Trivandrum		
1981	January 18	Impaired Glucose Tolerance	Nagpur		

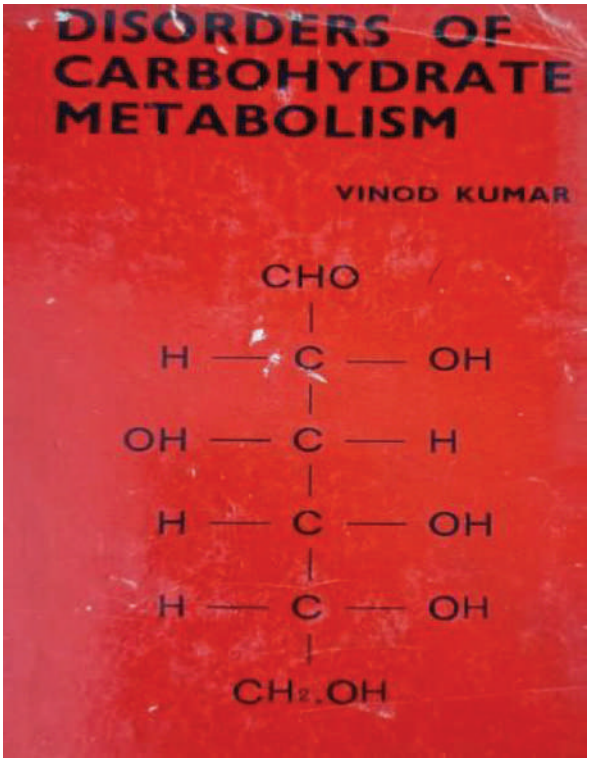
RSSDI Monographs (i) Disorders of Carbohydrate Metabolism (ii) Diabetes Mellitus

1. Disorders of Carbohydrate Metabolism: A multi author book of 161 pages edited by Vinod Kumar and published in 1974 by Research Society for Study of Diabetes in India in association with M/S Arnold Heinemann Publishers (India) Private (Ltd.), New Delhi who had Associates in UK. It has 14 Chapters including some from eminent foreign authors of Sweden, Germany and England. This book was reviewed in International Diabetes Federation Bulletin (1976; XXI January (1): 28) by DR. Max Ellenberg, the then Immediate Past President of American Diabetes Association and the author of a 1962 Diabetes Book which was jointly written with Harold Rifkin.
2. Diabetes Mellitus: A multi author book of 320 pages edited by M Bhaskar Rao and published in 1977 by Research Society for Study of Diabetes in India in association with M/S Arnold Heinemann Publishers (India) Private (Ltd.), New Delhi who had Associates in UK. It has 25 Chapters by Indian authors on diverse areas such as epidemiology, aetiology, biochemistry, clinical and therapeutic aspects of diabetes and about diabetes in special situations.

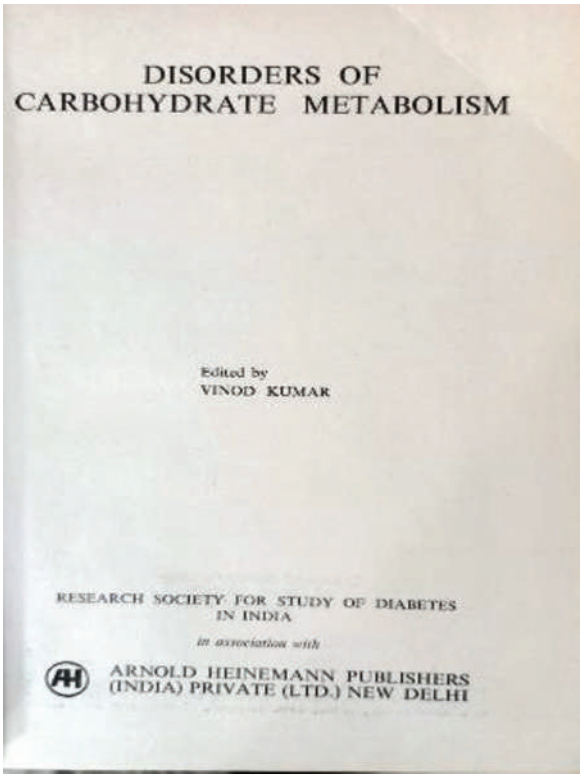
Resources

1. Minutes of various Executive and General Body meetings of RSSDI from 1972 onwards which were digitalised during later years of RSSDI by PV Rao.
2. RSSDI Secretary's Annual Reports during the initial 10 years of RSSDI.
3. Partial details about RSSDI income and expenditure available for the initial period.
4. Presentations made during the Session on Concept and Founding of RSSDI-Down the Memory Lane at the 37th Annual RSSDI Conference, Ahmedabad, 2009 by Vinod Kumar, OP Gupta and GS Mutalik.
5. Presentations made during the Session on Meet the Living Legends who founded RSSDI at the 42nd Annual RSSDI Conference, Bengaluru, 2014 by Vinod Kumar, OP Gupta and PV Rao.
6. Diabetes Bytes: The Insulin Story: Commemorating 100 years since insulin discovery. April 15 2021.
7. History of Diabetes in India. Presentation made at the Hybrid 49th Annual RSSDI Conference, Ahmedabad, 2021 by Vinod Kumar.
8. RSSDI Monograph on Disorders of Carbohydrate Metabolism. Ed. Vinod Kumar (Cover page and the main inside page-Appended below).
9. RSSDI Monograph on Diabetes Mellitus. Ed. M Bhaskar Rao. (Cover page and the main inside page-Appended below).

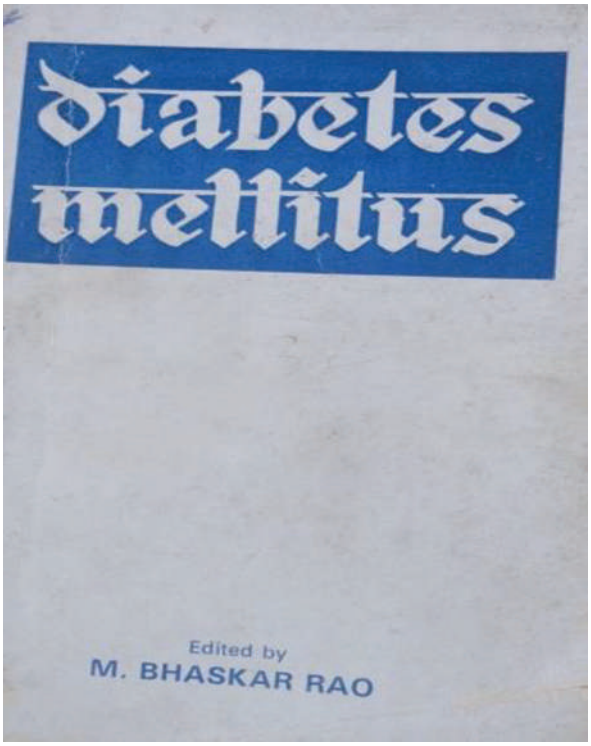
Acknowledgement to Lt. Gen. SP Kalra (Retd.) for his unceasing efforts in fetching the photograph of Lt. Gen. RS Hoon, the RSSDI President for 1976.



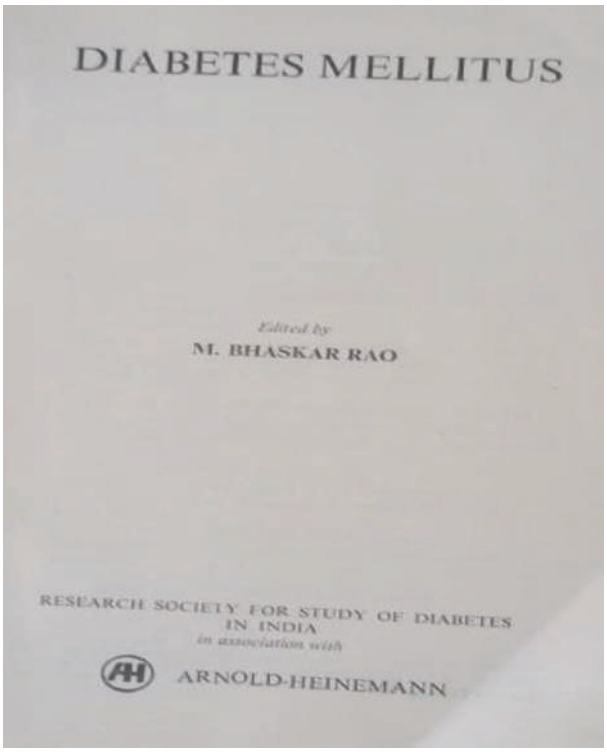
RSSDI BOOK 1 COVER PAGE



RSSDI BOOK 1 INSIDE PAGE



RSSDI BOOK 2 COVER PAGE



RSSDI BOOK 2 INSIDE PAGE

VISION STATEMENT

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

MISSION STATEMENT

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

NEW EXECUTIVE COMMITTEE AND OFFICE BEARERS 2021-2022

Patrons of RSSDI

Dr. H.B. Chandalia, Mumbai
Dr. C. Munichhoodappa, Bengaluru
Dr. Ashok K. Das, Puducherry
Dr. Binode K. Sahay, Hyderabad
Dr. V. Seshiah, Chennai
Dr. P.V Rao, Hyderabad
Dr. Jitendra Singh, New Delhi
Dr. V Mohan, Chennai
Dr. Vinod Kumar, New Delhi

President

Dr. Ch.Vasanth Kumar, Hyderabad

President Elect

Dr. Brij Makkar, New Delhi

Immediate Past President

Dr. Banshi Saboo, Ahmedabad

Secretary

Dr. Sanjay Agarwal, Pune

Vice-President

Dr. Anuj Maheshwari, Lucknow

Vice-President

Dr. Vijay Viswanathan, Chennai

Joint Secretary

Dr. Sujoy Ghosh, Kolkata

Treasurer

Dr. Sunil Gupta, Nagpur

Executive Committee

Dr. C R Anand Moses, Chennai
Dr. Sudhir Bhandari, Jaipur
Dr. J K Sharma, New Delhi
Dr. Bikash Bhattacharjee, Guwahati
Dr. Pratap Jethwani, Rajkot
Dr. L. Sreenivasa Murthy, Bengaluru
Dr. Sanjay Reddy, Bengaluru
Dr. Shalini Jaggi, New Delhi

Co-opted

Dr. Vijay Panikar, Mumbai
Dr. Rakesh Sahay, Hyderabad
Dr. Amit Gupta, Noida

TRAINEE GRANTS (Up to 10 grants)

Research Grants upto INR 200000 to support outstanding thesis/ research work by first year MD/DNB/ PHD students/Research fellows from India.

Eligibility Criteria
All Postgraduates in First year MD, DM /DNB from any of the institutions in the country are eligible to apply

How to apply?
Send in your Research proposals by email to the RSSDI Secy/ Chairman research committee by email/ apply directly on web site.

Research proposal should have following proofs-

1. A supporting letter from your guide/ head of department stating that this is a bonafide project for your thesis and also mentioning the dates of you joining the program and expected date of graduation. The guide must also state that he/she will stand guarantee for the work done
2. A detailed budget
3. Thesis proposal approved by the department/appropriate institutional authority
4. Approval by the ethics committee

Selection Process
Proposals will be reviewed by the research committee of the RSSDI.

Disbursement of Grant
A minimum of 50% of the grant amount will be disbursed initially. Further disbursement will be done annually based on submission of progress reports on the work done and utilisation of sanctioned amount. These reports must be filed to the secretary of the RSSDI

Responsibility:
All grant awardees are expected to present their work at RSSDI Annual Conference during research presentation's session. Failure to file progress reports annually and when requested by the RSSDI and failure to present progress at RSSDI Annual conf may result in the forfeiture of the grant.

All awardees are expected to follow the tenets of responsible and ethical conduct of research. Unethical or fraudulent use of RSSDI research funds will warrant adverse action from the society including

forfeiture of grant, black listing in the society's databases and other legal recourses that are available to the society.

Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

All awardees are encouraged to submit their work to the RSDDI Journal IJDDC

CALL for RESEARCH PROPOSALS for GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI

The proposals may of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization, of funding sought from RSSDI

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
All applications should be addressed to:
1. The Secretary , RSSDI
2. Soft copy of the research proposal should be sent to Secretary, RSSDI

When to apply
Proposals will be accepted Twice a year. Once between 1st Jan - 31st April & then July 1st to 30th Nov.

All research proposals will be reviewed by Research committee over a period of 4-6 weeks & approved proposals will be provided Research Grant after fulfilling all documentation by 30th June & then 31st December of each year.

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health-care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmacahri Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh
22.	Galaxy Speciality Centre	Sodala, Jaipur

carefully looked into all aspects of this course & has accredited & recognized 22 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

COURSE DETAILS

Name of the Course: Advanced Certificate Course in Diabetology

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

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

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

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

COURSE FEES:

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

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

ANNOUNCEMENTS

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

RSSDI 50th Golden Jubilee Year Celebrations
(look out for more details on our website)

RSSDI JNU certificate course in Diabetes:

Last date of submission of Application Form - 31st December, 2022
Screening Interview - 15th January 2023
Declaration of Exam Result - 22nd January 2023
Last date of payment of course fee - 31st January 2023
Commencement of course - 1st February 2023
Prospectus release date - 15th Nov 2022

Registered with Registrar of News Papers, India vide no. RNI-43019/85

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