VOLUME 43 | ISSUE 2
MARCH - APRIL 2023

ISSN 0973-3930

INTERNATIONAL JOURNAL OF DIABETES IN DEVELOPING COUNTRIES

OFFICIAL PUBLICATION OF RESEARCH SOCIETY FOR THE STUDY OF DIABETES IN INDIA

For Circulation in India only

Springer

International Journal of Diabetes in Developing Countries
Incorporating Diabetes Bulletin

Aims and Scope

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

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

Copyright Information

Subscription Information

International Journal of Diabetes in Developing Countries is published 6 times a year. Volume 43 (6 issues) of will be published in 2023.

ISSN 0973-3930 print version
ISSN 1998-3832 electronic version

For information on subscription rates please contact Springer Nature Customer Service Center: customerservice@springernature.com

The Americas (North, South, Central America and the Caribbean)
Springer Journal Fulfillment, 233 Spring Street, New York, NY, 10013-1578, USA
Tel. 800-SPRINGER (777-4643); 212-460-1500 (outside North America)

Outside the Americas
Springer Nature Customer Service Center GmbH
Tiergartenstr. 15, 69121 Heidelberg, Germany
Tel.: +49-6221-345-4303

Advertisements

E-mail contact: advertising@springer.com or anzeigen@springer.com (Germany)

Disclaimer

Springer Nature publishes advertisements in this journal in reliance upon the responsibility of the advertiser to comply with all legal requirements relating to the marketing and sale of products or services advertised. Springer Nature and the editors are not responsible for claims made in the advertisements published in the journal. The appearance of advertisements in Springer Nature publications does not constitute endorsement, implied or intended, of the product advertised or the claims made for it by the advertiser.

Journal Website

www.springer.com/13410
Electronic edition: link.springer.com/journal/13410

For the actual version of record please always check the online version of the publication.

Office of Publication

Springer Science+Business Media B.V., Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands

International Journal of Diabetes in Developing Countries

Volume 43 · Number 2 · March – April 2023

Further articles can be found at www.springerlink.com

Abstracted/Indexed in *Baidu, CAB Abstracts, CLOCKSS, CNKI, CNPIEC, Chemical Abstracts Service (CAS), Dimensions, EBSCO Academic Search, EBSCO CINAHL, EBSCO Discovery Service, EBSCO STM Source, EMBASE, EMCare, Google Scholar, IFIS Publishing, Japanese Science and Technology Agency (JST), Journal Citation Reports/Science Edition, Naver, OCLC WorldCat, Discovery Service, Portico, ProQuest-ExLibris Primo, ProQuest-ExLibris Summon, SCImago, SCOPUS, Science Citation Index Expanded (SCIE), Semantic Scholar, TD Net Discovery Service, UGC-CARE List (India), Wanfang*

Instructions for Authors for *Int J Diabetes Dev Ctries* are available at www.springer.com/13410.

Compliance with Ethical Requirements

International journal of Diabetes in Developing Countries requests that all authors comply with Springer's ethical policies. To view our ethics statements please visit the following:

· Conflict of Interest and Ethical Standards: http://www.springer.com/authors?SGWID=0-111-6-791531-0

· Informed Consent: http://www.springer.com/authors?SGWID=0-111-6-608209-0

Statement of Human and Animal Rights: http://www.springer.com/authors?SGWID=0-111-6-608309-0

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):171–172
https://doi.org/10.1007/s13410-023-01193-5

EDITORIAL

Hyperinsulenemia, Obesity, and T2 Diabetes: A continuum

Rajeev Chawla[1]

Published online: 6 April 2023

Insulin, an anabolic peptide hormone, is known to have a key role in carbohydrate, lipid, and fat metabolism. It has effects on almost every organ in the body, including adipose tissue, liver, muscle, brain, bone [1], kidneys [2], and vasculature [3]. A variety of mechanisms regulate insulin concentrations by affecting insulin clearance and secretion, through coordinated signals from the hypothalamic–pituitary–adrenal (HPA) axis, the liver–pancreas axis, the entero–osseous axis, and the bone–pancreas axis [4]. Hyperinsulinemia refers to chronically elevated insulin concentration without hypoglycemia, seen commonly in obese individuals as well as those with related metabolic disorders, due to dysregulation of insulin secretion and/or insulin clearance. A rise in fasting insulin levels is seen as an individual progresses from normal glucose tolerance to impaired glucose tolerance (IGT) to T2DM [5]. Obese subjects without diabetes or hypertension show an increased prevalence of hyperinsulinemia and insulin hypersecretion than insulin resistance [6] and hence in such subjects' hyperinsulinemia may rather precede and contribute to insulin resistance. Furthermore, cohort studies have demonstrated that individuals having similar degrees of insulin sensitivity may exhibit a wide range of insulin secretion.

The euglycemic glucose clamp (EGC) and oral glucose tolerance test (OGTT), both are important tools for the assessment of insulin clearance. Hyperinsulinemia is associated with both obesity and insulin resistance [7] and can result from increased insulin secretory capacity and/or reduced insulin clearance. Hyperinsulinemia, especially the insulin levels at 30 min during the OGTT, has demonstrated a causal relationship with obesity.

Prakash SS in his Letter to Editor "Hyperinsulinemia, obesity, and diabetes mellitus" published in this issue explored the difference in insulin resistance in obese and lean individuals. He diligently eluded that obese individuals demonstrate insulin resistance in the adipose tissue resulting in increased lipolysis and lipotoxicity in other tissues including muscle and liver [8, 9]. On the other hand, the progression of disease in the lean individuals could be driven by insulin resistance in the liver leading to inefficient insulin clearance and muscle insulin resistance. The difference between insulin resistance in the liver/muscles and that in adipose tissue could be related to the increased "capacity" of liver and muscle tissues to handle glucose and the increased "capability" of adipose tissue to efficiently store glucose as fat in the context of the whole-body glucose metabolism [9]. Based on these observations, it can be concluded that hyperinsulinemia in obese individuals could be due to increased insulin secretion to compensate for the adipose tissue insulin resistance, ultimately leading to β-cell failure and reduced insulin secretion, whereas in non-obese lean individuals, hyperinsulinemia could be due to reduced insulin clearance secondary to insulin resistance in the liver [8, 10–12].

Hence, the author summarises that in obese T2DM individuals, the main pathogenic mechanism could be the decrease in insulin secretory capacity, while in non-obese individuals, a reduction in insulin clearance leading to hyperinsulinemia is likely to play a major role in the pathogenesis of T2DM. This hypothesis that the onset of adipose insulin resistance in obese individuals and hepatic insulin resistance in non-obese individuals could be the critical events in the progression of NGT and IGT to T2DM lays grounds for future studies exploring these effects in both obese as well as non-obese subjects with or without diabetes.

The study by Vinay Kumar and Sudha Vidyasagar "Association of serum osteocalcin with beta cell function, insulin resistance and glycemic parameters in south Indian type 2 diabetic subjects" published in this issue has explored osteocalcin (OC), also known as bone Gla (gamma-carboxyglutamic acid) protein, which is a marker of bone formation, also currently purported to have a role in glucose and fat metabolism. This study looks at an association of this osteokine with parameters of glucose metabolism in Indian subjects, suggesting a possible role of OC in the pathogenesis of diabetes.

✉ Rajeev Chawla
rajeevaastikchawla@yahoo.com

[1] North Delhi Diabetes Centre, Delhi, India

Reduction in serum OC levels was demonstrated with an increase in insulin resistance (HOMA-IR); however, beta cell function did not show any relationship with OC levels. OC levels further showed a statistically insignificant negative association with FPG levels. Glycated haemoglobin (HbA1c) levels were shown to be significantly reduced in diabetic patients with higher serum OC levels. This study concluded that serum OC was significantly associated with insulin resistance and HbA1c in subjects with T2DM, suggesting a possible role of serum OC in glucose metabolism in T2DM. This study adds another perspective to our understanding of the complex mechanisms involving insulin resistance and opens vistas for future studies looking at the link between bone markers and glucose homeostasis.

The study by Sevil Karahan Yılmaz et al. "Comparison of Inflammation-Related Hematologic Indices For Predicting Metabolic Syndrome in Adults" published in this issue evaluated different hematologic indices (neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio (LMR), lymphocyte/high-density lipoprotein to cholesterol ratio (LHR), neutrophil/high-density lipoprotein to cholesterol ratio (NHR), and monocyte/high-density lipoprotein to cholesterol ratio (MHR) associated with inflammation in predicting metabolic syndrome in adults. This study concluded that the NHR index is a strong predictor of metabolic syndrome. In men, the NHR index is a better predictor of metabolic syndrome than the LHR index, and in women, the NHR index is better than both LHR and LMR.

Hyperinsulinemia is commonly associated with obesity, metabolic syndrome, and the early stage of T2DM. There is a need for larger long-term studies to further test the role of hyperinsulinemia as a key driving force in these conditions and to determine its net contribution, which is most likely to be context-dependent. The traditional paradigm, where hyperinsulinemia was considered to be an adaptation to obesity-induced insulin resistance seems to be changing with mounting evidence showing that hyperinsulinemia can precede and eventually cause obesity and insulin resistance.

Summing up, while insulin is an anabolic peptide hormone essential for maintaining normal life and metabolism, the negative consequences of hyperinsulinemia in causation as well as contribution to conditions such as obesity and T2DM shed light on the importance of keeping insulin levels within a healthy target range. Lifestyle interventions or therapeutics with mild insulin-suppressing actions can provide new opportunities to prevent and treat certain disorders like obesity, chronic inflammation, and cancers that have been found to be associated with hyperinsulinemia.

References

1. Shanbhogue VV, Finkelstein JS, Bouxsein ML, Yu EW. Association between insulin resistance and bone structure in nondiabetic postmenopausal women. J Clin Endocrinol Metab. 2016;101(8):3114–22.
2. Brands MW, Manhiani MM. Sodium-retaining effect of insulin in diabetes. Am J Physiol Regul Integr Comp Physiol. 2012;303(11):R1101–9.
3. Guo J, Breen DM, Pereira TJ, Dalvi PS, Zhang H, Mori Y, Ghanim H, Tumiati L, Fantus IG, Bendeck MP, Dandona P, Rao V, Dolinsky VW, Heximer SP, Giacca A. The effect of insulin to decrease neointimal growth after arterial injury is endothelial nitric oxide synthase dependent. Atherosclerosis. 2015;241(1):111–20.
4. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, Faugere MC, Aja S, Hussain MA. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010;142(2):309–19.
5. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. b-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab. 2005;90(1):493–500.
6. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G; European group for the study of insulin resistance (EGIR). Insulin resistance and hypersecretion in obesity. J Clin Invest. 1997; 100(5):1166–1173.
7. Prakash SS. Models that explain the cause of obesity. Indian J Endocrinol Metab. 2018;22:569–70.
8. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med. 2016;280:465–75.
9. Fazakerley DJ, Krycer JR, Kearney AL, Hocking SL, James DE. Muscle and adipose tissue insulin resistance: malady without mechanism? J Lipid Res. 2019;60:1720–32.
10. Gastaldelli A, Ghani MA, DeFronzo RA. Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. Diabetes. 2021;70:377–85.
11. Bojsen-Møller KN, Lundsgaard AM, Madsbad S, Kiens B, Holst JJ. Hepatic insulin clearance in regulation of systemic insulin concentrations—role of carbohydrate and energy availability. Diabetes. 2018;67:2129–36.
12. Gastaldelli A, Gaggini M, DeFronzo RA. Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the San Antonio Metabolism Study. Diabetes. 2017;66:815–22.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):173–183
https://doi.org/10.1007/s13410-022-01133-9

REVIEW ARTICLE

Prevalence of diabetes mellitus in Indian tribal population: a systematic review and meta-analysis

Saravanan Chinnaiyan[1] · Bharathi Palanisamy[1] · Lavanya Ayyasamy[2]

Received: 9 December 2021 / Accepted: 20 September 2022 / Published online: 3 October 2022

Abstract

Background Non-communicable diseases are the most serious public health threat of the twenty-first century. Diabetes is becoming a major public health issue worldwide and is associated with a slew of potentially fatal comorbidities.

Aim Based on the available literature, our systematic review and meta-analysis aimed to understand the prevalence of diabetes mellitus in the Indian tribal community.

Materials and methods Following PRISMA guidelines, studies reporting the prevalence of diabetes mellitus among tribes in India were retrieved by independent investigators who electronically conducted a literature search by exploiting searches conducted in PubMed, Science Direct, and Google Scholar. We estimated pooled prevalence with 95% confidence intervals using the R Studio statistical tool in a random-effects model. This review includes twenty-three studies from 2000 to 2020 conducted in all five regions of India.

Results The pooled prevalence rate across the twenty-three studies of 35,985 participants was 6% (95% confidence intervals [CI] = 5–7%). High heterogeneity was observed in prevalence estimates. The prevalence of diabetes mellitus in different subgroups ranged from 4% (95% CI = 0.00, 0.05%) to 10% (95% CI = 0.00, 0.19) (North, 5%; South, 5%; East, 10%; West, 4%; and Central, 7%).

Conclusion As a result, concentrated efforts aimed at filling awareness gaps as well as operational research or other data gaps would aid in the prevention and control of diabetes, as well as filling these gaps in tribal areas.

Keywords Diabetes mellitus · Prevalence · Tribal population · Non-communicable diseases · Systemic review

Introduction

A tribe is an endogamous social group with no functional specializations, ruled by hereditary or non-hereditary tribal officers, united in language or dialect, and assuming social distance and association with other tribes or clans but without any accompanying discrimination in the case of caste structures, adherence to traditions, beliefs, and customs, and anti-naturalization ideas from external sources. Tribal communities account for nearly 8.6% of the Indian population [1]. As a result, they make up a significant portion of our population. However, despite its large number, it has been largely ignored by policymakers. Modernization affected each group, increasing conflicts with sedentary villagers, who no longer seemed to have a symbiotic relationship.

The elderly of the tribes lived in their traditional way of life and living. Scheduled tribes have long relied on traditional methods of food preparation. Personal health care was primarily based on local and traditional healing patterns. Access to their health care facilities was poor due to issues of distance, language, and discrimination [2]. But due to globalization, people have been upgraded to their lifestyle by adapting semi-urban and semi-rural, leading to several lifestyle diseases.

Furthermore, the value of their occupation had decreased as a result of changing times. Tribal people were increasingly looking for work in labour markets or on construction

✉ Saravanan Chinnaiyan
drsharav13@gmail.com

Bharathi Palanisamy
bharathi.vmp@gmail.com

Lavanya Ayyasamy
lavanyanandhuam@gmail.com

[1] SRM School of Public Health, SRM Institute of Science and Technology, Chengalpattu District, Kattankulathur, Tamil Nadu, India

[2] ICMR-National Institute of Epidemiology, Chennai, India

sites [3]. Their lifestyle and dietary habits have changed as a result of modernization. Those populations have also seen their livelihoods and lifestyles adjusted as they have no longer previous employment and lifestyle.

Diabetes is a dangerous and prolonged disease that affects the lives and well-being of individuals, families, and societies worldwide. It is one of the top ten causes of death in adults, accounting for four million deaths globally in 2017. The global health costs associated with diabetes are estimated at $727 billion in 2017 [4]. Diabetes mellitus is one of the leading causes of heart attack, blindness, lower limb amputation, kidney failure, stroke, and other CVD diseases. The WHO also reports that diabetes directly causes 1.6 million deaths, almost half of all deaths from hyperglycemia before the age of seventy [5].

According to the World Health Organization, diabetes is increasing at a rate of approximately 6% per year, and it is predicted that by 2030, there will be 9.4 million people with diabetes in the world. Diabetes is the biggest problem in India and the world, with a 35% increase in mortality, according to a survey of the industrial population in India [6]. The prevalence of diabetes is already increasing at both rural and urban levels [7, 8].

Recent epidemiological evidence shows a similar or even greater vulnerability of persons of the lower socio-economic groups in India to diabetes. [9–11]. Family history is a strong risk factor for developing diabetes mellitus; for people who have an affected parent, the lifetime risk is about 40% and is about 70% when both parents are affected [12].

The overall prevalence of diabetes mellitus in India is estimated to be around 7.3%, with higher rates in cities (11.2%) and rural regions (5.2%) [13]. Numerous studies show that diabetes is becoming a major health issue in India. Diabetes is becoming more prevalent in both rural and tribal areas. Type 2 diabetes management in India is a unique and complex health concern, where it is more expensive than industrialized countries but might be concealed by weak reporting and significantly fewer diabetic registers. Clinical lethargy, poor adherence to medicinal products, and inadequate awareness of disease are critical hurdles for reaching glycemic objectives, especially in the real clinical world [8]. This systematic review and meta-analysis estimate the overall prevalence of diabetes among Indian tribes.

Methods

Our systematic review and meta-analysis followed the "Preferred Reporting Items for Systematic Review and Meta-Analysis" (PRISMA) guidelines. A systematic literature search was carried in three scientific databases, suitable studies were extracted, and the methodology was evaluated using the Newcastle–Ottawa scale (NOS) checklist. A meta-analysis was used to compile the findings. The protocol has been registered with the PROSPERO (registration number CRD42021257645) international prospective registry of systematic reviews.

Search strategy

Using electronic methods, two authors (SC and LA) independently performed systematic searches. Electronic searches were conducted using PUBMED, Scopus, and Google Scholar. We used search strategies such as Medical Subject Headings (MeSH) and keywords such as diabetes, insulin dependence, impaired fasting glucose, impaired glucose tolerance, insulin dependence, metabolic syndrome, diabetes complications, prevalence, population studies, study-level community, tribe, and ethnicity group, Indigenous Peoples, India, with English as a language restriction.

Inclusion and exclusion of studies

The study was included only to account for the prevalence of diabetes among tribes in India. Only studies with participants whose blood sugar was measured were included. The self-reporting of participants has been excluded. Studies published in English were selected. Studies published between January 2000 and December 2020 were considered. It includes cross-sectional studies, prospective or retrospective studies, case–control studies, and clinical studies, with no additional age limits for tribal populations was added. We screened the titles and abstracts to find papers that were relevant. After this screening process, we looked over the abstracts of the papers. The results of twenty-three of them are summarized in Table 1. The study excluded review articles, conference abstracts, and case studies.

Study screening and study selection

During the first stage, the titles and abstracts of all articles discovered were individually reviewed by two authors (SC and LA) in accordance with the inclusion criteria. Based on the inclusion criteria, the full texts of eligible studies were reviewed further. For this course, relevant studies were chosen.

Quality assessment

The Newcastle–Ottawa scale was used to assess the methodological quality of observational studies (NOS). To assess the risk of bias, two authors assessed independently. Three characteristics were investigated using NOS checklists (SC and LA) (i.e. selection, comparability, and outcome). There are two versions of the checklist for evaluating cross-sectional studies (eight components) and case–control studies

Table 1 Characteristics of the studies included

References	Year	State	Region	Study design	Inclusion criteria	Exclusion criteria	Name of tribes	Study period	NOS score
R P Agrawal et al. [14]	2004	Rajasthan	West	Cross-sectional design	Aged 20 years and above	NA	Raica	September to December 2002	6
Sobhanjan Sarkar et al. [15]	2006	Sikkim	East	Cross-sectional design	Aged 12 years and above	NA	(I) Toto (ii) Bhutia	October 2002 to March 2003	4
Lau et al. [16]	2009	Tripura	East	Cross-sectional design	Above 25 of age	NA	NA	NA	4
Satwanti Kapoor et al. [17]	2010	Madhya Pradesh	Central	Cross-sectional design	Age group 18–60	NA	Saharia	NA	3
Bandana Sachdev [18]	2011	Rajasthan	West	Cross-sectional design	Above 18 of age	(i) Except pregnant women (ii) Seriously ill respondents (iii) Those who using medication or drugs like oral contraceptive pill (OCP), corticosteroids etc	NA	September to November 2009	3
Bandana Sachdev [19]	2012	Rajasthan	West	Cross-sectional design	Men and women ≥ 18 years of age	(i) Pregnant women (ii) Seriously ill patients (iii) Herbal medication (iv) Corticosteroids and oral contraceptive pills	(i) Natt tribe (39.2%) ($N=418$) (ii) Banjara tribe (33.3%) ($N=435$) (iii) Sapara tribe (11.8%) ($N=150$) (iv) Bawariya ($N=55$) (v) Sansui ($N=72$) (vi) Bhopa ($N=120$) (vii) Gujjar ($N=46$)	NA	4
Ashita Singh et al. [20]	2012	Arunachal Pradesh	East	Cross-sectional Study	NA	NA	NA	NA	5
S. Achanta et al. [21]	2013	Andhra Pradesh	South	Cross-sectional design	(i) All patients with TB registered in the tertiary care unit at the ten peripheral health institutions (PHIs) (ii) Aged > 30 years	NA	NA	January to September 2012	3
Mungreiphy and Satwanti Kapoor [22]	2014	Manipur	North	Cross-sectional design	NA	NA	Tangkhul Nagas	NA	3

Table 1 (continued)

References	Year	State	Region	Study design	Inclusion criteria	Exclusion criteria	Name of tribes	Study period	NOS score
Kapoor et al. [23]	2014	Himachal Pradesh	North	Cross-sectional design	NA	NA	Gaddi	2008–2010	6
Nikkin T and Meriton Stanly [24]	2015	Tamil Nadu	South	Cross-sectional design	(i) Individuals aged 25 to 65 (ii) Participants residing in the area at least for 1 year only were included	(i) Pregnant women are omitted (ii) Patients with severe illness	NA	December 2014 to 08th January 2015	3
Basavanagowdappa Hathur et al. [25]	2015	Karnataka	South	Cross-sectional design	NA	NA	Jenu Kuruba	NA	5
Shankar Radhakrishnan and Manivanan Ekambaram [26]	2015	Tamil Nadu	South	Cross-sectional design	Age group above 40	NA	NA	NA	5
Pangi Vijaya Nirmala et al. [27]	2016	Andhra Pradesh	South	Cross-sectional design	All men and women aged 15–68	NA	NA	NA	5
Magna Manjareeka et al. [28]	2016	Odisha	Central	Cross-sectional design	(i) Adults who are diagnosed for the first time with pulmonary TB (ii) No past event of smoking and alcohol disorders	(i) With sickle cell diseases (ii) With thalassaemia (iii)Smokers and alcoholic subjects (iv) Patients with extra-pulmonary TB and severely ill (v) History of TB was excluded	NA	January to September 2014	6
Negi et al. [29]	2016	Himachal Pradesh	North	Cross-sectional design	20–70 years	NA	NA	May 2014 to July 2014	5
Bhat et al. [30]	2017	Madhya Pradesh	Central	Case–control	All ages above 18	NA	Saharia	March to November 2013	6
Deo et al. [31]	2017	Maharashtra	Central	Cross-sectional design	All ages above 18	NA	Katkari	2012 and 2014	5
Vijayakumar et al. [32]	2018	Karnataka	South	Cross-sectional design	Age of < 25 years	NA	NA	NA	4
Deo et al. [33]	2018	Maharashtra	Central	Cross-sectional design	All ages above 18	NA	(i) Sakri (ii) Khed (iii) Roha (iv) Mangaon (v) Mhasal	July 2012 and August 2016	4
Sathiyanarayanan et al. [34]	2019	Tamilnadu	South	Cross-sectional design	Participants of age 18 and above from both genders	(i) Severe chronic illness (ii) Physical disability (iii) Mental disability	Malayalee	January 2018 to March 2018	6
Madhu et al. [35]	2019	Karnataka	South	Cross-sectional design	NA	NA	Soliga tribe	NA	6

Table 1 (continued)

References	Year	State	Region	Study design	Inclusion criteria	Exclusion criteria	Name of tribes	Study period	NOS score
Mohd Ashraf Ganie et al. [36]	2020	Kashmir	North	Cross-sectional design	All men and women	(i) Confinement to bed (ii) Pregnancy (iii) Mental Illness (iv) Refuse to participate	(i) Gujjars (ii) Bakerwals	December 2014 and December 2016	6

(eight components). As a result, each study receives a maximum quality score of eight. Studies with less than three points are ranked as high-risk bias, studies with less than five points are ranged as medium risk of bias, and studies with more than five points are ordered as low risk of bias.

Data extraction

Pre-designed forms have been created to extract data from related studies. Each study was assigned a unique identification number, and the following descriptive information was collected: about authors, including year of publication, region, tribe type, duration, study design, sex of study participants, sample size, and prevalence of diabetes. Two reviewers (SC and LA) worked independently on study selection, quality assessment, and data extraction. The controversy was resolved through discussion with the third author (BP).

Data analysis

With the final set of studies, we performed a meta-analysis using the R software (R Foundation for Statistical Computing, Vienna, Austria). The random-effects model was used due to the anticipated heterogeneity. The final data were reported as pooled prevalence with a 95% confidence interval (CI) for outcomes such as the prevalence of diabetes mellitus. A forest plot was used to represent the estimated pooled prevalence, and a funnel plot was used to evaluate and visually represent publication bias. We also used Egger's test to determine the asymmetry of the plot. p values of < 0.10 were considered statistically significant in terms of publication bias.

Heterogeneity was assessed by chi-square of heterogeneity and I^2 statistic. p value less than 0.10 in the chi-square test indicates significant heterogeneity, while I^2 value was used to quantify the heterogeneity using the following criteria: less than 25% = mild heterogeneity, 25–75% = moderate heterogeneity, and > 75% = substantial heterogeneity. We conducted a subgroup analysis based on the geographical regions in India (North, South, East, West, Central). We also performed a sensitivity analysis for indicating influential studies in our review (Table 2).

Overview of studies

Study selection process

We found 261 studies through the systematic literature search (PubMed, Science Direct, Google scholar), and after removing duplicates, 131 records were screened during the primary screening stage. We deemed 48 of those studies relevant for the full-text retrieval. Twenty-three studies met

Table 2 Sensitivity analysis of included studies

Omitted studies	Summary effect size
R P Agrawal et al	6% (5–8%)
Sobhanjan Sarkar et al	5% (4–6%)
Lau et al	6% (5–7%)
Satwanti Kapoor et al	6% (5–7%)
Bandana Sachdev	6% (5–7%)
Bandana Sachdev	6% (5–7%)
Ashita Singh et al	6% (5–7%)
S. Achanta et al	6% (5–7%)
N. K. Mungreiphy and Satwanti Kapoor	6% (5–7%)
Kapoor et al	5% (5–7%)
Nikkin T and Meriton Stanly	6% (5–7%)
Basavanagowdappa Hathur et al	6% (5–9%)
Shankar Radhakrishnan and Manivanan Ekambaram	6% (4–7%)
Pangi Vijaya Nirmala et al	6% (5–7%)
Magna Manjareeka et al	6% (4–7%)
Negi et al	6% (5–7%)
Bhat et al	6% (5–7%)
Deo et al	6% (5–7%)
Vijayakumar et al	6% (5–7%)
Deo et al	6% (5–7%)
S. Sathiyanarayanan et al	6% (4–7%)
Madhu et al	6% (4–7%)
Mohd Ashraf Ganie et al	6% (5–7%)
Overall effect size	**6% (5 – 7%)**

the eligibility criteria during the second screening stage, and 25 studies were excluded, and the reason for the exclusion was provided in the PRISMA flowchart (Fig. 1).

Characteristics of studies included

The characteristics of the included studies are listed in Table 1. Overall, 35,985 individuals have been recruited in 23 studies. In terms of study design, 22 studies were cross-sectional, and one was case–control. In terms of quality, all the studies had a low risk to unclear risk of bias, and all the studies satisfied the criteria to be a high-quality study.

Prevalence of diabetes mellitus rate

Over the course of 23 studies, a total of 35,985 people were examined. The pooled prevalence of diabetes mellitus rate in India was 6.00% (95% CI, 5.00–7.00%) (Fig. 2). However, heterogeneity was substantial ($I^2 = 98\%$; chi-square test for heterogeneity = $p < 0.001$). The funnel plot (Fig. 3) of pooled prevalence rates did not show any significant evidence of publication bias. However, Egger's ($p < 0.0001$) and Harbord ($p = 0.0002$) tests revealed publication bias. Subgroup analysis was conducted based on regions (North, South, East, West, Central) in India. The estimated pooled prevalence rate in East India was 10% (95% CI, 0–19%); it was 7% (95% CI, 0–9%) in Central India, it was 5% (95% CI, 0–10%) in North India, it was 5% (95% CI, 0–7%) in South India, and it was 4% (0–5%) in West India. Hence, the pooled prevalence of diabetes mellitus is higher in eastern India and lower in western India (Fig. 4). A sensitivity analysis was performed excluding one study at a time in order to assess the robustness of the observed outcomes. The overall estimates were ranged from 5% (95% CI, 4–6%) to 6% (95% CI, 5–9%) which is not overlapping actual findings of summary effect measure (6.00% (95% CI, 5.00–7.00%).

Discussion

The ongoing demographic and epidemiological transition has changed the healthcare needs of the people globally. Infectious diseases are rapidly losing ground to non-communicable diseases as the leading cause of disability and death.

In the current review, the burden of diabetes mellitus among Indian tribals was estimated. Twenty-three studies in a span of 20 years have shown that chronic illness of diabetes is a neglected problem in the tribal population. In a country with over a billion people, about 8% of whom are tribal, the prevalence of diabetes in tribal communities has been estimated at 6%, implying that more than four million people are affected by diabetes. A systematic review of diabetes in the tribal population by Upadhyay et al. [37] diabetes was found to be prevalent in 5.9% between 2000 and 2011, similar to our present results, indicating a secular trend of diabetes among the tribes.

In India, there is a disparity within the country, with the prevalence of diabetes mellitus ranging from 5.9 to 12.1% in urban areas (North, 8.6% to 11.6%; South, 13.5% to 19.5%) [38], which is higher than our study findings 6% (North, 5%; South, 5%; East, 10%; West, 4%; and Central, 7%).

The prevalence of diabetes mellitus in this review of the tribal population is a significantly lower prevalence of diabetes at 6% comparing to a national prevalence of 7.3% [39]. The tribal population of other countries like Sudan, Taiwan, and the United Arab Emirates have seen an increase in the prevalence of diabetes, and metabolic syndrome has been discovered in the population [40–42].

The major risk factors for diabetes include economic boom, sedentary lifestyle, sophistication, and environmental factors. The association between low-level education and diabetes was statistically significant, with some studies showing the highest prevalence among illiterate individuals [43]. There was also a statistically significant association

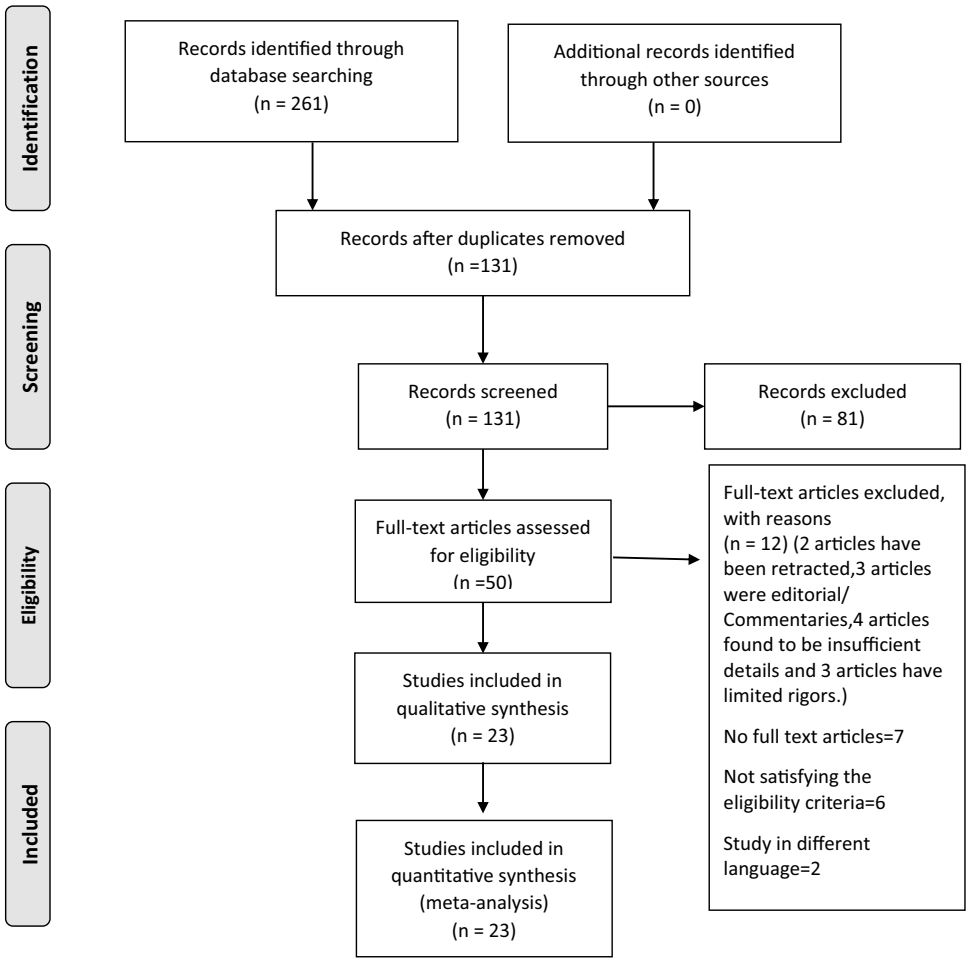

Fig. 1 Process of study selection and assessment of inclusion and exclusion criteria

between diabetes and socio-economic class. A study by Arora et al. [44] revealed a higher prevalence of diabetes in the lower and upper economic class than the upper-middle class.

Migration can also contribute to the increased rates of type 2 diabetes. Tribes migrated to cities as populations grew, and they began to lead a largely dependent lifestyle on a paid workforce and low salary. Relocation to these urban areas in search of work is believed to be the leading cause of lifestyle change and cultural and social changes [45]. Tribal populations have moved into and around villages due to growing urbanization and sedentary lifestyles and have adopted a similar lifestyle to that of the rural population, as evidenced by other studies [46].

The nutritional transition has been explained by a rapid and recent change in the diets of people in many countries, with high consumption of foods of animal origin, artificial sweeteners, fat, and fast food [47]. Several factors, such as behavioral, cultural, and dietary habits related to daily life, are associated with diabetes. The elderly population of the tribes are usually illiterate and from a low socio-economic background [48]. More detailed evaluation needs to be done in these tribal groups about diabetic groups, and more numbers have to be identified for better understanding the pathogenesis involved.

Strengths

Our review adds to the limited knowledge about the prevalence of diabetes among Indian tribes. It follows PRISMA guidelines. We performed several quality assessments, including assessing the risk of bias, subgroup analysis, and sensitivity analysis in included studies. On performed sensitivity analysis, none of the studies influencing the overall estimate showed the robustness of our overall findings.

Limitations

There are some limitations to the study as well. The heterogeneity among studies is the main limitation of this review. This could be due to studies that were conducted in various geographical areas and targeted diverse tribal populations. The majority of these studies did not include data on diabetes prevalence by gender. Gender-specific estimates could not

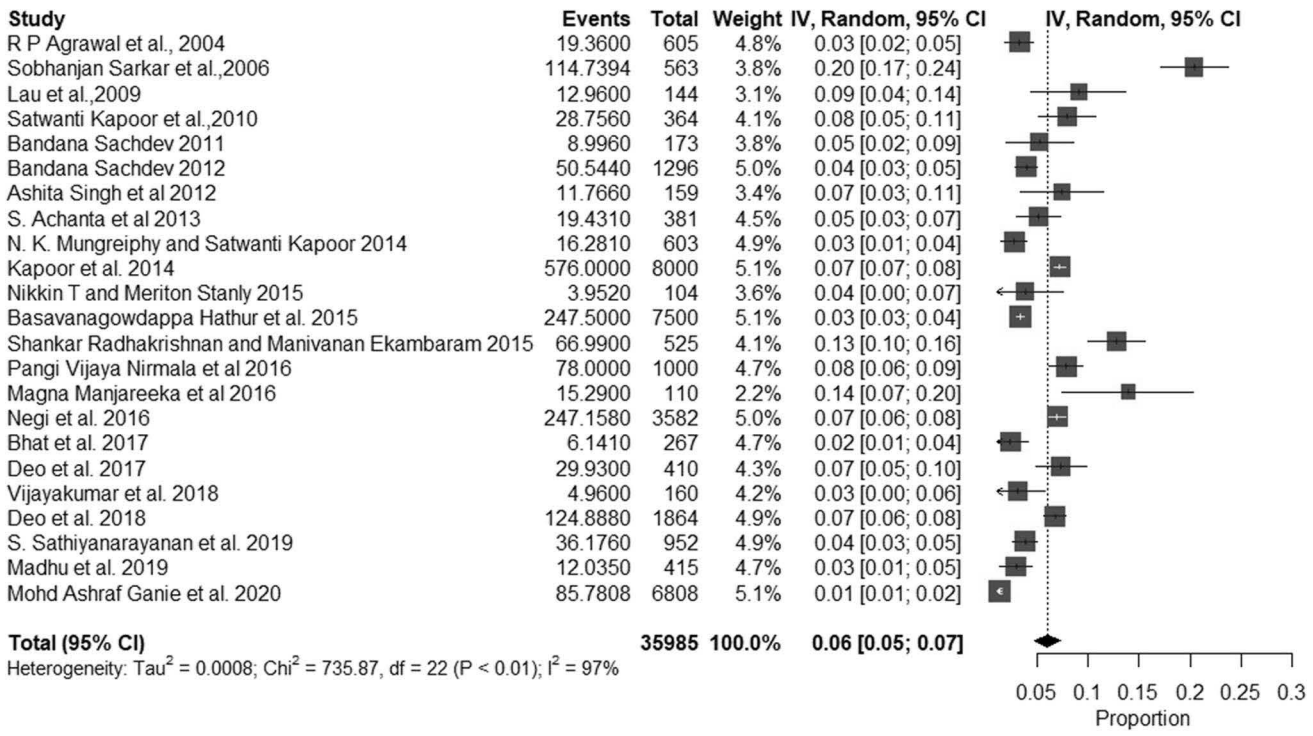

Fig. 2 Pooled estimated of selected studies

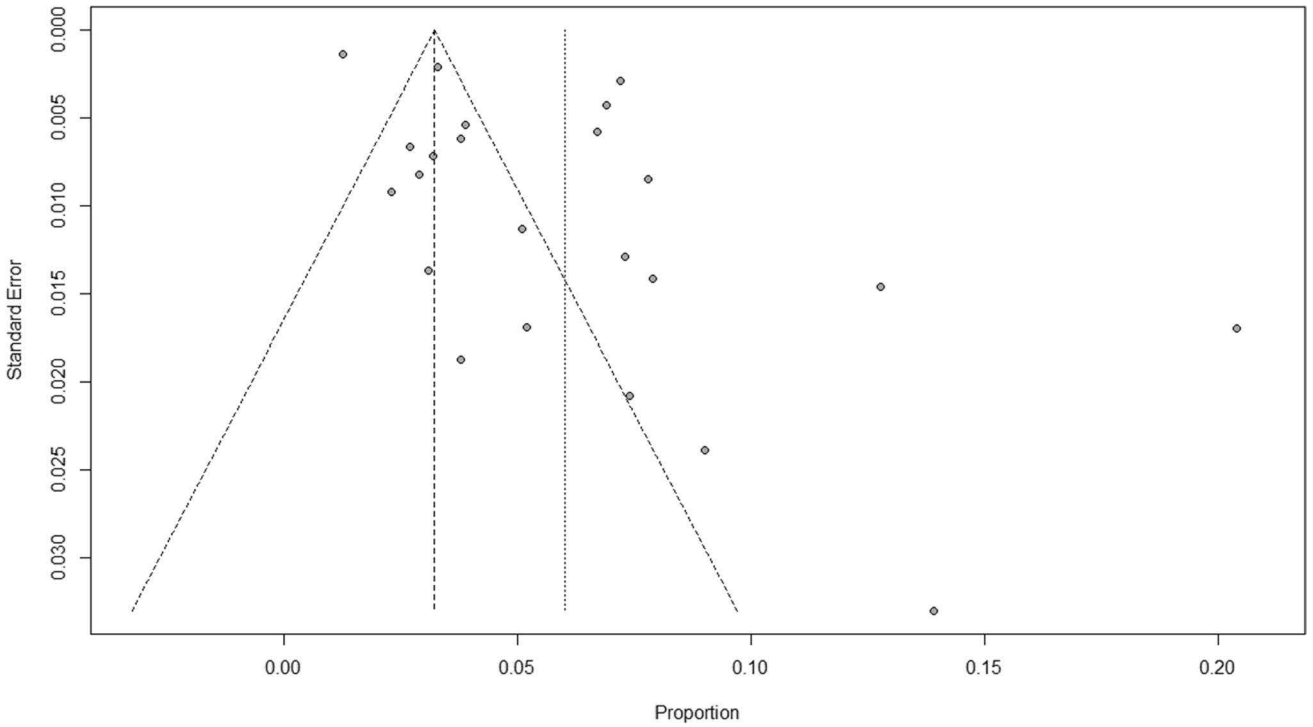

Fig. 3 Funnel plot

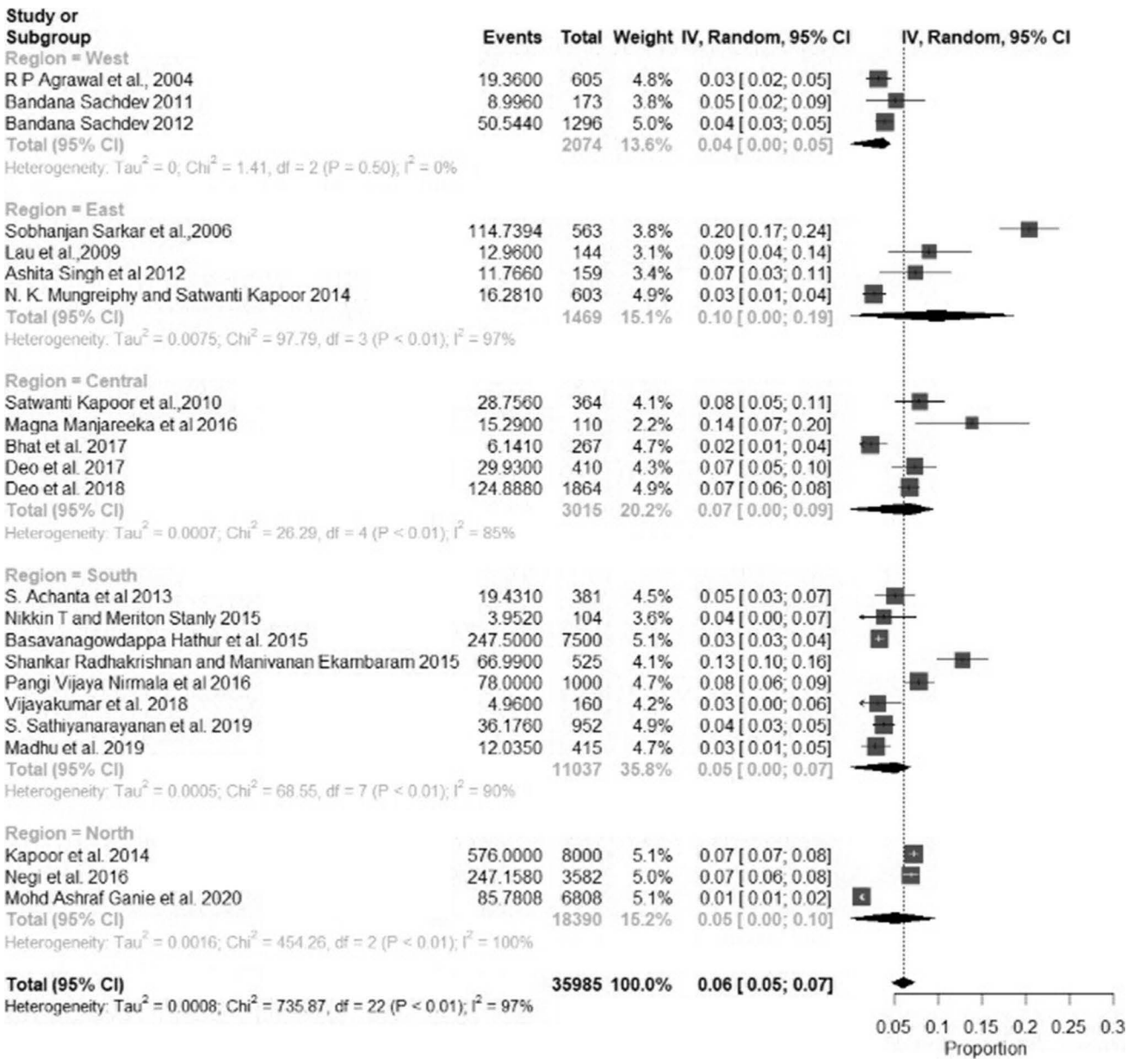

Fig. 4 Subgroup analysis

be computed as a result. It was not possible to determine whether the difference between males and females is statistically significant. Only about a quarter of the included studies were of high enough quality (low risk of bias).

Furthermore, most of the articles included in this review were cross-sectional studies. As a result, other confounding variables can affect the outcome variables. Most of the studies included in this review were small sample sizes that might have influenced the reported estimated prevalence. In addition, this meta-analysis included only studies from thirteen states indicating under-representation due to the small number of studies included.

Conclusion

Reliable data on diabetes prevalence among indigenous peoples of India are lacking. Efforts should be made to obtain relevant data that can be used to formulate future policies and plans. Tribal health should be a priority, and tribal areas should have an adequate medical infrastructure. Programmes should be put in place to educate the public about the disease, its signs and symptoms, the importance of early diagnosis and treatment, and the availability of trained staff and health care facilities. Screening for all diseases should be encouraged in mobile clinics.

Policymakers must pay attention to the changing health needs of tribal communities.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-022-01133-9.

Data availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Kumar MM, Pathak VK, Ruikar M. Tribal population in India: a public health challenge and road to future. J Fam Med Prim Care [Internet]. 2020 [cited 2021 Jul 24];9(2):508. Available from: /pmc/articles/PMC7113978/
2. Papier K, Jordan S, D'Este C, Bain C, Peungson J, Banwell C, et al. Incidence and risk factors for type 2 diabetes mellitus in transitional Thailand: results from the Thai cohort study. BMJ Open [Internet]. 2016 Dec 1 [cited 2021 Aug 14];6(12):e014102. Available from: https://bmjopen.bmj.com/content/6/12/e014102
3. Nageswara Rao Ambati, Anand S. Changes in occupational patterns and its impact on health seeking behaviour of denotified tribal women. Int J Psychosoc Rehabil. 2020;24(02):5353–78.
4. Williams R, Karuranga S, Malanda B, Saeedi P, Basit A, Besançon S …, Colagiuri S. Global and regional estimates and projections of diabetes-related health expenditure: results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2020;162:108072.
5. Diabetes [Internet]. [cited 2021 Aug 14]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/diabetes
6. Ks R, Prabhakaran D, Chaturvedi V, Jeemon P, Kr T, Ramakrishnan L, et al. Bulletin -Reddy. 2006;027037(05).
7. Ranasinghe P, Jayawardena R, Gamage N, Sivanandam N, Misra A. Prevalence and trends of the diabetes epidemic in urban and rural India: a pooled systematic review and meta-analysis of 1.7 million adults. Ann Epidemiol. 2021;58:128–148. https://doi.org/10.1016/j.annepidem.2021.02.016.
8. Bajaj S. RSSDI clinical practice recommendations for the management of type 2 diabetes mellitus 2017. Int J Diabetes Dev Ctries [Internet]. 2018 [cited 2021 Aug 14];38(Suppl 1):1. Available from: /pmc/articles/PMC5838201/
9. Mendenhall E, Narayanan G, Prabhakaran D. Depression and diabetes in India: perspectives and recommendations. Diabet Med. 2012;29(9):e308–11. https://doi.org/10.1111/j.1464-5491.2012.03708.x.
10. Gupta V, Millett C, Walia GK, Kinra S, Aggarwal A, Prabhakaran P, Bhogadi S, Kumar A, Gupta R, Prabhakaran D, Reddy KS, Smith GD, Ben-Shlomo Y, Krishna KV, Ebrahim S. Socioeconomic patterning of cardiometabolic risk factors in rural and peri-urban India: Andhra Pradesh children and parents study (APCAPS). Z Gesundh Wiss. 2015;23(3):129–136. https://doi.org/10.1007/s10389-015-0662-y.
11. Laverty AA, Palladino R, Lee JT, Millett C. Associations between active travel and weight, blood pressure and diabetes in six middle income countries: a cross-sectional study in older adults. Int J Behav Nutr Phys Act. 2015;20(12):65. https://doi.org/10.1186/s12966-015-0223-3.PMID:25986001;PMCID:PMC4443597.
12. Ali O. Genetics of type 2 diabetes. World J Diabetes [Internet]. 2013 [cited 2021 Sep 2];4(4):114. Available from: /pmc/articles/PMC3746083/
13. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (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.
14. Agrawal RP, Singh G, Nayak KC, Kochar DK, Sharma RC, Beniwal R, et al. Prevalence of diabetes in camel-milk consuming 'Raica' rural community of North-. Science (80-). 2004;24(August 2014):109–14.
15. Sarkar S, Das M, Mukhopadhyay B, Chakrabarti CS, Majumder PP. High prevalence of metabolic syndrome & its correlates in two tribal populations of India & the impact of urbanization. Indian J Med Res. 2006;123(5):679–86.
16. Lau SL, Debarm R, Thomas N, Asha HS, Vasan KS, Alex RG, et al. Healthcare planning in north-east India: a survey on diabetes awareness, risk factors and health attitudes in a rural community. J Assoc Physicians India. 2009;57:305–9.
17. Kapoor S, Tyagi R, Saluja K, Chaturvedi A, Kapoor aK. Emerging health threats among a primitive tribal group of Central India. J Public Health Epidemiol. 2010;2(2):13–9.
18. Sachdev B. Community based study on incidence of type 2 diabetes and hypertension among nomad tribal population of Rajasthan, India. Int J Sci Nat [Internet]. 2011;2(2):296–301.
19. Sachdev B. Screening of {type} 2 {diabetes} {mellitus} and {its} {associated} {risk} {factors} among {select} {tribes} of {Rajasthan}. Int J Heal Sci Res [Internet]. 2012;2(1). Available from: https://www.semanticscholar.org/paper/Screening-of-Type-2-Diabetes-Mellitus-and-Its-Risk-Sachdev/b1975449bcf1128ed8070727ccf63dac5cefd42e. Accessed 22 Jul 2022.
20. Thomas N, Singh A, Milton P, Nanaiah A, Samuel P. Awareness and attitude toward diabetes in the rural population of Arunachal Pradesh, Northeast India. Indian J Endocrinol Metab. 2012;16(7):83.
21. Achanta S, Tekumalla RR, Jaju J, Purad C, Chepuri R, Samyukta R, et al. Screening tuberculosis patients for diabetes in a tribal area in South India. Public Heal Action. 2013;3(1):43–7.
22. Mungreiphy NK, Kapoor S. Efficacy of obesity indices and age in predicting diabetes: study on a transitional tribe of northeast India. Ethn Dis. 2014;24(3):342–8.
23. Kapoor D, Bhardwaj AK, Kumar D, Raina SK. Prevalence of diabetes mellitus and its risk factors among permanently settled tribal individuals in tribal and urban areas in northern state of sub-Himalayan region of India. Int J Chronic Dis. 2014;2014:1–9.
24. Nikkin T, Stanly MM. Diabetes and hypercholesterolemia among a tribal population in Tamilnadu, India. Int J Community Med Public Health. 2016;3:47–50.
25. Hathur B, Basavegowda M, Kulkarni P, Ashok NC. Metabolic syndrome among diabetics and pre-diabetics of Jenu Kuruba tribe in Mysore district (JKDHS-2) - an evidence of metabolic abnormalities leading to increase in CVD's among Jenu Kuruba tribal population. Diabetes Metab Syndr Clin Res Rev [Internet]. 2015;9(4):205–9. Available from: https://doi.org/10.1016/j.dsx.2015.08.004
26. Radhakrishnan S, Ekambaram M. Prevalence of diabetes and hypertension among a tribal population in Tamil Nadu. Arch Med Heal Sci. 2015;3(1):66.
27. Nirmala P. Comparative study of prevalence of type-2 diabetes mellitus in various demographic regions of Andhra Pradesh, India: a population-based study. Int J MCH AIDS. 2016;5(2):103–11.
28. Manjareeka M, Palo SK, Swain S, Pati S, Pati S. Diabetes mellitus among newly diagnosed tuberculosis patients in tribal Odisha: an exploratory study. J Clin Diagnostic Res. 2016;10(10):LC06–8.

29. Negi PC, Chauhan R, Rana V, Vidyasagar, Lal K. Epidemiological study of non-communicable diseases (NCD) risk factors in tribal district of Kinnaur, HP: a cross-sectional study. Indian Heart J [Internet]. 2016;68(5):655–62. Available from: https://doi.org/10.1016/j.ihj.2016.03.002
30. Bhat J, Rao VG, Sharma RK, Muniyandi M, Yadav R, Bhondley MK. Investigation of the risk factors for pulmonary tuberculosis: a case–control study among Saharia tribe in Gwalior district, Madhya Pradesh, India. Indian J Med Res. 2017;146(1):97.
31. Deo, Pawar P, Kanetkar S, Kakade S. Prevalence and risk factors of hypertension and diabetes in the Katkari tribe of coastal Maharashtra. J Postgrad Med [Internet]. 2017 [cited 2021 Jul 22];63(2):106. Available from: https://www.jpgmonline.com/article.asp?issn=0022-3859;year=2017;volume=63;issue=2;spage=106;epage=113;aulast=Deo
32. Vijayakumar V, Balakundi M, Metri KG. Challenges faced in diabetes risk prediction among an indigenous South Asian population in India using the Indian Diabetes Risk Score. Public Health [Internet]. 2019;176:114–7. Available from: https://doi.org/10.1016/j.puhe.2018.09.012
33. Deo M, Pawar P, Kanetkar S, Kakade S. Multicentric study on prevalence and risk factors for hypertension and diabetes in tribal communities in Western and Northern Maharashtra. J Postgrad Med [Internet]. 2018 [cited 2021 Jul 22];64(1):23. Available from: /pmc/articles/PMC5820811/
34. Sathiyanarayanan S, Muthunarayanan L, Devaparthasarathy T. Changing perspectives in tribal health: rising prevalence of lifestyle diseases among tribal population in India. Indian J Community Med [Internet]. 2019 Oct 1 [cited 2021 May 19];44(4):342–6. Available from: https://www.ijcm.org.in/article.asp?issn=0970-0218;year=2019;volume=44;issue=4;spage=342;epage=346;aulast=Sathiyanarayanan
35. Madhu B, Prathyusha K, Prakruthi P, Srinath KM. Comparison of prevalence of life style risk factors and 10 year risk of CVD event among rural and tribal population of Kollegal Taluk, Chamrajanagar district, South India. Diabetes Metab Syndr Clin Res Rev [Internet]. 2019;13(5):2961–6. Available from: https://doi.org/10.1016/j.dsx.2019.07.056
36. Ganie MA, Sahar T, Rashid A, Baba MS, Ahmad N, Bhat H, et al. Prevalence of diabetes and prediabetes in tribal population of Kashmir: lessons for the future. Diabetes Res Clin Pract [Internet]. 2020;169:108457. Available from: https://doi.org/10.1016/j.diabres.2020.108457
37. Upadhyay RP, Misra P, Chellaiyan VG, Das TK, Adhikary M, Chinnakali P, et al. Burden of diabetes mellitus and prediabetes in tribal population of India: a systematic review. Diabetes Res Clin Pract [Internet]. 2013;102(1):1–7. Available from: http://dx.doi.org/https://doi.org/10.1016/j.diabres.2013.06.011
38. Ramachandran A, Snehalatha C. Current scenario of diabetes in India. J Diabetes. 2009;1(1):18–28.
39. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR–INDIAB population-based cross-sectional study. Lancet Diabetes Endocrinol. 2017;5(8):585–96.
40. Elbagir MN, Eltom MA, Elmahadi EMA, Kadam IMS, Berne C. A high prevalence of diabetes mellitus and impaired glucose tolerance in the Danagla community in Northern Sudan. Diabet Med. 1998;15(2):164–9.
41. Huang TL, Hsu SY, Tsai RK, Sheu MM. Etiology of ocular diseases in elderly Amis aborigines in Eastern Taiwan (The Amis Eye Study). Jpn J Ophthalmol. 2010;54(4):266–71.
42. Hamoudi R, Saheb Sharif-Askari N, Saheb Sharif-Askari F, 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):17437. https://doi.org/10.1038/s41598-019-53505-7.PMID:31767874;PMCID:PMC6877520.
43. Gikas A, Sotiropoulos A, Panagiotakos D, Peppas T, Skliros E, Pappas S. Prevalence, and associated risk factors, of self-reported diabetes mellitus in a sample of adult urban population in Greece: MEDICAL Exit Poll Research in Salamis (MEDICAL EXPRESS 2002). BMC Public Health. 2004;4:1–9.
44. Arora E, Shenoy S, Sandhu JS. Effects of resistance training on metabolic profile of adults with type 2 diabetes. Indian J Med Res. 2009;129(5):515–9.
45. Prabhakar H, Manoharan R. The Tribal Health Initiative model for healthcare delivery: a clinical and epidemiological approach. Natl Med J India. 2005;18(4):197–204.
46. Gupta R, Misra A. Type 2 diabetes in India: regional disparities. Br J Diabetes Vasc Dis. 2007;7(1):12–6.
47. Maletnlema T. A Tanzanian perspective on the nutrition transition and its implications for health. Public Health Nutr. 2002;5(1a):163–8.
48. Manojan KP. Indigenous knowledge in education: a study among Paniya tribes in Kerala. J Soc Work Educ Pract. 2018;3(1):43–55.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):184–190
https://doi.org/10.1007/s13410-022-01093-0

ORIGINAL ARTICLE

Comparison of inflammation-related hematologic indices for predicting metabolic syndrome in adults

Sevil Karahan Yilmaz[1] · Fatih Özçiçek[2]

Received: 30 October 2021 / Accepted: 17 May 2022 / Published online: 7 June 2022

Abstract

Aim It was planned to evaluate different hematologic indices (neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio (LMR), lymphocyte/high-density lipoprotein to cholesterol ratio (LHR), neutrophil/high-density lipoprotein to cholesterol ratio (NHR), and monocyte/high-density lipoprotein to cholesterol ratio (MHR)) associated with inflammation in predicting metabolic syndrome in adults and to predict which marker is the better predictor.

Materials and methods This study comprised 399 adults between the ages of 18 and 65. Bodyweight, height, waist circumference, and blood pressure were measured; fasting blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, and hemogram values were analyzed. The International Diabetes Federation criteria were used to define metabolic syndrome.

Results The study included 133 (33.3%) males and 266 (66.4%) females with an average age of 54.3 ± 11.8 years. The prevalence of metabolic syndrome is 58.6% (male 44.3%; female 65.7%). For both men (AUC = 0.730, cutoff = 4.5) and women (AUC = 0.669, cutoff = 4.2), the NHR index has the highest AUC. LHR has the second-highest metabolic syndrome determination in men (AUC = 0.647, cutoff = 6.9). Women's LHR (AUC = 0.626, cutoff = 6.4) and LMR (AUC = 0.757, cutoff = 4.9) had the second and third highest AUCs, respectively, while NLR and PLR were not significant in either gender ($p > 0.05$).

Conclusion The NHR index is a strong predictor of metabolic syndrome. In men, the NHR index is a better predictor of metabolic syndrome than the LHR index, and in women, the NHR index is better than the LHR and LMR.

Keywords Metabolic syndrome · Lymphocyte/monocyte ratio · Lymphocyte/high-density lipoprotein to cholesterol ratio · Neutrophil/high-density lipoprotein to cholesterol ratio · Monocyte/high-density lipoprotein to cholesterol ratio

Introduction

Metabolic syndrome refers to metabolic disorders such as elevated blood pressure, a high waist circumference, dyslipidemia, and insulin resistance [1, 2]. The prevalence of metabolic syndrome (MetS) increases with socioeconomic factors and lifestyle changes in both developed and developing countries [3]. It is critical to understand how to lower the prevalence of MetS and successfully avoid it [4].

Metabolic syndrome, a global public health problem, is a chronic, low-grade inflammatory condition [5]. Prothrombotic and proinflammatory states are the main features of MetS and are associated with increased activity of inflammatory cytokines [6]. It is reported that indices such as white blood cell count, red blood cell count, lymphocyte count, and platelet count from hemogram parameters and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) from biochemical parameters are indicators of inflammation. Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), lymphocyte/monocyte ratio (LMR), lymphocyte/high-density lipoprotein to cholesterol ratio (LHR), neutrophil/high-density lipoprotein to cholesterol ratio (NHR), and monocyte/high-density lipoprotein to cholesterol ratio (MHR) are novel inflammatory markers and associated with many cardiovascular risk factors [5, 7, 8].

✉ Sevil Karahan Yilmaz
karahany.sevil12@gmail.com

Fatih Özçiçek
fozcicek@hotmail.com

[1] Department of Nutrition and Dietetics, Faculty of Health Sciences, Erzincan Binali Yıldırım University, 24030 Erzincan, Turkey

[2] Department of Internal Medicine, Faculty of Medicine, Erzincan Binali Yıldırım University, 24100 Erzincan, Turkey

Metabolic syndrome is a low-grade inflammatory state characterized by alterations in the MHR, NLR, and LMR indices [9]. It has an important and powerful effect in predicting the progression and development of LHR and NHR with MetS, which are easy to achieve and evaluate in patients [8]. LHR is a useful marker of inflammation to assess the presence and severity of MetS [5]. The risk of MetS rises as NLR rises; therefore, NLR values are a useful tool for predicting MetS development [10].

As a novel inflammatory marker, LHR is an effective predictor of newly diagnosed MetS and should be widely used in epidemiological studies [4]. The MHR could be an available and useful inflammatory marker to assess patients with MetS and the severity of the disease [11].

Insulin resistance is linked to increased platelet activity and plays a key role in the pathogenesis of metabolic syndrome. It has been reported that there is a possible relationship between mean platelet volume (MPV) and MetS [12–14].

The metabolic syndrome is diagnosed using a variety of criteria. The prevalence of metabolic syndrome is increasing day by day, it is crucial to decrease and prevent this condition, and new predictors are needed to diagnose metabolic syndrome. The literature uses the World Health Organization (WHO), Adult Treatment Panel (ATP III), and International Diabetes Federation (IDF) criteria for the diagnosis of metabolic syndrome. In the evaluation of inflammation, C-reactive protein (CRP) results are routinely reviewed using biochemical parameters. Some studies use NLR, PLR, LMR, LHR, NHR, MHR, and MPV indices to predict metabolic syndrome. Given the current literature data, this study aimed to assess individuals with metabolic syndrome using more specific markers (NLR, PLR, LMR, LHR, NHR, MHR, and MPV) to predict which marker is more predictive.

Methods

This is a descriptive, cross-sectional, and correlational study.

Population and sample

Individuals who applied to Erzincan Provincial Health Directorate Binali Yıldırım University Mengücek Gazi Training and Research Hospital Internal Medicine Polyclinic between June 2021 and September 2021 constitute the study population. No sample selection method will be used in the study, and participants who voluntarily decided to engage in the study with the complete count method were included. While individuals aged 18–65 years were enrolled in the study, pregnant and lactating women and individuals with malignant and inflammatory diseases, liver and kidney failure, and hematological diseases were not enrolled. Power analysis was performed, and it was planned to conduct the study with 200 subjects.

Data collection tools

In the study, descriptive characteristics, anthropometric measurements, biochemical parameters, and hematologic indices were used to collect data.

Descriptive characteristics

The researchers' information form includes questions regarding the subjects' sociodemographic characteristics, introductory characteristics, and disease status.

Anthropometric measurements

Bodyweight, height, and waist circumference Bodyweight was measured with a precision scale calibrated at regular intervals (±0.1 kg sensitive) without shoes and wearing thin clothes, body height was measured with the feet side by side, and the head in the Frankfort plane (eye triangle and the upper edge of the auricle are at the same height, parallel to the floor), and the heels of the feet touched the wall, according to the method with a non-flexible tape measure. The waist circumference was measured using a non-flexible circumference tape measure by finding the middle of the lowest rib bone and the crista iliaca.

Biochemical parameters and blood pressure measurement

The results of biochemical parameters requested by the patients' physicians (fasting blood sugar (FPG), total cholesterol, triglycerides (TG), LDL-C and HDL-C, leukocytes, hemoglobin, CRP, lymphocytes, platelets, mean platelet volume (MPV), monocytes, neutrophils) were used. Blood pressure was measured on the left arm by the physician using a manual blood pressure monitor.

Metabolic syndrome was defined using the criteria of the Metabolic Syndrome International Diabetes Federation. To diagnose MetS, two of the following criteria and the presence of abdominal obesity (waist circumference: > 94 cm in men, > 80 cm in women) are required: (1) TG > 150 mg/dl; (2) HDL-C (< 50 mg/dl in men, 40 mg/dl in women); (3) hypertension (≥ 130/85 mmHg); and (4) FPG ≥ 100 mg/dl or type 2 diabetes mellitus.

Hematologic indices

NLR, PLR, LMR, LHR, NHR, and MHR values were calculated.

Statistical analysis

In the analysis of data, the IBM SPSS ver 22.0 (IBM Corp. Released 2013, IBM SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp.) package program was used. The normality of the distribution of numeric variables was evaluated using the Kolmogorov–Smirnov test. Mean, standard deviation, percentage, and independent *T*-test were used in the evaluation of the data. Binary logistic regression was used to evaluate the unadjusted and adjusted associations between the hematological index and MetS components. Adjustments were made for age and smoking status. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. When testing the diagnostic value of hematological indices, receiver operating characteristic (ROC) curve analysis was used. ROC curve analysis was used to determine the area under the curve (AUC), cutoff point, sensitivity, and specificity. A p-value of less than 0.05 indicates statistical significance.

Results

One hundred thirty-three (33.3%) males and 266 (66.4%) females with a mean age of 54.3 ± 11.8 years participated in the study. The prevalence of metabolic syndrome is 58.6% (male 44.3%; female 65.7%). Table 1 shows the demographic and clinical characteristics of patients based on their metabolic syndrome status. Age, body mass index (BMI), waist

Table 1 Demographic and clinical characteristics of the study participants classified by the presence of MetS

	Men (n = 133)			Women (n = 266)		
	MetS (n = 59)	Non-MetS (n = 74)	p	MetS (n = 175)	Non-MetS (n = 91)	p
Age (years)	57.7 ± 13.6	49.4 ± 19.9	**0.007**	54.6 ± 13.0	49.4 ± 18.3	**0.006**
Smoking status (%)						
Yes	2 (22.2%)	7 (77.8%)	0.150	12 (54.5%)	10 (45.5%)	0.176
No	57 (46.0%)	67 (54.0%)		163 (66.8%)	81 (33.2%)	
BMI (kg/m^2)	28.7 ± 4.2	25.9 ± 4.8	**0.001**	31.4 ± 3.4	28.2 ± 5.3	**< 0.001**
Waist circumference (cm)	105.6 ± 10.6	97.3 ± 11.7	**< 0.001**	113.9 ± 9.8	100.6 ± 13.6	**< 0.001**
SBP (mmHg)	139.2 ± 14.8	124.6 ± 13.0	**< 0.001**	13.4 ± 1.6	12.1 ± 1.3	**< 0.001**
DBP (mmHg)	77.1 ± 13.1	62.8 ± 12.5	**< 0.001**	7.2 ± 1.2	6.3 ± 1.0	**< 0.001**
FBG (mg/dl)	183.4 ± 93.2	113.5 ± 35.2	**< 0.001**	181.8 ± 88.5	110.5 ± 39.3	**< 0.001**
Cholesterol (mg/dl)	234.5 ± 44.6	180.1 ± 42.1	**< 0.001**	231.1 ± 50.4	188.3 ± 51.6	**< 0.001**
TG (mg/dl)	188.9 ± 84.0	108.7 ± 56.4	**< 0.001**	171.9 ± 66.6	101.8 ± 35.5	**< 0.001**
LDL-C (mg/dl)	147.1 ± 37.7	105.9 ± 31.3	**< 0.001**	146.5 ± 40.5	118.1 ± 38.6	**< 0.001**
HDL-C (mg/dl)	47.1 ± 8.2	54.1 ± 7.6	**< 0.001**	49.7 ± 10.5	57.3 ± 19.1	**< 0.001**
CRP (mg/dl)	2.4 ± 0.5	1.5 ± 0.3	0.199	2.3 ± 0.8	1.2 ± 0.7	0.041
Hemoglobin (g/l)	14.1 ± 2.1	13.5 ± 2.3	0.136	13.5 ± 1.7	12.9 ± 1.8	**0.013**
Nötrofil (×10^3/µl)	4.2 ± 1.4	3.9 ± 1.3	0.189	4.1 ± 1.2	4.0 ± 1.3	0.563
Lenfosit (×10^3/µl)	2.6 ± 0.6	2.3 ± 0.6	**0.005**	2.8 ± 0.8	2.6 ± 0.7	**0.023**
Monosit (×10^3/µl)	0.5 ± 0.2	0.5 ± 0.3	0.912	0.5 ± 0.1	0.5 ± 0.1	0.853
NLR	1.6 ± 0.6	1.7 ± 0.6	0.424	1.6 ± 0.1	1.6 ± 0.1	0.282
PLR	100.6 ± 28.1	118.6 ± 45.2	0.062	105.1 ± 28.9	111.0 ± 41.1	**0.041**
LMR	5.2 ± 2.1	4.6 ± 1.9	0.092	5.6 ± 1.9	5.1 ± 1.8	**0.038**
MPV	10.2 ± 0.7	10.2 ± 1.0	0.986	10.3 ± 0.9	10.1 ± 1.1	0.218
LHR	8.2 ± 3.3	6.5 ± 2.5	**0.002**	7.6 ± 2.8	6.4 ± 2.3	**< 0.001**
NHR	5.1 ± 1.4	3.8 ± 1.3	**<0.001**	5.2 ± 1.9	4.1 ± 1.6	**< 0.001**
MHR	10.5 ± 3.3	11.2 ± 4.9	0.514	10.8 ± 3.9	7.6 ± 2.8	**0.024**

*$p > 0.05$, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fast blood glucose, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *CRP* C-reactive protein, *NLR* neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *LMR* lymphocyte/monocyte ratio, *MPV* mean platelet volume, *LHR* lymphocyte/high-density lipoprotein to cholesterol ratio, *NHR* neutrophil/high-density lipoprotein to cholesterol ratio, *MHR* monocyte/high-density lipoprotein to cholesterol ratio

Table 2 Odds ratio (95% confidence intervals) for hematological indices and metabolic syndrome components

	NLR	PLR	LMR	LHR	NHR	MHR
Men						
High blood glucose						
Unadjusted OR (95% Cl)	0.97 (0.55–1.72)	0.99 (0.98–1.00)	1.17 (0.98–1.45)	1.03 (0.90–1.18)	1.21 (0.92–1.59)	1.01 (0.87–1.56)
Adjusted OR (95% Cl)	0.98 (0.51–1.88)	0.99 (0.98–1.00)	1.14 (0.89–1.45)	1.05 (0.90–1.21)	1.23 (0.90–1.68)	0.86 (0.46–1.46)
High triglycerides						
Unadjusted OR (95% Cl)	0.79 (0.47–1.34)	0.99 (0.98–1.00)	0.96 (0.81–1.15)	**1.17 (1.03–1.32)**	**1.66 (1.27–2.18)**	0.96 (0.90–1.23)
Adjusted OR (95% Cl)	0.70 (0.40–1.22)	0.99 (0.97–1.00)	0.99 (0.82–1.20)	**1.16 (1.02–1.33)**	**1.78 (1.31–2.41)**	0.67 (0.59–0.98)
Low HDL-C						
Unadjusted OR (95% Cl)	0.60 (0.34–1.05)	1.18 (0.55–2.50)	0.89 (0.67–1.18)	**1.46 (1.22–1.75)**	**2.89 (1.71–4.87)**	0.95 (0.89–1.17)
Adjusted OR (95% Cl)	1.07 (0.47–2.39)	0.99 (0.98–1.01)	0.84 (0.60–1.18)	**1.46 (1.18–1.80)**	**3.55 (1.87–6.56)**	0.84 (0.78–1.10)
High blood pressure						
Unadjusted OR (95% Cl)	1.50 (0.90–2.50)	0.99 (0.98–1.00)	1.09 (0.92–1.29)	**1.14 (1.01–1.29)**	1.16 (0.92–1.46)	0.84 (0.76–1.02)
Adjusted OR (95% Cl)	1.54 (0.88–2.70)	0.99 (0.98–1.00)	1.06 (0.88–1.28)	**1.16 (1.01–1.33)**	1.15 (0.89–1.48)	0.88 (0.81–1.13)
Metabolic syndrome						
Unadjusted OR (95% Cl)	1.23 (0.74–2.04)	1.01 (0.99–1.02)	1.86 (1.72–2.02)	**1.82 (1.72–1.93)**	**1.54 (1.40–1.72)**	1.66 (1.56–1.87)
Adjusted OR (95% Cl)	1.28 (0.76–2.17)	1.01 (0.99–1.02)	1.86 (1.71–2.04)	**1.79 (1.69–2.92)**	**1.49 (1.36–1.68)**	1.79 (1.69–2.00)
Women						
High blood glucose						
Unadjusted OR (95% Cl)	1.17 (0.75–1.82)	1.00 (0.99–1.03)	1.06 (0.91–1.22)	1.07 (0.96–1.19)	1.05 (0.91–1.23)	0.64 (0.57–0.99)
Adjusted OR (95% Cl)	1.26 (0.78–2.01)	1.00 (0.99–1.01)	1.03 (0.88–1.19)	1.06 (0.95–1.19)	1.01 (0.87–1.18)	0.83 (0.79–1.12)
High triglycerides						
Unadjusted OR (95% Cl)	0.80 (0.54–1.19)	0.99 (0.98–1.00)	**1.16 (1.02–1.32)**	**1.17 (1.06–1.30)**	**1.43 (1.22–1.67)**	0.63 (0.60–0.97)
Adjusted OR (95% Cl)	0.82 (0.54–1.17)	0.99 (0.98–1.00)	**1.18 (1.04–1.34)**	**1.18 (1.07–1.30)**	**1.45 (1.24–1.69)**	0.74 (0.67–1.11)
Low HDL-C						
Unadjusted OR (95% Cl)	0.80 (0.54–1.18)	**0.99 (0.98–1.00)**	1.08 (0.95–1.22)	**1.46 (1.30–1.65)**	**2.14 (1.74–2.63)**	**1.61 (0.99–1.72)**
Adjusted OR (95% Cl)	0.76 (0.51–1.14)	**0.99 (0.98–1.00)**	1.08 (0.95–1.23)	**1.46 (1.29–1.65)**	**2.24 (1.79–2.81)**	**1.69 (1.66–2.72)**
High blood pressure						
Unadjusted OR (95% Cl)	0.79 (0.54–1.16)	0.99 (0.98–1.00)	**1.17 (1.03–1.33)**	1.04 (0.95–1.14)	**1.16 (1.02–1.33)**	0.68 (0.65–0.97)
Adjusted OR (95% Cl)	0.85 (0.56–1.27)	0.99 (0.98–1.00)	**1.14 (1.00–1.30)**	1.03 (0.94–1.13)	1.13 (0.98–1.30)	0.68 (0.65–0.97)
Metabolic syndrome						
Unadjusted OR (95% Cl)	1.24 (0.83–1.83)	1.05 (0.99–1.01)	**1.86 (1.75–1.99)**	**1.68 (1.57–1.81)**	**1.82 (1.73–1.91)**	**1.10 (1.00–1.45)**
Adjusted OR (95% Cl)	1.24 (0.82–1.89)	1.03 (0.99–1.01)	**1.88 (1.77–2.02)**	**1.69 (1.57–1.83)**	**1.82 (1.73–1.92)**	**1.12 (1.00–1.46)**

The odds ratios were adjusted for age and smoking use. *NLR* neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *LMR* lymphocyte/monocyte ratio, *LHR* lymphocyte/high-density lipoprotein to cholesterol ratio, *NHR* neutrophil/high-density lipoprotein to cholesterol ratio, *MHR* monocyte/high-density lipoprotein to cholesterol ratio

Table 3 The area under the receiver operating characteristic curve, cutoff value, sensitivity, and specificity of the hematological indices predicting MetS in men

Predictors	AUC	*p*	Cutoff	Sensitivity	Specificity	OR (95% CI)
NLR	0.446	0.287	1.555	0.407	0.581	0.347–0.545
PLR	0.438	0.217	107.725	0.492	0.527	0.339–0.536
LMR	0.588	0.083	4.660	0.525	0.473	0.490–0.685
LHR	0.647	**0.038**	6.950	0.610	0.405	0.554–0.741
NHR	0.730	**0.044**	4.540	0.661	0.662	0.644–0.815
MHR	0.543	0.051	0.005	0.983	0.973	0.443–0.642

NLR neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *LMR* lymphocyte/monocyte ratio, *LHR* lymphocyte/high-density lipoprotein to cholesterol ratio, *NHR* neutrophil/high-density lipoprotein to cholesterol ratio, *MHR* monocyte/high-density lipoprotein to cholesterol ratio

Table 4 The area under the receiver operating characteristic curve, cutoff value, sensitivity, and specificity of the hematological indices predicting MetS in women

Predictors	AUC	*p*	Cutoff	Sensitivity	Specificity	OR (95% CI)
NLR	0.469	0.401	1.45	0.480	0.527	0.396–0.541
PLR	0.484	0.663	102.35	0.827	0.763	0.410–0.557
LMR	0.575	**0.043**	4.990	0.561	0.441	0.503–0.648
LHR	0.626	**0.001**	6.430	0.584	0.419	0.557–0.695
NHR	0.669	**< 0.001**	4.281	0.980	0.768	0.602–0.737
MHR	0.551	**0.036**	0.002	1.000	0.994	0.481–0.621

NLR neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *LMR* lymphocyte/monocyte ratio, *LHR* lymphocyte/high-density lipoprotein to cholesterol ratio, *NHR* neutrophil/high-density lipoprotein to cholesterol ratio, *MHR* monocyte/high-density lipoprotein to cholesterol ratio

circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, total cholesterol, TG, LDL-C, lymphocyte, LHR, and NHR levels were higher and HDL-C levels lower in men with metabolic syndrome than in those without metabolic syndrome ($p < 0.05$). Age, body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, total cholesterol, TG, LDL-C, hemoglobin, lymphocytes and PLR, LMR, LHR, NHR, and MHR values were higher and HDL-C values lower in female subjects with metabolic syndrome than in subjects without metabolic syndrome ($p < 0.05$).

Table 2 demonstrates the associations of hematological indices with MetS and its components for both sexes. After adjustments for age and smoking status, LHR and NHR were significantly associated with high triglycerides, low HDL-C, high blood pressure, and MetS in men. In women, PLR was significantly associated with low-HDL-C; LMR was significantly associated with high triglycerides, high blood pressure, and MetS; LHR was significantly associated with high triglycerides, low-HDL-C, and MetS; NHR was significantly associated with high triglycerides, low-HDL-C, high blood pressure, and MetS; and MHR was significantly associated with low-HDL-C and MetS.

Table 3 and Fig. 1 for men and Table 4 and Fig. 2 for women show the areas under the ROC curve of inflammation-related indices in determining MetS in men and women, as well as cutoff points, sensitivity, and specificity values. For both men (AUC = 0.730, cutoff = 4.5) and women (AUC = 0.669, cutoff = 4.2), the NHR index has the highest AUC. LHR has the second-highest MetS determination in men (AUC = 0.647, cutoff = 6.9). Women's LHR (AUC = 0.626, cutoff = 6.4) and LMR (AUC = 0.757, cutoff = 4.9) had the second and third highest AUCs, while NLR and PLR were not significant in either gender ($p > 0.05$).

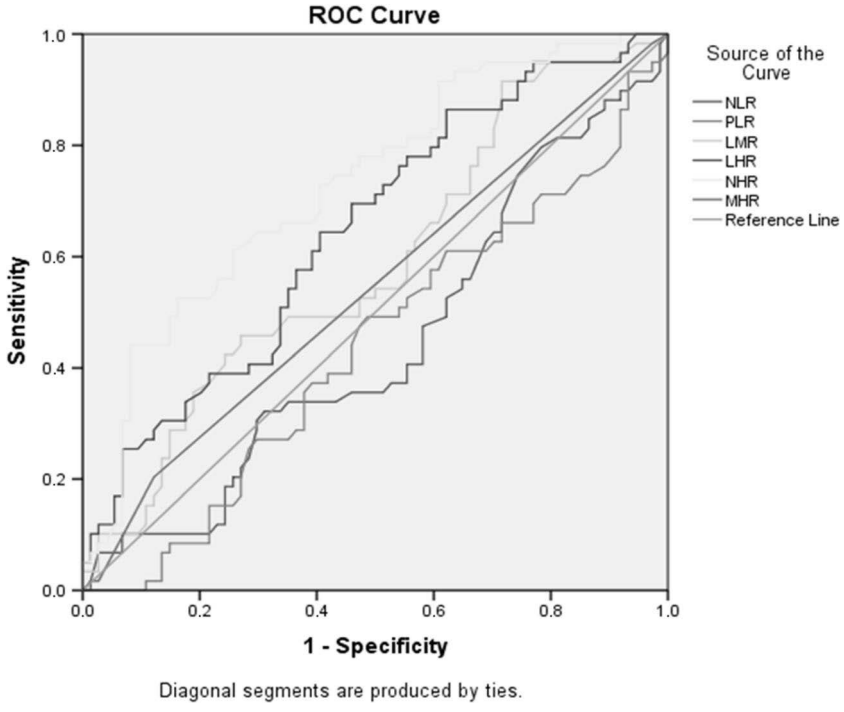

Fig. 1 The area under the receiver operating characteristic curve of the hematological indices to predict MetS in men ($n = 133$)

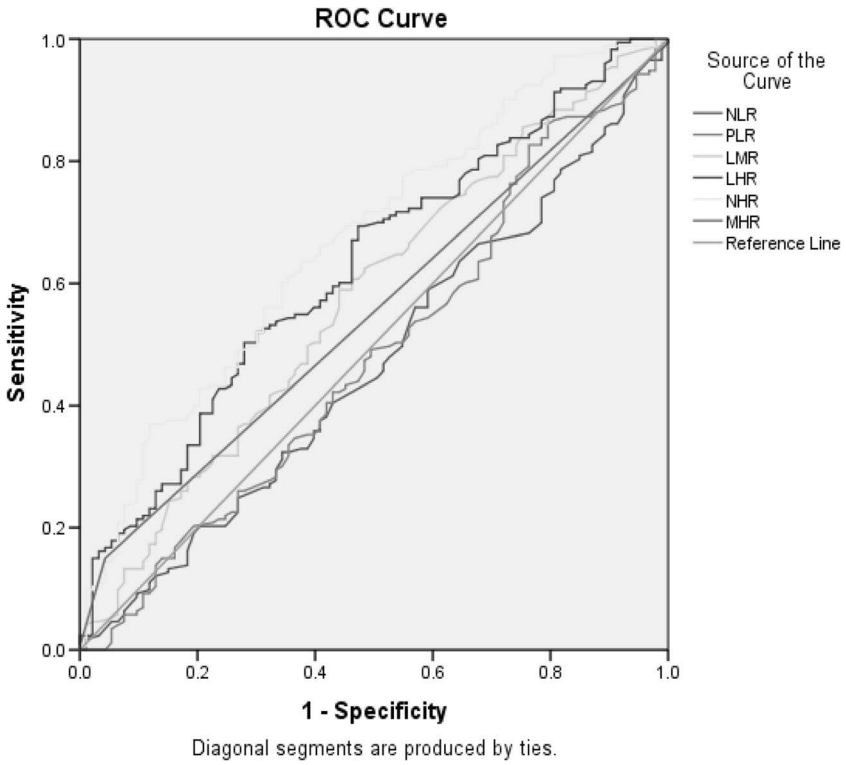

Fig. 2 The area under the receiver operating characteristic curve of the hematological indices to predict MetS in women (n = 133)

Discussion

Metabolic syndrome is a characteristic condition that includes various risk factors such as central obesity, dyslipidemia, hypertension, hyperglycemia, and low-grade inflammation. Analysis of parameters such as NLR, PLR, LMR, LHR, NHR, and MHR may be useful to assess chronic inflammatory status because they can be easily measured in patients with MetS using a complete blood count.

This study examined the predictors and cutoff values of 7 hematologic indices associated with inflammation, including NLR, PLR, LMR, LHR, NHR, MHR, and MPV, to predict MetS in adults. A high rate of metabolic syndrome (58.6%) was observed in the adult subjects. First, the NHR index in both genders, the LHR index in men, and the LHR, LMR, and MHR indexes in women were statistically significant in determining MetS. It was found that the NHR index was a better predictor of MetS in both genders.

The CRP and NLR levels, which are indicators of inflammation, are associated with MetS components [15, 16]. In contrast, NLR [5, 7–10, 17, 18], a reliable indicator of inflammation and associated with the presence and severity of MetS in studies, showed no relationship with MetS in our study. Similar to the findings of this study, no relationship was discovered between CRP, NLR, and MetS in other studies [19, 20].

The PLR value [5, 7, 8], a new inflammatory index associated with cardiovascular disease, could not be associated with MetS in this study, like the study by Yu et al. [4].

Lymphocytes/HDL-C and neutrophils/HDL-C are novel inflammatory markers that can predict the presence and severity of MetS [3–6, 8]. In a study conducted in China, LHR and NHR were reported to have a strong effect on predicting MetS, especially in females [8]. In this study, NHR and LHR were found to be better predictors of MetS in both men and women.

Lymphocyte/monocyte ratio and MHR indices are new and useful indicators of MetS [21]. The MHR could be an available and useful inflammatory marker to evaluate patients with MetS and the severity of the disease [11]. In a study conducted with 771 individuals to predict MetS, the MHR index was a better predictor compared to the PLR, NLR, and LMR indices [9]. In this study, LMR and MHR values were found to be higher in females with MetS, and LMR and MHR were found to be significant in predicting MetS in females.

Hematological indexes which can be easily measured from peripheral complete blood count and hematological indicators such as PLR, NLR, LMR, LHR, NHR, and MHR could be useful to evaluate “chronic inflammatory state” in MetS patients. These indexes can be considered a simple, cost-effective, fast, easily obtainable, and useful tool that can suggest some important clinical and metabolic features of MetS patients. These parameters are easily accessible markers that are less expensive than other inflammation markers (e.g., cytokines, adipokines, CRP, interleukin [IL]-1, interleukin [IL]-6, tumor necrosis factor-a [TNF-a], monocyte chemoattractant protein 1 (MCP-1), and serum amyloid A (SAA)).

Determination of metabolic syndrome in adults will be an effective step to reduce its prevalence and prevent it.

There are some limitations to our study. This is primarily a cross-sectional study, so a causal relationship between the indices and metabolic syndrome cannot be established. Second, a small group of patients was studied, and data were collected from a single center. Studies with a large group of patients from many centers are needed.

Conclusion

NHR index is a strong predictor of metabolic syndrome. NHR index is a better predictor of MetS than LHR index in men and NHR index in women than LHR and LMR.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval The study was approved by the Erzincan Binali Yildirim University Clinical Research Ethics Committee.

Consent to participate Written informed consent was obtained from all participants before study commencement.

Ethics Committee Approval dated 24/05/2021 and numbered 07/01 was obtained from Erzincan Binali Yıldırım University Clinical Research Ethics Committee for the study.

Informed Consent Patients who volunteered to participate in the study were enrolled after reading and signing the informed consent form.

References

1. Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL. Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Ther Adv Cardiovasc Dis. 2017;11:215–25. https://doi.org/10.1177/1753944717711379.
2. Gluvic Z, Zaric B, Resanovic I, Obradovic M, Mitrovic A, Radak D, Isenovic E. Link between metabolic syndrome and insulin resistance. Curr Vasc Pharmacol. 2017;15:30–9. https://doi.org/10.2174/1570161114666161007164510.
3. Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;20:12. https://doi.org/10.1007/s11906-018-0812-z.
4. Yu S, Guo X, Li G, Yang H, Zheng L, Sun Y. Lymphocyte to high-density lipoprotein ratio but not platelet to lymphocyte ratio effectively predicts metabolic syndrome among subjects from rural China. Front Cardiovasc Med. 2021;8:583320. https://doi.org/10.3389/fcvm.2021.583320.
5. Chen H, Xiong C, Shao X, Ning J, Gao P, Xiao H, Chen Y, Zou Z, Hong G, Li X, Shao M, Sheng J, Yang X, You X, Zou H. Lymphocyte to high-density lipoprotein ratio as a new indicator of inflammation and metabolic syndrome. Diabetes Metabol Syndr Obes. 2019;12:2117–23. https://doi.org/10.2147/DMSO.S219363.
6. McCracken E, Monaghan M, Sreenivasan S. Pathophysiology of the metabolic syndrome. Clin Dermatol. 2018;36:14–20. https://doi.org/10.1016/j.clindermatol.2017.09.004.
7. Akboga MK, Canpolat U, Balci KG, Akyel A, Sen F, Yayla C, Cay S, Aras D, Aydogdu S. Increased platelet to lymphocyte ratio is related to slow coronary flow. Angiology. 2016;67:21–6. https://doi.org/10.1177/0003319715574625.
8. Chen T, Chen H, Xiao H, Tang H, Xiang Z, Wang X, Wang X, Zou H. Comparison of the value of neutrophil to high-density lipoprotein cholesterol ratio and lymphocyte to high-density lipoprotein cholesterol ratio for predicting metabolic syndrome among a population in the southern coast of China. Diabetes Metabol Syndr Obes. 2020;13:597–605. https://doi.org/10.2147/DMSO.S238990.
9. Battaglia S, Scialpi N, Berardi E, Antonica G, Suppressa P, Diella FA, et al. Gender, BMI and fasting hyperglycaemia influence monocyte to-HDL ratio (MHR) index in metabolic subjects. PLoS ONE. 2020;15(4):e0231927. https://doi.org/10.1371/journal.pone.0231927.
10. Liu CC, Ko HJ, Liu WS, Hung CL, Hu KC, Yu LY, et al. Neutrophil-to-lymphocyte ratio as a predictive marker of metabolic syndrome. Medicine. 2019;98(43):e17537.
11. Uslu AU, Sekin Y, Tarhan G, Canakcı N, Gunduz M, Karagulle M. Evaluation of monocyte to high-density lipoprotein cholesterol ratio in the presence and severity of metabolic syndrome. Clin Appl Thromb Hemost. 2018;24(5):828–33.
12. Barale C, Russo I. Influence of cardiometabolic risk factors on platelet function. Int J Mol Sci. 2020;21:623. https://doi.org/10.3390/ijms21020623.
13. Nardin M, Verdoia M, Barbieri L, Luca GD. Impact of metabolic syndrome on mean platelet volume and its relationship with coronary artery disease. Platelets. 2019;30:615–23.
14. Ding Q, Wang F, Guo X, Liang M. The relationship between mean platelet volume and metabolic syndrome in patients with type 2 diabetes mellitus: a retrospective study. Medicine. 2021;100(13):e25303. https://doi.org/10.1097/MD.0000000000025303.
15. Sun M, Zhang L, Chen S, Liu X, Shao X, Zou H. Association of C-reactive protein and metabolic disorder in a Chinese population. Int J Environ Res Public Health. 2015;12:8228–42. https://doi.org/10.3390/ijerph120708228.
16. Syauqy A, Hsu CY, Rau HH, Chao JCJ. Association of dietary patterns, anthropometric measurements, and metabolic parameters with C-reactive protein and neutrophil-to-lymphocyte ratio in middle-aged and older adults with metabolic syndrome in Taiwan: a cross-sectional study. Nutr J. 2018;17(1):1–12.
17. Surendar J, Indulekha K, Mohan V, Pradeepa R. Association of neutrophillymphocyte ratio with metabolic syndrome and its components in Asian Indians (CURES-143). J Diabetes Complicat. 2016;30:1525–9. https://doi.org/10.1016/j.jdiacomp.2016.08.006.
18. Buyukkaya E, Karakas MF, Karakas E, Akçay AB, Tanboga İH, Kurt M, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clin Appl Tromb Hemost. 2014;20:159–63. https://doi.org/10.1177/1076029612459675.
19. Adams-Huet B, Jialal I. The neutrophil count is superior to the neutrophil/lymphocyte ratio as a biomarker of inflammation in nascent metabolic syndrome. Ann Clin Biochem. 2019;56:715–6. https://doi.org/10.1177/0004563219866221.
20. Lin HY, Zhang XJ, Liu YM, Geng LY, Guan LY, Li XH. Comparison of the triglyceride glucose index and blood leukocyte indices as predictors of metabolic syndrome in healthy Chinese population. Sci Rep. 2021;11(1):1–7.
21. Vahit D, Akboga MK, Samet Y, Hüseyin E. Assessment of monocyte to high density lipoprotein cholesterol ratio and lymphocyte-to-monocyte ratio in patients with metabolic syndrome. Biomark Med. 2017;11(7):535–40.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):191–198
https://doi.org/10.1007/s13410-022-01097-w

ORIGINAL ARTICLE

Shear wave elastography in evaluation of carotid elasticity in the type 2 diabetes mellitus patients with nonalcoholic fatty liver disease

Mengmeng Zhang[1] · Lian Tang[1] · Xiuxiu Cui[1] · Tingting Yu[1] · Zhen Li[1] · Xiya Li[1] · Guangsen Li[1]

Received: 21 December 2021 / Accepted: 23 May 2022 / Published online: 24 June 2022

Abstract

Purpose Using shear wave elastography (SWE) to assess carotid elasticity in the type 2 diabetes mellitus (T2DM) patients with non-alcoholic fatty liver disease (NAFLD).

Methods There were 98 patients diagnosed T2DM in our hospital, including 35 cases without NAFLD (group A), 33 cases with mild NAFLD (group B), and 30 cases with moderate to severe NAFLD (group C) according to the classification standard of fatty liver. There was no plaque by carotid ultrasound in all the selected patients. The left carotid intima-media thickness (IMT), standard carotid arterial systolic diameter (Ds), standard carotid arterial diastolic diameter (Dd), and systolic peak velocity (PSV) were measured by routine two-dimensional and M-mode ultrasound, and the stiffness coefficient (β) is obtained by calculation. Shear wave elastography (SWE) was used to measure the anterior wall of the left carotid artery values of longitudinal elastic modulus, including the mean values of mean elastic modulus (MEmean), the maximum elastic modulus (MEmax), and minimum elastic modulus (MEmin) at the end of diastole.

Results There was no significant difference in Ds, Dd, and PSV among the groups (all $p > 0.05$). In group C, IMT, β, MEmean, MEmax, and MEmin increased significantly compared with groups A and B (all $p < 0.05$). Compared with group A, MEmean, MEmax, and MEmin increased in group B (all $p < 0.05$), while IMT and β were no significant difference (both $p > 0.05$).

Conclusion SWE can quantitatively evaluate the carotid elasticity of the T2DM patients with NAFLD.

Keywords Shear wave elastography · Type 2 diabetes mellitus · Non-alcoholic fatty liver disease · Carotid elasticity

Introduction

NAFLD, the fatty infiltration of liver tissues and hepatocytes, is related to visceral obesity, dyslipidemia, insulin resistance, and type 2 diabetes mellitus. The pathogenesis of patients with metabolic syndrome also contains insulin resistance. Although NAFLD is not defined as metabolic syndrome, it can be recognized as another component of metabolic syndrome according to the same pathological mechanism between them [1, 2]. With an increasing prevalence, NAFLD has become one of the common liver diseases around the world. Owing to the complex pathogenesis of NAFLD, the multiple systems and organs of patients with NAFLD were often involved. Patients with NAFLD exhibit high-risk for diabetes and cardiovascular disease (CVD); studies have shown that the seriousness of liver histopathology in patients with NAFLD is closely linked with early stage of atherosclerosis, and mild NAFLD can lead to the occurrence of atherosclerosis [3, 4]. T2MD and NAFLD, the mutually susceptible factors, have the same pathological mechanisms, which may cause severe lipid metabolism disorder and chronic inflammation, and accelerate the occurrence of atherosclerosis. Cardiovascular diseases play an important role in the morbidity and mortality of patients with diabetes, and the prognosis is often worse when patients are concomitant with other diseases such as non-alcoholic fatty liver disease, hypertension, and obstructive sleep apnea [5, 6]. Carotid artery is representative in reflecting systemic atherosclerosis and the occurrence of carotid atherosclerosis is earlier than the coronary artery [7]. Consequently, early assessment of the carotid elasticity in

Mengmeng Zhang and Lian Tang contributed equally to this work.

✉ Guangsen Li
liguangsen009@163.com

[1] Department of Ultrasound, The Second Affiliated Hospital of Dalian Medical University, Dalian 116027, China

patients is of great significance in preventing cardiovascular diseases.

The lesions of human tissues are often accompanied by the variations in the elasticity. Providing doctors with accurate tissue elastic coefficient values can help them conduct pathological research and clinical diagnosis of diseases effectively. Recently, ultrasound techniques, including ultra-fast pulse wave technique (UFPWV) and vascular echo tracking technique (ET), have certain advantages in evaluating the carotid elasticity, and they can evaluate carotid elasticity quantitatively and qualitatively. Despite promising results obtained in the early diagnosis of atherosclerosis, the complicated calculation methods and influence of blood pressure will lead to errors with the measurement results [8]. SWE, a new elastography method, measures the elasticity of tissues and organs quantitatively by tracking shear waves passing through tissues [9]. SWE realized the quantitative analysis of the elasticity coefficient of tissues, avoiding the shortcomings of traditional elastography technology. As a non-invasive tool suitable for evaluating organs, SWE is feasible and effective in the diagnosis of thyroid, breast, liver, testis, and other organ lesions [10]. The elasticity measurement of SWE for vascular diseases can quantitatively measure the stiffness of plaques. The present study aimed to assess the carotid elasticity of T2DM patients with NAFLD by SWE.

Materials and methods

Study population

The patients diagnosed with T2MD in the Endocrinology Department of our hospital from January 2020 to September 2021 were selected. No plaque was detected by carotid ultrasound in all the selected patients and IMT ≤ 1.2 mm. According to the grading criteria for NAFLD [11], we divided all the 98 patients into three groups by liver ultrasound examination. Group A including 35 patients without NAFLD (age 27–58 years; average age 46.1 ± 8.0 years; male–female ratio 22:13), group B including 33 mild fatty liver patients (age 25–59 years; average age 47.6 ± 7.4 years; male–female ratio 19:14), and group C including 30 moderate-severe fatty liver patients (age 32–58 years; average age 45.4 ± 7.4 years; male–female ratio 20:10). The course of T2DM is 5 to 10 years. The diagnostic criteria of NAFLD complied with the clinical diagnosis and treatment Guidelines for NAFLD issued by the USA in 2017 [12]. All the selected subjects with the left ventricular ejection fraction > 50% were enrolled.

Exclusion criteria included recent acute complications of diabetes such as ketoacidosis, severe hypoglycemia, infection, and so on; abnormal thyroid hormone level; coronary heart disease, hypertension, stroke and other cardiovascular diseases; a history of viral hepatitis, autoimmune hepatitis, alcoholic hepatitis, drug-induced liver injury, and other liver diseases; rheumatic immune disease, malignant tumor, and other diseases. Furthermore, we depleted the patients with excessive carotid artery pulsation and excessive respiratory movement, which may cause the elastic images displayed blurred.

Instruments and methods

We recorded gender, age, duration of diabetes, body mass, and then calculated the body mass index (BMI, kg/m^2) of all the selected subjects. After a 15-min rest, the heart rate (HR) and blood pressure (BP) of each patient were measured. After an overnight fast, vein blood samples were drawn in the morning to measure fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). All the above biochemical indexes were detected by an automatic biochemical analyzer.

Conventional ultrasound examination of the carotid artery

All patients underwent a conventional ultrasound examination of the carotid artery using a Mindray ultrasound diagnostic instrument equipped with a 14-15WU linear probe (10–12 MHz). The technique was performed with the patients in a horizontal recumbent position. The patients exposed the carotid artery scanning area of patients by tilting their heads and necks back as far as possible, and were asked to tilt their heads to the opposite side of the examination. When the long axial section of the left common carotid artery was shown by the two-dimensional ultrasound, we adjusted the probe slightly to ensure that the carotid intima-media were presented clearly. The measurement site was selected 1.0–1.5 cm below the carotid bifurcation, then we used the instrument's built-in measurement program to measure the carotid intima-media thickness (IMT) of the posterior wall of the common carotid artery at the end of the diastolic [13]. Common carotid artery systolic inner diameter (Ds), common carotid artery diastolic internal diameter (Dd), and peak systolic velocity (PSV) were measured using M-mode and color flow Doppler. Then we calculated wall motion degrees (ΔD, ΔD=Ds-Dd) and stiffness coefficient (β, β=ln(systolic/diastolic blood pressure)/[(Ds—Dd)/Dd]). Each parameter was measured three times, and the average value was taken.

SWE examination of the elastic modulus of the anterior wall of carotid artery

When the wall structure of carotid artery was displayed clearly in the long axial section at the same site as above, we switched

to SWE mode avoiding oppression, and the patients were asked to hold their breath and avoid swallowing and coughing. When the color code signal region covered the anterior and posterior of the intima-media and moving index was better, the operator froze and stored the image. Repeating this operation three times, the operator used the program coming with the instrument to measure the intima-media central part of the anterior wall at the end of diastolic. The sampling frame was round; the diameter was set to l mm; we counted every 2 mm alongside the intima-media. The average value was taken for ten consecutive measurements. We repeated to measure the three stored pictures, and took samples in the same parts of each graph. Finally, we calculated the mean of the average Young's modulus of each graph to obtain the final MEmean, MEmin and MEmax.

Repetition test

Twenty-five patients were randomly selected to test the elastic modulus of the left carotid artery including MEmean, MEmax, and MEmin, using SWE by two ultrasound doctors. After a week, the same observers conducted the same measurements by ultrasound doctors; then we assessed the interobserver and intraobserver correlation coefficients.

Statistical analysis

This study used SPSS 19.0 for statistical analysis. All variables conforming to normal distribution were expressed in the form of average ± standard deviation($\overline{x} \pm s$). We used χ^2 test to test the quantitative data among the three groups, used one-way ANOVA to compare the statistical difference among the three groups, and compared the two groups using the Lsd-t test. The correlation between the elastic modulus of cervical shear wave and IMT, β, HbA1c was analyzed through Pearson correlation. $p < 0.05$ was considered to be statistically significant.

Results

General parameters

There was no significant difference in age, gender, HR, BP, FBG, TC, HDL-C, and LDL-C among the three groups (all $p > 0.05$). In group C, the BMI and HbA1 were higher than groups A and B, the difference was statistically significant (all $p < 0.05$), and there was no significant difference between groups A and B (all $p > 0.05$). There were significant differences in ALT, AST, and TG among the three groups (all $p < 0.05$); group C was higher than groups A and B, and group B was higher than group A (Shown in Table 1).

Table 1 Comparison of general clinical data between the three groups ($\overline{x} \pm s$)

Parameters	Group A (n = 35)	Group B (n = 33)	Group C (n = 30)
Age, years	46.1 ± 8.0	47.6 ± 7.4	45.4 ± 7.4
Male: female ratio	22/13	19/14	20/10
HR, rates/min	73.71 ± 7.73	75.88 ± 9.50	76.93 ± 10.23
BMI, kg/m^2	24.38 ± 2.08	25.50 ± 2.47	27.14 ± 3.34*$^{\#}$
Systolic BP, mmHg	125.46 ± 5.80	126.48 ± 6.47	128.13 ± 7.44
Diastolic BP, mmHg	75.54 ± 7.61	77.30 ± 5.80	78.97 ± 7.92
FBG, mmol/L	8.52 ± 1.63	8.86 ± 1.82	8.95 ± 2.07
HbA1c, %	7.97 ± 1.89	8.58 ± 2.10	9.85 ± 2.00*$^{\#}$
ALT, U/I	21.02 ± 6.21	34.00 ± 9.94*	51.18 ± 21.84*$^{\#}$
AST, U/I	17.48 ± 4.87	29.23 ± 5.27*	35.87 ± 10.99*$^{\#}$
TC, mmol/L	5.52 ± 1.02	5.20 ± 1.10	4.81 ± 1.30
TG, mmol/L	1.55 ± 0.60	2.03 ± 1.10*	2.60 ± 1.20*$^{\#}$
HDL-C, mmol/L	1.52 ± 0.64	1.35 ± 0.37	1.30 ± 0.32
LDL-C, mmol/L	2.82 ± 0.50	2.93 ± 0.77	3.04 ± 0.87

HR heart rate, *BMI* body mass index, *BP* blood pressure, *FBG* fasting blood glucose, *HbA1c* glycosylated hemoglobin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Compared with group A, *$p < 0.05$; compared with group B, $^{\#}p < 0.05$

Conventional ultrasound parameters

There was no difference in Ds, Dd, ΔD, and PSV among the three groups. In group C, IMT and β were higher than groups A and B (all $p < 0.05$), while there was no significant difference between groups A and B (all $p > 0.05$) (shown in Table 2).

SWE parameters

In groups B and C, the elastic modulus of the anterior wall of the carotid artery including MEmean, MEmax, and MEmin significantly increased than group A (all $p < 0.05$), and group C was higher than group B ($p < 0.05$) (shown in Table 3, Fig. 1). Correlation analysis showed that MEmax, MEmean, and MEmin were positively correlated with β ($r = 0.537$, 0.543, 0.525, $p < 0.01$), and MEmax, MEmean, and MEmin were also positively correlated with HbA1c ($R = 0.456$, 0.483, 0.438, $p < 0.01$) in each group, but there was no correlation among MEmax, MEmean, MEmin, and IMT (all $p > 0.05$).

Repetition test

The intraobserver ICC values of MEmax, MEmean, and MEmin measured by SWE ranged from 0.847 to 0.887, and interobserver ICC values of MEmax, MEmean, and MEmin by SWE ranged from 0.791 to 0.934, indicating good repeatability (Shown in Table 4).

Discussion

As an essential organ for fat metabolism, the liver fat content of healthy people was less than 3%. In clinical practice, NAFLD is usually defined as the abnormality of fat metabolism and ≥ 5% fatty infiltration of hepatocytes and liver tissues in the case of patients lacking other recognized causes of fatty liver (e.g., alcohol, virus, drugs, autoimmunity, etc.) [14]. The incidence of NAFLD in T2DM patients is up to three times higher than those ordinary people, and NAFLD also affects the occurrence and development of T2DM. There exists a complex bidirectional relationship between T2DM and NAFLD. Associated with a modest increase prevalence of cardiovascular disease, NAFLD is independent of traditional risk factors for cardiovascular diseases [4, 15]. The extent of carotid atherosclerosis can predict cardiac and macrovascular disease. Through various mechanisms, the T2DM patients with NAFLD can not only lead to carotid atherosclerosis, but also result in changes of carotid artery structure and function [16]. SWE, a new ultrasonic technology for non-invasive measurement of tissue hardness, mainly uses focused acoustic pulse to cause tissue displacement, thus inducing shear waves [17]. Ultrasound can capture these shear waves in real-time, and express tissue hardness by elastic modulus finally. Stiff tissue has a high modulus of elasticity. Previous studies have shown that SWE can be used to evaluate the elastography in carotid arterial in patients with hypertension [18], Behcet's disease [19], acute ischemic stroke [20], spontaneous coronary artery dissection [21], and other diseases.

Carotid atherosclerosis is the accumulation of fatty tissue in the artery wall, leading to the arterial wall thickens and stiffens, and the main manifestations of it were increased intima-media thickness (IMT), plaque formation, and decreased elasticity [22–24]. It is well known that carotid IMT is representative in responding to the occurrence and progression of early atherosclerosis. Targher et al. [25] used ultrasound to measure carotid IMT in 85 patients with biopsy-proven NAFLD, and the results showed that compared with healthy control group, the IMT of carotid artery in NAFLD patients increased obviously. Silaghi et al. [26] noted that compared with patients suffering NAFLD alone, patients with NAFLD and T2DM have higher prevalence of carotid atherosclerosis and their IMTs were significantly thicker. Our study found that the IMT in group C was thicker than those in groups A and B, and there exists statistical difference among

Table 2 Comparison of carotid artery conventional ultrasound parameters between the three groups ($\bar{x} \pm s$)

Parameters	Group A ($n = 35$)	Group B ($n = 33$)	Group C ($n = 30$)
IMT, mm	0.63 ± 0.11	0.69 ± 0.13	0.84 ± 0.18*#
Ds, mm	6.93 ± 0.50	7.04 ± 0.45	7.15 ± 0.37
Dd, mm	6.33 ± 0.44	6.46 ± 0.43	6.51 ± 0.37
ΔD, mm	0.61 ± 0.14	0.58 ± 0.09	0.57 ± 0.10
β	5.53 ± 1.83	5.58 ± 1.32	6.39 ± 1.30*#
PSV, cm/s	60.43 ± 10.70	58.90 ± 10.53	57.14 ± 10.00

IMT left carotid intima-media thickness, *Ds* standard carotid arterial systolic diameter, *Dd* standard carotid arterial diastolic diameter, ΔD Ds-Dd, *β* stiffness coefficient, *PSV* systolic peak velocity

Compared with group A, *$p < 0.05$; compared with group B, #$p < 0.05$

Table 3 Comparison of elastic modulus of carotid ultrasonic shear wave among the three groups ($\bar{x} \pm s$)

Parameters	Group A ($n = 35$)	Group B ($n = 33$)	Group C ($n = 30$)
MEmax, kPa	77.78 ± 11.36	87.20 ± 15.79*	101.70 ± 16.87*#
MEmean, kPa	62.73 ± 10.86	73.68 ± 12.23*	85.33 ± 13.38*#
MEmin, kPa	50.78 ± 10.67	61.65 ± 12.17*	71.85 ± 12.43*#

MEmean the mean values of mean elastic modulus, *MEmax* the maximum elastic modulus, *MEmin* the minimum elastic modulus

Compared with group A, *$p < 0.05$; compared with group B, #$p < 0.05$

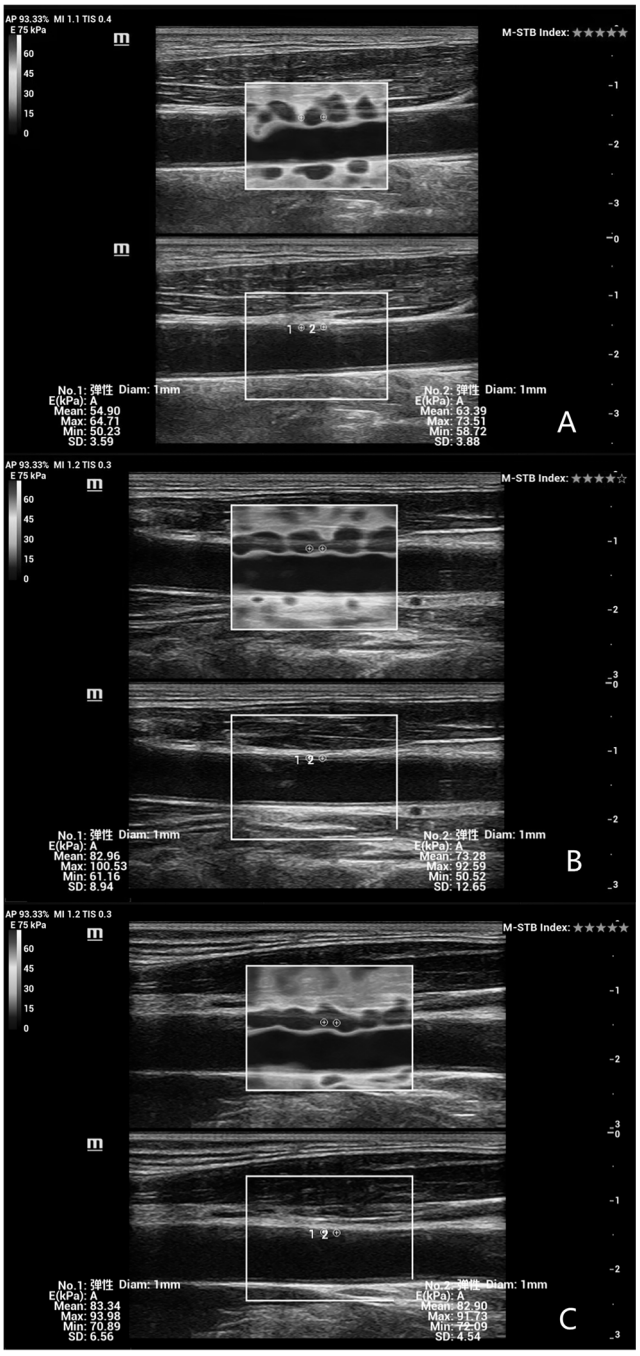

Fig. 1 The carotid shear wave elastic diagrams of the three groups are obtained by SWE. **A**, **B**, **C** represents groups A, B and C. Compared with groups A and B, the elastic modulus of the anterior wall of the carotid artery including MEmean, MEmax, and MEmin increased in group C. The high elastic modulus reflects the decline of carotid elasticity

the three groups. However, the IMT was still within the normal range, while there was no statistical difference between groups A and B. The result suggests that the carotid intima-media thickening in patients with T2DM and NAFLD is a long-term subclinical process, and carotid IMT tends to increase with the aggravation of NAFLD.

The carotid artery is divided into three layers: intima, media, and adventitia. The media is composed of thicker elastic fibers, there is no clear boundary between the intima and the media, and the adventitia is composed of connective tissue without elastic membrane. The early stage of carotid atherosclerosis is characterized by elastic degeneration of the intima-media membrane, vascular endothelial dysfunction, and arterial hyperelasticity, which usually precede the structural changes of IMT and others. The common mechanism of carotid atherosclerosis caused by T2DM and NAFLD is insulin resistance [27, 28]. The interaction between T2DM and NAFLD can lead to a vicious cycle of insulin resistance [29], accelerating carotid atherosclerosis. Insulin resistance can not only lead to blood glucose and lipid metabolism disorders seriously, but also release more inflammatory stimulators, causing vascular endothelial cell damage, vascular smooth muscle cells and connective tissue hyperplasia, and arterial wall stiffness ultimately. Salvi et al. [30], using PWV to evaluate the elasticity of carotid artery in fatty liver patients, noted that the elasticity of carotid artery could be reduced in patients with mild fatty liver. This study suggested that in groups B and C, MEmax, MEmean, and MEmin of carotid artery were higher than those in group A, and those in group C were higher than group B, indicating that the elasticity of carotid artery could be increased in T2DM patients with mild NAFLD. With the aggravation of NAFLD, the elasticity of carotid artery increased more significantly [31, 32], and was earlier than carotid artery IMT thickening. This result is consistent with the foreign literatures, suggesting that the elastic function changes of carotid artery were earlier than the structural changes [33]. In addition, compared with groups A and B, carotid artery β in group C was higher, and there was no statistical difference between groups A and B. Correlation analysis showed that MEmax, MEmean, and MEmin were positively correlated with β in each group, indicating that SWE can evaluate the carotid elasticity in T2DM patients with NAFLD with good repeatability and it is an effective new method for quantitative evaluation of the carotid elasticity.

Table 4 Reliability analysis of carotid elastic modulus measurement

	Intraobservers			Interobservers		
Parameters	ICC	95% consistency limit	*p*	ICC	95% consistency limit	*p*
MEmax	0.860	0.707–0.936	< 0.001	0.878	0.743–0.945	< 0.001
MEmaen	0.887	0.763–0.949	< 0.001	0.934	0.857–0.971	< 0.001
MEmin	0.847	0.683–0.930	< 0.001	0.791	0.585–0.902	< 0.001

NAFLD is defined as the liver manifestation of metabolic syndrome. The primary pathogenesis of NAFLD is based on insulin resistance, which is also considered to be essential pathogenesis of T2DM [34]. Therefore, glucose metabolism, lipid metabolism disorder, and body mass index show a significantly increasing trend with the increase of NAFLD grade [35]. Patients with NAFLD generally have no obvious clinical manifestations, most of them performed that ALT and AST elevated during liver function tests, mainly ALT [36]. Studies [37] have proven that serum ALT levels, significantly related to liver fat content and insulin resistance, can increase with the liver fat infiltration, and it is a reliable indicator for the diagnosis of NAFLD. Our study showed that there were significant statistical differences in ALT, AST, TG, BMI, and HbA1c among the three groups, and group C was higher than groups A and B. In indicating abnormal blood glucose, the level of HbA1c is more stable than FPG. Besides, HbA1c also can better reflect the average blood glucose level of human body. Studies suggested that HbA1c is not only an independent risk factor for NAFLD, but also strongly linked with the occurrence and development of carotid atherosclerosis [38, 39]. This may be because HbA1c can increase blood viscosity and slow down the dissociation rate of oxygenated hemoglobin, resulting in tissue hypoxia, aggravating the further injury of arterial endothelium, releasing more endothelial contractile factors, and ultimately causing diastolic endothelial dysfunction [40]. Furthermore, HbA1c causes the disorder of glucose and lipid metabolism, which can stimulate endothelial cells and vascular smooth muscle cells proliferation, promote lipid deposition, and accelerate the occurrence of atherosclerosis [41–43]. Correlation analysis showed that MEmax, MEmean, and MEmin were positively correlated with HbA1c, suggesting that HbA1c could promote the occurrence of carotid atherosclerosis in T2DM patients with NAFLD.

This study showed that SWE can quantitatively evaluate the carotid elasticity of T2DM patients with NAFLD.

Study limitations

The present study has some limitations. ① SWE is affected by respiratory movement, so it requires a high degree of coordination between operators and patients. ② The gold standard for diagnosing NAFLD is liver biopsy, while we used ultrasound in this study, meaning that hepatic steatosis may be missed when liver fat is less than 30%. ③ In our study, the sample size of patients was small. And due to rapid heart rate and curved carotid artery, there were about 20% patients not selected. The sample size should be expanded for further study in the future.

Conclusions

All in all, the carotid elasticity of patients is closely related to the occurrence and development of NAFLD and T2DM. The elasticity of carotid artery could be increased in T2DM patients with mild NAFLD. SWE, as a new technique, can quantitatively evaluate the carotid elasticity of the T2DM patients with NAFLD, which is of great significance to prevent cardiovascular diseases.

Acknowledgment We thank Prof. Li for guidance in study design and manuscript revision. In addition, we gratefully acknowledge the support and help from the Department of Ultrasound of the Second Affiliated Hospital of Dalian Medical University.

Declarations

Ethics approval The study acquired the agreement of the local ethics committee and all participants had given written informed consent.

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

References

1. Qi JC, Huang JC, Lin QC, Zhao JM, Lin X, Chen LD, Huang JF, Chen X. Relationship between obstructive sleep apnea and nonalcoholic fatty liver disease in nonobese adults. Sleep Breath. 2016;20:529–35. https://doi.org/10.1007/s11325-015-1232-9.
2. Zamirian MM, Samiee EM, Moaref AM, Abtahi FM, Tahamtan MM. Assessment of subclinical myocardial changes in nonalcoholic fatty liver disease: a case-control study using speckle tracking echocardiography. Iran J Med Sci. 2018;43:466–72.
3. Mavrogiannaki AN, Migdalis IN. Nonalcoholic Fatty liver disease, diabetes mellitus and cardiovascular disease: newer data. Int J Endocrinol. 2013;2013:450639. https://doi.org/10.1155/2013/450639.
4. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015;62:S47–64. https://doi.org/10.1016/j.jhep.2014.12.012.
5. Devaraj NK. Knowledge, attitude, and practice regarding obstructive sleep apnea among primary care physicians. Sleep Breath. 2020;24:1581–90. https://doi.org/10.1007/s11325-020-02040-1.
6. Lee KW, Loh HC, Ching SM, Devaraj NK, Hoo FK. Effects of vegetarian diets on blood pressure lowering: a systematic review with meta-analysis and trial sequential analysis. Nutrients. 2020;12. https://doi.org/10.3390/nu12061604.
7. Lee DG, Han JH, Kwon KY, Kim JH, Han KH, Lee EJ. Association of 10-year atherosclerotic cardiovascular disease risk score with carotid intima-media thickness and plaque. Korean J Fam Med. 2015;36:310–5. https://doi.org/10.4082/kjfm.2015.36.6.310.
8. Hou JS, Wang CH, Lai YH, Lin YL, Kuo CH, Subeq YM, Hsu BG. Negative correlation of serum adiponectin levels with carotid-femoral pulse wave velocity in patients treated with hemodialysis. Biol Res Nurs. 2018;20:462–8. https://doi.org/10.1177/1099800418768887.
9. Hekimoglu K, Yildirim Donmez F, Arslan S, Ozdemir A, Demir C, Yazici C. The role of shear wave elastography in the diagnosis of

chronic autoimmune thyroiditis. Med Ultrason. 2015;17:322–6. https://doi.org/10.11152/mu.2013.2066.173.khu.
10. Kim JR, Suh CH, Yoon HM, Lee JS, Cho YA, Jung AY. The diagnostic performance of shear-wave elastography for liver fibrosis in children and adolescents: a systematic review and diagnostic meta-analysis. Eur Radiol. 2018;28:1175–86. https://doi.org/10.1007/s00330-017-5078-3.
11. Zeng MD, Fan JG, Lu LG, Li YM, Chen CW, Wang BY, et al. Guidelines for the diagnosis and treatment of nonalcoholic fatty liver diseases. J Dig Dis. 2008;9:108–12. https://doi.org/10.1111/j.1751-2980.2008.00331.x.
12. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328–57. https://doi.org/10.1002/hep.29367.
13. Wang Y, Zhao C, Meng P, Yu Y, Li G, Kong F, Mu L, Yang J, Ma C. Incremental value of carotid elasticity modulus using shear wave elastography for identifying coronary artery disease in patients without carotid plaque. J Hypertens. 2021;39:1210–20. https://doi.org/10.1097/hjh.0000000000002773.
14. Khneizer G, Rizvi S, Gawrieh S. Non-alcoholic fatty liver disease and diabetes mellitus. Adv Exp Med Biol. 2021;1307:417–40. https://doi.org/10.1007/5584_2020_532.
15. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. Curr Diab Rep. 2015;15:607. https://doi.org/10.1007/s11892-015-0607-4.
16. George JM, Bhat R, Pai KM, Arun S, Jeganathan J. The carotid intima media thickness: a predictor of the clincal coronary events. J Clin Diagn Res. 2013;7:1082–5. https://doi.org/10.7860/jcdr/2013/4767.3029.
17. Tada T, Kumada T, Toyoda H, Ito T, Sone Y, Okuda S, Tsuji N, Imayoshi Y, Yasuda E. Utility of real-time shear wave elastography for assessing liver fibrosis in patients with chronic hepatitis C infection without cirrhosis: Comparison of liver fibrosis indices. Hepatol Res. 2015;45:E122–9. https://doi.org/10.1111/hepr.12476.
18. Marais L, Pernot M, Khettab H, Tanter M, Messas E, Zidi M, Laurent S, Boutouyrie P. Arterial stiffness assessment by shear wave elastography and ultrafast pulse wave imaging: comparison with reference techniques in normotensives and hypertensives. Ultrasound Med Biol. 2019;45:758–72. https://doi.org/10.1016/j.ultrasmedbio.2018.10.032.
19. Alis D, Durmaz ESM, Civcik C, Tutuncu M, Saip S, Kocer N, et al. Assessment of the common carotid artery wall stiffness by Shear Wave Elastography in Behcet's disease. Med Ultrason. 2018;20:446–52. https://doi.org/10.11152/mu-1565.
20. Li Z, Du L, Wang F, Luo X. Assessment of the arterial stiffness in patients with acute ischemic stroke using longitudinal elasticity modulus measurements obtained with Shear Wave Elastography. Med Ultrason. 2016;18:182–9. https://doi.org/10.11152/mu.2013.2066.182.wav.
21. Al-Mutairi FF, Al-Hussaini A, Marsh AM, Samani N, McCann G, Adlam D, et al. Ultrasound shear wave elastography imaging of common carotid arteries in patients with Spontaneous Coronary Artery Dissection (SCAD). J Ultrasound. 2022. https://doi.org/10.1007/s40477-021-00627-2.
22. Simon A, Megnien JL, Levenson J. Coronary risk estimation and treatment of hypercholesterolemia. Circulation. 1997;96:2449–52. https://doi.org/10.1161/01.cir.96.7.2449.
23. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. J Hepatol. 2008;49:600–7. https://doi.org/10.1016/j.jhep.2008.06.012.
24. Abdallah LR, de Matos RC, YPDM ES, Vieira-Soares D, Muller-Machado G, Pollo-Flores P. Non-alcoholic fatty liver disease and its links with inflammation and atherosclerosis. Curr Atheroscler Rep. 2020;22:7. https://doi.org/10.1007/s11883-020-0820-8.
25. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, Cigolini M, Falezza G, Arcaro G. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care. 2006;29:1325–30. https://doi.org/10.2337/dc06-0135.
26. Silaghi CA, Silaghi H, Crăciun AE, Fărcaş A, Colosi HA, Cosma DT, et al. Age, abdominal obesity, and glycated hemoglobin are associated with carotid atherosclerosis in type 2 diabetes patients with nonalcoholic fatty liver disease. Med Ultrason. 2015;17:300–7. https://doi.org/10.11152/mu.2013.2066.173.cas.
27. Fruci B, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic fatty liver: a possible new target for type 2 diabetes prevention and treatment. Int J Mol Sci. 2013;14:22933–66. https://doi.org/10.3390/ijms141122933.
28. Mazzotti A, Caletti MT, Marchignoli F, Forlani G, Marchesini G. Which treatment for type 2 diabetes associated with non-alcoholic fatty liver disease? Dig Liver Dis. 2017;49:235–40. https://doi.org/10.1016/j.dld.2016.12.028.
29. Tanase DM, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, Ouatu A, Floria M. The intricate relationship between type 2 diabetes mellitus (T2DM), insulin resistance (IR), and nonalcoholic fatty liver disease (NAFLD). J Diabetes Res. 2020;2020:3920196. https://doi.org/10.1155/2020/3920196.
30. Salvi P, Ruffini R, Agnoletti D, Magnani E, Pagliarani G, Comandini G, Praticò A, Borghi C, Benetos A, Pazzi P. Increased arterial stiffness in nonalcoholic fatty liver disease: the Cardio-GOOSE study. J Hypertens. 2010;28:1699–707. https://doi.org/10.1097/HJH.0b013e32833a7de6.
31. Sinn DH, Cho SJ, Gu S, Seong D, Kang D, Kim H, et al. Persistent nonalcoholic fatty liver disease increases risk for carotid atherosclerosis. Gastroenterology. 2016;151:481–8.e1. https://doi.org/10.1053/j.gastro.2016.06.001.
32. Oni E, Budoff MJ, Zeb I, Li D, Veledar E, Polak JF, Blankstein R, Wong ND, Blaha MJ, Agatston A, Blumenthal RS, Nasir K. Nonalcoholic fatty liver disease is associated with arterial distensibility and carotid intima-media thickness: (from the Multi-Ethnic Study of Atherosclerosis). Am J Cardiol. 2019;124:534–8. https://doi.org/10.1016/j.amjcard.2019.05.028.
33. Zhang L, Yin JK, Duan YY, Liu X, Xu L, Wang J, Yang YL, Yuan LJ, Cao TS. Evaluation of carotid artery elasticity changes in patients with type 2 diabetes. Cardiovasc Diabetol. 2014;13:39. https://doi.org/10.1186/1475-2840-13-39.
34. Watt MJ, Miotto PM, De Nardo W, Montgomery MK. The liver as an endocrine organ-linking NAFLD and insulin resistance. Endocr Rev. 2019;40:1367–93. https://doi.org/10.1210/er.2019-00034.
35. Utzschneider KM, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2006;91:4753–61. https://doi.org/10.1210/jc.2006-0587.
36. Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, Obermayer-Pietsch B, Stauber RE. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. Liver Int. 2012;32:845–51. https://doi.org/10.1111/j.1478-3231.2011.02735.x.
37. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab. 2007;92:3490–7. https://doi.org/10.1210/jc.2007-0482.
38. Bae JC, Cho YK, Lee WY, Seo HI, Rhee EJ, Park SE, Park CY, Oh KW, Sung KC, Kim BI. Impact of nonalcoholic fatty liver disease on insulin resistance in relation to HbA1c levels in nondiabetic subjects. Am J Gastroenterol. 2010;105:2389–95. https://doi.org/10.1038/ajg.2010.275.
39. Mo Y, Zhou J, Ma X, Zhu W, Zhang L, Li J, Lu J, Hu C, Bao Y, Jia W. Haemoglobin A1c variability as an independent correlate of

atherosclerosis and cardiovascular disease in Chinese type 2 diabetes. Diab Vasc Dis Res. 2018;15:402–8. https://doi.org/10.1177/1479164118778850.

40. James PE, Lang D, Tufnell-Barret T, Milsom AB, Frenneaux MP. Vasorelaxation by red blood cells and impairment in diabetes: reduced nitric oxide and oxygen delivery by glycated hemoglobin. Circ Res. 2004;94:976–83. https://doi.org/10.1161/01.Res.0000122044.21787.01.
41. Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes. 1989;38:1539–43. https://doi.org/10.2337/diab.38.12.1539.
42. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005;111:3481–8. https://doi.org/10.1161/circulationaha.105.537878.
43. Li H, Cui Y, Zhu Y, Yan H, Xu W. Association of high normal HbA1c and TSH levels with the risk of CHD: a 10-year cohort study and SVM analysis. Sci Rep. 2017;7:45406. https://doi.org/10.1038/srep45406.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):199–207
https://doi.org/10.1007/s13410-022-01079-y

ORIGINAL ARTICLE

Basal-bolus insulin therapy for the treatment of non-critically ill patients with type 2 diabetes in Vietnam: effectiveness and factors associated with inpatient glycemic control

Mai Ngoc Thi Tran[1,2] · Khiem Tran Dang[3,4] · Luong Dai Ly[2,5] · Nam Quang Tran[1,6]

Received: 15 October 2021 / Accepted: 20 April 2022 / Published online: 7 May 2022

Abstract

Purpose This study assessed the effectiveness of basal-bolus insulin therapy (BBIT) in non-critically ill patients with type 2 diabetes mellitus (DM) and the factors associated with optimal inpatient glycemic control (IGC) with BBIT.

Methods This prospective study included 103 patients who were admitted to the University Medical Center and were treated with BBIT. Clinical characteristics, glucose, and glycated hemoglobin (HbA1c) levels at admission, renal function tests, basal-bolus insulin dosing, and other treatments were recorded. The optimal IGC was defined and classified for the analysis.

Results The mean age of the patients was 67.2 ± 12.0 years. The blood glucose and HbA1c levels at admission were 319.2 ± 184.8 mg/dL and 10.7 ± 2.6%, respectively. Optimal IGC was defined as patients with ≥60% of in-hospital blood glucose values within the target range (3.9–10 mmol/L). Of the 103 patients, 66 patients (64%) achieved optimal IGC and only 5 patients (4.9%) had at least one hypoglycemic episode. The number of patients consuming snacks was higher in the poor than in the optimal IGC group whereas an estimated glomerular filtration rate (eGFR) <45-mL/min/1.73 m^2 was predominant in the optimal IGC group. Multivariate analysis revealed that snack consumption and glucocorticoid (GC) use were factors associated with poor IGC, while eGFR <45 mL/min/1.73 m^2 was a favorable factor for optimal IGC.

Conclusion BBIT is safe and effective for the treatment of IGC in non-critically ill patients. Moreover, eGFR <45 mL/min/1.73 m^2 at admission, snack consumption, and GC therapy were independent factors associated with IGC outcomes.

Keywords Type 2 diabetes mellitus · Inpatient glycemic control · Basal–bolus insulin therapy · Snack consumption · Glucocorticoid use

Introduction

Patients diagnosed with diabetes mellitus (DM) have a three-fold higher rate of hospitalization than those without DM [1]. Hyperglycemia (blood glucose [BG] level > 140 mg/dL [7.8 mmol/L]) has been reported in 22–46% of non-critically ill-hospitalized patients [2, 3]. Inpatient hyperglycemia is associated with increased morbidity and mortality, longer hospital stay, higher transfer rate to the intensive care unit, and greater need for transitional or nursing home care after discharge [3].

Insulin regimens are important for inpatient glycemic control (IGC) in non-critically ill patients with type 2 DM [2, 4].

Protocol identifying number: code 584/ University of Medicine and Pharmacy – BOARD, October 31, 2019.

✉ Nam Quang Tran
nam.tq@umc.edu.vn

[1] Department of Endocrinology, University of Medicine and Pharmacy at Ho Chi Minh City, 217 Hong Bang, Ward 11, District 5, Ho Chi Minh City, Vietnam

[2] Endocrinology Clinic, My Duc Hospital, Ho Chi Minh City, Vietnam

[3] Department of Surgery, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

[4] Grant and Innovation Center, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

[5] Department of Endocrinology, Vietnam National University, Ho Chi Minh City, Vietnam

[6] Department of Endocrinology, University Medical Center at Ho Chi Minh City, Ho Chi Minh City, Vietnam

Subcutaneous insulin therapy, which comprises a basal long-acting insulin dose (glargine, detemir, or degludec) once daily or an intermediate-acting insulin (neutral protamine Hagedorn) twice daily alone or in combination with short- (regular) or rapid-acting insulin (lispro, aspart, or glulisine) before meals, is preferred for IGC in these patients [2, 5]. This combination regimen results in better BG levels [6–8], lower frequency of hypoglycemic events [9], and fewer hospital complications [7] than other insulin regimens. This regimen, also called basal-bolus insulin therapy (BBIT), is the potential treatment of choice for maintaining BG levels in the range of 6.1–10 mmol/L in non-critically ill patients.

Although BBIT has been studied and used worldwide for over a decade and its effectiveness is universally accepted, it has not yet been widely applied in Vietnamese hospitals. In many Asian countries in general, and Vietnam in particular, premixed insulin is still predominantly used for inpatient glycemic control [10, 11]. BBIT has gained popularity over the last 5 years in some large medical centers. Therefore, no report on BBIT outcomes has been published in Vietnam. To date, there are recommendations only for the BG target for inpatient hyperglycemia management, but no criteria are available for optimal IGC in any guidelines or consensus, especially when the BBIT was utilized [2]. In practice, the threshold for optimal IGC is often chosen by each author. In our own experience, optimal IGC was confirmed if patients had more than or equal to 60% of their in-hospital BG values falling within the target range (3.9–10 mmol/L). We aimed to determine the effectiveness of BBIT using a new definition of optimal IGC in non-critically ill patients with type 2 DM in Vietnam.

In general, IGC seems to be significantly associated with preadmission diabetes control (HbA1c and admission BG) and inpatient glucocorticoid use [12, 13]. However, there are not many studies on the predictors of IGC when using BBIT in non-critically ill patients with type 2 DM both worldwide and in Vietnam.

Hence, we conducted this study to assess the effectiveness of BBIT with a new definition of optimal IGC and to identify the factors that affect IGC in Vietnamese patients with type 2 DM in a non-intensive care setting.

Materials and methods

Study design

This prospective study was approved by the University of Medicine and Pharmacy at Ho Chi Minh city Ethics Committee on October 31, 2019, and conducted from November 2019 to June 2020 at the Endocrinology Department of the University Medical Center (UMC) in Ho Chi Minh City, Vietnam.

Patients aged ≥18 years were enrolled if type 2 DM diagnosis was confirmed and they had been previously treated with diet management, hypoglycemic agents, and insulin. All patients had experienced a hospital stay of at least 6 days. The exclusion criteria included type 1 DM diagnosis, pregnancy, acute hyperglycemic emergencies (diabetic ketoacidosis and hyperosmolar hyperglycemic syndrome), severe hyperglycemia requiring intravenous insulin infusion on admission, continuous enteral and parenteral nutrition, change in nutritional support during the study period, hypoglycemia on admission, surgical interventions, ongoing corticosteroid therapy (>40 mg/day of equivalent hydrocortisone), and mental health conditions which prevented patients from providing informed consent (Fig. 1).

Procedures

The patients were initially treated with BBIT according to an institutionally approved protocol on admission (Appendix). All oral hypoglycemic agents, including metformin and dipeptidyl peptidase-4 inhibitors, were discontinued. The daily insulin dose (DID) was based on the total DID before admission or the patient's body weight. BBIT was administered subcutaneously as follows:

Patients treated with insulin before admission were administered their previous total DID.

The starting DID for patients treated with diet management or oral hypoglycemic agents before admission was as follows.

- Insulin-sensitive (thin stature, poor appetite, elderly, presence of chronic kidney disease, and no history of insulin treatment): 0.3 units/kg
- Insulin-resistant (obese, presence of infection, ongoing glucocorticoid [GC] treatment): 0.5–1 units/kg
- No characteristics of insulin sensitivity/resistance: 0.4 units/kg

Half of the DID was prescribed as basal insulin glargine (100 units/mL; LANTUS® SoloStar®, Sanofi S.A., Paris, France) once daily at the same time. The other half was prescribed regular insulin (100 units/mL; Actrapid® HM, Novo Nordisk A/S, Bagsværd, Denmark), divided into equal doses administered before meals. Preprandial rapid-acting insulin was administered until oral food intake could prevent hypoglycemia in patients who were unable to eat. Patients received supplemental (correction) doses of insulin before meals and at bedtime, according to a previously used protocol (Appendix).

Capillary BG was measured four times a day if the patients could consume three meals per day: fasting, 5 a.m.–6 a.m.; preprandial, 10 a.m.–11 a.m.; afternoon, 4 p.m.–5 p.m.; and postprandial or at bedtime, around 9 p.m.–10 p.m. Pre-meal capillary BG level was measured in patients receiving supported enteral nutrition. The BG test was performed using a

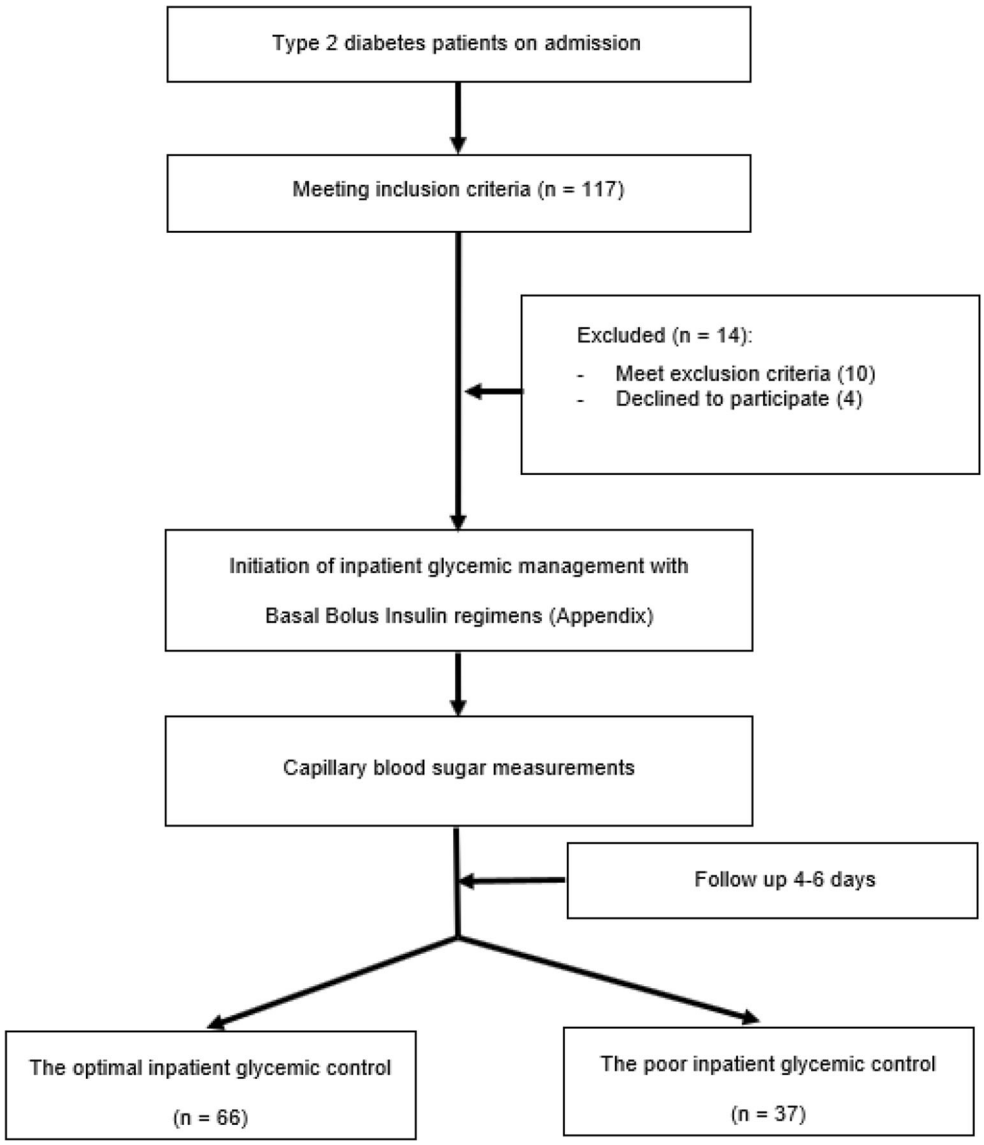

Fig. 1 Study flowchart

point-of-care glucose meter (AccuChek Performa, Roche Diagnostics, Basel, Switzerland). Additionally, BG was checked whenever a patient showed signs of hypoglycemia, according to the Endocrine Society 2012 guidelines [2]. Laboratory tests, including basic biochemical studies, lipid profiles, and HbA1c tests, were conducted for all patients on the first day.

Capillary BG of the patients was monitored for at least 4 days and up to 6 days after admission. The capillary BG levels recorded on the first day after admission (day 0) were excluded from the dataset because of multiple confounding factors that might affect BG concentration on admission, such as stress, acute illnesses, decompensated conditions of chronic diseases, changes in nutritional support, and pre-hospitalized medical treatments. Day 0 represents an unstable period of BG control and might not reflect the quality of inpatient care. Moreover, a supplemental (correction) insulin dose was not administered on day 0. Capillary BG levels on the second day (day 1) were included in the analyses.

Definition of variables

The target BG level in the range of 3.9–10 mmol/L was defined as the optimal IGC.

Hypoglycemia and hyperglycemia were defined as BG < 3.9 mmol/L and > 10.0 mmol/L, respectively. Severe hypoglycemia was defined as the loss of consciousness and/or seizures.

BBIT effectiveness was assessed using a "patient model." It was an analytic model that employed all the BG values of a patient collected during their hospitalization, except for day 0, to calculate the following parameters: mean BG measurement during each patient's in-hospital stay, percentage of BG values within the optimal range

(3.9–10 mmol/L), and percentage of BG values < 3.9 mmol/L and >10.0 mmol/L. In another model, the "patient-day model," the BG values of each patient were categorized according to the calendar days. The mean patient-day glucose level was calculated as the mean BG per day. The percentage of BG values between 3.9 mmol/L and 10.0 mmol/L per patient per day was also included. These events were confirmed by a time point fingerstick BG monitoring method rather than using the mean values.

Patients were divided into optimal and poor IGC groups after receiving BBIT. The former included patients with ≥60% of their in-hospital BG values within the target range (3.9–10 mmol/L).

To assess the factors associated with IGC in patients treated with BBIT, we analyzed factors including the duration of diabetes, HbA1c, admission blood glucose, infection, high waist circumference, estimated glomerular filtration rate, inpatient glucocorticoid therapy, and snack consumption.

A snack in a hospital is any carbohydrate-containing meal that is not part of the "in-hospital diet" formulated by dieticians and endocrinologists. Snack consumption was confirmed by caregiver observations or patient statements during in-hospital care. Snack consumption was defined as consumption of snacks for more than 40% of the total follow-up days in the hospital.

Inpatient glucocorticoid therapy was defined as an inpatient receiving glucocorticoid therapy for more than 40% of the total follow-up days in the hospital.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range) depending on the data distribution. Categorical variables were presented as frequencies and percentages. The Kolmogorov–Smirnov test was used to determine the normality of the distributions. Comparisons between continuous variables were performed using two-tailed independent Student's t-test or Mann–Whitney *U* test. Categorical variables were analyzed using chi-square tests with Yates correction or Fisher's exact test. Logistic regression was performed to explore independent risk factors associated with poor IGC.

All statistical analyses were performed using STATA IC 14 (StataCorp LLC, College Station, TX, USA); *p*-values < 0.05 were considered statistically significant.

Results

Table 1 presents the demographic characteristics. The mean age of enrolled patients was 67.2 ± 12.0 years. The mean duration since DM diagnosis was 12.5 ± 9.4 years. Nearly two-thirds of the patients were overweight or obese (body mass index [BMI] ≥ 23 kg/m^2 for Asians), with an average BMI of 24 kg/m^2. Patients using insulin before hospitalization accounted for 62.1% of the sample, and premixed insulin therapy was the most common treatment (49.5%). The BG and HbA1c levels at admission were 319.2 ± 184.8 mg/dL and 10.7 ± 2.6%, respectively. Infectious diseases in 68 patients (66%) were the main diagnoses during admission.

Glycemic control

The mean BG level in this patient model was 10.7 ± 3.1 mmol/L. The percentage distribution of BG values < 3.9 mmol/L, 3.9–10.0 mmol/L, and > 10.0 mmol/L were 0.3 ± 1.4%, 57.3 ± 31.5%, and 42.4 ± 31.8%, respectively (Table 1).

The patient-day model revealed each patient's BG level on the calendar day. The daily BG levels showed a downward trend, wherein the BG level on day 3 was significantly lower than that of day 0 (10.4 ± 3.1 vs. 12.5 ± 3.6 mmol/L; $p < 0.001$) (Fig. 2). However, the percentage of BG values within the target range on day 3 was higher than that on day 0 (60.6 ± 36.3% vs. 34.9 ± 36.1%; $p < 0.001$) (Fig. 3).

Regarding BG control, 64.1% (66/103) of the patients presented optimal IGC. Additionally, 4.9% (5/103) of the patients had at least one episode of hypoglycemia during hospitalization, and all of them belonged to the optimal IGC group (7.6%). No hypoglycemic cases were observed in the poor IGC group. Moreover, no severe hypoglycemia-related complications (unconsciousness or seizures) were observed in either group.

Differences between Optimal IGC and Poor IGC groups

The optimal and poor IGC groups included 66 and 37 patients, respectively (Fig. 1).

The BG and HbA1c levels on admission did not differ statistically between the two groups ($p = 0.33$ and $p = 0.11$, respectively) (Table 1). The percentage of patients with estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m^2 was significantly lower (13.5% vs. 36.4%, $p = 0.01$) and that of patients consuming snacks was four-fold higher (67.6% vs. 15.2%, $p < 0.001$) in the poor IGC group than in the optimal IGC group. According to the optimal IGC subgroups' analysis, patients with eGFR < 45 mL/min/1.73 m^2 required lower DID than those with eGFR ≥ 45 mL/min/1.73 m^2 (0.74 ± 0.04 vs. 0.65 ± 0.05 units/kg/day; $p = 0.21$). Inpatients received nutritional support or diabetes-care education equally, regardless of group ($p = 0.416$ and $p = 1.000$, respectively). Regarding the use of GCs, the poor IGC patients outnumbered the optimal IGC patients (40.5% vs. 25.8%), but without a

Table 1 Clinical and paraclinical characteristics of inpatients receiving basal-bolus insulin therapy

Variables	All patients (n = 103)	Optimal IGC (n = 66)	Poor IGC (n = 37)	P-value
Demographic and preadmission data				
Age (years)	67.2 ± 12.0	68.8 ± 10.5	64.4 ± 14.1	0.291
Female (%)	63 (61.2)	38 (57.6)	25 (67.6)	0.318
Duration of diabetes (years)	12.5 ± 9.4	13.3 ± 9.3	10.9 ± 9.5	0.162
BMI (kg/m^2)	24 ± 4.9	23.9 ± 3.4	25.7 ± 5.0	0.178
High waist circumference[a]	52 (50.5)	30 (45.5)	22 (59.5)	0.173
Diabetes therapy before hospital admission				0.541
Diet management alone	9 (8.7)	4 (6.1)	5 (13.5)	
Oral hypoglycemic agents	30 (29.1)	19 (28.8)	11 (29.7)	
Insulin alone	33 (32.0)	21 (31.8)	12 (32.4)	
Insulin with oral hypoglycemic agents	31 (30.1)	22 (33.3)	9 (24.3)	
Insulin dose before hospitalization (units/kg/day)[b]	0.7 ± 0.3	0.6 ± 0.4	0.7 ± 0.3	0.327
In-hospital care characteristics				
BG on admission (mg/dL)	319.2 ± 184.8	300.2 ± 165.9	353.1 ± 212.6	0.334
HbA1c (%)	10.7 ± 2.6	10.4 ± 2.6	11.3 ± 2.5	0.106
eGFR <45 mL/min/1.73 m^2, (%)	29 (28.2)	24 (36.4)	5 (13.5)	0.013*
I nfectious diseases (%)	68 (66.0)	45 (68.2)	23 (62.2)	0.536
Nutritional regimen				0.416
Consume three meals	97 (94.2)	61 (92.4)	36 (97.3)	
Enteral nutrition by nasogastric tube	6 (5.8)	5 (7.6)	1 (2.7)	
Snack consumption	35 (34.0)	10 (15.2)	25 (67.6)	<0.001*
Glucocorticoid therapy	32 (31.1)	17 (25.8)	15 (40.5)	0.120
Diabetes-care education	99 (96.1)	63 (95.5)	36 (97.3)	1.000
Mean BG (mmol/L)	10.7 ± 3.1	8.8 ± 1.2	14.1 ± 2.5	<0.001*
Mean percentage of BG values (%)				
3.9–10.0 mmol/L	57.3 ± 31.5	78.3 ± 11.7	19.9 ± 17.6	<0.001*
>10.0 mmol/L	42.4 ± 31.8	21.2 ± 12.0	80.1 ± 17.6	<0.001*
<3.9 mmol/L	0.3 ± 1.4	0.5 ± 1.7	0 ± 0	<0.001*
Insulin treatment (BBIT protocol)				
Total DID (units/kg/day)	0.72 ± 0.27	0.70 ± 0.25	0.75 ± 0.31	0.505
Total DID (units/day)	43.6 ± 15.1	41.6 ± 14.1	47.3 ± 16.3	0.091
Basal DID (units/day)	18.3 ± 6.5	17.8 ± 6.6	19.2 ± 6.2	0.256
Percentage of basal DID (%)	42.1 ± 4.9	42.8 ± 5.2	40.9 ± 4.0	0.039*
Bolus DID (units/day)	25.3 ± 9.3	23.8 ± 8.2	28.1 ± 10.6	0.073
Percentage of bolus DID (%)	57.9 ± 4.9	57.2 ± 5.2	59.1 ± 4.0	0.039*

Categorical and continuous variables are presented as numbers (percentages) and mean ± standard deviation, respectively. *BMI* body mass index, *BG* blood glucose, *eGFR* estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration equation, *HbA1c* glycated hemoglobin, *IGC* inpatient glycemic control, *BBIT* basal-bolus insulin therapy, *DID* daily insulin dose. Statistical significance was set at $p < 0.05$

[a] Waist circumference >90 cm in males and >80 cm in females

[b] Calculated for 64 patients treated with insulin before admission

statistically significant difference in the univariate analysis (p = 0.12). However, in the multivariate analysis, the GC use was significantly associated with the poor IGC outcomes (odds ratio [OR] = 2.6, 95% confidence interval, 1.0–6.8; p = 0.048) (Table 2).

Regarding insulin administration, patients with poor IGC required a higher total DID, basal DID, bolus DID, and DID (units/kg/day) than the optimal IGC group, but the difference was not statistically significant ($p > 0.05$) (Table 1). Furthermore, the percentage of bolus DID was higher and that

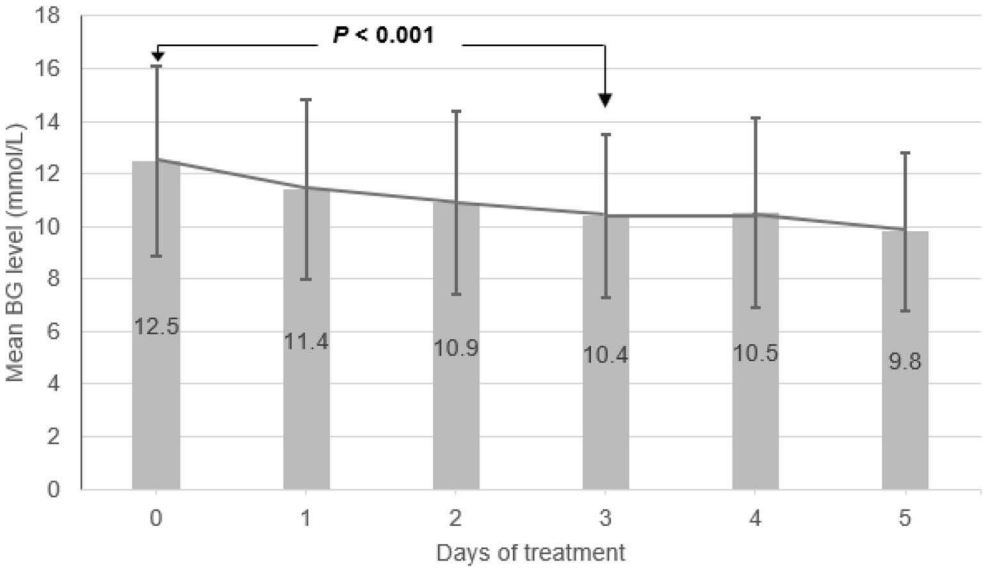

Fig. 2 Changes in the mean daily blood glucose level over the treatment days. The daily BG levels showed a downward trend, wherein the BG level on day 3 was significantly lower than that of day 0 (10.4 ± 3.1 vs. 12.5 ± 3.6 mmol/L; p <0.001). *BG: blood glucose*

of basal DID was lower in the poor IGC group than in the optimal IGC group (p = 0.04) (Table 1).

Discussion

Despite its worldwide application in recent decades, BBIT has only been introduced in Vietnamese healthcare centers recently. UMC is one of the few hospitals in which this insulin regimen has been applied for inpatient treatment. Thus, this is a pioneering study on the effectiveness of BBIT and the factors associated with IGC when using this regimen in non-critically ill patients with type 2 DM in Vietnam.

The principal standard of effective glycemic treatment is the outcome of IGC. Previous researchers have selected various thresholds to determine IGC. Goldberg et al. set a very high cutoff (≥ 85% of BG values should be in the target range) to qualify as optimal IGC [14]. Pasquel et al. included those patients with approximately 60% of BG values in the 3.9–10.0 mmol/L range in the IGC group [15]. Our center chose a cutoff of 60% of BG values within the target range to qualify for the optimal IGC group. In our opinion, this threshold is not too high to accomplish, and is a reasonable treatment goal. In clinical practice, there are recommendations only for the BG target for inpatient hyperglycemia management, whereas there are no criteria available for optimal IGC in any guidelines or consensus [2]. Thus, the IGC cutoff depends mainly on the experience of each research team.

Based on our definition of optimal IGC, 64.1% of our patients achieved optimal IGC during hospitalization, of whom more than half had no hypoglycemic events. The Randomized Study of BBIT (RABBIT 2) trial reported 66%

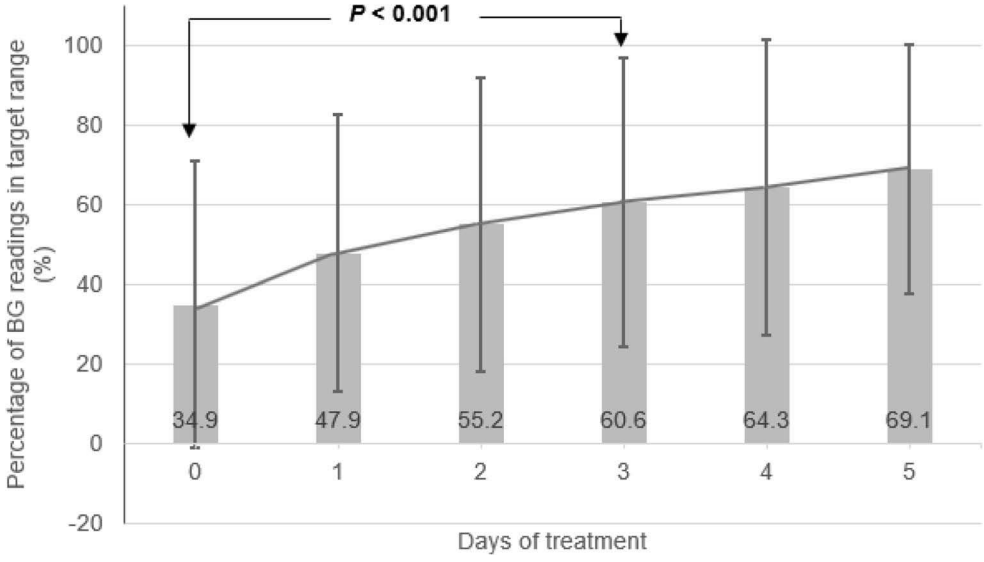

Fig. 3 Changes in the mean daily percentage of blood glucose values in the range of 3.9–10.0 mmol/L. The percentage of BG values in the range of 3.9–10.0 mmol/L showed an upward trend, wherein the percentage of BG within the target range on day 3 was higher than that on day 0 (60.6 ± 36.3% vs. 34.9 ± 36.1%; p <0.001). *BG: blood glucose*

Table 2 Independent predictors of poor inpatient glycemic control after multivariate analysis

Variables	Odds ratio	(95% confidence interval)		*P*-value
		Lower	Upper	
Duration of diabetes (years)	1.0	0.95	1.1	0.783
HbA1c (%)	2.4	0.9	6.3	0.074
Infection				
No	(Reference)			
Yes	0.9	0.3	2.3	0.78
High waist circumference[a]				
No	(Reference)			
Yes	2.5	0.9	6.2	0.056
eGFR <45 mL/min/1.73 m^2 at admission				
No	(Reference)			
Yes	0.3	0.1	0.8	0.026*
Glucocorticoid therapy				
No	(Reference)			
Yes	2.6	1.0	6.8	0.048*
Snack consumption				
No	(Reference)			
Yes	11.1	3.4	26.3	<0.001*

IGC inpatient glycemic control, *eGFR* estimated glomerular filtration rate using Chronic Kidney Disease Epidemiology Collaboration equation, *HbA1c* glycated hemoglobin. Statistical significance was set at $p < 0.05$

[a] Waist circumference >90 cm in males and >80 cm in females

of BBIT-induced patients had optimal BG levels (< 140 mg/dL) [8]. There was little difference in the optimal IGC results between the RABBIT 2 trial and our study, except for the optimal IGC definition. The RABBIT 2 trial compressed each patient's BG values during the entire hospital stay into a single mean value. This maneuver did not reflect the BG variability throughout the follow-up period and underestimated the relative impact of the extended length of stay on the patient's outcomes. In contrast, our research used the "patient model" to calculate the percentage of in-hospital BG values for each patient in the target range. This model provided a balanced approach that attributed a patient's IGC to each BG value, BG monitoring frequency, and length of stay. The IGC outcomes in our study seemed to be better than the outcomes in Pérez's study, which demonstrated that 47.7% of the patients achieved a fasting BG between 90 and 130 mg/dL, and 30.2% presented postprandial BG ≤ 180 mg/dL [16].

In our study, 4.9% of the patients had at least one hypoglycemic episode, which was not very high as compared to the 3% recorded in the RABBIT 2 trial (hypoglycemic cutoff < 60 mg/dL) [8]. Zaman Huri et al. presented similar results: 2.5% of patients had hypoglycemia (< 60 mg/dL) and 5.7% of patients had BG levels < 70 mg/dL [17]. The variation of "optimal" BG outcomes and hypoglycemic rates could be attributed to the different optimal IGC definitions and the different hypoglycemic cutoffs used in certain studies. Regardless of the hypoglycemic cutoff, the incidence of hypoglycemia was low in BBIT-treated populations. This conclusion is similar to that of previous BBIT studies. Based on our institutional BBIT protocol, regular BG monitoring and correction of bolus insulin dose before each meal contributed to reducing the hypoglycemic incidence in patients with poor appetite (see Appendix). The low incidence of hypoglycemia in our department could be attributed to many factors. First, the fear of hypoglycemia seemed to be a major barrier to improving IGC. Physicians tend to prescribe lower doses of starting insulin and correction doses to ensure that their patients do not experience a hypoglycemic event. Second, up to one third of the study population consumed snacks during their hospital stay, although it was not recommended to consume additional food between the main repasts. Snack consumption protected DM patients from hypoglycemia but negatively affected IGC. Finally, patients might have had asymptomatic hypoglycemia during periods when capillary BG levels were not checked. Such hypoglycemic episodes can be detected through continuous glucose monitoring.

Similar to other Asian population studies, such as the study by Yamamoto et al [18], our study proved that bolus DID accounted for the majority of the total DID. Such a high bolus DID is derived from the adjustment of the pre-prandial DID. This insulin dose was often higher than the basal DID if we

refer to the guideline for BBIT initiation that recommends a 1:1 basal-to-bolus ratio. The high bolus DID could be attributed to elevated postprandial glycemia owing to the carbohydrate-rich ingredients used in Asian recipes.

Multivariate analyses showed that there were some independent factors associated with IGC outcomes in BBIT-treated patients, including eGFR < 45 mL/min/1.73 m^2 (p = 0.026), in-hospital snack consumption (p < 0.001), and GC use (p = 0.048). These outcomes nearly matched those of the univariate analyses, wherein snack consumption was a predictive factor associated with poor IGC, while eGFR < 45 mL/min/1.73 m^2 was a favorable factor for optimal IGC. In patients diagnosed with diabetic kidney disease (DKD), a large proportion of exogenous insulin (30–80%) is metabolized and degraded by the kidneys as compared to the non-DKD patients [19]. Therefore, it is necessary to decrease the total DID to avoid hypoglycemia in these patients [20, 21]. Type-2 DM patients with reduced eGFR tend to achieve the BG target with a lower total DID than those with normal eGFR (0.65 ± 0.05 vs. 0.74 ± 0.04 units/kg/day; p = 0.21).

Furthermore, the use of GCs, considered ineffective for IGC in univariate analysis (p = 0.120), was a notable predictor of poor IGC in multivariate analysis. The use of GCs in patients with pre-existing diabetes will undoubtedly worsen IGC [11]. Another study with a large number of participants showed that GC use was an important predictor of poor IGC [11]. GCs aggravate hyperglycemia in patients with known DM [22], leading to a hyperglycemia incidence of up to 46% and an increase in the BG levels by approximately 68% as compared to the baseline values [23, 24]. Additionally, there can be acute complications such as non-ketotic hyperosmolar state, diabetic ketoacidosis [25], and even death in patients with pre-existing DM.

Our study had some limitations. First, this study was merely a descriptive study that introduced a new insulin regimen (BBIT) and analyzed IGC outcomes in hospitalized Vietnamese patients. As a pilot study, we lacked a control group that used other insulin regimens for comparison with BBIT. Second, the correction of bolus insulin before meals depended only on capillary BG levels because there were no ingredient analytic platforms (especially carbohydrate estimation) assessing inpatients' meals. Finally, the study did not investigate the impact of BBIT on long-term outcomes, including length of stay, diabetes-related complications, or mortality. We have not yet performed a follow-up after discharge to evaluate the outpatient glycemic control for mid- and long-term periods, which is an essential evidence to not only calibrate a safe BBIT dosage but also ensure crucial surveillance of homecare patients.

Conclusion

In conclusion, type-2 DM inpatients who received BBIT achieved a high optimal IGC with acceptable hypoglycemic rates in a noncritical care department. Supplementary meals, especially snacks, and GC therapy were factors associated with poor IGC, whereas decreased eGFR was identified as a favorable factor for optimal IGC when using BBIT to manage in-hospital hyperglycemia. Further studies on factors associated with glycemic control with BBIT should be conducted in non-critically ill patients with type 2 DM, especially homecare patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-022-01079-y.

Acknowledgements We would like to express our gratitude to the medical staff of the Endocrinology Department of University Medical Center, Ho Chi Minh City, for their support in data collection.

Code availability Not applicable.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mai Ngoc Thi Tran, Nam Quang Tran, and Khiem Tran Dang. The first draft of the manuscript was written by Mai Ngoc Thi Tran and all authors thoroughly commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability Not applicable.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Council for Medical Research of the University of Medicine and Pharmacy at Ho Chi Minh City (Protocol number: 584, University of Medicine and Pharmacy, BOARD, October 31, 2019).

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

Consent for publication The authors have consented to have their data published in a journal article.

Conflict of interest The authors declare no competing interests.

References

1. Jiang HJ, Stryer D, Friedman B, Andrews R. Multiple hospitalizations for patients with diabetes. Diabetes Care. 2003;26(5):1421–6.
2. Umpierrez GE, Hellman R, Korytkowski MT, Kosiborod M, Maynard GA, Montori VM, Seley JJ, van den Berghe G. Management of hyperglycemia in hospitalized patients in non-critical care setting: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(1):16–38.
3. Umpierrez GE, Isaacs SD, Bazargan N, You X, Thaler LM, Kitabchi AE. Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes. J Clin Endocrinol Metab. 2002;87(3):978–82.
4. Moghissi ES, Korytkowski MT, DiNardo M, Einhorn D, Hellman R, Hirsch IB, Inzucchi SE, Ismail-Beigi F, Kirkman MS, Umpierrez GE, American Association of Clinical Endocrinologists, American Diabetes Association. American Association of Clinical Endocrinologists and American Diabetes Association consensus statement on inpatient glycemic control. Diabetes Care. 2009;32(6):1119–31.
5. King AB, Armstrong DU. Basal bolus dosing: a clinical experience. Curr Diabetes Rev. 2005;1(2):215–20.
6. Umpierrez GE, Smiley D, Hermayer K, Khan A, Olson DE, Newton C, Jacobs S, Rizzo M, Peng L, Reyes D, Pinzon I, Fereira ME, Hunt V, Gore A, Toyoshima MT, Fonseca VA. Randomized study comparing a Basal-bolus with a basal plus correction insulin regimen for the hospital management of medical and surgical patients with type 2 diabetes: basal plus trial. Diabetes Care. 2013;36(8):2169–74.
7. Umpierrez GE, Smiley D, Jacobs S, Peng L, Temponi A, Mulligan P, Umpierrez D, Newton C, Olson D, Rizzo M. Randomized study of basal-bolus insulin therapy in the inpatient management of patients with type 2 diabetes undergoing general surgery (RABBIT 2 surgery). Diabetes Care. 2011;34(2):256–61.
8. Umpierrez GE, Smiley D, Zisman A, Prieto LM, Palacio A, Ceron M, Puig A, Mejia R. Randomized study of basal-bolus insulin therapy in the inpatient management of patients with type 2 diabetes (RABBIT 2 trial). Diabetes Care. 2007;30(9):2181–6.
9. Bellido V, Suarez L, Rodriguez MG, Sanchez C, Dieguez M, Riestra M, Casal F, Delgado E, Menendez E, Umpierrez GE. Comparison of Basal-Bolus and Premixed Insulin Regimens in Hospitalized Patients With Type 2 Diabetes. Diabetes Care. 2015;38(12):2211–6.
10. Sheu WH, Ji L, Lee WJ, Jabbar A, Han JH, Lew T. Efficacy and safety of premixed insulin analogs in Asian patients with type 2 diabetes: A systematic review. J Diabetes Investig. 2017;8(4):518–34. https://doi.org/10.1111/jdi.12605.
11. Nguyen KT, Diep BTT, Nguyen VDK, Van Lam H, Tran KQ, Tran NQ. A cross-sectional study to evaluate diabetes management, control and complications in 1631 patients with type 2 diabetes mellitus in Vietnam (DiabCare Asia). Int J Diabetes Dev Ctries. 2020;40:70–9. https://doi.org/10.1007/s13410-019-00755-w.
12. Bender M, Smith TC, Thompson J, Koucheki A, Holdy K. Predictors of Suboptimal Glycemic Control for Hospitalized Patients with Diabetes: Targets for Clinical Action. J Clin Outcomes Manag. 2015;22(4):159–67.
13. Alaqil A, Alkahtani M, Algahtani M. Prevalence and Predictors of Glycemic Control in Hospitalized Patients with Diabetes. Indian J Public Health Res Dev. 2020;48(11):1449–53.
14. Goldberg PA, Bozzo JE, Thomas PG, Mesmer MM, Sakharova OV, Radford MJ, Inzucchi SE. Glucometrics-assessing the quality of inpatient glucose management. Diabetes Technol Ther. 2006;8(5):560–9.
15. Pasquel FJ, Gianchandani R, Rubin DJ, Dungan KM, Anzola I, Gomez PC, Peng L, Hodish I, Bodnar T, Wesorick D, Balakrishnan V, Osei K, Umpierrez GE. Efficacy of sitagliptin for the hospital management of general medicine and surgery patients with type 2 diabetes (Sita-Hospital): a multicentre, prospective, open-label, non-inferiority randomised trial. Lancet Diabetes Endocrinol. 2017;5(2):125–33.
16. Perez A, Reales P, Barahona MJ, Romero MG, Minambres I, Group H S. Efficacy and feasibility of basal-bolus insulin regimens and a discharge-strategy in hospitalised patients with type 2 diabetes-the HOSMIDIA study. Int J Clin Pract. 2014;68(10):1264–71.
17. Zaman Huri H, Permalu V, Vethakkan SR. Sliding-scale versus basal-bolus insulin in the management of severe or acute hyperglycemia in type 2 diabetes patients: a retrospective study. PLoS One. 2014;9(9):e106505.
18. Yamamoto S, Hayashi T, Ohara M, Goto S, Sato J, Nagaike H, Fukase A, Sato N, Hiromura M, Tomoyasu M, Nakanishi N, Lee S, Osamura A, Yamamoto T, Fukui T, Hirano T. Comparison of liraglutide plus basal insulin and basal-bolus insulin therapy (BBIT) for glycemic control, body weight stability, and treatment satisfaction in patients treated using BBIT for type 2 diabetes without severe insulin deficiency: A randomized prospective pilot study. Diabetes Res Clin Pract. 2018;140:339–46.
19. Rajput R, Sinha B, Majumdar S, Shunmugavelu M, Bajaj S. Consensus statement on insulin therapy in chronic kidney disease. Diabetes Res Clin Pract. 2017;127:10–20.
20. Charpentier G, Riveline JP, Varroud-Vial M. Management of drugs affecting blood glucose in diabetic patients with renal failure. Diabetes Metab. 2000;26(Suppl 4):73–85.
21. Snyder RW, Berns JS. Use of insulin and oral hypoglycemic medications in patients with diabetes mellitus and advanced kidney disease. Semin Dial. 2004;17(5):365–70.
22. Trence DL. Management of patients on chronic glucocorticoid therapy: an endocrine perspective. Prim Care. 2003;30(3):593–605.
23. Donihi AC, Raval D, Saul M, Korytkowski MT, DeVita MA. Prevalence and predictors of corticosteroid-related hyperglycemia in hospitalized patients. Endocr Pract. 2006;12(4):358–62.
24. Tamez-Perez HE, Quintanilla-Flores DL, Rodriguez-Gutierrez R, Gonzalez-Gonzalez JG, Tamez-Pena AL. Steroid hyperglycemia: Prevalence, early detection and therapeutic recommendations: A narrative review. World J Diabetes. 2015;6(8):1073–81.
25. Cagdas DN, Pac FA, Cakal E. Glucocorticoid-induced diabetic ketoacidosis in acute rheumatic fever. J Cardiovasc Pharmacol Ther. 2008;13(4):298–300.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):208–213
https://doi.org/10.1007/s13410-022-01047-6

ORIGINAL ARTICLE

Cost of diabetes treatment in private facilities for low resource urban community in South India

Anu Maria Jacob[1] · Arutselvi Devarajan[1] · Sukanya Nachimuthu[1] · Manjula Datta[1] · Vijay Viswanathan[2]

Received: 12 January 2021 / Accepted: 8 February 2022 / Published online: 22 March 2022

Abstract

Background and aim Diabetes has become a public health threat. The cost of diabetes care varies in different communities and depends on health-seeking behavior and socio-economic condition of the people. Data on economic and social impact of diabetes treatment among socially weaker section is scanty. Hence, this study was conducted to estimate the direct and indirect cost of treating diabetes among people living in low resource urban setting.

Methods A cross-sectional study was conducted among 1065 households in the two selected areas of North Chennai. Data was collected from individuals who were taking diabetes treatment in private health care facilities. The cost toward diabetes treatment for each participant was calculated using their medical bills, tablet strips and also self-reported cost details.

Results A total of 341 individuals were found to have diabetes and out of them, 230 (M:F, 63:167) individuals who attended private health facilities for their diabetes treatment were studied. Around 73% of them earned a monthly income of < INR 10,000. The study revealed that median annual direct cost of treating diabetes among the study participants was amounted to INR 7540. Annual median indirect cost amounted to INR 1650.

Conclusion People living with diabetes in low resource urban setting spent more toward direct medical cost. The out of pocket expenditure was mainly on hospitalization followed by medicines and investigations. The strengthening and provision of appropriate primary public health care services for diabetes in this setting may reduce out of pocket expenditure toward treatment of diabetes in private health care facilities.

Highlights

- Nearly 3/4th of study participants who earn INR 10,000/month monitor their diabetes status rarely, but take medicines alone.
- They spend INR 6000 on medicines annually while they spend only INR 400 on lab investigations.
- More than 1/4th (28.3%) of them were irregular in taking medication and buying medicines with previous tablet strips from the medical shop.
- Hypertension (24.7%) was the most common co-morbid condition prevalent among them than any other diabetic complications.

Keywords Health-care · Treatment cost · Low resource setting · Urban poor · Diabetes

Introduction

Diabetes is one of the fastest growing diseases in the world, which causes morbidity and mortality [1]. It makes families poor and reduces the life expectancy of people. Diabetes is a common threat which affects anyone irrespective of their class or caste [2]. Every year, around 5.8 million Indians die due to non-communicable diseases such as heart disease, lung disease, stroke, cancer, and diabetes [3]. According to IDF Atlas, "diabetes is a global emergency of the 21st

✉ Vijay Viswanathan
drvijay@mvdiabetes.com

[1] Department of Epidemiology, Prof. M. Viswanathan Diabetes Research Centre (WHO Collaborating Centre for Research, Education and Training in Diabetes) (IDF Centre of Excellence in Diabetes Care), Royapuram, Chennai 600013, India

[2] M.V. Hospital for Diabetes & Prof. M. Viswanathan Diabetes Research Centre (WHO Collaborating Centre for Research, Education and Training in Diabetes) (IDF Centre of Excellence in Diabetes Care), Royapuram, Chennai 600013, India

century" [4]. An estimated number of 425 million people are living with diabetes worldwide. Currently, there are 77 million people living with diabetes in India and this number is expected to increase to 152.8 million by 2045 [5]. It is also estimated that 7.8% of the population above 18 years in our country either experience raised blood glucose level or on treatment for diabetes [6]. India stands to lose 0.15 trillion US $ [15 lakhs in INR] to diabetes alone according to the world economic forum report on economics of non-communicable diseases [7]. A recent study conducted in India shows that the prevalence of diabetes is higher among urban poor compared to other sections [8]. Study by Vigneswari et al. from South India reported that the prevalence of obesity and central obesity was higher among urban poor, especially among women [9]. Hence, it is also important to study the cost of diabetes care among the urban poor. The present study was conducted to find the direct and indirect cost of treating diabetes among economically backward group.

Materials and methods

A community based cross-sectional study was conducted in two economically disadvantaged communities (Panamarathotti and Jeevarathnam Nagar) in North Chennai, Tamil Nadu, the southern state of India. Northern region of Chennai has more of slum settlements (470 slums) compared to Southern (272 slums) and central (389 slums) regions of Chennai [10]. Hence, North Chennai was selected and listed all the areas and selected first two areas Panamarathotti and Jeevarathnam Nagar in Royapuram based on computer-generated random numbers and ensured existence of mixed groups such as fishing communities, migrant laborers from other districts of Tamil Nadu, and also other parts of India that represents poor resource setting. All the households were surveyed in the selected study areas. Out of a total of 1065 households surveyed, 341 individuals were found to have diabetes. The history of diabetes was confirmed with the medical records and treatment prescriptions. Out of 341 individuals, 230 who satisfied the inclusion criteria of (1) age > 18 years; (2) treatment taken only in private health care centres for a minimum period of 1 year; and (3) patients with records such as doctor's prescription for diabetes, medical bills, or tablet (OHAs) strips/insulin vials and willingness to participate in the study were included.

All the study participants were interviewed by trained field investigators using pretested, structured interview schedule. The cost details were recorded by seeing their medical bills, tablet strips, and self-reported cost details. The annual cost was calculated by multiplying total visits to the doctor or medical shop with out of pocket expenditure for medicines, laboratory investigations such as fasting blood sugar, HbA1c or lipid profile, and any other additional tests including kidney function test, Eye examination, ECG, Echo, foot examination if done.

Statistical analysis

Data analysis was done using SPSS version 20. Median and range (minimum, maximum) were given for the continuous variables. Frequencies and percentages were given for the categorical variables.

Results

Socio-economic profile

The majority of the study participants were females (72.6%). Mean age of the study participants was 54 ± 11.8 years. Around 55.6% of the study participants were homemakers. Monthly household income of 73% of the participants was less than INR 10,000 and 43% of the participants were the primary income earner of the family. The detailed summary of the socio-demographic description is shown in Table 1.

History of diabetes, its management, and other co-morbid conditions

About 52.6% of the participants had reported living with diabetes for more than 5 years. The majority (89.1%) of them was treated with oral hypoglycemic agent (OHA). Around 38% of the participants reported with presence of complications with high proportion of retinopathy (26.5%). Hypertension was the most common co-morbid condition (46%) prevalent among the study participants. Various behavioral risk factors such as smoking, alcoholism, tobacco chewing, and usage of snuff were reported by 17.3% of the participants. The study found that 28.3% of the study participants were irregular in taking medication and were also buying medicines with previous tablet strips from the nearby medical shops. Irregular medication and irregular follow-up visits were common in this group. About 41.5% of the study participants reported that they could not follow the regular medication due to financial constraints and other reasons including lack of knowledge about the impact of discontinuing medicines and its complications (38.5%), stopped taking medicine because they felt better (15.4%), and fear of side effects (6.2%) etc.

Table 1 Socio-demographic profile of the study participants

Variables	Number (%)
Total participants	230
Mean age	54±11.8
Age in years	
> 18–29 years	2 (0.9)
30–49 years	77 (33.5)
49 and above	151 (65.6)
Gender	
Male	63 (27.4)
Female	167 (72.6)
Education	
Illiterate	78 (34)
School	137 (59.5)
College	14 (6)
Professional	1 (0.5)
Occupation	
Unskilled worker	29 (12.6)
Skilled worker/Formal sector	31 (13.5)
Business	22 (9.6)
Retired	15 (6.5)
House wife	128 (55.6)
Unemployed/Student	5 (2.2)
Income	
< 10,000	168 (73.0)
10,001–20,000	54 (23.5)
20,001–40,000	8 (3.5)
> 40,000	0 (0)
Duration of diabetes	
< 5 years	109 (47.4)
5–10 years	71 (30.9)
> 15 years	50 (21.7)
Co-morbidities/ complications	
Retinopathy	61 (26.5)
Neuropathy	6 (2.6)
Foot complications (amputation and ulcer)	10 (4.3)
Hypertension	106 (46.1)
Heart diseases	6 (2.6)
Don't know	4 (1.7)

Health-seeking behavior

Out of 230 participants, around 70% of them used to go to general practitioners, 25.7% to private hospitals, and 4.3% were visiting multi-specialty hospitals for their diabetes treatment.

Direct cost measures

The annual direct medical cost of the study participants per year was estimated at INR 7368. Other than the expenditure for inpatient care, people spent more money on medicines. The annual cost of buying tablets and insulin alone was INR 6000 (INR 805-INR 62,400) while the annual cost of lab investigation was INR 400 (INR 30-INR 16,000). The detailed summary of direct medical cost is shown in Table 2.

The non-medical cost was estimated at INR 320. Among the total participants, 54.3% had no cost of transportation charge. The details of direct non-medical cost are summarized in Table 3.

The total direct cost for treating diabetes per annum was estimated at INR 7540 which included both total medical and non-medical cost of treating diabetes. This study also showed that 60% of the total medical cost spent only for the purchase of medicines followed by lab investigations (18%) and consultation fee (7.4%) per OP visit.

Indirect cost measures

The annual indirect cost was calculated at INR 1650 (INR 200-INR 8000), which included the reported loss of income of the patient as well as the care taker during their outpatient visits (OP) or in-patient (IP) admissions. Only 13.04% (30) of the participants had to bear the indirect cost while 86.9% did not report this income loss. Among them, 35.6% were found to be housewives, retired/unemployed, and students. Information on annual indirect cost spent on diabetes treatment is provided in Table 3.

It was found that the average expenditure toward diabetes treatment per person per annum was INR 7925. The details of the total cost for treating diabetes are provided in Table 4.

Mode of payment

Most of the expenses were met through out of pocket money (91%) and 8% reported that they had borrowed loan to spend for their treatment. Only 1% mentioned they had medical insurance. Responding to a question about the escalating cost scenario in the treatment for diabetes and its impact, nearly 47% of the participants said that they would continue the same treatment even if there would be a cost escalation. Among the remaining, 25.2% responded that they would prefer cheaper treatment options, 14.3% would go to Government hospital, 9.1% might stop treatment, and 4.3% responded that they don't know what they would do.

Discussion

The economic burden of diabetes on persons affected with diabetes and their households was highlighted in several studies earlier [11]. This study has revealed the

Table 2 Annual direct medical cost for treating diabetes (in INR)

Various Cost	*N*	Median	Range	
			Minimum	Maximum
Annual cost of consultation	230	600	100	6000
Annual cost of Lab Investigation	230	400	30	16,000
Annual cost of tablets	230	6000	805	62,400
Annual cost of Insulin	25	6000	858	24,000
Annual cost of Hospitalization	17	18,000	250	2,30,000
Annual cost of other Investigations	6	2750	2000	5000
Annual Direct medical cost	230	7368	510	2,72,800

N number of participants, *INR* Indian Rupees

Total direct medical cost per person per annum (in INR) = 7368

economic burden of diabetes care among people living in low resource urban setting. In this study, we surveyed the people who were attending private health care centres in order to find out the medical, non-medical, and indirect cost for diabetes care. Previous studies from India showed that people with diabetes were spending more on hospitalization and lab investigations [12–14]. It was observed that both urban and rural dwellers spent around 40% of the total expenditure toward medications [15]. A similar proportion (38.8%) of expenditure on medications was also shown in a study conducted among the urban poor in Karnataka [16]. Whereas, our study showed 60% of the total expenditure was for buying medicines.

The annual expenses toward medicines (OHAs and insulin) and lab investigations were found to be INR 6000 (INR 805-INR 62,400) and INR 400 (INR 30-INR 16,000) respectively. From this result, it may be concluded that the less priority was given for monitoring of the blood glucose levels. Non-medical expenses were estimated as INR 320 per annum. It was observed that the majority of participants did not spend on transportation as they used to access nearby medical facilities by walk for their treatment.

A study on the economic burden of diabetes mellitus and its socio-economic impact on household expenditure for the urban poor in Mumbai showed that the mean indirect cost of diabetes management for 1 year was INR 4185. In contrast, our study results showed less annual indirect cost of INR 1650. But the direct cost in both Mumbai and Chennai are more or less a same amount [17]. A study conducted among urban population in Karnataka [16] reported INR 359 as indirect cost per visit with 5 average visits per year. Hence, the total annual indirect cost would cost around INR 1795 that is closely similar to our findings, whereas the direct cost incurred was found to be relatively lesser than the direct cost exhibited by our study. The possible reasons for the notable difference observed in the indirect cost for Mumbai and Chennai could be due to the availability, proximity, accessibility, and affordability of the health facilities and also the cost of living in these cities. It also depends on the number of visits made by patients to the health facility for treatment. While, another study conducted in coastal Karnataka showed expenses of INR 4282 that was lesser than expenses made by our study

Table 3 Annual direct and indirect non-medical cost for treating diabetes (in INR)

Various Cost	*N*	Median	Range	
			Minimum	Maximum
Direct non-medical cost				
Annual cost of transportation	105	300	60	3600
Annual cost of food expenses	20	180	40	1800
Direct non-medical cost	109	320	60	3600
Indirect non-medical cost				
Loss of income	30	1650	200	8000

N number of participants, *INR* Indian Rupees

Total direct non-medical cost per person per annum (in INR) = 320

Total indirect non-medical cost per person per annum (in INR) = 1650

Table 4 Total cost of diabetes care (in INR)

Total cost	*N*	Median	Range	
			Minimum	Maximum
Total direct cost	230	7540	510	2,73,040
Total indirect cost	30	1650	200	8,000
Total Cost	230	7925	700	2,77,040

N number of participants, *INR* Indian Rupees

participants. The indirect cost also varied much from their study as they showed very less indirect cost of INR 462 accounted to 28% of the indirect cost incurred by our study participants [18]. A clinic-based study showed less absenteeism by patients who visited private hospitals [19]. This is consistent with our study findings as study participants were observed to prefer private hospitals as they could visit these health facilities in their convenient timing and also to avoid long queue and waiting hours in the public health facilities.

Another study from Chennai conducted in 2013, on the economic burden of middle income groups with diabetes reported that the median annual direct medical cost was INR 6550 and median annual direct non-medical cost was INR 250 [20]. When we compare this medical cost (INR 6550), urban poor of the same city spends almost the same amount for diabetes care now. A community-based cost of illness study conducted in 2009 [14] among the South Indian population reported that one has to spend INR 25,391 toward the direct cost for diabetes care and INR 4970 toward the indirect cost. When we compare the direct and indirect cost with present study, the urban poor are spending three times lesser than the general population [15]. The findings of the current study revealed that urban poor spends lesser amount on lab investigations or monitoring of blood sugar, other investigation such as ECG, ECHO, Eye test, etc., compared to the general population. This study also demonstrated that a majority of them had less absenteeism and did not lose their daily wage income unlike the people attending government health facilities that they faced more indirect expenses toward their diabetes treatment [19]. But the inability to spend on investigation costs may also results in suboptimal care.

Another key finding was the practice of irregular medication and irregular follow-up visit to doctor and absence of regular monitoring of the blood sugar. Not only the financial constraints, but also lack of knowledge on the importance of proper medication and severity of disease may be the reasons for this. A routine check-up is necessary to prevent the diabetic complications. As most of the people living in this setting used to visit general practitioners, it is necessary for those general practitioners to be aware of this fact and keep a track on their health status regularly to prevent the development of the complications.

The urban poor spend a high proportion of their income on diabetes care [21]. In the year 2000, one could treat diabetes with less than INR 5000 per annum [13]. It grew to INR 10,000 in 2007 in urban settings and INR 6260 in rural settings in India [15]. In the present study, people with diabetes from a low resource urban setting were found to spend an average of INR 8000 for diabetes care per annum. This amount is equal to their one and half month's salary, and hence they would prefer not affording to health and they would prioritize to meet their daily life expenses.

Our study findings showed that 67.4% of the participants were taking treatment in private health centers. Several other studies also reported similar results that over 65% of the urban poor in India are depending on various private healthcare providers [22–24].

Here is an urgent need for more studies on health seeking behavior, and the pattern of health care provided to the poor with respect to diabetes. It is also important to have Government-run investigation units with subsidized price for every one lakh population in urban areas. Even though the urban poor live in cities and towns that are surrounded by high-standard health care facilities and technologies, they could not afford those facilities. Other than free treatment, government has to set up some collaboration with disease specific private health care centres or introduce public-private partnership (PPP) system to improve the accessibility of the health care facilities among poor people.

Limitations

Information such as consultation fee, lab investigation charges toward few hospital visits, number of hospital visits were self-reported by some of the study participants as there were no bills or records available for the same. Hence, there was a chance for the occurrence of recall bias. The gender distribution showed that high proportion of women participated in this study. This might be due to non-availability of men in the households who have left for work during the survey. This would have compromised the equal representation of both genders for the study.

Acknowledgments We thank our epidemiology team members for data collection and entry. We are indebted to all our study participants who were willing to spend their valuable time for the interview process.

Funding This study did not receive any fund from external sources.

Declarations

Conflict of interest The authors declare no conflict of interest.

Informed Consent Ethical clearance was taken from the Institutional Ethics Committee (EC. No.: IEC/N-001/IN/03/2017) and a written informed consent was obtained from each participant before starting the study.

References

1. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al KJ. Epidemiology of Type 2 Diabetes - Global Burden of disease and Forecasted trends. J Epidemiol Glob Health. 2020;10(1):107–

11. https://doi.org/10.2991/jegh.k.191028.001. PMID: 32175717; PMCID: PMC7310804
2. World Health Organization. Geneva: Global report on diabetes 2016. http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf. Accessed 10 September 2019.
3. World Health Organization. Geneva. Global status report on non-communicable diseases. 2014. https://apps.who.int/iris/bitstream/handle/10665/148114/9789241564854_eng.pdf Accessed 12 July 2019
4. International Diabetes Federation [Internet]. Belgium: IDF-Diabetes Atlas 7th edition 2015. https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/13-diabetes-atlas-seventh-edition.html. Accessed 06 December 2019.
5. International Diabetes Federation [Internet]. Prevalence of Diabetes in India. https://diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf. Accessed 08 February 2020.
6. World Health Organization [Internet]. India: World Health Day Report- 2016. http://www.searo.who.int/india/mediacentre/events/world_health_day/brief_note_on_diabetes_in_india-6april.pdf?ua=1. Accessed 02 August 2019.
7. Bloom DE, Cafiero-Fonseca ET, Candeias V, Adashi E, Bloom L, Gurfein L, *et al*. Economics of non-communicable diseases in India: the costs and returns on investment of interventions to promote healthy living and prevent, treat, and manage NCDs. World Economic Forum, Harvard School of Public Health, 2014. http://www3.weforum.org/docs/WEF_EconomicNonCommunicableDiseasesIndia_Report_2014.pdf. Accessed 10 April 2020.
8. Anjana RM, Deepa M, Pradeepa R, Jagadish M, Kanwar N, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR–INDIAB population-based cross-sectional study. Lancet Diabetes Endocrinol. 2017;5(8):585–96. https://doi.org/10.1016/S2213-8587(17)30174-2. Epub 2017 Jun 7. Erratum in: Lancet Diabetes Endocrinol. 2017;5(8):e5
9. Vigneswari A, Manikandan R, Satyavani K, Archana S, Rajeswari R, Viswanathan V. Prevalence of risk factors of diabetes among urban poor south Indian population. JAPI. 2015;63(10):32–3.
10. Slums in Chennai increased by 50% in a single decade, Times of India, January 18, 2016 <https://timesofindia.indiatimes.com/city/chennai/slums-in-chennai-increase-by-50-in-a-single-decade/articleshow/50618951.cms>. Accessed 19 July 2020.
11. Oberoi S, Kansra P. Economic menace of diabetes in India: a systematic review. Int J Diabetes Dev Ctries. 2020;40:464–75. https://doi.org/10.1007/s13410-020-00838-z.
12. Tharkar S, Satyavani K, Viswanathan V. Cost of medical care among type 2 diabetic patients with a co-morbid condition - hypertension in India. Diabetes Res Clin Pract. 2009;83(2):263–7. https://doi.org/10.1016/j.diabres.2008.11.027.
13. Kapur A. Economic analysis of diabetes care. World Diabetes Foundation, Lyngby, Denmark. Indian J Med Res. 2007;125(3):473–82.
14. Tharkar S, Devarajan A, Kumpatla S, Viswanathan V. The socio-economics of diabetes from a developing country: A population based cost of illness study. Diabetes Res Clin Pract. 2010;89(3):334–40. https://doi.org/10.1016/j.diabres.2010.05.009.
15. Ramachandran A, Ramachandran S, Snehalatha C, Augustine C, Murugesan N, Viswanathan V. Increasing expenditure on health care incurred by diabetic subjects in a developing country. Diabetes Care. 2007;30(2):252–6. https://doi.org/10.2337/dc06-0144.
16. Javalkar SR. The economic burden of health expenditure on diabetes mellitus among urban poor: a cross sectional study. Int J Community Med Public Health. 2019;6:1162–6. https://doi.org/10.18203/2394-6040.ijcmph20190604.
17. Fernandes SD, Fernandes SDA. Economic burden of diabetes mellitus and its socio- economic impact on household expenditure in an urban slum area. Int J Res Med Sci. 2017;5(5):1808–13. https://doi.org/10.18203/2320-6012.ijrms20171585.
18. Eshwari K, Kamath VG, Rao CR, Kamath A. Annual cost incurred for the management of type 2 diabetes mellitus—a community-based study from coastal Karnataka. Int J Diabetes Dev Ctries. 2019;39(3):590–5. https://doi.org/10.1007/s13410-018-0691-5.
19. Sharma KM, Ranjani H, Zabetian A, Datta M, Deepa M, Moses CRA, Narayan KMV, Mohan V, Ali MK. Excess cost burden of diabetes in Southern India: a clinic-based, comparative cost-of-illness study. Glob Health Epidemiol Genom. 2016;1:e8. https://doi.org/10.1017/gheg.2016.2.
20. Loganathan ACV, John KR. Economic burden of diabetes in people living with the disease: a field study. J Diabetology. 2013;4(3):1–8.
21. Jitendra S. Economic burden of Diabetes. Medicine Update. 2015:1-4. http://apiindia.org/wp-content/uploads/medicine_update_2013/chap45.pdf. Accessed 4 April 2021.
22. Banerjee A, Bhawalkar JS, Jadhav SL, Rathod H, Khedkar DT. Access to health services among slum dwellers in an industrial township and surrounding rural areas: a rapid epidemiological assessment. J Family Med Prim Care. 2012;1(1):20–6. https://doi.org/10.4103/2249-4863.94444.
23. Singh S, Kalaskar S. Health care seeking behavior and utilization pattern in an urban slum of Mumbai: A cross sectional study. Int J Curr Res. 2017;9(4):49342–5.
24. Tharkar S, Devarajan A, Barman H, Mahesh U, Viswanathan V. How far has translation of research been implemented into clinical practice in India? Are the recommended guidelines adhered to? Int J Diabetes Mellit. 20154;3(1):25–30. https://doi.org/10.1016/j.diabres.2010.05.009. Epub 2010 Jun 9

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):214–225
https://doi.org/10.1007/s13410-022-01074-3

ORIGINAL ARTICLE

Real-world assessment of effectiveness and safety profile of remogliflozin etabonate in management of type 2 diabetes mellitus

Bipin Sethi[1] · Subhankar Chowdhury[2] · Supratik Bhattacharya[3] · Sagar Katare[4] · Sachin Suryawanshi[4] · Hanmant Barkate[4]

Received: 8 April 2021 / Accepted: 17 April 2022 / Published online: 19 May 2022

Abstract

Aim To assess the real-world effectiveness and safety of remogliflozin in the management of type 2 diabetes mellitus (T2DM) in a large uncontrolled population.

Methods A retrospective cohort analysis was conducted at 1578 sites across India. Medical records of all patients who had received a remogliflozin-based regimen for a 3-month duration as per routine practice for the management of T2DM were analysed for effectiveness and safety. The efficacy assessments included mean change in HbA1c, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), bodyweight, BMI, and blood pressure from baseline to 3 months. Safety assessments included incidence of adverse events reported.

Results A total of 5452 eligible patients' data were analysed. The mean change of HbA1c level from baseline (8.63%) to 3-month follow-up (7.68%) was − 0.95%. The mean change in FPG and PPG from baseline to the end of follow-up was − 42.4 mg/dL and − 69.1 mg/dL, respectively. A significant reduction in glycemic parameters was observed from baseline to follow-up. The overall incidence of adverse events (AEs) was about 25.9%. Genito-urinary tract infections (12.6%) were more frequently reported AEs, and no severe AEs were reported.

Conclusion Remogliflozin etabonate was effective in improving glycemic parameters. It was well-tolerated in the real-world setting used for glycemic management of T2DM.

Keywords Remogliflozin etabonate · Type 2 diabetes mellitus · HbA1c · Adverse events

Background

Type 2 diabetes mellitus (T2DM) is one of the most important and prevalent chronic non-communicable diseases today, in India, which is anticipated rise to 101 million by 2030 [1]. As improved glycemic control is associated with reduced risk of micro and macrovascular complications, optimal therapy for patients with T2DM requires an appropriate selection of glucose-lowering therapies considering side effects like hypoglycemia and weight gain with some other agents.

However, initial metformin monotherapy, as recommended by clinical guidelines, most often are insufficient to achieve or maintain glycaemic targets. Consequently, treatment with additional glucose-lowering agents to existing anti-diabetic therapy is required [2, 3].

SGLT2i represents a new class of anti-diabetic drugs that recent guidelines have recommended as one of the first-line anti-diabetic agents in the management of T2DM [4]. SGLT2 inhibitors exhibit additional unique benefits of reduction in both body weight and blood pressure (BP) [5].

Remogliflozin, available as prodrug remogliflozin etabonate (RE), is a novel potent selective inhibitor of SGLT2 that has been approved recently in India [6]. Recent phase III pivotal study of RE in Indian subjects demonstrated RE to be an efficacious and tolerable agent in Indian T2DM

✉ Sagar Katare
Sagar.Katare@glenmarkpharma.com

[1] Department of Endocrinology, Care Hospital, Hyderabad, India

[2] Department of Endocrinology, IPGME&R and SSKM Hospital, Kolkata, India

[3] Department of Endocrinology, SKN Diabetes Center, Kolkata, India

[4] Global Medical Affairs, Glenmark Pharmaceuticals Limited, Mumbai, India

patients and was found to be non-inferior to dapagliflozin in the management of T2DM [7].

The real-world evidence (RWE) is the clinical evidence which demonstrates the usage and potential benefits and/or risks of a medical product derived from analysis of real-world data (RWD). RWD has potential to complement the knowledge available from conventional randomized clinical trials (RCTs), whose design limitations make it difficult to generalize findings to population at large uncontrolled settings. The present study was planned to be as retrospective cohort analysis of RWD of Indian T2DM patients from routine clinical settings to further characterize the clinical effectiveness and safety profile.

Methodology

This multi-centric retrospective cohort analysis was planned with data retrieved from medical records of patients treated in routine practice with remogliflozin regimens at multiple centres across India. The study protocol was approved by the Independent Ethics Committee. The study conduct was in compliance with study protocol, the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines E6 (R2), the Indian Council of Medical Research (ICMR), the National Ethical Guidelines for Biomedical and Health Research Involving Human Participant (2017), and applicable regulations. The data for this study was retrieved from medical records collected from 1578 sites from all over India between October 2019 and March 2020.

At each study centre, the medical records of adult (≥ 18 years) patients of either gender were screened for fresh initiation of remogliflozin for the management of T2DM as per routine practice and had completed 3-month follow-up after initiation of remogliflozin. A maximum of up to first 20 patients at each study centre were considered for analysis.

The day of initiation of remogliflozin was considered to be the index date (day 0, baseline). The assessment day was considered 3 months from the index date (day 90, follow-up). The investigators retrieved the available clinical data from the medical records of eligible patients treated at the respective centres and captured in de-identified manner in the case report forms (CRFs). The following data was planned for retrieval designed on day 0: baseline demographics (age, gender, height, BMI, duration of DM, family history of DM, co-morbid conditions, and concomitant medications). The data planned for retrieval on both day 0 and day 90 included body weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and glycosylated hemoglobin (HbA1c), estimated glomerular filtration rate (eGFR), serum creatinine, urine albumin-to-creatinine ratio (UACR), serum uric acid, and serum electrolytes (Na, K, Cl, Ca). The incident adverse events reported in medical records were to be captured for the entire observation period of 3 months from day 0.

The study intended to assess the effectiveness as well as the safety of remogliflozin in the real-world setting. The endpoints considered for assessment of clinical effectiveness included mean change from baseline to 3-month follow-up in HbA1c, FPG, and PPG levels for glycemic parameters. The mean change from baseline at 3 months in total body weight, BMI, SBP, and DBP was assessed in non-glycemic parameters from the available real-world data. Safety assessment included the incidence of adverse events in terms of symptoms and signs as transcribed from medical records. The safety assessment also included assessment of renal function, viz., eGFR, serum creatinine, serum uric acid, UACR, and serum electrolytes as available in the medical records. Further subgroup analyses were planned on the basis of baseline HbA1c and baseline BMI. The endpoints assessed in the subgroups included glycemic parameters (HbA1c, FPG, PPG) and body weight.

Statistical analysis

The CRFs, with data transcribed from medical records as available, from all centres were pooled together. Only CRFs with non-missing essential demographic characteristics (age, gender, weight, height) and availability of baseline and follow-up measurements of any one of 3 glycemic parameters were selected for data analysis. All statistical analyses were done using the software STATA. All characteristics were summarized descriptively. For continuous variables, data were represented using means ± SD. For categorical data, the number and percentage were used in the data summaries. The intragroup change from baseline was tested by paired *t*-test. For intragroup assessment, medical records wherein both the baseline and 3-month measurements for corresponding parameter were available were considered. For subgroup analysis, only patients' records with baseline and follow-up assessment available for all the parameters to be assessed were considered. All *p* values were two-tailed, and the values were considered statistically significant if $p < 0.05$.

Results

A total of 5452 eligible patient's data were analysed in this study. The mean age at diagnosis was 54.9 ± 10.27 years, with male predominance (61.4%). At the time of presentation, 61.5% of patients had positive family history of diabetes (FHD), and the average duration of diabetes was found to be 6.2 ± 4.29 years. Out of total 5452 patients, approximately 64.5% of patients were presented with co-morbid conditions at diagnosis. Hypertension (54.9%) and dyslipidemia (46.2%)

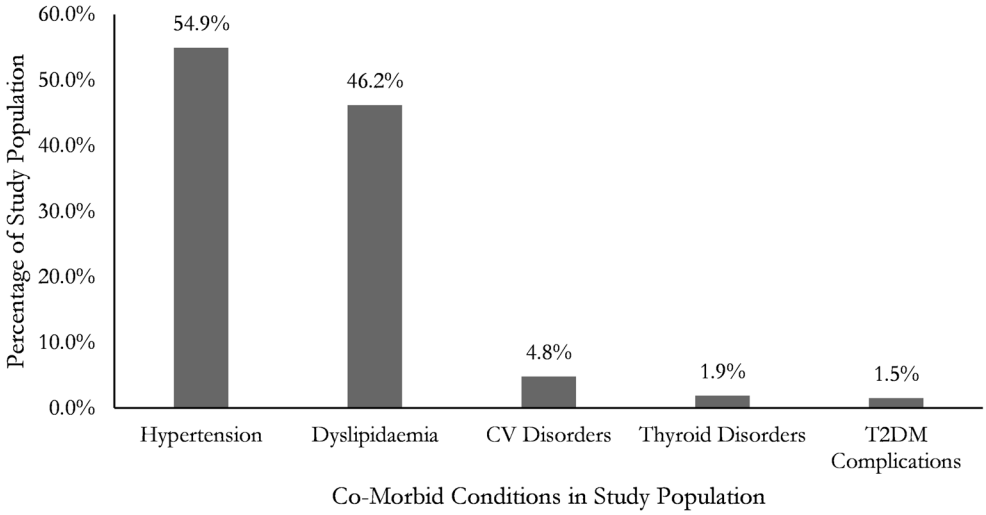

Fig. 1 Prevalence of comorbid conditions in total population. **Legend:** N=5452; CV=Cardiovascular; T2DM=Type 2 diabetes mellitus

were the most frequently observed co-morbid diseases among all patients (Fig. 1).

In the present study, more than half of the patients (55%) were on two concomitant oral anti-diabetic drugs (OADs), followed by 27.4% and 16.7% of patients on one and > 2 concomitant OADs, respectively, along with remogliflozin treatment. The most common concomitant regimen was metformin plus sulfonyl urea combination (33.8%). Figure 2 presents the summary statistics of concomitant OADs along with remogliflozin treatment. Approximately 55% of patients were on concomitant medication other than anti-diabetic drugs (ADDs), which are most common as being anti-hypertensive (59%) agents.

The mean baseline characteristics of the patients in terms of HbA1c levels, FPG, and PPG presentation were found to be 8.6 ± 1.04%, 181.4 ± 36.29 mg/dL, and 257.7 ± 59.56 mg/dL, respectively. The average body weight and BMI were noticed to be 74.6 kg and 28.3 kg/m^2 at diagnosis. Table 1 describes the baseline characteristics of the study participants.

Effect of remogliflozin on glycemic parameters

The mean change from baseline in HbA1c levels at 3 months was − 0.95% (Fig. 3) which was found to be statistically significant ($p < 0.05$). The mean reduction in FPG level from baseline level of 181.4 mg/dL was − 42.4 mg/dL, while the mean reduction in PPG from baseline of 257.7 mg/dL was − 69.1 mg/dL at the 3-month follow-up ($p < 0.05$ for both) (Fig. 4). The changes in glycemic parameters from baseline to follow-up are shown in Table 2.

The mean body weight reduced by 2.6 kg from 74.4 to 71.8 kg in 3 months, with a mean change of – 1 kg/m^2 in BMI. As > 50% of patients were on concomitant antihypertensive medications, the change in blood pressure was assessed only in the patients who had normal BP at baseline and were not receiving any BP-altering medications. The reduction in systolic and diastolic BP from baseline to 3-month follow-up was − 3.3 mmHg and − 2.3 mmHg.

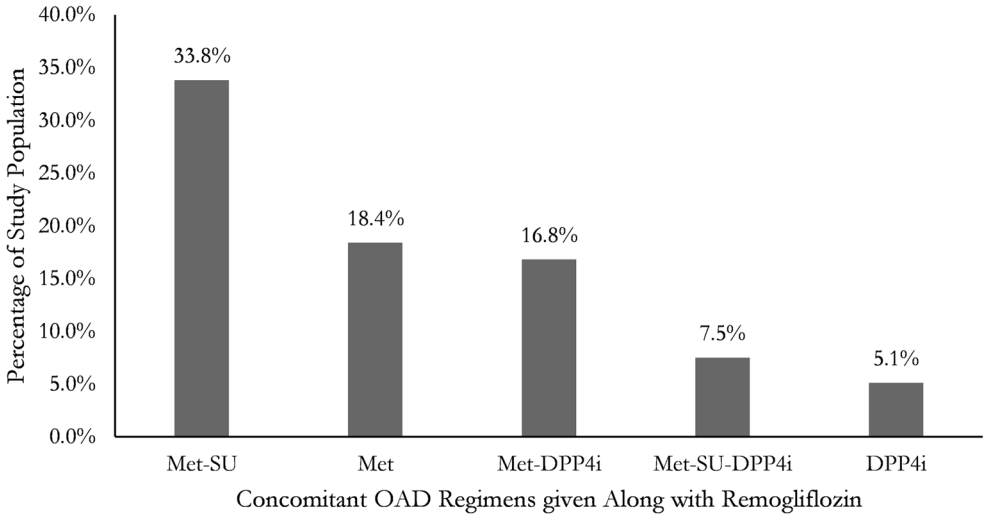

Fig. 2 Proportion of OAD regimens concomitantly administered with remogliflozin. **Legend:** DPP4i - Dipeptidyl Peptidase-4 inhibitor; Met – Metformin; SU – Sulfonyl Urea

Table 1 Baseline characteristics of the study population

Baseline characteristics		*N* = 5452
Age (years)		54.9 ± 10.27
Gender (*n*, %)	Males	3350 (61.4%)
	Females	2102 (38.6%)
Duration of T2DM (years)[1]		6.2 ± 4.29
Family history of T2DM[2] (*n*, %)		2913 (61.5%)
Height (cms)		162.7 ± 9.54
Weight (kg)		74.6 ± 9.77
BMI (kg/m^2)		28.3 ± 4.67
HbA1c (%)[3]		8.6 ± 1.04
FPG (mg/dL)[4]		181.4 ± 36.29
PPG (mg/dL)[5]		257.7 ± 59.56
Co-morbid conditions (> 2%) (n, %)		***3518 (64.5%), N (%)***
Hypertension^		2993 (54.9)
Dyslipidaemia^		2520 (46.2)
Cardiovascular disorders^		265 (4.8)
Concomitant medications (n, %)		
One concomitant OAD		1495 (27.4)
*Metformin^*		*1007(67.4)*
*DPP4i^*		*276 (18.5)*
Two concomitant OADs		3000 (55.0)
*Met+SU^*		*1846 (61.5)*
*Met+DPP4i^*		*915 (30.5)*
More than 2 concomitant OADs		914 (16.7)
*Met+SU+DPP4i^*		*409 (44.7)*
*Met+SU+AGI^*		*269 (29.4)*

[1] *n* = 5069; [2] *n* = 4734; [3] *n* = 5358; [4] *n* = 4785; [5] *n* = 4962

All values are mean ± SD unless specified otherwise

^The proportion on subitems derived considering the total n from the parent list item

AGI, α-glucosidase inhibitor; *BMI*, body mass index; *DPP4i*, dipeptidyl peptidase-4 inhibitor; *FPG*, fasting plasma glucose; *HbA1c*, glycated haemoglobin; *Met*, metformin; *SU*, sulfonyl urea; *TZD*, thiazolidinedione; *T2DM*, type 2 diabetes mellitus; *PPG*, postprandial plasma glucose; *OAD*, oral anti-diabetic drug

Assessment of non-glycemic parameters

The change in all non-glycemic parameters was statistically significant. Non-glycemic parameters have been exploratorily analysed as they are available pre- and postdata assessment. The changes in non-glycemic parameters from baseline to follow-up are shown in Table 2 and Figs. 5, 6, and 7.

Subgroup analysis

The subgroup analysis was conducted on the basis of baseline levels of HbA1c and BMI, as per availability of parameter at baseline and follow-up. Accordingly, 4445 patients were divided into 3 subgroups of < 8.5%, 8.5–10%, and > 10% as per baseline HbA1c, and 4145 patients were divided into 3 subgroups of < 25 kg/m^2, 25–30 kg/m^2, and > 30 kg/m^2 as per baseline BMI.

Subgroup analysis as per baseline HbA1c

To evaluate the effects of remogliflozin treatment, patients were classified into 3 subgroups on baseline HbA1c levels. At baseline, 2162 patients had HbA1c < 8.5%, 1957 patients had HbA1c 8.5–10%, and 326 patients had HbA1c > 10%. Baseline demographic characteristics of the patients at baseline HbA1c are given in Table 3. With remogliflozin treatment, patients across the 3 baseline subgroups had significant reduction in HbA1c, FPG, PPG, and body weight from baseline to follow-up. The change in HbA1c showed proportional reduction as per baseline HbA1c. The same trend was observed with FPG, PPG, and weight loss. Larger HbA1c reductions (− 1.76%) were seen among patients with higher baseline HbA1c (> 10%) (Figs. 8, 9, and 10).

Subgroup analysis as per baseline BMI

In the overall sample, at baseline, 945 patients had BMI < 25 kg/m^2, 1961 had BMI 25–30 kg/m^2, and 1239 patients had BMI > 30 kg/m^2. Demographic characteristics of the patients at baseline BMI are described in Table 4. With the use of remogliflozin, the subgroups of BMI at baseline had significant reduction in HbA1c, FPG, PPG levels, and body weight from baseline to 3-month follow-up. The greater reduction in body weight was observed in patients with higher baseline BMI. The reduction of 2.0 kg and 3.3 kg in body weight was seen in subgroup of BMI 25–30 kg/m^2 and BMI > 30, respectively (Figs. 11, 12, and 13).

Non-glycemic parameters have been observed in low set of study population where data was available. Hence, this data is not representative of study to be considered.

Safety assessment of remogliflozin treatment

The reporting in safety section of the CRFs was observed to be low (437 CRFs), and hence, only these were considered for safety analysis. Of the 437 records, 25.9% (113) patients reported total of 136 events. Table 5 shows the incidences of adverse events with remogliflozin-based regimens. Genitourinary tract infections (GTIs) (12.5%) were most frequent AE associate with the use of remogliflozin.

The mean eGFR of 83.8 mL/min at baseline decreased by − 1.8 mL/min at 3 months, while serum creatinine decreased by − 0.07 mg/dL ($p > 0.05$ for both; Table 6). The serum uric acid and UACR showed statistically significant change from baseline of − 0.35 mg/dL and − 6.1 mg/g, respectively, at 3 months. The changes in serum sodium, potassium, chloride,

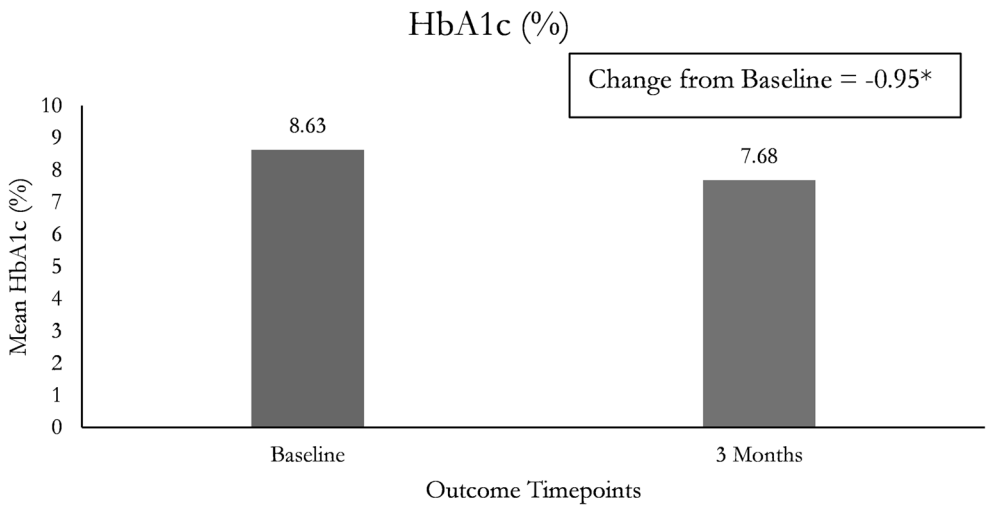

Fig. 3 Change in glycemic parameters (HbA1c). **Legend:** N=5358; HbA1c = glycosylated haemoglobin; * = P<0.05, statistically significant

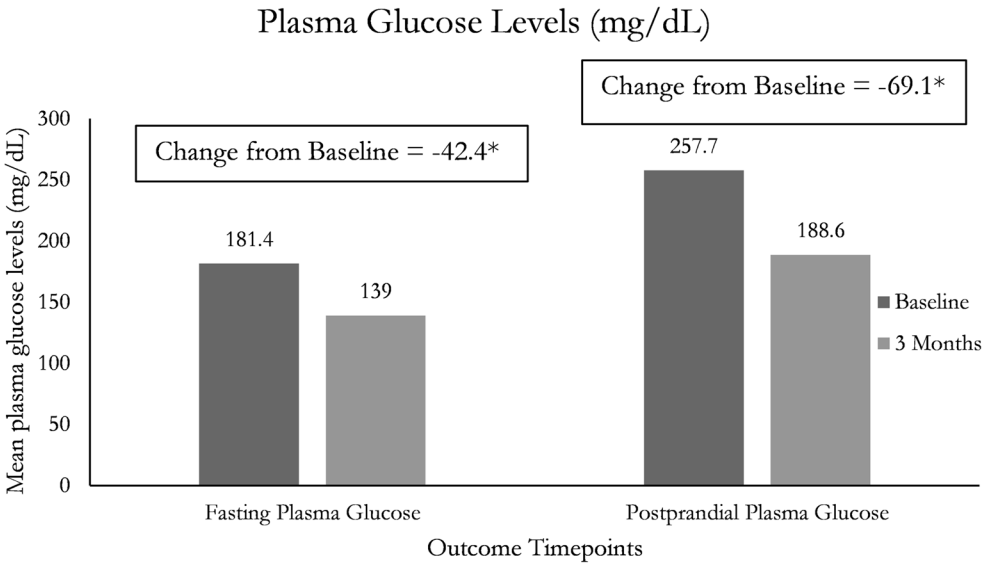

Fig. 4 Change in glycemic parameters (FPG and PPG). **Legend:** Change from baseline in Mean FPG levels, (N=4785); PPG (N=4962), FPG =Fasting Plasma Glucose; PPG=Postprandial Plasma Glucose; * = P<0.05, statistically significant

Table 2 Change in glycemic parameters and non-glycemic parameters

Parameters	*N*	Baseline	Follow-up (3 months)	Mean change from baseline
Glycemic parameters				
HbA1C (%)	5358	8.6 ± 1.04	7.68 ± 0.84	− 0.95*
FPG (mg/dL)	4785	181.4 ± 36.29	139 ± 27.15	− 42.4*
PPG (mg/dL)	4962	257.7 ± 59.56	188.6 ± 42.03	− 69.1*
Non-glycemic parameters				
Body weight (kg)	5241	74.6 ± 9.77	71.8 ± 9.51	− 2.6*
BMI (kg/m^2)	5241	28.3 ± 4.67	27.3 ± 4.53	− 1.0*
SBP (mmHg)	1886	126.9 ± 6.92	123.6 ± 7.54	− 3.3*
DBP (mmHg)	1886	82.4 ± 7.10	80.1 ± 7.29	− 2.3*

All values are mean ± SD unless specified otherwise; *statistically significant, $p < 0.05$; *BMI*, body mass index; *DBP*, diastolic blood pressure; *FPG*, fasting plasma glucose; *HbA1c*, glycated haemoglobin; *PPG*, postprandial plasma glucose; *SBP*, systolic blood pressure

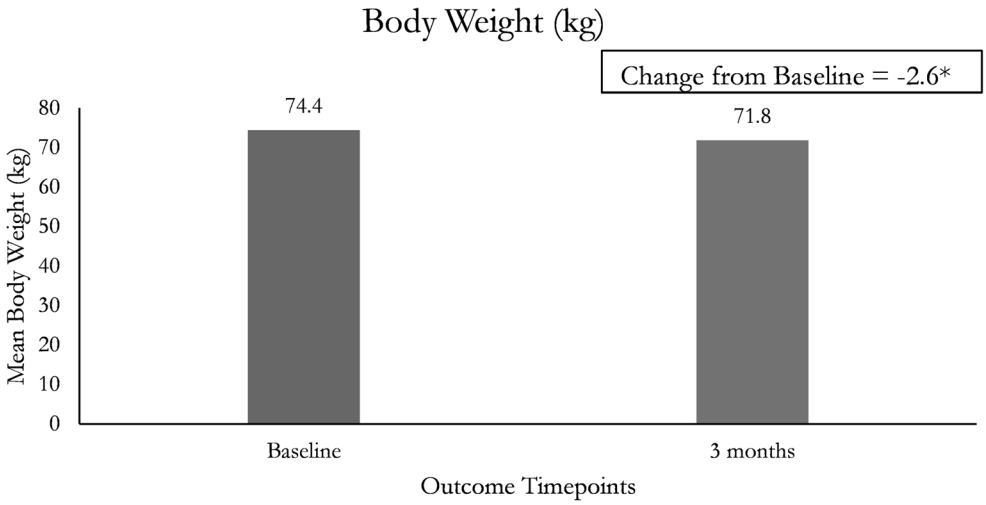

Fig. 5 Change in non-glycemic parameters (body weight). Legend: change from baseline in mean body weight values (N = 5241); *$p < 0.05$, statistically significant

and calcium from baseline to 3 months were − 1.7 mEq/L, − 0.1 mEq/L, 1 mEq/L, and − 0.1 md/L, respectively.

Discussion

The intensification of treatment of T2DM patients with addition of one or more ADDs for adequate glycemic control in routine practice is a well-established fact. This current analysis planned to assess the real-world effectiveness and safety of remogliflozin demonstrated significant reduction in glycemic (HbA1c, FPG, PPG) parameters after 3 months of treatment but also good safety and tolerability profile with remogliflozin-based anti-diabetic regimens.

The baseline demographics, comorbidities, and anti-diabetic therapy observed in the study population were comparable to epidemiology of T2DM patients and prescriptions practices in India. The age, BMI, and gender distribution in study population were similar to that reported by Singla et al. [8]. Though occurrence of comorbidities observed in study population was similar as reported by Iglay et al. [9], the incidence rate was lower. This could plausibly be due to reporting bias in retrospective data collection. It could also be due to use in low co-morbid patients on account of its recent launch and no experience with remogliflozin in treating physicians. The popular choice of metformin and sulfonylureas utilization as evident in Indian practice [8, 10] was also observed in the current study.

A significant decrease in HbA1c (− 0.95%, $p < 0.001$) was noticed after 3 months of treatment with remogliflozin added to ongoing background anti-diabetic therapy in uncontrolled settings of routine clinical practice. A real-world assessment in an uncontrolled clinical setting, resembling the current study, was conducted by Viswanathan et al. [11], Chakravorty et al. [12], and Munk et al. [13] to investigate the effectiveness of dapagliflozin, canagliflozin, and empagliflozin, respectively. The FOREFRONT study [11] by Viswanathan et al. reported a significant reduction of − 1% in HbA1c after 3 months of treatment with add-on Dapagliflozin to ongoing therapy. Similarly, Chakravorty et al. [12] have reported reduction of − 0.9% in HbA1c with

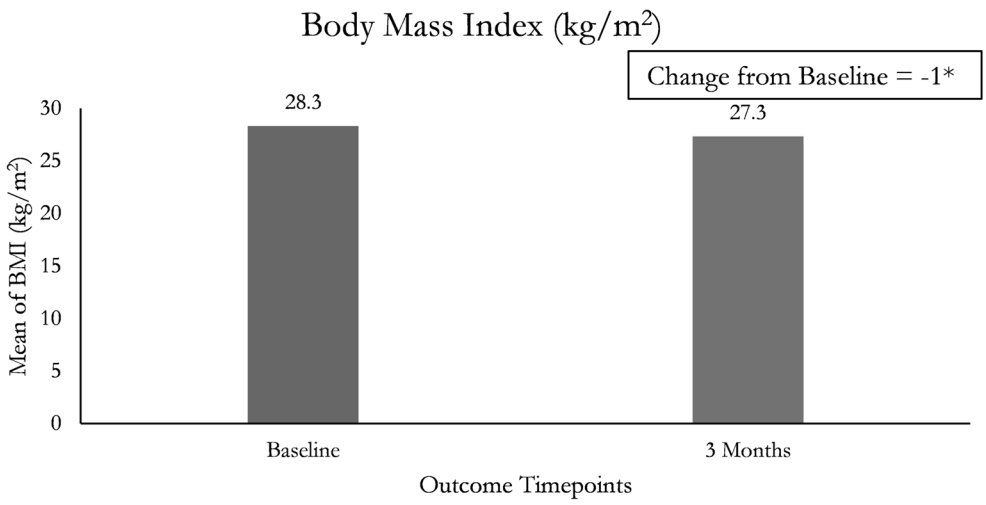

Fig. 6 Change in non-glycemic parameters (BMI). Legend: change from baseline in mean body mass index (N = 5241); *$p <$ 0.05, statistically significant

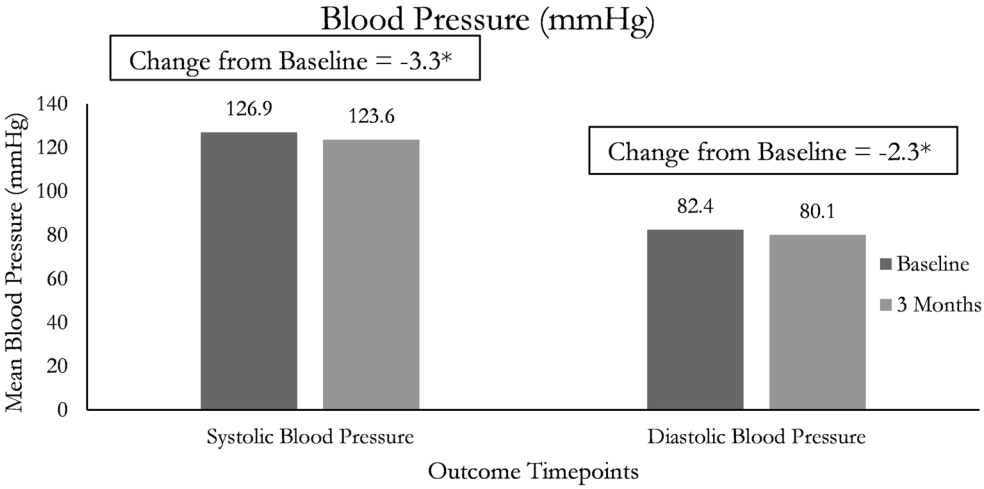

Fig. 7 Change in non-glycemic parameters (blood pressure). Legend: change from baseline in mean SBP and DBP ($N = 1886$). SBP, systolic blood pressure; DBP, diastolic blood pressure; *$p < 0.05$, statistically significant

Table 3 Baseline glycemic and metabolic characteristics of the patients in subgroups as baseline HbA1c

HbA1c subgroups	< 8.5%	8.5–10%	> 10%
N	2162	1957	326
HbA1c (%)	7.86 ± 0.39	9.11 ± 0.47	10.9 ± 0.74
Weight (kg)	73.6 ± 9.49	74.9 ± 9.48	77.5 ± 10.53
BMI (kg/m^2)	27.9 ± 4.33	28.4 ± 4.55	29.8 ± 5.18
FPG (mg/dL)	168.5 ± 29.28	186.7 ± 33.81	213.3 ± 39.87
PPG (mg/dL)	239.7 ± 54.31	272.8 ± 53.49	314.9 ± 50.40

All values are mean ± SD unless specified otherwise; *BMI*, body mass index; *FPG*, fasting plasma glucose; *HbA1c*, glycated haemoglobin; *PPG*, postprandial plasma glucose

canagliflozin, and Munk et al. [13] have reported reduction of − 0.91% in HbA1c with empagliflozin after 3 months of treatment when added on to existing therapy of patients. A real-world assessment of SGLT2i as add-on therapy that included both empagliflozin and dapagliflozin by Hong et al. [14] also reported − 0.94% reduction in HbA1c at 12 weeks. The results in the current study are in good agreement with these reports on other SGLT2i suggestive of similar glycemic reduction with remogliflozin as compared to other SGLT2i in routine practice. The phase III study of RE observed − 0.49% and − 0.72% reduction in HbA1c at 12 and 24 weeks in patients uncontrolled on metformin monotherapy [7], whereas the phase II study of RE observed change from baseline of − 0.96% in HbA1c at 12 weeks in drug naïve T2DM patients [15].

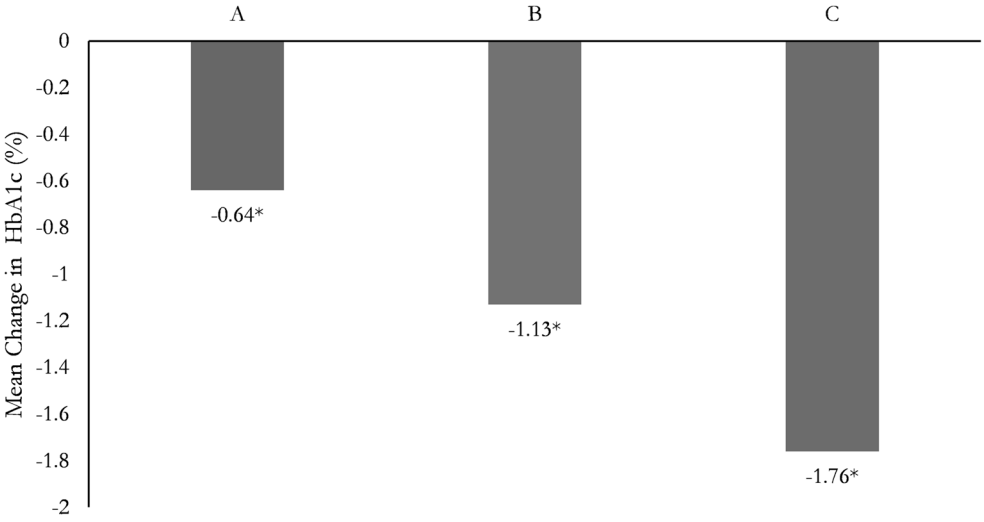

Fig. 8 Change in mean HbA1c levels in subgroups based on baseline HbA1c levels. Legend: Change from baseline in Mean HbA1c levels. ***A***= Subgroup with baseline HbA1c <8.5%, (N=2162); ***B***= Subgroup with baseline HbA1c 8.5-10%, (N = 1957); ***C***= Subgroup with baseline HbA1c >10%, (N = 326); HbA1c = Glycosylated Haemoglobin; * = P<0.05, statistically significant

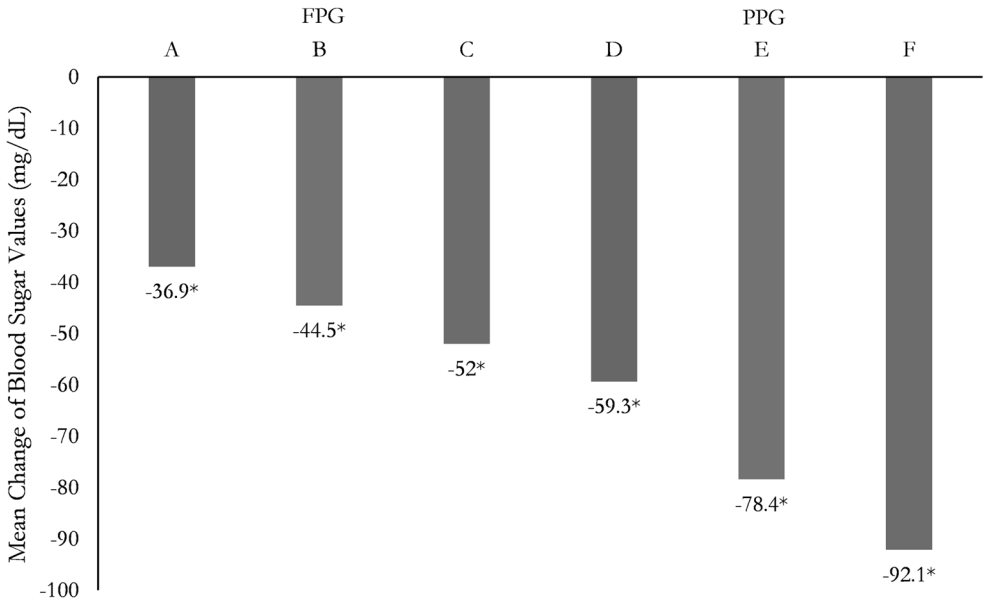

Fig. 9 Change in mean plasma glucose levels in subgroups based on baseline HbA1c levels. **Legend**: Change from baseline in FPG & PPG levels. ***A,D***= Subgroup with baseline HbA1c <8.5%, (N= 2162); ***B,E***=Subgroup with baseline HbA1c 8.5-10%, (N = 1957); ***C,F***=Subgroup with baseline HbA1c >10%, (N = 326); FPG = Fasting Plasma Glucose; PPG = Postprandial Plasma Glucose; HbA1C = Glycosylated Haemoglobin; * = P<0.05, statistically significant.

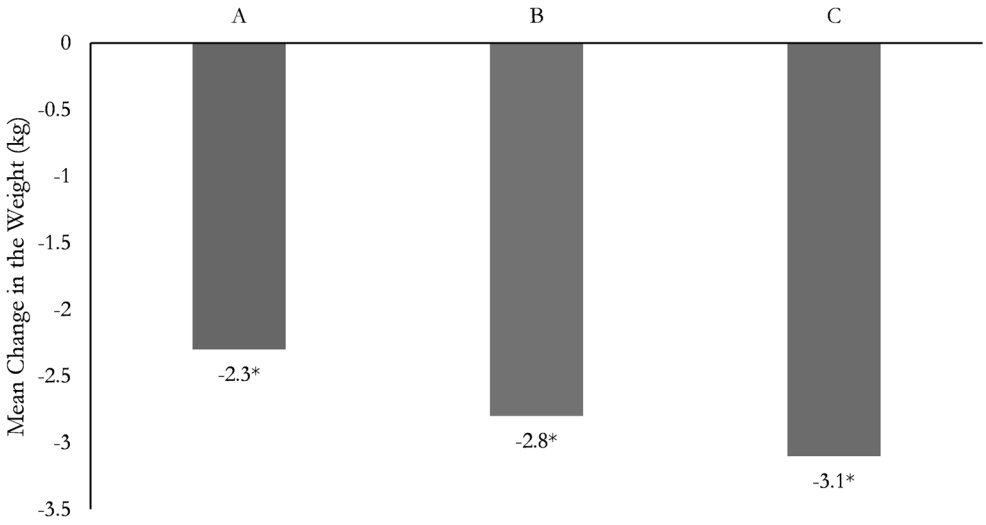

Fig. 10 Change in mean body weight in subgroups based on baseline HbA1c levels. **Legend:** Change from baseline in Mean Body weight values. ***A***= Subgroup with baseline HbA1c <8.5%, (N=2162); ***B***= Subgroup with baseline HbA1c 8.5-10%, (N = 1957); ***C***= Subgroup with baseline HbA1c >10%, (N = 326); HbA1c = Glycosylated Haemoglobin;* = P<0.05, statistically significant

The mean change in FPG and PPG levels was − 42.4 mg/dL and − 69.1 mg/dL, respectively, at 3-month postbaseline. A real-world assessment of SGLT2i (including empagliflozin and dapagliflozin) by Hong et al. [14] observed − 30.3 mg/dL reduction in FPG at 3 months. A retrospective analysis of canagliflozin add-on to teneligliptin reported FPG reduction of − 27.3 mg/dL at 3 months [16]. Though reports of PPG reduction with SGLT2i in the real-world setting are

Table 4 Baseline glycemic and metabolic characteristics of the patients in subgroups as baseline BMI

BMI subgroups	< 25 kg/m^2	25–30 kg/m^2	> 30 kg/m^2
N	945	1961	1239
BMI (kg/m^2)	22.9 ± 1.54	27.5 ± 1.39	33.5 ± 3.67
Weight (kg)	65.4 ± 5.77	73.7 ± 7.36	82.2 ± 8.72
HbA1c (%)	8.4 ± 0.91	8.6 ± 0.98	8.8 ± 1.01
FPG (mg/dL)	174.8 ± 31.44	181.1 ± 34.96	182.0 ± 35.89
PPG (mg/dL)	255.2 ± 54.35	259.2 ± 56.94	262.1 ± 61.30

All values are mean ± SD unless specified otherwise; *BMI*, body mass index; *FPG*, fasting plasma glucose; *HbA1c*, glycated haemoglobin; *PPG*, postprandial plasma glucose

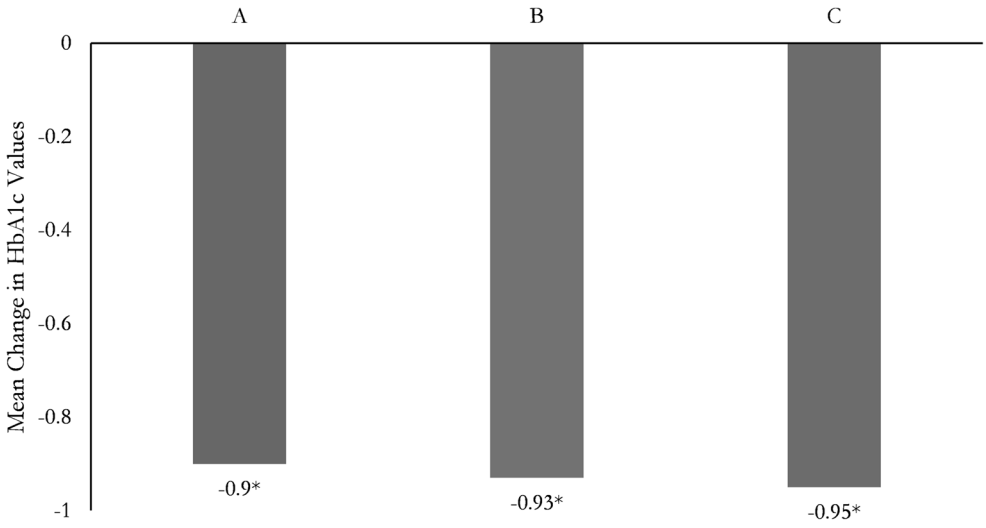

Fig. 11 Change in mean of HbA1c in subgroups based on baseline BMI values. **Legend:** Change from baseline in Mean HbA1c levels. ***A***= Subgroup with baseline BMI < 25, (N=945); ***B***= Subgroup with baseline BMI 25-30, (N = 1961); ***C***= Subgroup with baseline BMI >30, (N = 1239); * = P<0.05, statistically significant

limited, Chakravorty et al. [12] have observed − 53.6 mg/dL reduction in PPG after 3 months of add-on therapy with canagliflozin. The results are in good agreement with study results, and the marginal variance can be attributed to labile nature of plasma glucose levels.

The body weight and BMI reduction observed after 3-month follow-up was − 2.6 kg and – 1 kg/m^2, respectively. The weight reduction observed at 3 months of add-on SGLT2i therapy ranged from 1.4 to 2.2 kg [12, 17]. However, a study performed by Sykes et al. [15] demonstrated reduction in bodyweight, ranging from 1.36 to 3.51 kg at week 12 in patients receiving RE. A meta-analysis of 34 randomized clinical trials with 9154 patients showed that SGLT2i including canagliflozin, dapagliflozin, and empagliflozin were associated with a loss of body weight within a range of − 2.0 to − .2.3 kg [18]. Similarly, the BMI reduction at 3 months after initiation of SGLT2i ranged from 0.5 to 2.7 kg/m^2 in real-world studies [12, 19]. The results observed in study are comparable to reported reductions with other SGLT2i.

The reduction in systolic and diastolic BP from baseline to 3-month follow-up was − 3.3 mmHg and − 2.3 mmHg, respectively. Limited studies [14, 17, 19] have evaluated effect of SGLT2i on blood pressure in the real-world setting; these reported SBP reduction ranging from − 2.2 to − 4.7 mmHg, while DBP reduction ranges from − 1.3 to − 1.5 mmHg after 3-month therapy. A systemic review effect of SGLT2i on BP by Storgaard et al. [18] has estimated SBP and DBP reduction of − 3.9 mmHg and − 2.0 mmHg. The BP reduction observed in the current study is similar to BP reduction observed in phase III study of remogliflozin [7] in line with reductions observed with the use of SGLT2i.

A systematic review which included data from sixty-eight randomized controlled trials showed that addition of SGLT2i to other OADs resulted a significant impact on the reduction

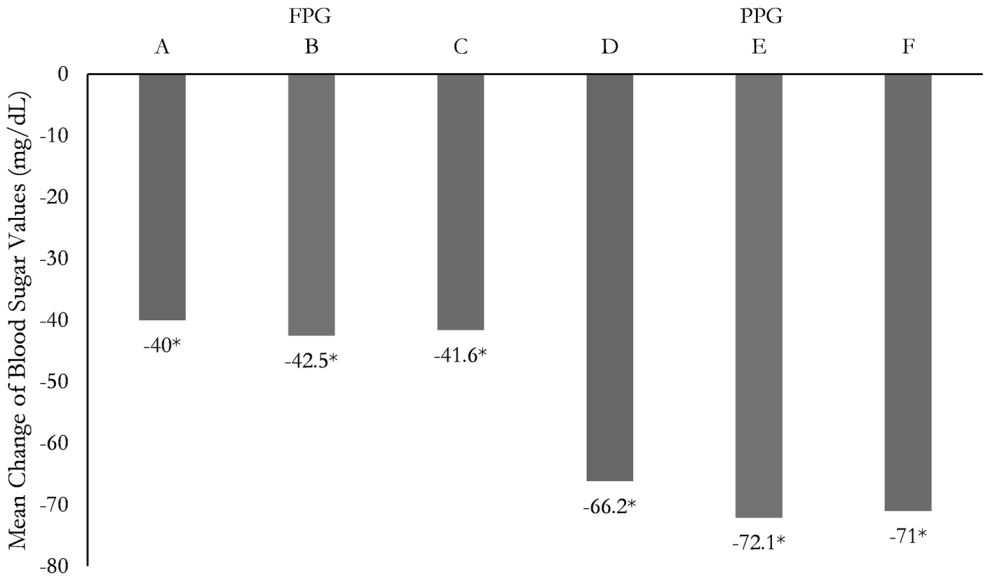

Fig. 12 Change in Mean of FPG and PPG in subgroups based on baseline BMI values. **Legend:** Change from baseline in Mean FPG and PPG levels. ***A,D***= Subgroup with baseline BMI < 25, (N=945); ***B,E***= Subgroup with baseline BMI 25-30, (N = 1961); ***C,F*** = Subgroup with baseline BMI >30, (N = 1239); FPG = Fasting Plasma Glucose; PPG = Postprandial Plasma Glucose; * = P<0.05, statistically significant

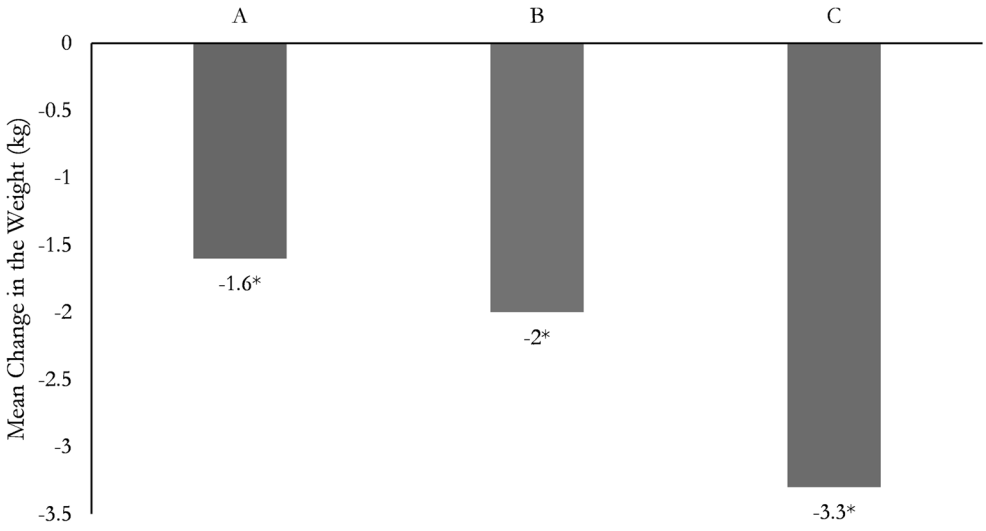

Fig. 13 Change in mean of weight from in subgroups based on baseline BMI values. **Legend:** Change from baseline in Mean Body Weight values. ***A***= Subgroup with baseline BMI < 25, (N=945); ***B***= Subgroup with baseline BMI 25-30, (N = 1961); ***C***= Subgroup with baseline BMI >30, (N = 1239); * = P<0.05, statistically significant

of HbA1c levels, weight loss, and blood pressure [20]. In the subgroup analysis, which was based on baseline HbA1c and BMI, patients treated with RE demonstrated to have clinically relevant and statistically significant reductions in HbA1c, body weight, FPG, PPG, and systolic and diastolic BP regardless of baseline HbA1c and BMI. Larger HbA1c reductions were seen among patients with higher baseline HbA1c. These findings were consistent with observations from various clinical trials of other SGLTs inhibitors [21–23]. Similarly, the reduction in body weight was greater in patients with higher baseline BMI which too has been observed in various trials with SGLT2i [24]

Administration of remogliflozin as add-on therapy was well-tolerated in patients with T2DM. Though present study had a limitation of low safety reporting, the observed incidence rate of treatment emergent adverse event (TEAE) of 25.9% was in agreement with TEAE of 32.6% observed in phase III study with remogliflozin 100 mg BID [7]. The most common AE was genito-urinary tract infections (12.6%) which is cumulative incidence of UTI and GTIs. These have been cumulatively reported due to inability of specific classification on account of non-standardized reporting. The pattern and incidence of adverse events observed in the study are in accordance to known safety profile of SGLT2 inhibitors [25, 26].

Table 5 Reported incidences of adverse events (> 1%)

Adverse event	*N* = 437	Percentage
Genito-urinary tract infections*	55	12.6%
Acidity	13	2.6%
Body pain/cramps	11	2.5%
Bowel disturbances	9	2.0%
Vomiting	7	1.6%
Giddiness/fatigue	5	1.1%
Others	36	5.4%

*Includes cumulative incidence of GTI and UTI

The reduction of − 1.8 mL/min in eGFR and − 0.07 mg/dL in serum creatinine was found to be statistically significant in the current study. The reduction in eGFR after initiation of SGLT2i is a known phenomenon, with a biphasic pattern of initial and transient dip over the first 2 weeks followed by recovery to baseline and stabilization during the subsequent months [27]. Hong et al. observed similar reduction of − 1.9 mL/min at 12 weeks in the real-world assessment of SGLT2i therapy [14]. Hence, reduction observed in the current study probably coincides with recovery phase of eGFR as was also observed in phase III study of RE [7]. This could plausibly explain low serum creatinine whose quantum of change can be reasonably considered to be clinically insignificant.

Conclusion

Remogliflozin etabonate in the real-world setting was found to be an effective agent for improvement of glycemic parameters when used as add-on therapy in the management of Indian T2DM patients, without any new safety concerns. The effectiveness and safety profile are similar to profile observed with other SGLT2i. The results were in accordance to earlier conducted developmental studies with RE.

Limitations of the study

Our findings of the study need to be interpreted within the limitations of this study. The study did not control for modification in background therapy; thereby, the reduction observed needs to be attributed to collective efficacy of

Table 6 Change in renal laboratory parameters and serum electrolyte

Parameters	*N*	Baseline	Follow-up (3 months)	Mean change from baseline
Renal parameters				
eGFR (mL/min)	533	83.8 ± 21.05	82 ± 19.07	− 1.8*
Sr creatinine	627	1.03 ± 0.26	0.96 ± 0.23	− 0.07*
Sr uric acid	698	5.55 ± 1.47	5.2 ± 1.24	− 0.35
UACR	209	19.8 ± 68.12	13.7 ± 34.22	− 6.1
Sr electrolyte concentrations				
Sr sodium	505	139.5 ± 4.36	137.8 ± 3.97	− 1.7
Sr potassium	492	4.1 ± 0.43	4 ± 0.41	− 0.1
Sr calcium	324	9.2 ± 0.51	9.1 ± 0.53	− 0.1
Sr chloride	302	100.9 ± 3.59	101.9 ± 4.18	1

All values are mean ± SD unless specified otherwise; *statistically significant, $p < 0.05$; *eGFR*, estimated glomerular filtration rate; *Sr*, serum; *UACR*, urine albumin to creatinine ratio

remogliflozin and concomitant changes in therapy, if any. The reporting and diagnoses of comorbidities were not validated; therefore, prevalence may have been underestimated. The laboratory data was limited only to patients in whom they were performed and reported, in CRFs, with the possibility of reporting biases. Other limitations include lack of electronic medical records and limited inclusion criteria. Our study results are real-world observations with no parameter being attributed to study drug, and the requirement of well-controlled clinical studies for confirmation of observed trends is necessary.

Acknowledgments The authors acknowledge the support of QREC Clinical Research LLP's statistical team and medical writing team aid in the preparation of this manuscript.

Code availability Not applicable

Author contribution All authors contributed to the study conception and design. Dr Sagar Katare, Dr Sachin Suryawanshi, and Dr Hanmant Barkat were involved in study conduct, analysis, study report preparation, manuscript preparation, and review. Dr Bipin Sethi, Dr Subhankar Chowdhury, and Dr Supratik Bhattacharya were involved in study report review and manuscript review. All authors read and approved the final manuscript.

Funding The study was sponsored and conducted by Glenmark Pharmaceuticals Limited, Mumbai, India. Glenmark Pharmaceuticals Limited has also supported in statistical analysis and publication of the manuscript.

Data availability Confidentiality of the patient's data was maintained during data handling.

Declarations

Ethics approval This is an observational study. The study conduct was approved by an Independent Ethics Committee.

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, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
2. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes - 2020. Diabetes Care 2020;43(Suppl. 1):S98–S110
3. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of diabetes. Diabetologia. 2015;58:429–42.
4. Tamez-Perez HE, Gonzalez-Guajardo EE, Tamez-Pena AL. Consensus statement by the American Association of Clinical Endocrinologists and American College of endocrinology on the comprehensive type 2 diabetes management algorithm - 2019 executive summary. Endocr Pract. 2019;25(6):622.
5. Ferrannini E, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol. 2012;8:495–502.
6. Mohan V, Mithal A, Joshi S, Aravind S, Chowdhury S. Remogliflozin etabonate in the treatment of type 2 diabetes: design, development, and place in therapy. drug design. Dev Ther. 2020;14:2487–501.
7. Dharmalingam M, Aravind S, Thacker H, Paramesh S, Mohan B, Chawla M, et al. Efficacy and safety of remogliflozin etabonate, a new sodium glucose co-transporter-2 inhibitor, in patients with type 2 diabetes mellitus: a 24-week, randomized, double-blind, active-controlled trial. Drugs. 2020;80(6):587–600.
8. Singla R, Bindra J, Singla A, Gupta Y, Kalra S. Drug prescription patterns and cost analysis of diabetes therapy in India: audit of an endocrine practice. Ind J Endocrinol Metab. 2019;23(1):40–5.
9. Iglay K, Hannachi H, Joseph Howie P, Xu J, Li X, Engel S, et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. Curr Med Res Opin. 2016;32(7):1243–52.
10. Rosenstock J, Seman LJ, Jelaska A, Hantel S, Pinnetti S, Hach T, et al. Efficacy and safety of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as add-on to metformin in type 2 diabetes with mild hyperglycaemia. Diabetes Obes Metab. 2013;15:1154–60.

11. Viswanathan V, Singh K. Use of Dapagliflozin in the management of type 2 diabetes mellitus: a real-world evidence study in Indian patients (FOREFRONT). Diabetes Technol Ther. 2019;21(8):415–22.
12. Chakravorty S, Patel V. 2359-PUB: clinical effectiveness of canagliflozin 100mg in T2DM patients—a real-world study. Diabetes. 2019;68(Supplement 1):2359-PUB.
13. Munk N, Knudsen J, Pottegård A, Witte D, Thomsen R. Differences between randomized clinical trial participants and real-world empagliflozin users and the changes in their glycated hemoglobin levels. JAMA Netw Open. 2020;3(2):e1920949.
14. Hong A, Koo B, Kim S, Yi K, Moon M. Efficacy and safety of sodium-glucose cotransporter-2 inhibitors in Korean patients with type 2 diabetes mellitus in real-world clinical practice. Diab Metab J. 2019;43(5):590–606.
15. Sykes AP, O'Connor-Semmes R, Dobbins R, et al. Randomized trial showing efficacy and safety of twice-daily remogliflozin etabonate for the treatment of type 2 diabetes. Diabetes Obes Metab. 2015;17:94–7.
16. Fushimi Y, Obata A, Sanada J, Iwamoto Y, Mashiko A, Horiya M, et al. Effect of combination therapy of canagliflozin added to teneligliptin monotherapy in Japanese subjects with type 2 diabetes mellitus: a retrospective study. J Diab Res. 2020;2020:1–7.
17. Scheerer M, Rist R, Proske O, Meng A, Kostev K. Changes in HbA1c, body weight, and systolic blood pressure in type 2 diabetes patients initiating dapagliflozin therapy: a primary care database study. Diab, Metab Syndrome Obes: Targets Ther. 2016;9:337–45.
18. Storgaard H, Gluud LL, Bennett C, Grøndahl MF, Christensen MB, Knop FK, et al. Benefits and harms of sodium-glucose cotransporter 2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2016;11:e0166125.
19. Puli K, Vanjari N. A 12 week prospective clinical evidence of empagliflozin efficacy in uncontrolled type 2 diabetes mellitus treated with metformin and a sulfonylurea. Int J Basic Clin Pharmacol. 2019;8(12):2639–44.
20. Kalra S, Kesavadev J, Chadha M, Kumar G. Sodium-glucose cotransporter-2 inhibitors in combination with other glucose-lowering agents for the treatment of type 2 diabetes mellitus. Ind J Endocrinol Metabol. 2018;22(6):827–36.
21. Buysman EK, Chow W, Henk HJ, Rupnow MF. Characteristics and short-term outcomes of patients with type 2 diabetes mellitus treated with canagliflozin in a real-world setting. Curr Med Res Opin. 2015;31:137–43.
22. WildingJP BL. LeiterLA, et al. efficacy and safety of canagliflozin bybaselineHbA1c and known durationoftype2diabetes mellitus. J Diabetes Complicat. 2015;29:438–44.
23. Rosenstock J. ChuckL, Gonzalez-OrtizM, etal. Initial combination therapy with canagliflozin plus metformin versus each component as monotherapy in drug-naïve type 2diabetes. Diabetes Care. 2016;39:353–62.
24. Lee P, Ganguly S, Goh S. Weight loss associated with sodium-glucose cotransporter-2 inhibition: a review of evidence and underlying mechanisms. Obes Rev. 2018;19(12):1630–41.
25. INVOKANA (canagliflozin) [package insert]. Titusville, NJ: Janssen Pharmaceuticals; 2020
26. FARXIGA® (dapagliflozin) [package insert]. Princeton, NJ: AstraZeneca Pharmaceuticals LP; 2020
27. De Nicola L, Gabbai F, Garofalo C, Conte G, Minutolo R. Nephroprotection by SGLT2 inhibition: back to the future? J Clin Med. 2020;9(7):2243.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):226–234
https://doi.org/10.1007/s13410-022-01061-8

ORIGINAL ARTICLE

Assessment of executive functions in subjects with type 2 diabetes mellitus

Anandhalakshmi Swaminathan[1] · Rajkumar Ramanathan[2] · Thirunavukarasu Manickam[3]

Received: 17 August 2021 / Accepted: 18 February 2022 / Published online: 25 March 2022

Abstract

Background Type 2 diabetes mellitus (T2DM) is one of the leading health problems in India and its effect on the eyes, heart, etc. has been demonstrated but its effect on neuronal processing has been neglected. The evidence for executive function impairment in patients with T2DM has been suggested by few studies.

Aim To examine the executive functions in subjects with T2DM and non-diabetic subjects and to detect the effect of glycemic control and diabetic duration.

Methods This cross-sectional study involved 124 subjects with T2DM aged between 30 and 60 years and 124 age-matched non-diabetic controls. After history taking, glycosylated hemoglobin, fasting and post-prandial blood sugar levels were tested. The neuropsychological assessment included the Mini-Mental State Examination (MMSE), PGI memory scale, Trial Making test (TMT) A and B, and Wisconsin Card Sorting Test (WCST).

Results We found a significant decrease in MMSE scores, attention concentration, and retention of dissimilar pairs of PGI memory scale, TMT A and B scores among the subjects with T2DM. In WCST, the mean values of total number of errors, percent errors, perseverative responses, perseverative errors, the number of categories completed, and trials to complete first category were significantly increased, and total number of correct, conceptual level responses were significantly decreased among the subjects with T2DM. These test score subsets were also significantly correlated with metabolic control and diabetic duration.

Conclusion Our study results support an association between executive dysfunction and type 2 DM. The poor glycemic control and longer duration of diabetes show a positive correlation with executive dysfunctions.

Keywords Executive function · Glycated hemoglobin · Trial Making Test · Type 2 diabetes mellitus · Wisconsin Card Sorting Test

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has been increasing in India and all over the world with India contributing a considerable part of global burden [1, 2]. T2DM is associated with reduced quality of life and chronic long-term complications and the implications for the Indian healthcare system are enormous. Many epidemiological studies have registered an association between T2DM and increased cognitive decline [3, 4]. Among the cognitive variables studied, one form of cognitive function that seems to be exceptionally affected among individuals with T2DM is executive function (EF). EF consists of a set of complex cognitive control mechanisms responsible for higher cortical functions such as planning, decision-making, and target-directed behavior that are crucial for self-governance and self-control [5].

People with T2DM need a lot of self-care activities to maintain their blood sugar level. EF is related to treatment adherence, and the impairment of EF may adversely affect the subject's ability to self-care such as difficulty in identifying and correcting hypoglycemic episodes by poor virtue of adaptive responses and sluggish processing speed [6].

The impairment in EF in subjects with type 2 diabetes mellitus would symbolize a potential barrier to efficient

✉ Anandhalakshmi Swaminathan
dranandhalakshmismd@gmail.com

[1] Department of Physiology, All India Institute of Medical Sciences, Kalyani, West Bengal 741235, India

[2] Department of Psychiatry, Sree Balaji Medical College, Chrompet, Chennai, India

[3] Department of Psychiatry, SRM Medical College, Kattangulathur, Chennai, India

disease management and may warrant special clinical consideration. However, to our knowledge, only a very few studies have been conducted examining executive abilities in subjects with type 2 diabetes mellitus and whether deterioration in EF is a direct outcome of poor glycemic control has not yet been determined. If chronic hyperglycemia is linked to poor EF, one might predict that efforts to improve glycemic control would reduce its decline. With these considerations in mind, the present study was directed to examine the memory and EF in subjects with T2DM and age-matched non-diabetic subjects as controls using the PGI Memory Scale for assessing memory [7] and Wisconsin Card Sorting Test (WCST) which is the best characterized measure of EF [8] along with the trial making tests [9] and to detect the effect of glycemic control and duration of DM on memory and EF.

Material and methods

Study design

This was a cross-sectional observational study conducted from January 2016 to January 2018 at a tertiary care Medical College Hospital and Research Centre at Tamil Nadu, India.

Study setting and participants

We included 124 subjects with type 2 DM and 124 non-diabetic controls. The participants were selected by convenient non-random sampling. The diabetic patients attending the diabetic outpatient department during the specified time period consenting for interview were included in the study. Similarly, the controls were the subjects without diabetes attending the master health check-up.

Patients with psychiatric illness, liver dysfunction, thyroid disorder, subjects with type 1 diabetes mellitus, hypertension, history of previous head injury, stroke, epilepsy, etc. were excluded from the study.

The diagnosis of subjects with type 2 diabetes mellitus was determined according to the WHO criteria, fasting blood sugar ≥126 mg/dl, and 2 h post-load glucose test ≥200 mg/dl. Blood glucose levels were estimated by glucose oxidase-peroxidase (GOD/POD) method using Beckman Coulter auto-analyzer. HbA1C was measured by turbid metric immunoassay.

Data collection

All the participants were subjected to a structured interview in the out-patient department to collect demographic information such as age, sex, literacy level, and occupation. From the subjects with diabetes, the following details such as duration of diabetes, presence of co-morbidities such as hypertension, dyslipidemia, any symptoms of retinopathy, nephropathy, neuropathy, etc., and personal details such as smoking and alcoholism were also enquired and recorded. The details of treatment such as taking insulin or oral hypoglycemic agents or both the regularity of treatment and history of hospitalization for diabetes complications were also enquired and noted.

Cognitive function tests

We used Folstein Mini Mental State examination (MMSE) to assess global cognition. The total test score for MMSE test is 30 points, and an individual with a score of below 24 is identified to have cognitive impairment. MMSE has overall sensitivity 64% and specificity 96% and it was shown to have both good test-retest reliability (0.80–0.95) to identify dementia in the stages of mild to moderate [10]. It continues to be used for obtaining a standard index of cognitive dysfunction severity that is easily understood by clinicians across the spectrum of neurocognitive disorders' management. The memory of the subjects was tested by using the PGI Memory Scale [7] which consists of 10 subtests standardized for adult subjects such as remote memory, recent memory, mental balance, attention and concentration, delayed recall, immediate recall, verbal retention for similar pairs, verbal retention for dissimilar pairs, visual retention, and recognition. The test was developed and validated by Postgraduate Institute of Medical Education and Research, Chandigarh, India [7]. For remote, memory simple questions relating to current information and for recent memory questions that assess the patient's ability to recall events in the recent past were asked. For testing mental balance, alphabet and numbers were recalled in backward and forward series. Digit span forward and backward repetition was used to test attention and concentration. For testing delayed recall, the patient was instructed to recall after 1-min time, the names of common objects those the investigator has read out. The test for immediate recall included immediate sequential reproduction of the sentences read out by examiner in verbatim. For testing the verbal retention for similar pairs, a series of similar associative pairs of words were administered and the patient has to mention the associate words in response to the stimulus word. In the test for verbal retention for dissimilar pairs, the associate pair of words was unrelated and dissimilar.

For visual retention test, the investigator displayed five cards containing geometrical figure and patient was instructed to reproduce the drawing from memory. For testing recognition, the investigator showed a card containing common objects. Two minutes later, a second card containing another set of pictures having some picture appeared in first card was shown to the patient. Patient was asked to identify the picture that appeared in both the cards. Scores were allotted accordingly.

The EF was assessed by TMT [9] and the Wisconsin Card Sorting Test (WCST) [8]. The TMT has part A and part B. In both, the subject has to connect 25 successive objectives on a paper. The objectives in part A are all numerical and in part B alternates between numerical and alphabets. The participant's goal is to complete the trail as fast as they can and the time taken for completing the test is noted. The cognitive resilience and psychomotor speed (motor speed and visual search) can be assessed by the TMT. For the Trail Making Test, the test-retest scores range from 0.70 to 0.78, and the inter-rater reliabilities from 0.96 to 0.98 [9].

Next, we used the WCST which a classical reasoning test used in clinical neuropsychological practice, designed by Heaton et al. The highly developed cognitive functions (EF) mainly related to frontal lobes such as working memory, abstract thinking, set shifting, attention, perseverance, intellectual flexibility, inhibition of impulsive responses, strategic planning, and ability in concept formation can be assessed by this assessment tool [8]. A good performance in WCST also involves temporal and parietal along with frontal lobe. The failure on performance of WCST indicates executive dysfunction. The WCST is a complex task and requires 4 stimulus cards and 128 response cards and 45 min to administer.

The subject was instructed to match 128 response cards to one of four stimulus cards on the principle of a sorting rule that is decided by the examiner. Every response card had a design characterized by the following features: color (yellow, green, red, blue), number (1–4), and figure (circles, stars, triangles, crosses). After the participants pair each response card with stimulus card, he is given feedback whether the matching is correct or incorrect, but the sorting principle is not revealed. After the participant has done ten consecutive right matches in accordance with sorting principle, the sorting principle is changed without caution and the participant has to figure out the new rule and successfully make use of it. This test was continued until the subject has finished six categories or when all the 128 cards were used.

After administration of the test, the responses were entered in the system software WCST: CV4TM devised by Robert K. Heaton, PhD, and purchased from PAR, Inc., and scores were obtained. WCST summary scores indicate the total number of categories accomplished, the total number of correct or total number of errors, indicating overall level of achievement, the number and percentage of preservative errors reflecting inhibitory control and set shifting, the percentage of conceptual level responses reflecting apprehension or intuitiveness into the correct sorting principles, and learning-to-learn, reflecting effectiveness in learning the task, failure to maintain set, etc.

Reliability and validity The construct validity of the WCST as a measure of executive function has been established by Shute and Huertas, V [11]. With the exclusion of the learning to learn score (r=.658), interscorer reliability coefficient varied from 0.895 to 1.000 for the 11 scores of the WCST [8].

Statistical analysis

For statistical analysis, we used the statistical software SPSS 18 for windows. Normality was tested with frequency histograms and Shapiro-Wilk test. Data were presented as mean ± standard deviation. Students' test was used to compare the parameters between the groups. Correlation of score of MMSE and different subsets of PGI memory scale with biochemical parameters and diabetes duration was assessed by using Pearson's correlation. A two-sided p-value less than 0.05 was considered as significant.

Results

Table 1 shows the characteristics of the subjects with type 2 diabetes and controls at the baseline. The groups were comparable and there was no significant difference in physical characteristics like age, height, weight, and body mass index.

Figure 1 shows the scores of MMSE and two subsets of PGI memory scale, i.e., attention concentration (AT/CT) and retention for dissimilar pairs (RT dissimilar) of the subjects with type 2 diabetes and the controls. The values of the column represent the mean and standard deviation of scores of MMSE and attention concentration and retention for dissimilar pairs of both the groups. * Indicates a p value of less than 0.05 which was considered to indicate statistical significance. There was a significant decrease in the MMSE score, attention concentration, and retention for dissimilar pairs in the subjects with type 2 diabetes when compared to the controls.

r indicates the Pearson correlation coefficient. p value of less than 0.05 was considered to indicate statistical significance. Figure 2 shows a significant negative correlation between HbA1C level and MMSE score. There is also a significant negative correlation between HbA1C levels and different subsets of PGI memory scale such as mental balance, attention/concentration, delayed recall, retention of dissimilar pairs, and visual retention.

r indicates the Pearson correlation coefficient. p value of less than 0.05 was considered to indicate statistical significance. Figure 3 shows significant negative correlation of MMSE score with diabetic duration. Also, the visual recognition score has a significant negative correlation with the duration of diabetes.

Table 2 compares the scores of Trail Making Tests between the two groups. There was a significant increase in the Trail Making Tests A and B scores among the subjects with type 2 diabetes.

WCST performance indexes in two groups are shown in the Table 3. All mean values were significantly different ($p <$

Table 1 Comparison of physical characteristics between diabetics and controls

Parameters	Group 1 (subjects with type 2 diabetes) mean ± SD	Group II (controls) mean ± SD	*p* value
Age (years)	51 ±7.8	50 ±5.6	0.231
Height (cm)	154±8.2	159±7.2	0.516
Weight (kg)	63.48 ± 8.4	61.12 ± 8.7	0.1
Body mass index(BMI)	26.72 ±3.78	25.76±4.23	0.273

Values are expressed in mean and standard deviation

SD standard deviation

0.05), except for trails administered, non-preservative error, percentage non-preservative error, and learning to learn.

r indicates the Pearson correlation coefficient. *p* value of less than 0.05 was considered to indicate statistical significance. Table 4 shows the correlation of Trail Making Tests with HBA1C level and duration of diabetes. Both TMT A and B test scores were positively correlated with $HbA1_C$ levels indicating that the subjects with poor blood glucose control take more time to complete the test.

r indicates the Pearson correlation coefficient. *p* value of less than 0.05 was considered to indicate statistical significance. Table 5 shows the correlation of various parameters of WCST tests with HBA1C level and duration of diabetes. Trails administered and total number of errors, % errors, perseverative responses and its percentage, perseverative errors and its percentage, and trails to complete first category have a significant positive correlation with $HbA1_C$ levels. Total number correct, conceptual level response, and its percentage and number of categories completed have a significant negative correlation with $HbA1_C$ levels. Trails administered and total numbers of errors have a significant positive correlation with diabetic duration.

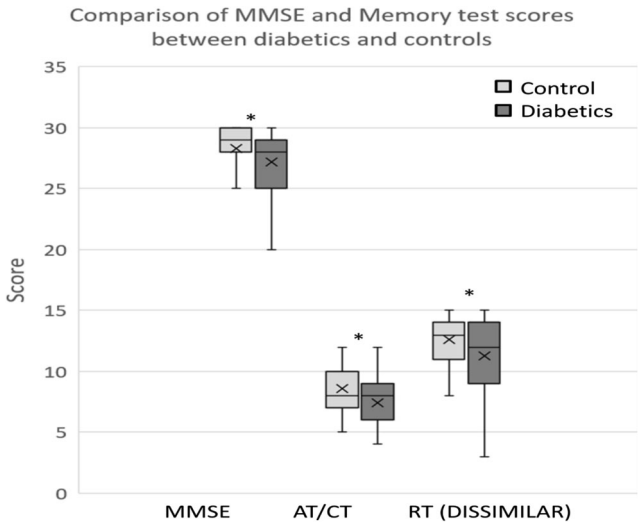

Fig 1 Comparison of MMSE and memory test scores between subjects with type 2 diabetes and controls

Discussion

In the present study, we examined the executive functions in subjects with type 2 diabetes mellitus and those of subjects without diabetes and both the groups were comparable in the baseline characteristics like age, height, weight, and BMI. We found that the mean MMSE score which is a measure of global cognition was significantly decreased among subjects with diabetes when compared to the controls. This finding was consistent with those of various studies, which showed a mild to moderate cognitive impairment in subjects with diabetes [12, 13]. Diabetes mellitus also increases the risk of the progression from such an impairment to dementia. The study by Cukierman and colleagues reported 1.2 to 1.5-fold more alteration over time in domains of cognition and 1.6-fold increase in the risk of developing Alzheimer's disease in subjects with diabetes [13]. We found that the diabetic subjects revealed impairment on attention/concentration which is a measure of mental control and working memory. Also, the verbal retention for dissimilar pairs which tests the capability of acquiring new information is also reduced in the subjects with diabetes. Earlier studies indicate that the poor immediate memory in diabetes is attributed to hippocampal atrophy [14].

In our study, there is a significant negative correlation between MMSE score and HBAIC levels among the subjects with type 2 diabetes. In our study, the MMSE scores are reduced for those with higher HbA1C levels and thus having a poor blood glucose control which is in concordance with the results of Ebady et al. [15]. On the contrary, Lindeman et al. [16] compared participants having diabetes and those with normal glucose tolerance and did not demonstrate any cognitive impairment in diabetes after adjusting the factors like ethnic background, gender, and age.

We have found a negative correlation between HbA1C levels of the subjects with diabetes and the scores of mental balance, attention/concentration, delayed recall, retention

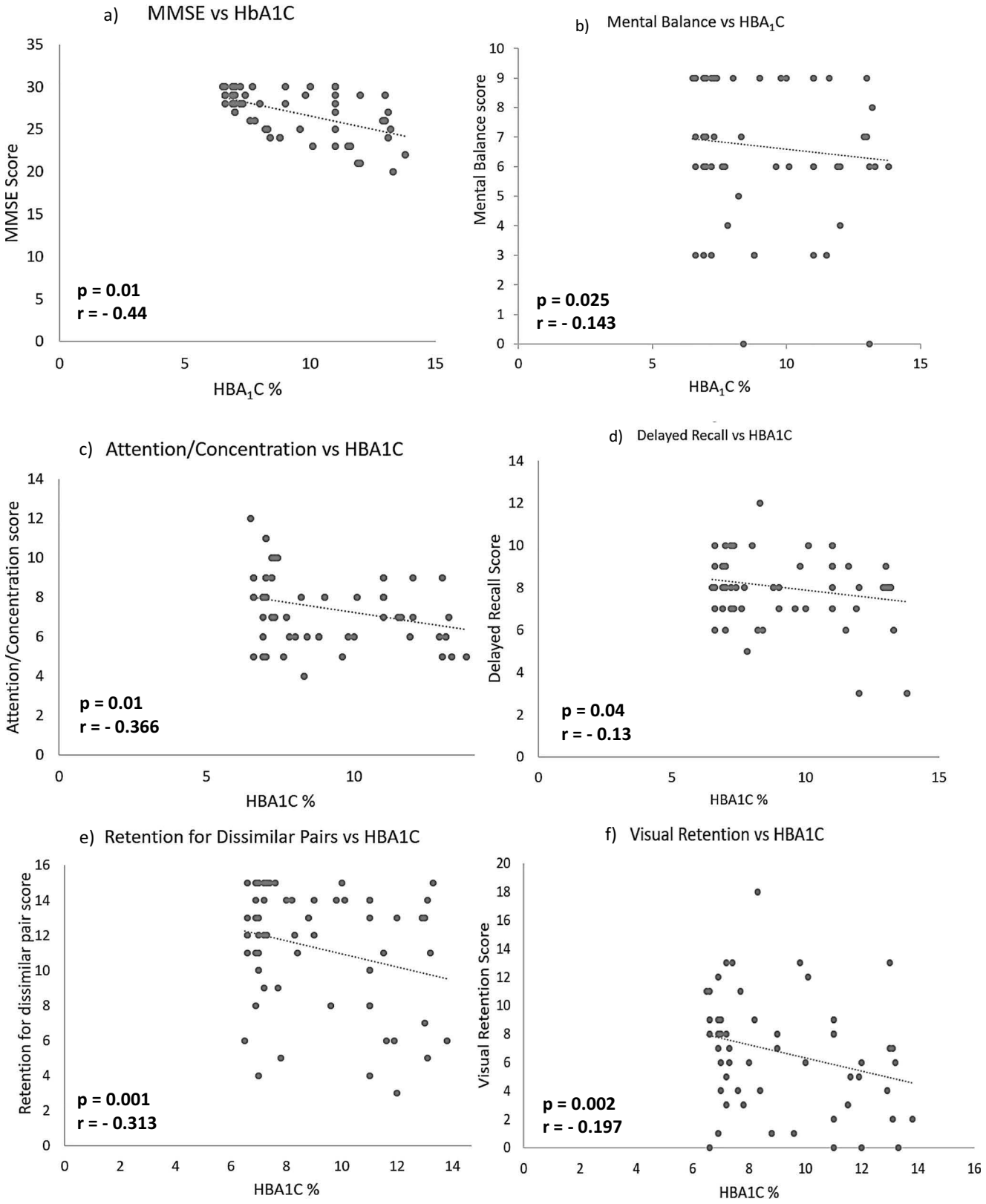

Fig 2 Correlation of MMSE and different subsets of PGI memory scale scores with HBA1C levels among the subjects with diabetes

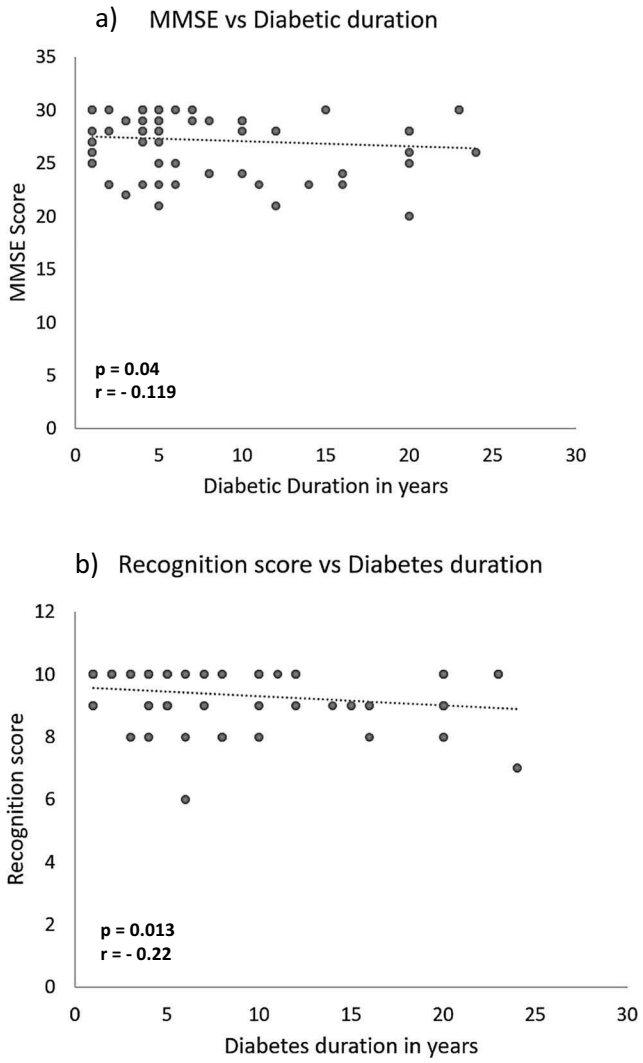

Fig 3 Correlation of MMSE score and visual recognition scores with the duration of diabetes

(dissimilar pairs), and visual retention scores. These findings support that the poor control of blood glucose has detrimental effect on the cognition of the subjects. We have found that there was a significant increase in the scores of the subjects with diabetes than the controls regarding Trail Making Tests (TMT) A and B scores. The Trail Making Test (TMT) is a two-part neuropsychological test, in which TMT A evaluates visual-conceptual and visual-motor tracking and sustained attention and TMT B evaluates task shifting abilities which is an executive function [17]. Although few studies investigated the brain regions engaged by TMT through functional neuroimaging demonstrating that TMT performance is related to frontal regions activity, there is also evidence that the brain-behavior correlations for the TMT are versatile and not limited to the frontal lobe [18, 19].

This proves that subjects with diabetes have reduced rate of cognitive processing and executive functioning. Our findings are compatible with observations in the study by Suresh et al. [20]. As Bell-McGinty et al. have suggested that performance in Trail Making Test (TMT) conveys information on EF domain that is appropriate to mobility and independence in activities of daily living [21], a reduced performance as shown in our study predicts that the quality of life will be affected.

WCST test demands the participants to make use of the investigator's comments for accurately matching the series of response cards with one of four stimulus cards. WCST was categorized as a task for working memory by researchers like Berman et al. [22] by virtue of the reason that participants need to hold preceding response information over short periods to direct their upcoming response. WCST performances involve aspects of both spatial orientation and object working memory as mentioned in previous research [23].

Functional imaging research involving WCST generally indicates judicious activation of prefrontal cortex mainly the dorsolateral region. We noted in our study that among the WCST performance indexes, the mean values of total number of errors, percent errors (total number of errors divided by number of trials administered), total number correct, perseverative responses, percent perseverative responses (total number of perseverative response divided by number of trials administered), perseverative errors (number of errors in which a subject continuously respond incorrectly using the same pattern), and percent perseverative errors (reflect the density or concentration of perseverative error in relation to overall test performance) were significantly different between the two groups.

Perseverations measure the perseverative behavior. In this study, we followed the scoring and recording methods suggested by Heaton et al., who considered whether or not the response card matches the "perseverated-to" principle. In some situations, the response might be both correct and

Table 2 Comparison of Trail Making Tests among diabetics and controls

Parameters	Subjects with diabetes (n=124) Mean ± SD	Controls (n=124) Mean ± SD	Test statistic	p value
TMT A	69.87 ± 27.183	49.18 ±17.128	7.172	<0.001*
TMT B	122.39 ± 48.433	76.50 ±36.122	8.457	<0.001*

*$p < 0.05$ indicates statistical significance

Table 3 Wisconsin Card Sorting Test (WCST) parameters among the study groups

Parameters	Subjects with diabetes (n=124) Mean ± SD	Controls (n=124) Mean ± SD	Test statistic	p value
Trails administered	115.37 ± 22.17	114.05 ± 18.11	0.514	0.607
Total number of errors	53.03± 30.16	38.23 ± 19.14	4.615	<0.001*
Total number correct	59.44 ± 18.16	75.82±10.69	−8.657	<0.001*
% errors	45.45 ± 21.26	31.97 ±13.09	6.012	<0.001*
Perseverative responses	48.98 ± 37.47	22.47 ± 13.69	7.401	<0.001*
% Perseverative responses	39.18 ± 28.36	18.74 ± 10.01	7.566	<0.001*
Perseverative errors	39.13 ± 27.56	19.87 ± 11.39	7.189	<0.001*
% Perseverative errors	31.37 ± 20.62	14.06 ± 9.83	8.433	<0.001*
Non perseverative error	16.81 ± 11.29	18.19 ± 11.78	−0.947	0.345
% Non-perseverative errors	14.05 ± 8.52	15.40 ± 8.59	−1.246	0.214
Conceptual level response	43.66 ± 24.76	65.44 ± 13.75	−8.559	<0.001*
% Conceptual level response	41.71 ± 27.61	59.65 ± 17.67	−6.093	<0.001*
Number of categories completed	2.76 ± 2.38	4.79 ± 1.49	−8.053	<0.001*
Trails to complete first category	53.12 ± 48.46	29.53 ± 23.11	4.894	<0.001*
Inability to maintain set	1.00 ± 1.31	1.42 ± 1.13	2.692	0.008*
Learning to learn	3.69 ± 10.54	−1.90 ± 9.64	−1.398	0.163

*$p < 0.05$ indicates statistical significance

perseverative, and, in that condition, it is possible that the judgment "right" given by the examiner augments the perseverative behavior presented by the subject. According to Heaton et al., incorrect responses that do not match the perseverated-to principle are scored as non-perseverative errors. WCST perseverative errors have been elucidated as a failure to restrict a learned response in spite of receiving error clues and have been described as the key feature of frontal lobe impairment [24, 25].

In the present study, the mean values of conceptual level responses and percent CLR (percentage of correct response occurring in runs of three or more), the number of categories completed, trials to complete first category, and inability to maintain set were significantly different between the two groups. In WCST, a total number of trials to successfully finish the first category and the number of CLR are associated to the degree of primary apprehension and the capacity for abstraction. These cognitive capabilities need the effective functioning of the frontal lobe [26]. The CLR score is the total number of consecutive correct responses in a sequence of three or more. This definition is based on the principle that a subject succeeding on three consecutive trials is considered to have at least an insight concerning the suitable strategy for the sorting task at hand and that the correct order produced is not a result of random responding.

The total number of categories accomplished indicates overall success, whereas the number of trials to complete the first category provides an index of conceptual capability. A failure to maintain the set occurs whenever an incorrect response follows a consecutive series of correct matches. The failure to maintain set (FTMS) index is a measure of the lack of the correct sorting principle during testing. The FTMS sheds light on conceptual instability. This index also construed as a measure of working memory. The FTMS score is the number of sequences of five correct responses or more, followed by an error, before achieving the 10 necessary for a set change. According to Heaton, in 1981, failure to complete four stages may be treated as a good index of impaired functioning. The EF deficits as measured by Wisconsin card sorting test revealed that diabetic subjects performed poorer than normal control subjects.

Our study results show that both TMT A and B test scores were positively correlated with $HbA1_C$ levels and people with poor blood sugar control need more time to complete the test. Also in WCST, the trails administered and total number of errors, % errors, perseverative responses and its percentage, perseverative errors and its percentage, and trails to complete

Table 4 Correlation of Trail Making Tests with diabetic parameters

Parameters		$HbA1_C$	DM duration
TMT A	r-value	0.439**	0.141
	p value	0.000	0.118
TMT B	r-value	0.433**	0.121
	p value	0.00*	0.182

Table 5 Correlation of WCST parameters with FBS, PPBS, $HbA1_C$, and diabetes duration

Parameters		$HbAI_C$	DM duration
Trails administered	*r*-value	0.182**	0.214**
	p value	0.004*	0.017*
Total number of errors	*r*-value	0.301**	0.222**
	p value	0.00*	0.013*
Total number correct	*r*-value	−0.378**	−0.013
	p value	0.00*	0.887
% errors	*r*-value	0.389**	0.138
	p value	0.00*	0.126
Perseverative responses	*r*-value	0.391**	0.123
	p value	0.00*	0.173
% perseverative responses	*r*-value	0.392**	0.109
	p value	0.00*	0.228
Perseverative errors	*r*-value	0.396**	0.144
	p value	0.00*	0.112
% perseverative errors	*r*-value	0.431**	0.130
	p value	0.00*	0.150
Non-perseverative error	*r*-value	0.086	0.090
	p value	0.179	0.322
% non-perseverative errors	*r*-value	0.053	0.031
	p value	0.407	0.735
Conceptual Level response	*r*-value	−0.417**	−0.079
	p value	0.00*	0.385
% Conceptual level response	*r*-value	−0.391**	−0.143
	p value	0.00*	0.113
Number of categories completed	*r*-value	−0.463**	−0.132
	p value	0.00*	0.145
Trails to complete first category	*r*-value	0.364**	0.151
	p value	0.00*	0.095
Failure to maintain set	*r*-value	−0.045	0.094
	p value	0.484	0.301
Learning to learn	*r*-value	−0.025	−0.008
	p value	0.700	0.928

first category have a significant positive correlation with $HbA1_C$ levels. Total number correct, conceptual level response, and its percentage and number of categories completed have a significant negative correlation with $HbA1_C$ levels. Trails administered and total numbers of errors have a significant positive correlation with diabetic duration. This shows that due to hyperglycemia or poorly controlled diabetes, the performance was significantly impaired.

WCST is sensitive (though not specific) to effects of lesions in prefrontal cortex and white matter. The most prominent result is increase in perseverative errors, i.e., tendency to persist in a specific response despite feedback indicating that it is no longer correct [27].

The Wisconsin Card Sorting Test (WCST) is commonly observed as a task that requires general EF. It is used to examine such cognitive capabilities as set-shifting, and concept formation (e.g., the creation of a sorting rule), the inhibition of improper habitual or prepotent responses, and responses to task-irrelevant information using response from examiner, and working memory, which the subject uses to keep the current sorting principle in mind [28, 29].

Inadequate performance on the WCST is considered to be a result of deficiency in various cognitive processes, including learning from feedback, maintaining a task goal in mind, noticing that a change in plan of action is necessary, inhibiting a previous motor response, switching to a different response, and maintaining a response process over time [30]. These functions can be regarded as elements of EF and have been considered as specific to the frontal region of the brain. Since CA is interpreted as a measure of flexibility in set-shifting, the poor performance by the diabetic group could be considered an indication of inadequate function in these individuals concerning concept formation, working memory, and set-shifting. Individuals with diabetes have been reported to make more perseverative responses on the WCST compared to controls.

The limitations in our study were that being a cross-sectional study, the causal relationship between T2DM and EF impairment cannot be determined. Since we used verbal questionnaire and self-report regarding diabetic complications, adherence to treatment, etc., we could not address the relationship between intervention, complications, and cognition in the current study. Our study did not evaluate the magnetic resonance images of brain. The study was conducted not in the community level but among the patients attending one hospital, and other aspects like racial/ethnic factors that might influence diabetic control could not be evaluated. Long-term longitudinal studies may be needed to substantiate and confirm our results.

Conclusion

T2DM individuals showed reduced performance in executive functions such as set shifting ability, cognitive flexibility, psychomotor speed, working memory, and new learning ability which negatively correlates with hyperglycemia. They should be screened for executive dysfunction as a routine.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Institutional ethical committee approval was obtained and informed written consent was obtained from all the included study subjects.

References

1. International Diabetes Federation.IDF Diabetes Atlas.8th edn. International Diabetes Federation, 2017 http://diabetesatlas.org/resources/(2017)-atlas.html
2. Global report on diabetes. WHO - World Health Organization, 2016
3. Nicolae H, Panea C, Gurgu R. Banjenaru Subclinical cognitive impairment in middle aged patients with type II diabetes mellitus. Romanian J Neurol. 2012;11:184–8.
4. Kataria L, Pandya H, Shah S, Shah H, Gerg R. Prevalence and pattern of cognitive dysfunction in type 2 diabetes mellitus. Int J Med Appl Sci. 2013;2(4):240–6.
5. Royall DR, Lauterbach EC, Cummings JL, Reeve A, Rummans TA, Kaufer DI, LaFrance WC Jr, Coffey CE. Executive control function: a review of its promise and challenges for clinical research. A report from the Committee on Research of the American Neuropsychiatric Association. J Neuropsychiatr Clin Neurosci. 2002;14(4):377–405.
6. Nguyen HT, Grzywacz JG, Arcury TA, Chapman C, Kirk JK, Ip EH, Bell RA, Quandt SA. Linking glycemic control and executive function in rural older adults with diabetes mellitus. J Am Geriatr Soc. 2010;58(6):1123–7.
7. Pershad D, Verma SK. Handbook of PGI battery of brain dysfunction (PGI- BBD) Agra. National Psychological Corporation 1990.
8. Heaton RK, Chelune GJ, Talley JL, et al. Wisconsin card sorting test manual. Odessa: Psychological Assessment Resources, Inc; 1993.
9. Reitan RM. Trail Making Test: manual for administration and scoring. Tuscon: Reitan Neuropsychology Laboratory; 1992.
10. Lomholt RK, Jurgensen KS. The mini mental state examination in screening of cognitive dysfunction and dementia. Uges kr Laeger. 1998;160:7251–4.
11. Shute GE, Huertas V. Developmental variability in frontal lobe function. Dev Neuropsychol. 1990;6(1):1–11.
12. Eze CO, Ezeokpo BC, Kalu UA, Onwuekwe IO. The prevalence of cognitive impairment amongst type 2 diabetes mellitus patients at Abakaliki South-East Nigeria. J Metabolic Synd. 2015;4:171. https://doi.org/10.4172/2167-0943.1000171.
13. Cukierman T, Gerstein HC, Williamson JD. Cognitive decline and dementia in diabetes–systematic overview of prospective observational studies. Diabetologia. 2005;48(12):2460–9.
14. Old SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, et al. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. Diabetologia. 2007;50(4):711–9.
15. Ebady SA, Arami MA, Shafigh MH. Investigation on the relationship between diabetes mellitus type 2 and cognitive impairment. Diabetes Res Clin Pract. 2008;82:305–9.
16. Lindeman RD, Romero LJ, LaRue A, Yau CL, Schade DS, Koehler KM, Baumgartner RN, Garry PJ. A biethnic community survey of cognition in participants with type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance: the New Mexico Elder Health Survey. Diabetes Care. 2001;24:1567–72.
17. Lezak MD. Neuropsychological Assessment. 3rd. New York: Oxford University Press; 1995.
18. Zakzanis KK, Mraz R, Graham SJ. An fMRI study of the Trail Making Test. Neuropsychologia. 2005;43(13):1878–86.
19. Jacobson SC, Blanchard M, Connolly CC, Cannon M, Garavan H. Brain and cognition an fMRI investigation of a novel analogue to the Trail Making Test. Brain Cogn. 2011;77(1):60–70.
20. Suresh Kumar P, Greeshma C, Sreekumaran. Study of dementia in diabetic patients with particular reference to Cerad and Trail Making Test. Int J Sci Eng Res. 2014;5(10):74–7.
21. Bell-McGinty S, Podell K, Franzen M, Baird AD, Williams MJ. Standard measures of executive function in predicting instrumental activities of daily living in older adults. Int J Geriatr Psychiatry. 2002;17(9):828–34.
22. Berman KR, Ostrem JL, Randolph C, Gold J, Goldberg TE, Coppola R, Carson RE, Herscovitch P, Weinberger DR. Physiological activation of a cortical network during performance of the Wisconsin Card Sorting Test: a positron emission tomography study. Neuropsychologia. 1995;33:1027–46.
23. Wilson FA, Scalaidhe SP, Goldman-Rakic PS. Dissociation of object and spatial processing domains in primate prefrontal cortex. Science. 1993;260(5116):1955–8.
24. Gold JM, Harvey PD. Cognitive deficits in schizophrenia. Psychiatr Clin North Am. 1993;16:295–312.
25. Burgess PW, Shallice T. Response suppression, initiation and strategy use following frontal lobe lesions. Neuropsychologia. 1996;34:263–72.
26. Stuss DT, Alexander MP. Executive functions and the frontal lobes: a conceptual view. Psychol Res. 2000;63:289–98.
27. Demakis GJ. A meta-analytic review of the sensitivity of the Wisconsin Card Sorting Test to frontal and lateralized frontal brain damage. Neuropsychology. 2003;17(2):255–64.
28. Castellanos FX, Sonuga-Barke EJS, Milham MP, Tannock R. Characterizing cognition in ADHD: beyond executive dysfunction. Trends Cogn Sci. 2006;10:117–23.
29. Robinson S, Goddard L, Dritschel B, Wisley M, Howlin P. Executive functions in children with autism spectrum disorders. Brain Cogn. 2009;71:362–8.
30. Geurts HM, Corbett B, Solomon M. The paradox of cognitive flexibility in autism. Trends Cogn Sci. 2009;13:74–82.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):235–243
https://doi.org/10.1007/s13410-022-01071-6

ORIGINAL ARTICLE

Impact of microbiological characteristics on the costs of treating diabetic foot infection

Julianne Soares Jardim Lacerda Batista[1] · Bil Randerson Bassetti[2] · Thaís Dias Lemos Kaiser[2] · Creuza Rachel Vicente[3] · Ricardo Pinto Schuenck[1]

Received: 21 October 2021 / Accepted: 17 April 2022 / Published online: 10 May 2022

Abstract

Objective To investigate the impact of the microbiological profile on the costs of treating diabetic foot infections (DFI).

Methods Data was accessed from electronic medical records of a Brazilian public tertiary hospital. The PEDIS score was used to classify ulcers and the risk of subsequent events (non-healing ulcer, need of amputation, death). Microbiological samples were obtained from deep tissue in surgical procedures and identified by standard methods. Information on actual treatment costs was collected in the financial sector.

Results We analyzed 117 medical records of 97 patients. The median PEDIS score was 9, and most patients (94.02%) presented a high risk of subsequent events. Most of the 226 microorganisms isolated were Gram-negative (68.14%). Longer hospitalization length and higher costs involved polymicrobial infections (31.92 days; Int $ 21,755.92), multidrug-resistant microorganism (MDR) (29.84 days; Int $ 20,219.99), and methicillin-resistant *Staphylococcus aureus* (30.25 days; Int $ 20,607.29). The costs with antimicrobials were significantly higher in polymicrobial infection than in monomicrobial and in the presence of MDR than non-multidrug-resistant microorganisms.

Conclusions The microbiological profile, number of microorganisms per wound, and bacterial resistance may increase the hospitalization time and costs of treating DFI, suggesting that bacterial culture may be more financially advantageous than applying empirical therapy for DFI at the beginning of the treatment.

Keywords Diabetic foot infection · Costs · Multidrug resistance · Polymicrobial infection

Introduction

Diabetes mellitus affected approximately 463 million people worldwide in 2019, and estimations indicate an increase to 700 million in 2045 [1]. Diabetic foot is one of the most frequent, costly, and limiting complications related to this disease [2]. This complication occurs due to poor glycemic control and vascular and neurological alterations. Diabetic foot alters the sensitivity of the feet, and injuries can originate from ulcers or contact with sharp objects. The wounds are a pathway open for developing infections that may progress to diabetic foot infection (DFI) [3].

Several characteristics may influence the microbiological profile of DFI, including geographic aspects, lesion severity, and previous antimicrobial use [4, 5]. Acute infections present mainly Gram-positive microorganisms, and *Staphylococcus aureus* is the primary agent. In chronic wounds, Gram-negative microorganisms are the majority, and infections are usually polymicrobial [4, 6], with virulence factors, metabolic cooperation, and quorum-sensing systems contributing to the chronicity [5]. In addition, studies have reported high rates of antimicrobial resistance in DFI worldwide, including resistance to multiple antimicrobials, resulting in high rates of amputations and deaths [7, 8].

Besides, the cost of treatment can be ten times higher in patients with diabetic foot than in diabetic people without this condition [9]. Other studies have shown the burden of this complication on health systems and out-of-pocket payment by those affected, mainly with costs related to hospitalization

✉ Ricardo Pinto Schuenck
ricardo.schuenck@ufes.br

[1] Laboratory of Molecular Biology and Bacterial Virulence (LABCAS), Department of Pathology, Center of Health Sciences, Federal University of Espírito Santo, Av. Marechal Campos, 1468, Maruípe, Vitória, ES CEP 29.043-900, Brazil

[2] Central State Hospital Benício Tavares Pereira, Vitória, Brazil

[3] Department of Social Medicine, Center of Health Sciences, Federal University of Espírito Santo, Vitória, Brazil

in middle and low-income countries [10, 11]. In Brazil, the estimated costs associated with the diabetic foot can reach 361 million dollars (Int$) per year [11]. However, there is still a dearth of studies on the actual cost of DFI, especially considering the microbiological characteristics. Therefore, this study presents the impact of the microbiological profile of DFI on the costs of treatment, indicating categories of expenditure to measure the actual therapeutical cost of this affection.

Research design and methods

Data were collected from medical records of adult patients (≥ 18 years) admitted to a tertiary public hospital from August 2016 to December 2019. This hospital is located in Vitória municipality, Espírito Santo state, Brazil, and is a reference center for the treatment of vascular and neurological diseases. The records included were those from patients with positive results in bacterial culture performed with deep tissue fragments, including readmissions. Therefore, the exclusion criteria encompassed the absence of clinical information and incomplete antibiogram results. The financial sector of the hospital provided information on the costs of antibiotics and the final total expenditure per patient.

The variables evaluated were age, sex, length of hospitalization (in days), antimicrobial therapy, surgical treatment (debridement, minor and major amputation, and plastic surgery), admission in the intensive care unit (ICU), bacterial culture, characterization of the isolate (monomicrobial or polymicrobial), antibiogram results, characterization of drug resistance (multidrug-resistant — MDR or non-multidrug resistant — NMDR), and costs with treatment.

An infectious disease specialist evaluated the ulcers and the risk of subsequent events (non-healing ulcer, need for amputation, death) using the classification of perfusion, extent, depth, infection, and sensation (PEDIS score). The risk of subsequent events was categorized as high (PEDIS score ≥ 7) and low (PEDIS score < 7) according to Chuan et al. [12].

MDR microorganisms were characterized by non-susceptibility to one or more agents of at least three different classes of antibiotics, according to the definition of Magiorakos et al. [13]. Microorganisms that did not fit in this condition were considered NMDR.

Polymicrobial infections involved more than one microorganism isolated per patient, and monomicrobial presented only one microorganism isolated.

The individual costs with DFI treatment included all expenses, such as hospitalization, procedures, surgeries, medications, materials, and antimicrobials. The cost of hospitalization includes hospitality services or daily room rates and considers staff costs, sanitation, and expenses with water, energy, and waste. All patients were admitted to the general ward with 3–6 beds. The procedures include laboratory tests and physiotherapy. The costs with surgery were related to all surgical procedures (debridement, minor and major amputation, plastic surgery). Medications and materials include all inputs (physiological solutions, needles, syringes, equipment, lint, and correlates) and medications used during the hospitalization, except antimicrobials. The antimicrobial therapy costs were calculated based on the dosage and time of use described in the medical records. All medications and antimicrobials used were generics. Costs were quoted in Brazilian real (BRL) and converted to International dollars (Int$) according to the 2016–2019 purchasing power parity (PPP) (1 Int$ = 2.190 BRL) [14].

The bacterial isolates were obtained from fragments of deep tissues of diabetic foot infection wounds (collected by debridement or other surgical procedure). All isolates were processed, isolated, and identified by standard methods [15]. Anaerobic culture was not performed in this study. The diffusion disc method was used to evaluate the susceptibility profile of the isolates to antimicrobials, according to the criteria established by the Clinical and Laboratory Standards Institute guidelines [16]. Besides, gradient method (to determine vancomycin and tigecycline susceptibility — E-test™ Biomérieux, France) and broth microdilution (to determine colistin susceptibility) were also employed.

Chi-square or Fisher's exact test was used to evaluate whether the type of infection (polymicrobial or monomicrobial, Gram-negative or Gram-positive microorganism, MDR or NMDR) affected the rates of amputation and the use of the ICU. Mann–Whitney *U* test was used to analyze whether the type of infection influenced the length of hospitalization and costs with the treatment. Statistical analyses were performed using GraphPad Prism (version 7.04). A p value < 0.05 was considered significant.

The Research Ethics Committee at the Federal University of Espírito Santo, in Vitória, Brazil, approved the study protocol (approval number 1.607.410).

Results

The study comprised 97 patients with DFI, primarily males (62.5%), with a mean age of 63.96 years (standard deviation = 11.08 years). Due to the readmission of 17 patients (14 readmitted once; three readmitted twice), 117 medical records were evaluated. The median PEDIS score was 9 (interquartile range = 10–8), ranging from 5 to 11, and most patients (94.02%) presented a high risk for a subsequent event (PEDIS score ≥7). The median length of hospitalization was 24.00 days (interquartile range = 15.00–40.00 days), and 56 (47.86%) patients were admitted to the ICU. The lower limb amputation occurred in 80 (68.37%) of hospitalizations, being 60 (75.0%) due to minor amputation and 20 (25.0%) due to major amputation. The median PEDIS score was 10

(interquartile range = 10–8) for patients who had an amputation and 9 (interquartile range = 10–8) for those who did not require amputation.

Debridements were performed in 33 cases (28.21%) and plastic surgery in two (1.71%). The main antimicrobials used in the 117 admissions were vancomycin (n = 58, 49.57%), ciprofloxacin (n = 48, 41.03%), piperacillin-tazobactam (n = 47, 40.17%), meropenem (n = 44, 37.61%), and ertapenem (n = 34, 29.06%). In addition, tigecycline was used in 11 cases (9.40%) and polymyxin B in six (5.13%).

A wide variety of bacterial species was identified among 226 microorganisms isolated. *Proteus mirabilis* (17.70%), *Staphylococcus aureus* (17.70%), and *Pseudomonas aeruginosa* (12.83%) were the most frequent (Table 1). The majority of the microorganisms were Gram-negative (n = 154, 68.14%). Most isolates were resistant to cephalosporins, penicillins, and quinolones. *P. mirabilis* was resistant to several classes, such as cephalosporins, e.g., cephalothin (n = 31, 77.50%) and ceftriaxone (n = 29, 72.50%); monobactam, e.g., aztreonam (n = 29, 72.50%); sulfonamides, e.g., sulfamethoxazole-trimethoprim (n = 23, 57.50%); and aminoglycosides, e.g., gentamicin (n = 22, 55.00%); and did not present resistance to carbapenems. Among the *S. aureus*, 24 (60.00%) were methicillin-resistant (MRSA), and the most of isolates were resistant to erythromycin (n = 32, 80.00%), ciprofloxacin (n = 25, 62.50%), and clindamycin (n = 19, 47.50%). *P. aeruginosa* showed more resistance to aztreonam (n = 16, 55.17%), ciprofloxacin (n = 16, 55.17%), and gentamicin (n = 14, 48.28%). Although less frequent, 24.14% (n = 7) of *P. aeruginosa* isolates were resistant to meropenem, and 13.79% (n = 4) to imipenem. All *A. baumannii* isolates were resistant to carbapenems, ceftazidime, and cefepime. In addition, *A. baumannii* were resistant to ciprofloxacin (n = 14, 93.33%), piperacillin-tazobactam (n = 14, 93.33%), gentamicin (n = 10, 66.67%), and sulfamethoxazole-trimethoprim (n = 10, 66.67%).

Table 1 Bacterial species isolated from diabetic foot infections

Bacterial species	N = 226	%
Proteus mirabilis	40	17.70
Staphylococcus aureus	40	17.70
Pseudomonas aeruginosa	29	12.83
Acinetobacter baumannii	15	6.64
Enterobacter cloacae	12	5.31
Enterococcus faecalis	11	4.87
Proteus vulgaris	10	4.42
Morganella morganii	9	3.98
Coagulase-negative Staphylococcus	9	3.98
Streptococcus agalactiae	6	2.65
Escherichia coli	5	2.21
Klebsiella aerogenes	5	2.21
Serratia marcescens	5	2.21
Other*	30	13.27

**Klebsiella pneumoniae*; *Proteus penneri*; *Enterobacter agglomerans*; *Klebsiella oxytoca*; *Citrobacter* spp.; *Staphylococcus intermedius*; *Betahemolytic Streptococcus* spp.; *Enterobacter aerogenes*; *Edwardsiella tarda*; *Pseudomonas putida*; *Providencia stuartii*; *Enterococcus faecium*; *Enterococcus* spp.; *Streptococcus viridans*

Most of the infections (n = 93, 79.48%) had at least one MDR bacteria, and 24 (20.52%) had only NMDR microorganisms. The median length of hospitalization in cases with MDR bacteria was 27 days (interquartile range = 16.00–41.00 days) while in patients with NMDR bacteria was 19 days (interquartile range = 13.25–29.75 days), without significant difference (p value = 0.14) (Fig. 1). Furthermore, there were no considerable differences between these groups regarding ICU admission (p value = 0.82), which occurred in 47.31% of MDR (n = 44) and 50.00% of NMDR (n = 12), as well as in amputation rates (p-value = 0.99), which affected 67.74% of MDR (n = 63) and 70.83% of NMDR (n = 17).

The median length of hospital stay in DFI caused by MRSA was 26.00 days (interquartile range = 16.25–48.75 days) and in infections with methicillin-sensitive *S. aureus* (MSSA) was 23.00 days (interquartile range = 13.25–34.75 days), without significant difference (p value = 0.25) (Fig. 1). Besides, there were no considerable differences regarding ICU admission, which occurred in 50.00% (n =12) of the patients with MRSA and 37.50% (n = 6) of MSSA cases (p value = 0.52). Amputation rates also did not differ between these groups (p value = 0.94), being 66.67% for MRSA (n = 16) and 62.50% for MSSA (n = 10).

Most of the infections were polymicrobial (n = 67, 57.26%): 62.68% (n = 42) caused by two bacterial species, 19.40% (n = 13) by three, 11.94% (n = 8) by four, 1.49% (n = 1) by five, and 4.47% (n = 3) by six. In the monomicrobial infections (n = 50, 42.74%), the main species isolated were *P. mirabilis* (n = 17, 34.0%) and *S. aureus* (n = 10, 20.0%). The median length of hospital stay was 28 days (interquartile range = 16.00–46.00 days) in patients with polymicrobial infection, being significantly higher than in those with monomicrobial infections (median = 21 days, interquartile range = 12.75–33.25 days) (p value = 0.03) (Fig. 1). There were no considerable differences between admission in ICU, which occurred in 50.00% (n = 25) of patients with monomicrobial infection and 46.26% (n = 31) of those with polymicrobial infection (p value = 0.68), as well as in amputation rate, which affected 68.00% (n = 34) of cases with monomicrobial infection, and 68.65% (n = 46) of those with polymicrobial infection (p value = 0.93).

Monomicrobial infections had predominance of Gram-negative bacteria (n = 33, 66.00%), and most of the monomicrobial infections with Gram-negative bacteria were MDR (n = 22, 66.67%). In monomicrobial infections caused by Gram-positive bacteria (n = 17, 34.00%), the rate of MDR

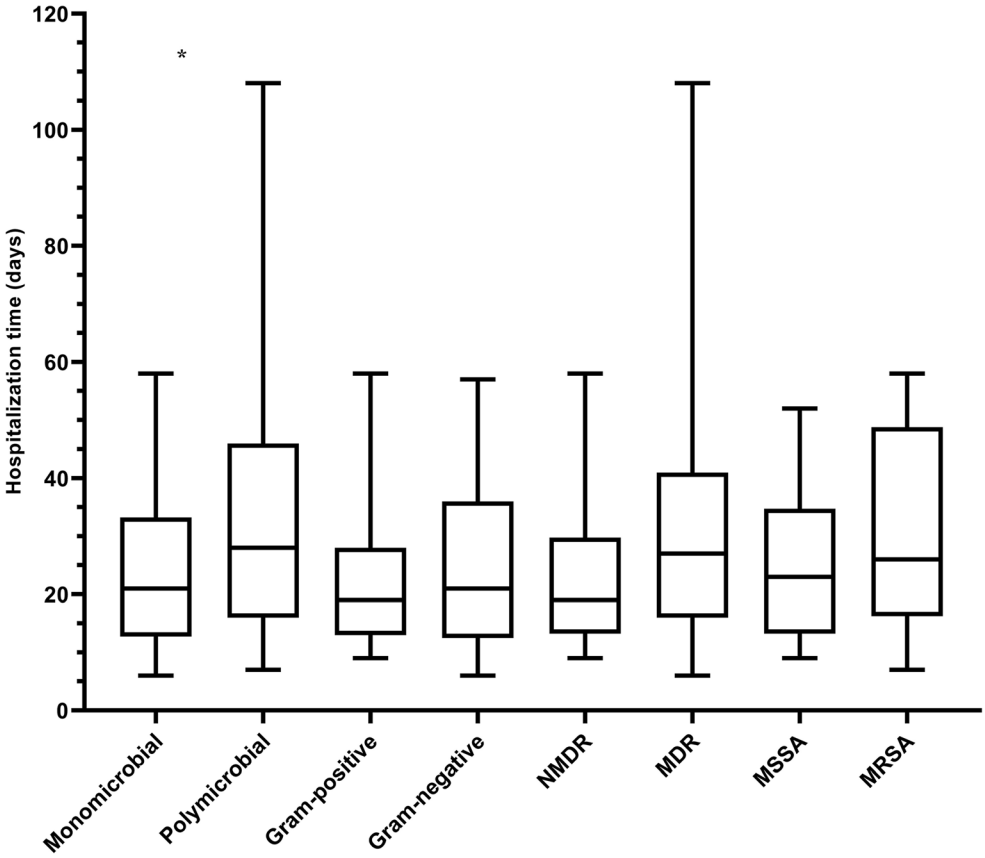

Fig. 1 Length of hospital stay of patients with diabetic foot infection in different groups according to the type of infection. Abbreviations: MDR — multidrug resistance; NMDR — non-multidrug resistance; MSSA — methicillin- sensitive *Staphylococcus aureus*; MRSA — methicillin-resistant *Staphylococcus aureus*. Mann–Whitney *U* test was used in all comparisons *p value < 0.05

was 52.94% (n = 9). Admission in ICU was necessary for 53.73% (n = 36) of patients with monomicrobial infection caused by Gram-negative bacteria, while the same occurred with 41.18% (n = 7) of patients with monomicrobial infection caused by Gram-positive bacteria, without significant differences (p value = 0.55). Regarding amputation, 73.13% (n = 49) of cases with monomicrobial infection caused by Gram-negative bacteria and 58.82% (n = 10) of those with monomicrobial infection caused by Gram-positive bacteria suffered this procedure, without significant difference (p value = 0.35). The median hospital stay for Gram-positive group was 19 days (interquartile range = 13.0–28.0 days), and in Gram-negative group was 21 days (interquartile range = 12.5–36.0 days), without a significant difference (p value = 0.57) (Fig. 1).

The median or mean PEDIS score by infection categories was similar: 9 (interquartile range = 10–9) and 9 (interquartile range = 10–8) for polymicrobial and monomicrobial infection (p value = 0.33); 9 (interquartile range = 10–8) and 8.58 (standard deviation = 1.38) for infections for MDR and NMDR microorganisms (p value = 0.05); 10 (interquartile range = 10–8.25) and 8.75 (standard deviation = 1.57) in MRSA and MSSA (p value = 0.54); 9 (interquartile range = 10–8) and 8.82 (standard deviation = 1.59) for infections caused by Gram-negative and Gram-positive microorganisms (p value = 0.94).

The cost of the 117 hospitalizations by DFI was Int$ 2,252,038.62. The average cost per patient was Int$ 19,248.19 (standard deviation = Int$ 15,243.22), with a minimum of Int$ 3122.76 and a maximum of Int$ 87,479.00. Daily room rates (mean = Int$ 9283.28, standard deviation = Int$ 8905.41) and surgery (mean = Int$ 4095.23, standard deviation = Int$ 3182.02) were the categories that most affected the final cost, followed by procedures and physiotherapy (mean = Int$ 2145.69, standard deviation = Int$ 2108.89), materials and medications (mean = Int$ 2103.56, standard deviation = Int$ 2051.09), and antimicrobials (mean = Int$ 1620.43, standard deviation = Int$ 2044.38).

Higher total expenditures occurred in infections with the following characteristics: polymicrobial, caused by Gram-negative bacteria, with MDR microorganisms, and in the presence of MRSA (Fig. 2). Comparing the mean cost of treatment according to the characteristics of the infections, polymicrobial was 1.37-fold more expensive than monomicrobial, MDR was 1.31-fold costlier than NMDR, with Gram-negative bacteria was 1.48-fold more expensive than in the presence of Gram-positive microorganisms, and with MRSA was 1.49-fold costlier than with MSSA. The total cost of treating polymicrobial infections was significantly higher than monomicrobial infections (p value = 0.03) (Table 2).

Analyzes by category of expenditure showed which ones most affected the final cost of the treatment. Expenditures in polymicrobial infections, in the presence of Gram-negative bacteria, and with MDR microorganisms were higher for almost all categories, except for materials and medications. The expenditure in the presence of MRSA was higher than in MSSA for all categories, especially costs related to materials and medications (1.66-fold higher) and antimicrobials (1.62-fold higher), although without significant differences. Comparing polymicrobial and monomicrobial infections, the categories with significant differences were procedure (1.53-fold higher), surgery (1.50-fold higher), and antimicrobials (2.16-fold higher), being costlier in the polymicrobial. Infections with Gram-negative bacteria were significantly more expensive than those with Gram-positive considering surgeries costs (1.71-fold higher). Infections with MDR bacteria were significantly costlier regarding antimicrobials expenditures (1.88-fold higher) than NMDR (Table 2).

Discussion

The present study demonstrated that the microbiological profile of DFI impacts its actual treatment cost. DFI classified as polymicrobial caused by Gram-negative and multidrug-resistant microorganisms presented higher hospitalization length and cost burden. Besides, expenditures with antimicrobials and surgeries had a higher impact on the final charge of hospital admissions.

Patients' general characteristics were similar to those described in DFI cases from different parts of the world, with a predominance of men, elderly, and typical comorbidities, such as arterial hypertension and heart diseases [7, 17]. The high risk of subsequent events and a PEDIS score similarly indicating high risk in all categories of infections may be related to the hospital's profile, which serves patients in more severe conditions.

The microbiological profile found was similar to that reported in other studies, being the most frequent species from the Enterobacteriaceae family, *S. aureus* and *P. aeruginosa* [18, 19]. Although *S. aureus* has been one of the main microorganisms isolated, Gram-negative bacteria were predominant. This fact confirms the trend observed in several countries where species of the Enterobacteriaceae family are frequently isolated from DFI [6, 20].

Most isolates showed a high antimicrobial resistance rate, including resistance to antibiotics used in the empirical treatment of DFI. Moreover, many isolates were MDR, as observed in other studies performed in tertiary hospitals [7, 21]. Use of broad-spectrum antimicrobials and admission of patients in an advanced stage of DFI, who previously used antimicrobials, may contribute to this profile. Here, patients with infections caused by MDR microorganisms had more extended hospitalization and higher final treatment costs than NMDR, without statistically significant difference. Other studies also showed an increase in hospitalization time and higher amputation rates in MDR due to the challenges in effectively treating cases presenting resistance to multiple drugs [8, 22].

In the present study, there were no significant differences regarding amputations, length of hospitalization, total costs with treatment, and costs in almost all categories between infections related to Gram-negative microorganisms and those caused by Gram-positive. Nevertheless, Gram-negative cases presented the worst outcomes, with a higher frequency of amputations, more extended hospital stay, and treatment more expensive, with a significant difference in costs with surgery. Monomicrobial infections with Gram-negative microorganisms in DFI have already been pointed out as a risk factor for lower limb amputation [23], which may be related to a large proportion of MDR microorganisms within this group. Previous studies demonstrated the burden of these infections on the health systems since blood infections caused by MDR Gram-negative bacilli acquired in hospitals [24] and MDR infections acquired in the community presented increased hospitalization time and treatment costs [24, 25].

S. aureus is a relevant DFI pathogen, and due to diverse virulence factors and its adaptation in polymicrobial communities, MRSA isolates have become an emerging global concern [26, 27]. In the present study, despite no statistically significant differences, DFI due to MRSA presented longer hospitalization length and higher costs in all categories than MSSA, corroborating a previous study that demonstrated more days of hospitalization (2–10 days) and higher costs (1.5 to 3 times more expensive) in MRSA infections than in MSSA [27].

The more significant differences in the present study occurred while comparing groups according to the diversity of bacterial species in DFI, with polymicrobial infections presenting higher treatment costs, including almost all categories, and longer length of hospitalization than monomicrobial. Polymicrobial infections can complicate chronic wounds and have been frequently reported in DFI [21, 28], although some studies indicate a higher prevalence of monomicrobial infections [5]. The majority of polymicrobial infections in the present study may be related to the hospital's profile, which receives more severe cases of DFI, including neuroischemic cases [20].

The costs associated with DFI treatment were high for all microbiological categories, mainly with daily room rates and surgeries, despite only polymicrobial infection versus monomicrobial infection presented a significant difference in total costs. These findings highlight the significant burden imposed by DFI on the health system and, considering the minimum monthly salary of Int $ 474.42 in Brazil,

Table 2 Cost of treating diabetic foot infections in different characteristics of infection and cost categories in the International dollar (Int$)

Characteristics of infection (*N*)	Median (IQR)	Mean (SD)	Mean cost difference	Mean cost ratio	*P*	Median cost by categories/(IQR)		
						Daily room rates	*P*	Procedures
Polymicrobial (67)	15,154.74 (29,563.63–9791.86)	21,755.92 (16,563.00)	5868.09	1.37	**0.03**	7167.99 (12,092.90–3729.25)	0.09	1634.55 (3033.90–1028.28)
Monomicrobial (50)	12,386.78 (20,420.51–7941.94)	15,887.83 (12,666.27)				5331.15 (9350.13–3574.65)		1063.96 (2353.04–484.71)
Gram-negative (33)	13,257.31 (23,510.36–9335.05)	17,879.85 (13,458.63)	5859.42	1.48	0.08	5810.00 (12.304.00–4.328.40)	0.10	1152.97 (2628.44–446.00)
Gram-positive (17)	10,115.68 (13,724.98–4938.92)	12,020.97 (10,246.43)				4758.80 (6853.45–2780.91)		792.24 (1266.91–573.84)
MDR (93)	13,901.88 (28,999.74–8861.73)	20,219.99 (16,082.83)	4737.53	1.31	0.26	7045.10 (12,005.70–3688.96)	0.16	1520.25 (2645.54–692.75)
NMDR (24)	12,600.66 (20,017.69–7999.23)	15,482.46 (10,891.99)				5247.78 (8873.30–3761.24)		1052.55 (2603.62–664.25)
MRSA (24)	13,960.78 (29,384.65–8835.27)	20,607.29 (15,108.58)	6738.28	1.48	0.19	15,796.87 (26,556.74–8117.81)	0.19	3029.02 (8782.40–1754.72)
MSSA (16)	12,784.10 (18,006.48–5216.29)	13,869.01 (9,521.64)				12,482.27 (18,678.00–6566.75)		2621.55 (5567.86–1421.23)

Characteristics of infection (*N*)	Median cost by categories/(IQR)							Total
	P	Surgery	*P*	Materials and medications	*P*	Antimicrobials	*P*	
Polymicrobial (67)	**<0.01**	3660.51 (6161.13–2171.27)	**<0.01**	1322.60 (3317.25–718.94)	0.26	1033.64 (3345.51–282.97)	**0.01**	1,457,646.94
Monomicrobial (50)		2768.44 (3954.53–1248.75)		1465.41 (2094.53–504.70)		406.29 (1167.13–186.65)		794,391.68
Gram-negative (33)	0.40	3038.86 (4670.25–2146.28)	**0.01**	1,459.01 (2,463.57- 579.73)	0.70	602.62 (1470.67–177.27)	0.22	590,035.14
Gram-positive (17)		1291.07 (3049.99–1134.08)		1723.70 (2041.64–378.12)		289.94 (581.28–203.07)		204,356.53
MDR (93)	0.18	3333.87 (5404.02–1722.97)	0.53	1322.60 (3081.73–693.97)	0.90	850.12 (2941.41–303.16)	**0.07**	1,880,459.47
NMDR (24)		2746.45 (4722.08–1752.46)		1479.78 (2447.50–535.82)		345.15 (1131.17–176.58)		371,579.15
MRSA (24)	0.48	7041.37 (12,924.50–4332.77)	0.12	3752.54 (7361.84–1811.02)	0.10	2051.22 (5109.11–804.82)	0.13	1,083,118.98
MSSA (16)		4568.12 (9302.19–2462.57)		2476.95 (4029.04–872.00)		939.89 (4192.19–271.31)		485.970,21

N number of cases analyzed, *IQR* interquartile range, *SD* standard deviation, "*P*" *p* value by Mann–Whitney *U*, *MDR* multidrug resistance, *NMDR* non-multidrug resistance, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-sensitive *Staphylococcus aureus*

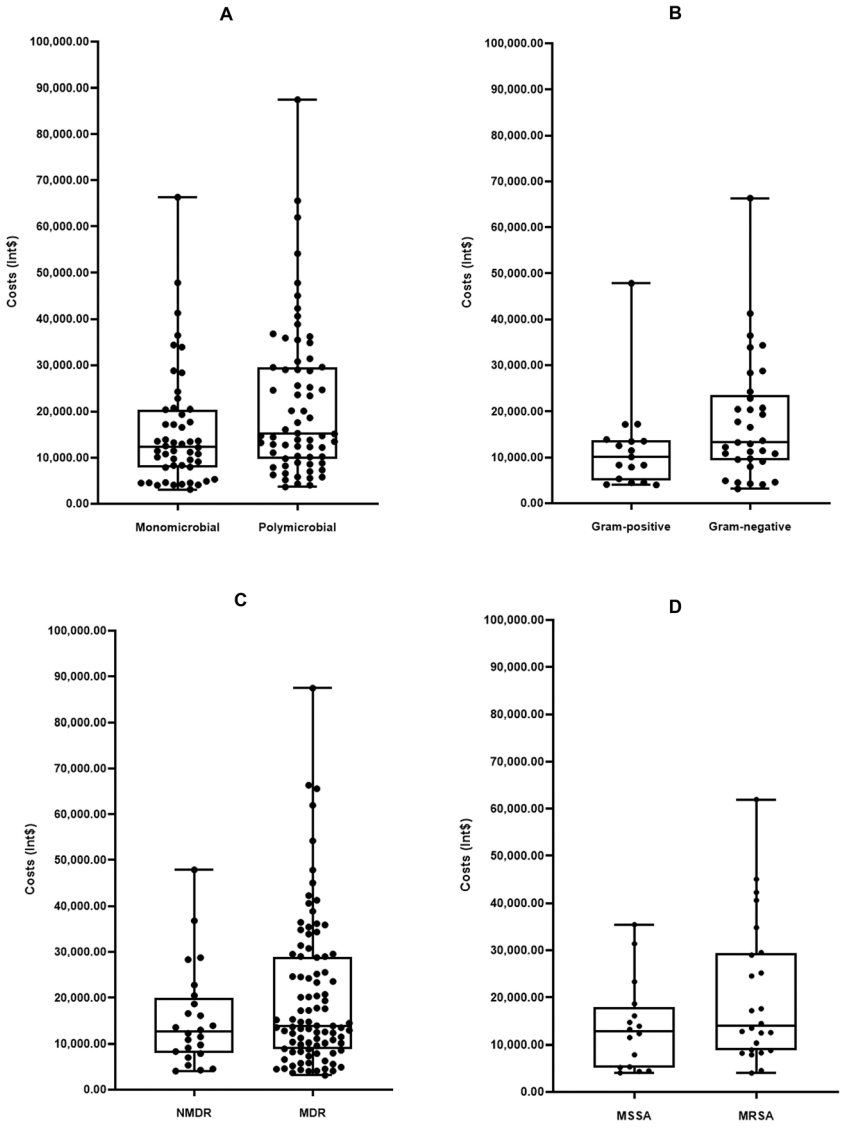

Fig. 2 Total costs with the treatment of diabetic foot infection in different categories of infection. Abbreviations: MDR — multidrug resistance; NMDR — non-multidrug resistance; MRSA — methicillin-resistant *Staphylococcus aureus*; MSSA — methicillin-sensitive *Staphylococcus aureus*

demonstrates how this complication can impact patients due to out-of-pocket payment. High expenditures with DFI treatment are a reality worldwide, as demonstrated in several studies [2, 29, 30]. However, the impact is much more significant in undeveloped and developing regions [10, 31]. For example, considering Brazil's minimum monthly salary, it would be necessary 2.5 years of working for paying one admission for treating a polymicrobial infection and 1.8 years for monomicrobial. Considering the average cost of infections caused by MDR microorganisms, they were equivalent to 6.6 additional months of working compared with those related to NMDR bacteria. Similarly, the average cost of treating infections caused by Gram-negative was equivalent to 8.2 months of working more than in cases with Gram-positive.

This study has some limitations. The analysis was restricted to aerobic bacteria, excluding anaerobic microorganisms that may cause DFI. In addition, the hospital is a reference center for the treatment of DFI, which may have influenced the microbiological profile and amputation rate found in the study due to the admission of more severe cases. Nevertheless, similar researches in other tertiary hospitals were employed for comparison. Despite the limitations, the present study was able to provide a detailed analysis of actual costs of treating DFI, including expenditures by categories, even those cheaper, such as gauze, probes, and needles, in a scenario with a scarcity of literature on the actual cost of hospitalizations for DFI.

The findings suggest that a bacterial culture may be more financially advantageous than applying empirical therapy for DFI at the beginning of the infection, contributing to better management of this complication. In addition, the results reiterate the importance of the rational use of antimicrobials in DFI and the need for adequacy of the empirical therapy the reality of each site, aiming to reduce amputations and costs associated with this morbidity [32].

Author contribution **J.S.J.L.B., B.R.B., and T.D.L.K.:** drafting the article, acquisition and interpretation of data, and **methodology**; **C.R.V. and R.P.S.:** supervision, analysis and interpretation of data, project administration, reviewing and editing the article. All authors contributed to the study conception and design.

Funding This work was supported by the Espírito Santo Research and Innovation Support Foundation (FAPES, grant no. 211/2018), National Council of Scientific and Technological Development (CNPq), Brazilian Ministry of Health, and in part by the Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001.

Declarations The Research Ethics Committee at the Federal University of Espírito Santo, in Vitória, Brazil, approved the study protocol (approval number 1.607.410).

Competing interest The authors declare no competing interests.

References

1. International Diabetes Federation. Diabetes Atlas 9th edition, IDF, Brussels, 2019. Available at: https://diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-eb.pdf; Accessed 08 July 2021.
2. Edmonds M, Manu C, Vas P. The current burden of diabetic foot disease. J Clin Orthop Trauma. 2021;17:88–93. https://doi.org/10.1016/j.jcot.2021.01.017.
3. Bandyk DF. The diabetic foot: pathophysiology, evaluation, and treatment. Semin Vasc Surg. 2018;31:43–8. https://doi.org/10.1053/j.semvascsurg.2019.02.001.
4. Miyan Z, Fawwad A, Sabir R, Basit A. Microbiological pattern of diabetic foot infections at a tertiary care center in a developing country. J Pak Med Assoc. 2017;57:665–9. http://www.jpma.org.pk/PdfDownload/8182.pdf
5. Macdonald KE, Jordan CY, Crichton E, Barnes JE, Harkin GE, Hall LML, Jones JD. A retrospective analysis of the microbiology of diabetic foot infections at a Scottish tertiary hospital. BMC Infect Dis. 2020;20:1–7. https://doi.org/10.1186/s12879-020-4923-1.
6. Rampal SRL, Devaraj NK, Yoganathan PR, Mahusin MA, Teh SW, Kumar SS. Distribution and prevalence of microorganisms causing diabetic foot infection in Hospital Serdang and Hospital Ampang for the year 2010 to 2014. Biocatal Agric Biotechnol. 2019;17:256–60. https://doi.org/10.1016/j.bcab.2018.11.019.
7. Najari HR, Karimian T, Parsa H, QasemiBarqi R, Allami A. Bacteriology of moderate-to-severe diabetic foot infections in two tertiary hospitals of Iran. Foot. 2019;40:54–8. https://doi.org/10.1016/j.foot.2019.05.001.
8. Matta-Gutiérrez G, García-Morales E, García-Álvarez Y, Álvaro-Afonso FJ, Molines-Barroso RJ, Lázaro-Martínez JL. The influence of multidrug-resistant bacteria on clinical outcomes of diabetic foot ulcers: a systematic review. J Clin Med. 2021;10:1948. https://doi.org/10.3390/jcm10091948.
9. Hicks CW, Selvarajah S, Mathioudakis N, Sherman RL, Hines KF, Black JH III, Abularrage CJ. Burden of infected diabetic foot ulcers on hospital admissions and costs. Ann Vasc Surg. 2016;33:149–58. https://doi.org/10.1016/j.avsg.2015.11.025.
10. Cavanagh P, Attinger C, Abbas Z, Bal A, Rojas N, Xu Z-R. Cost of treating diabetic foot ulcers in five different countries. Diabetes Metab Res Rev. 2012;28:107–11. https://doi.org/10.1002/dmrr.2245.
11. Toscano C, Sugita T, Rosa M, Pedrosa H, Rosa R, Bahia L. Annual direct medical costs of diabetic foot disease in Brazil: a cost of illness study. Int J Environ Res Public Health. 2018;15:89. https://doi.org/10.3390/ijerph15010089.
12. Chuan F, Tang K, Jiang P, Zhou B, He X. Reliability and validity of the perfusion, extent, depth, infection and sensation (PEDIS) classification system and score in patients with diabetic foot ulcer. PLoS One. 2015;10(4):e0124739. https://doi.org/10.1371/journal.pone.0124739.
13. Magiorakos A, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Bacteria : an international expert proposal for interim standard definitions for acquired resistance. Microbiology. 2011;18:268–81. https://doi.org/10.1111/j.1469-0691.2011.03570.x.
14. World Bank. International Comparison Program Database. GDP, PPP (Current International $). Available at: https://data.worldbank.org/indicator/PA.NUS.PPP?locations=BR. Accessed 08 July 2021.
15. Carroll KC, Pfaller MA, Landry ML, et al., editors. Manual of clinical microbiology. 12th ed. Washington, DC: ASM Press; 2019.
16. Clinical and Laboratory Standards Institute (CLSI). M100-S26. Performance standards for antimicrobial susceptibility testing: 26th informational supplement, CLSI, Wayne, PA, 2016.
17. Parisi MCR, Neto AM, Menezes FH, et al. Baseline characteristics and risk factors for ulcer, amputation and severe neuropathy in diabetic foot at risk: the BRAZUPA study. Diabetol Metab Syndr. 2016;50:18–25. https://doi.org/10.1186/s13098-016-0126-8.
18. Perim MC, Borges JC, Celeste SRC, et al. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. Rev Soc Bras Med Trop. 2015;48:546–54. https://doi.org/10.1590/0037-8682-0146-2015.
19. Jneid J, Cassir N, Schuldiner S, Jourdan N, Sotto A, Lavigne JP, la Scola B. Exploring the microbiota of diabetic foot infections with culturomics. Front Cell Infect Microbiol. 2018;8:282. https://doi.org/10.3389/fcimb.2018.00282.
20. Machado C, Teixeira S, Fonseca L, Abreu M, Carvalho A, Pereira MT, Amaral C, Freitas C, Ferreira L, Neto HR, Loureiro L, Martins J, Costa L, Gomes A, Pereira J, Guimarães R, Pinto S, Carvalho R. Evolutionary trends in bacteria isolated from moderate and severe diabetic foot infections in a Portuguese tertiary center. Diabetes Metab Syndr. 2020;14:205–9. https://doi.org/10.1016/j.dsx.2020.02.010.
21. Sekhar S, Vyas N, Unnikrishnan M, Rodrigues G, Mukhopadhyay C. Antimicrobial susceptibility pattern in diabetic foot ulcer: a pilot study. Ann Med Health Sci Res. 2014;4:742–5. https://doi.org/10.4103/2141-9248.141541.
22. Henig O, Pogue JM, Martin E, Hayat U, Ja'ara M, Kilgore PE, Cha R, Dhar S, Kaye KS. The impact of multidrug-resistant organisms on outcomes in patients with diabetic foot infections. Open Forum Infect Dis. 2020;7:1–6. https://doi.org/10.1093/ofid/ofaa161.
23. Sen P, Demirdal T, Emir B. Meta-analysis of risk factors for amputation in diabetic foot infections. Diabetes Metab Res Rev. 2019;35:e3165. https://doi.org/10.1002/dmrr.3165.
24. Thaden JT, Li Y, Ruffin F, Maskarinec SA, Hill-Rorie JM, Wanda LC, Reed SD, Fowler VG Jr. Increased costs associated with bloodstream infections caused by multidrug-resistant Gram-negative bacteria are due primarily to patients with hospital-acquired infections. Antimicrob Agents Chemother. 2017;61:e01709–16. https://doi.org/10.1128/AAC.01709-16.
25. López-Montesinos I, Domínguez-Guasch A, Gómez-Zorrilla S, Duran-Jordà X, Siverio-Parès A, Arenas-Miras MM, Montero MM, Sorli Redó L, Grau S, Horcajada JP. Clinical and economic burden of community-onset multidrug-resistant infections requiring hospitalization. J Infect. 2020;80:271–8. https://doi.org/10.1016/j.jinf.2019.12.021.
26. Viswanathan V, Pendsey S, Radhakrishnan C, Rege TD, Ahdal J, Jain R. Methicillin-Resistant *Staphylococcus aureus* in diabetic foot

infection in india: a growing menace. Int J Low Extrem Wounds. 2019;18:236–46. https://doi.org/10.1177/1534734619853668.

27. Antonanzas F, Lozano C, Torres C. Economic features of antibiotic resistance: the case of methicillin-resistant *Staphylococcus aureus*. Pharmacoeconomics. 2015;33:285–325. https://doi.org/10.1007/s40273-014-0242-y.
28. Saltoglu N, Ergonul O, Tulek N, Yemisen M, Kadanali A, Karagoz G, Batirel A, Ak O, Sonmezer C, Eraksoy H, Cagatay A, Surme S, Nemli SA, Demirdal T, Coskun O, Ozturk D, Ceran N, Pehlivanoglu F, Sengoz G, et al. Influence of multidrug resistant organisms on the outcome of diabetic foot infection. Int J Infect Dis. 2018;70:10–4. https://doi.org/10.1016/j.ijid.2018.02.013.
29. Cawich SO, Islam S, Hariharan S, Harnarayan P, Budhooram S, Ramsewak S, Naraynsingh V. The economic impact of hospitalization for diabetic foot infections in a Caribbean nation. Perm J. 2014;18:e101–4. https://doi.org/10.7812/TPP/13-096.
30. Ignatyeva VI, Severens JL, Ramos IC, Galstyan GR, Avxentyeva MV. Costs of hospital stay in specialized diabetic foot department in Russia. Value Heal Reg Issues. 2015;7:80–6. https://doi.org/10.1016/J.VHRI.2015.09.003.
31. Kumpatla S, Kothandan H, Tharkar S, Viswanathan V. The costs of treating long term diabetic complications in a developing country: a study from India. J Assoc Physicians India. 2013;61:102–9. http://japi.org/february_2013/03_oa_the_costs_of_treating.pdf
32. Lipsky BA, Senneville E, Abbas ZG, et al. IWGDF Guideline on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diab Metab Res Rev. 2020;36:e3280. https://iwgdfguidelines.org/wp-content/uploads/2020/11/Lipsky_et_al-2020-IWGDF-infection-guideline.pdf.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):244–251
https://doi.org/10.1007/s13410-022-01052-9

ORIGINAL ARTICLE

Maturity-onset diabetes of the young (MODY)-related genetic variants in a Turkish patient cohort with early-onset diabetes: a cross-sectional study

Asena Gökçay Canpolat · Çağlar Keskin · Berna İmge Aydoğan · Sevim Güllü · Özgür Demir · Mustafa Şahin, et al. ***[full author details at the end of the article]***

Received: 4 July 2021 / Accepted: 10 February 2022 / Published online: 30 March 2022

Abstract

Background There is no apparent data on the prevalence of maturity-onset diabetes of young (MODY) in adults in Turkey.
Aim We aimed to define the prevalence in our early-onset diabetes cohort and find the most prevalent types of MODY in this selected group.
Methods We included 92 patients who were diagnosed with either two types of diabetes under the age of 35 with a strong family history between 2014 and 2020. We excluded patients with low C-peptide levels and any positivity for beta-cell autoimmunity markers. A panel of fourteen genes of MODY was analyzed for each patient with a next-generation sequence analysis method.
Results The mean age for the diagnosis of diabetes was 27.9±8.0 years, and most of the patients were male (F/M:37/55). The median body mass index was 27 (19–32) kg/m^2, while the median HbA1c level was 6.4% (5–17%). Seven patients had eight pathogenic or likely pathogenic variants (7/92; 7.6%) of MODY genes with variants of GCK (MODY 2, n:1), HNF1A (MODY3, n:1), HNF1β (MODY5, n:1), ABCC8 (MODY12, n:2), INS (MODY 10, n:1), HNF4A (MODY 1, n:1), and PDX1 (MODY4, n:1).
Conclusion Molecular genetic diagnosis of MODY is necessary for optimal follow-up, treatment, prognosis, and genetic counseling because of the close relationship between phenotype and genotype. Even in our small-sample-sized cohort, we were able to detect MODY variants with appropriate patient selection, and we re-evaluated for additional clinical features for the newly diagnosed MODY patients according to their variants, modified their treatment, and screened their first-degree relatives. This result presents that diabetic patients below 35 age, without low C-peptide levels and any positivity for beta-cell autoimmunity markers, must be screened for MODY gene panel for diagnosis and genetic counseling.

Keywords Maturity-onset diabetes of young · Genetics · Next-generation sequencing

Introduction

Maturity-onset diabetes of the young (MODY) is a particular type of diabetes mellitus (DM), which is characterized by autosomal dominant inheritance in ≥2 consecutive generations. The early onset of DM (usually before 25 years of age) owing to impairment in glucose-stimulated insulin release as a consequence of altered pancreatic beta-cell function is present [1]. Because many patients with MODY are misdiagnosed as either type 1 or 2 DM, the exact incidence of the MODY is unknown. Its prevalence is estimated to be ~1–2 % of all diabetes cases in developed countries [2]. Monogenic variants of nuclear transcription factors and glucokinase enzyme are responsible for various distinct types of the disease spectrum. Depending on the population and the recruitment criteria, almost nearly 80% of all MODY cases are due to heterozygous variants in hepatocyte nuclear factor-1 alpha (HNF1A), hepatocyte nuclear factor-4 alpha (HNF4A), and glucokinase (GCK) [3, 4]. After being first described by Tattersall and Fajans in 1974, a total of 14 MODY genes have been sequenced and characterized to date worldwide [5, 6].

Both the prevalence and genetic variations of MODY may vary according to ethnic differences. Thus, the differences in clinical characteristics of the MODY patients in specific populations should be clarified. However, there were several reports about the prevalence and genetic variations of the MODY for Asian, European, African, and American countries, with no apparent data from Turkey in where one-third of the population was below 25 years of age [7–9]. Therefore,

we aimed to define the prevalence in our early-onset diabetes cohort and find the most prevalent types of MODY in this selected group.

Material and methods

Study population

The study was prospective and cross-sectional in design. All patients who were admitted to outpatient clinics of our tertiary center university hospital between 2014 and 2020 (outpatient clinic volume; 250 patients/day in total and 100 patients/day with type 1 or 2 DM) were screened for early-onset diabetes (diabetes onset age of below 35 years old) [10]. A total of 92 patients with early-onset diabetes were included in the study. All included patients had normal C-peptide levels (in reference ranges) and were negative for antibodies specific for type 1 diabetes. Subjects with genetically confirmed MODY or type 1 DM, history of diabetic ketoacidosis, low C-peptide levels (lower than reference range), and positive beta-cell autoantibodies were excluded.

The demographic, clinical, and laboratory data were recorded for each participant. Blood samples were obtained from each patient in the morning at an 8-h fasting state for measurement of the biochemistry panel, including lipid profiles, glycosylated hemoglobin A1c levels (HbA1c), C-peptide levels, islet cell autoantibodies, autoantibodies to insulin, and glutamic acid decarboxylase 65 (GAD65). HbA1c results were both reported as the National Glycohemoglobin Standardization Program (NGSP) units (%), and International Federation of Clinical Chemistry (IFCC) units (mmol/mol) fasting C peptide level was measured using the Roche® electrochemiluminescence assay and reported with a reference range of 1.1–4.4 ng/dl. An additional blood sample was also taken for genetic analysis.

The Institutional EC approved the study protocol, and all subjects signed informed consent forms. The research was funded by the Scientific Research Foundation of Ankara University (*Ankara, Turkey Project Number15B0230002*).

Variant screening

Next-generation sequencing (NGS)-based strategy was used for screening of the known and unknown MODY gene variants in subjects with early-onset diabetes, and the most prevalent MODY types in our Turkish patient cohort were presented. Polymerase chain reaction (PCR) primers were designed for sequencing of exons and exon-intron boundaries of HNF1A, HNF4A, GCK, NEUROD1, PDX1, KLF11, CEL, PAX4, HNF1B, INS (Insulin), BLK, ABCC8, KCNJ11, and APPL1, and sequencing was done for all patients. DNA was isolated with the magnetic bead method (MagPurix-Zinexts, Taiwan). PCR amplification was done in-house designed primers. Amplicons were checked by 2% agarose gel electrophoresis. Sequencing was done by the next-generation sequencing method by Miseq Illumina equipment (Illumina, San Diego, CA, USA) by following manufacturers' instructions. Data were evaluated by IGV 2.3 (Broad Institute) software. Variants were analyzed by using ACMG criteria. All variants were compared with GnomAD, ClinVar, PROVEAN, and the National Center for Biotechnology Information (NCBI) databases.

Statistical analyses

The Kolmogorov–Smirnov test assessed the normality of continuous data. Categorical variables such as HbA1c levels and treatment modalities were presented as numbers and percentages (%). Continuous data were displayed as means ± standard deviation for age and median (minimum-maximum) for body mass index. Statistical analyses were performed using the IBM SPSS Statistics for Windows (IBM Corp, Version 22.0. Armonk, NY).

Results

Among 92 patients, the mean age for diagnosis of diabetes was 27.9±8.0 years, and most of the patients were male (F/M:37/55). The median BMI was 27 (19–32) kg/m^2, while the median HbA1c level was 6.4% (46 mmol/mol) min–max [(5–17%) (31–119 mmol/mol)]. Treatment modalities among the study population were only diet (2%), monotherapy or combined oral anti-diabetic drugs (44%), basal insulin ± oral anti-diabetic drugs (15%), premixed insülin ± oral anti-diabetic drugs (15%), and intensive insulin ± oral anti-diabetic drugs (26%), respectively.

The oral anti-diabetic drugs used for the patient group were as follows: sulphonilureas (gliclazide, glimepiride; n:19), meglitinide analogues (repaglinide, nateglinide; n:4), metformin (n:64), and dipeptidyl peptidase-4 inhibitors (sitagliptin, vildagliptin, and saxagliptin; n:24).

Eighteen genetic variants were classified as pathogenic, likely pathogenic, and the variant of unknown significance (VUS) by using ACMG criteria. A total of 7 patients had eight pathogenic and likely pathogenic variants, while the remaining 10 had VUS. The details of the genetic variants were presented in Table 1.

The most frequent variations for HNF1A were found for c.293C>T (p.A98V) (p.Ala98Val) and HNF1A:c.293C>T (p.A98V) (p.Ala98Val) mutation. It was found heterozygous for 20 patients and homozygous for one patient in 92 patients. The mean allele frequency (MAF) of this mutation was recorded as 11.95 % in this group, while worldwide incidence is seen as 6%. The variants of HNF1A in our patient group

Table 1 Distribution of MODY variants

Patient	Gene	Variants	ACMG-classification	Clinical features	Laboratory	Treatment
N.G.	HNF4A	NM_175914.4:c.932G>A (p.Arg311His)	Likely pathogenic	Age at diagnosis 20 Chronic complicationsφ	HbA1c:7.6% Fasting insulin:5.1 µIU/mL Fasting C peptide:2.7 ng/ml	Gliclazide, metformin, and sitagliptin
Z.Y.	GCK	NM_033507.3:c.382T>C (p.Ser128Pro)	Likely pathogenic	Age at diagnosis 22 Chronic complicationsφ	HbA1c:6.3 % Fasting insulin:7.7 µIU/mL Fasting C peptide:1.8 ng/ml	Metformin
G.G.	GCK	NM_033508.3:c.490C>A (p.Leu164Ile)	Uncertain significance	Age at diagnosis 23 Chronic complicationsφ	HbA1c:6.5 % Fasting insulin: 10 µIU/mL Fasting C peptide: 3 ng/ml	Diet
Z.K.	HNF1A	NM_000545.8:c.273C>A (p.Asn91Lys)	Uncertain significance	Age at diagnosis 26 Chronic complicationsφ	HbA1c:7.5 % Fasting insulin: 10.3 µIU/mL Fasting C peptide: 2.3 ng/ml	Gliclazide, metformin, and sitagliptin
A.M.D	HNF1A	NM_000545.8:c.273C>A (p.Asn91Lys)	Uncertain significance	Age at diagnosis 22 Chronic complicationsφ	HbA1c: 6.8% Fasting insulin: 5.2 µIU/mL Fasting C peptide: 1 ng/ml	Glimepiride, metformin, and sitagliptin
G.B.	HNF1A HNF1A	NM_000545.8:c.273C>A (p.Asn91Lys) NM_000545.8:c.493T>C (p.Trp165Arg)	Uncertain significance Likely pathogenic	Age at diagnosis 28 Nephropathy+ retinopathy+ neuropathy +	HbA1c: 6.4% Fasting insulin: 7.9 µIU/mL Fasting C peptide: 2.7 ng/ml	Metformin and sitagliptin
S.S.	HNF1A	NM_000545.8:c.517G>A (p.Val173Met)	Uncertain significance	Age at diagnosis 23 Nephropathy+ retinopathy+ neuropathy +	HbA1c: 7.7 % Fasting insulin: 7.8 µIU/mL Fasting C peptide: 1.54 ng/ml	Gliclazide, metformin
H.K.	PDX1 INS	NM_000209.4:c.97C>A (p.Pro33Thr) NM_001291897.2:c.285C>A (p.Cys95Ter)	Likely pathogenic Pathogenic	Age at diagnosis 19 Nephropathy+, CKD/HD retinopathy+ neuropathy + CVE (at age 46)	HbA1c: 12 % Fasting insulin: 6 µIU/mL Fasting C peptide: 1.46 ng/ml	Insulin glargine and preprandial insulin lispro
S.C.	PDX1	NM_000209.4:c.226G>A (p.Asp76Asn)	Uncertain significance	Age at diagnosis 26 Chronic complicationsφ	HbA1c: 8.9 % Fasting insulin: 8.3 µIU/mL Fasting C peptide: 2.1 ng/ml	Insulin aspart/aspart protamine, metformin, and vildagliptin
O.A.	PDX1	NM_000209.4:c.418G>A (p.Ala140Thr)	Uncertain significance	Age at diagnosis 34 Chronic complicationsφ	HbA1c: 6.2 % Fasting insulin: 14 µIU/mL Fasting C peptide: 4.1 ng/ml	Metformin
A.Y.	HNF1B	NM_000458.4:c.755G>T (p.Arg252Leu)	Likely pathogenic	Age at diagnosis 33 Chronic complicationsφ CAD (at age 37) Urinary abnormalityφ	HbA1c: 7.9 % Fasting insulin: 10 µIU/mL Fasting C peptide: 2.4 ng/ml	Insulin aspart/aspart protamine, metformin, vildagliptin
E.Y.	NEUROD1	NM_002500.4:c.-71G>A	Uncertain significance	Age at diagnosis 33 Nephropathy+ retinopathy+ neuropathy +	HbA1c: 6.8 % Fasting insulin: 23 µIU/mL Fasting C peptide: 3.1 ng/ml	Gliclazide, metformin, and vildagliptin
S.B.	KLF11			Age at diagnosis 30	HbA1c: 7.5 %	Metformin and sitagliptin

Table 1 (continued)

Patient	Gene	Variants	ACMG-classification	Clinical features	Laboratory	Treatment
		NM_003597.5:c.1018A>T (p.Met340Leu)	Uncertain significance	Chronic complicationsφ	Fasting insulin: 9.6 µIU/mL Fasting C peptide: 2.64 ng/ml	
A.A.	ABCC8 ABCC8	NM_000352.6:c.1261G>A (p.Val421Ile) NM_000352.6:c.1818-6G>C	Likely pathogenic Uncertain significance	Age at diagnosis 16 Chronic complicationsφ	HbA1c: 6.3 % Fasting insulin: 11.5 µIU/mL Fasting C peptide: 2.8 ng/ml	Metformin
Z.A.	ABCC8	NM_000352.6:c.1616A>G (p.Tyr539Cys)	Likely pathogenic	Age at diagnosis 32 Nephropathy+ retinopathy+ neuropathy +	HbA1c: 6.7 % Fasting insulin: 5.2 µIU/mL Fasting C peptide: 3 ng/ml	Gliclazide and metformin
A.G.A.	ABCC8	NM_000352.6:c.1859G>A (p.Arg620His)	Uncertain Significance	Age at diagnosis 30 Chronic complicationsφ	HbA1c: 6.8 % Fasting insulin: 5 µIU/mL Fasting C peptide: 1.2 ng/ml	Gliclazide, metformin, and insulin NPH
V.S.	ABCC8	NM_000352.6:c.2805C>A (p.Asp935Glu)	Uncertain significance	Age at diagnosis 30 Chronic complicationsφ	HbA1c: 7.3 % Fasting insulin: 13.9 µIU/mL Fasting C peptide: 2 ng/ml	Repaglinide, metformin, and Insulin detemir

HNF4A hepatocyte nuclear factor-4 alpha, *GCK* glucokinase, *HNF1A* hepatocyte nuclear factor-1 alpha, *INS* insulin, *PDX1* pancreas-duodenum homeobox-1, *HNF1B* hepatocyte nuclear factor-1 beta, *NEUROD1* neurogenic differentiation factor 1, *KLF11* potassium channel, inwardly rectifying, subfamily J, member 11, *ABCC8* ATP-binding cassette, subfamily C, member 8, *CKD* chronic kidney disease, *HD* hemodialysis, *CVE* cerebrovascular event, *CAD* coronary artery disease, *HbA1c* glycosylated hemoglobin A1c, reference range for fasting insulin levels: 4–16 µIU/mL, reference range for fasting C peptide level: 1.1–4.4 ng/ml

were NM_000545.8:c.493T>C (p.Trp165Arg) and NM_000545.8:c.517G>A (p.Val173Met). Another frequent type of the MODY was MODY 2, and the variants of GCK genes were NM_033507.3:c.382T>C (p.Ser128Pro) and NM_033508.3:c.490C>A (p.Leu164Ile). NEUROD1: c.590C>A (p.P197H) (p.Pro197His) mutation was found heterozygote for 8 patients with a MAF of 4.34%. The most frequent variation for CEL was at 148 and found for 3 patients of the study group. The most frequent reported mutation for BLK was BLK: c.116C>T (p.P39L) (p.Pro39Leu) mutation and was heterozygote for 5 patients in this group. BLK: c.974A>C (p.K325T) (p.Lys325Thr) mutation was seen in 2 patients, and the clinical significance of the changes for the code rs77401687 is presented as an uncertain change in the ClinVar database. BLK: c.211G>A (p.A71T) (p.Ala71Thr) mutation was found in 4 patients, and it was evaluated as polymorphism. The most frequent KCNJ11 variations were at Y539C for 2 patients.

Other variants of the patient group were single gene variants of HNF4A NM_175914.4:c.932G>A (p.Arg311His), KLF NM_003597.5:c.1018A>T (p.Met340Leu), PAX4 ENST00000341640.2:c.497G>A (p.Arg166Gln), and BLK NM_001715.3:c.228C>G (p.Asp76Glu), respectively. BLK NM_001715.3:c.228C>G (p.Asp76Glu) and GCK NM_033508.3:c.490C>A (p.Leu164Ile) variants were found in the same patient, but she was diagnosed as MODY 2 according to her medical and laboratory features.

Two new mutations were discovered in this patient group, which are ABCC8: c.1616A>G (p.Tyr539Cys) (HET) at two sisters of a family and PDX1: c.228C>G (p.Asp76Glu) (HET). The first mutation was determined as pathogenic by the computational verdict because of eight pathogenic predictions from DANN, GERP, FATHMM, Mutation Assessor, Mutation Taster, PROVEAN, FATHMM-MKL, and SIFT. Only one benign prediction was achieved by LRT. ClinVar classifies this variant as likely pathogenic. The latter new mutation was a missense variant in gene PDX1 that has seven pathogenic missense variants out of 9 pathogenic variants. It was categorized as uncertain significance.

Discussion

The accurate diagnosis of MODY is essential because of the close genotype-phenotype correlations between the variant, co-morbidities, and clinical features of the MODY disease. Moreover, the identification of a MODY family will provide genetic counseling for the family and prediction for future generations. Also, searching for MODY, among other types of diabetes, has an impact on clinical practice and individualized treatment [5].

Although an accurate diagnostic approach for MODY has not been established clearly by recent guidelines, medical history, family history, physical examination, and laboratory testing are essential and complimentary for the diagnosis of the MODY. Traditional clinical features are only seen for less than half of the MODY patients. Therefore, molecular genetic testing is the primary diagnostic tool [1]. Molecular genetic testing can either be done with serial single-gene/multigene panel or genomic testing such as exome sequencing. The exome sequencing approach provides clinicians to analyze the MODY-related genes not included in the multigene groups and find possible new genotype-phenotype associations.

The general prevalence of MODY is approximately 1–2% of diabetics; however, the relative incidence of the disease varies by country according to national strategies for screening [11]. According to the literature, the percentage of MODY variants ranges from 0.65 to 6.3 in Europe [5, 12, 13]. Few studies have reported the presence of MODY in the Middle Eastern Population, and they also pointed out that the most prevalent forms are HNF1A, GCK, and HNF4A, similar to other parts of the world [14]. Also, there are a lot of studies from India with large sample sizes evaluating the most prevalent MODY types as HNF1A, GCK, and HNF4A [15–18]. Interestingly, Mohan et al. reported MODY 12 as the second most frequent mutation after HNF1A in India [19].

Although the most common causes of MODY are GCK (MODY2 30–50%), HNF1A (MODY3 30–65%), and HNF4A (MODY1 5–10%), respectively, worldwide [10], seven patients were found to have pathogenic or likely pathogenic variants (7/92; 7.6%) and were diagnosed as MODY with variants of GCK (MODY 2, n:1), HNF1A (MODY3, n:1), HNF1β (MODY5, n:1), ABCC8 (MODY12, n:2), INS (MODY 10, n:1), HNF4A (MODY 1, n:1), and PDX1 (MODY4, n:1) in our cohort. So, we did not observe a frequency ranking similar to that in the world or Asia. Maybe, it was due to the selective searching criteria of the study. The other studies were planned over an inclusion criterion for age and family history and search for prevalences of MODY with large genetic screening panels. We detected more selective cases by a strict screening plan for financial concerns. But, it may be a strategy for more economic screening in diabetes cases, especially in developing countries.

There are few sample-sized studies for both the pediatric and adult populations in our country, which also pointed out that GCK and HNF1A were the most frequent genes and were compatible with our results. Few studies reported the molecular spectrum of only some of the frequent mutations of GCK [20] and HNF1A [21], and some studies evaluated a panel of mutations of GCK, HNF1A, HNF4A, and HNF1B [22–24]. There is a relatively new study, which they performed in the similar time period with us, that reported a higher number of (n:182 patients) with a pre-diagnosis of MODY for 7 MODY-related genes [25]. They also found GCK and HNF1A

mutations 33% and 30%, respectively, in their cohort. There are only two comprehensive studies that evaluated 43 and 61 children with panels of 11 and 20 MODY genes, respectively, in Turkey [26, 27]. In another recent study from Turkey, with targeted NGS of 13 MODY-related genes, the missense mutations of *NEUROD1* p.D202E, *KFL11* p.R461Q, *BLK* p.G248R, and *KCNJ11* p.S385F were first associated with MODY [28].

In our study, we evaluated our early-onset diabetic patients with a new-generation sequence analysis method for 14 genes associated with MODY. We found seven patients who had pathogenic/likely pathogenic variants among a total of 92 patients. Moreover, we found two novel mutations associated with MODY. Our research is the first study investigating both novel and proven mutations of all 14 MODY genes by using targeted next-generation sequencing in a cohort of early-onset diabetes among adulthood in Turkey.

But, our study should be interpreted with some limitations. First of all, our study sample size was small, and the findings were confined to single-center data rather than the whole Turkish population. Also at the beginning of the study, we aimed to define the prevalence in our cohort and find the most prevalent types in this group, but we only could manage to define the point prevalence in this particular group as 7/92 (7.6%) according to pathogenic and likely pathogenic variants. But we could not speculate about the most prevalent types because of the small sample size of the group and cross-sectional design of the study. The larger-scale national studies will further determine a cost-effective MODY panel to be implemented particularly for our country.

Conclusion

Molecular genetic diagnosis of MODY is necessary for optimal follow-up, treatment, prognosis, and genetic counseling because of the close relationship between phenotype and genotype. Even in our small-sample-sized cohort, we were able to detect MODY variants with appropriate patient selection, and we re-evaluated for additional clinical features for the newly diagnosed MODY patients according to their variants, modified their treatment, and screened their first-degree relatives. This result presents that diabetic patients below 35 age, without low C-peptide levels, and any positivity for beta-cell autoimmunity markers must be screened for MODY gene panel for diagnosis and genetic counseling. Although molecular testing for MODY is expensive, correct identification of MODY has potential importance for the guidance of the optimal treatment and discontinuation of unnecessary treatments. By using our criteria, it is possible to screen fewer patients and detect more cases for our nation.

Data availability Data is available on request from the authors.

Declarations

Conflict of interest The authors declare no competing interests.

Informed Consent The Institutional Ethics Committee approved the study protocol, and all subjects signed informed consent forms.

References

1. Urbanova J, Brunerova L, Broz J. Hidden MODY-looking for a needle in a haystack. Front Endocrinol (Lausanne). 2018;9:355. https://doi.org/10.3389/fendo.2018.00355.
2. Firdous P, Nissar K, Ali S, Ganai BA, Shabir U, Hassan T, Masoodi SR. Genetic testing of maturity-onset diabetes of the young current status and future perspectives. Front Endocrinol (Lausanne). 2018;9:253. https://doi.org/10.3389/fendo.2018.00253.
3. Althari S, Gloyn AL. When is it MODY? Challenges in the Interpretation of sequence variants in MODY genes. Rev Diabet Stud. 2015;12(3-4):330–48. https://doi.org/10.1900/RDS.2015.12.330.
4. Thanabalasingham G, Owen KR. Diagnosis and management of maturity onset diabetes of the young (MODY). Bmj. 2011;343:d6044. https://doi.org/10.1136/bmj.d6044.
5. Schober E, Rami B, Grabert M, Thon A, Kapellen T, Reinehr T, Holl RW, DP-WIotGWGfPD. Phenotypical aspects of maturity-onset diabetes of the young (MODY diabetes) in comparison with type 2 diabetes mellitus (T2DM) in children and adolescents: experience from a large multicentre database. Diabet Med. 2009;26(5):466–73. https://doi.org/10.1111/j.1464-5491.2009.02720.x.
6. Porter JR, Rangasami JJ, Ellard S, Gloyn AL, Shields BM, Edwards J, Anderson JM, Shaw NJ, Hattersley AT, Frayling TM, Plunkett M, Barrett TG. Asian MODY: are we missing an important diagnosis? Diabet Med. 2006;23(11):1257–60. https://doi.org/10.1111/j.1464-5491.2006.01958.x.
7. Ming-Qiang Z, Yang-Li D, Ke H, Wei W, Jun-Fen F, Chao-Chun Z, Guan-Ping D. Maturity onset diabetes of the young (MODY) in Chinese children: genes and clinical phenotypes. J Pediatr Endocrinol Metab. 2019;32(7):759–65. https://doi.org/10.1515/jpem-2018-0446.
8. Chapla A, Mruthyunjaya MD, Asha HS, Varghese D, Varshney M, Vasan SK, Venkatesan P, Nair V, Mathai S, Paul TV, Thomas N. Maturity onset diabetes of the young in India - a distinctive mutation pattern identified through targeted next-generation sequencing. Clin Endocrinol. 2015;82(4):533–42. https://doi.org/10.1111/cen.12541.
9. Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia. 2010;53(12):2504–8. https://doi.org/10.1007/s00125-010-1799-4.
10. Naylor R, Johnson AK, del Gaudio D, Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2022. 2018;24.
11. Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. Diabetes Care. 2011;34(8):1878–84. https://doi.org/10.2337/dc11-0035.
12. Malachowska B, Borowiec M, Antosik K, Michalak A, Baranowska-Jazwiecka A, Deja G, Jarosz-Chobot P, Brandt A, Mysliwiec M, Stelmach M, Nazim J, Peczynska J, Glowinska-Olszewska B, Horodnicka-Jozwa A, Walczak M, Malecki MT, Zmyslowska A, Szadkowska A, Fendler W, Mlynarski W. Monogenic diabetes prevalence among Polish children-summary

of 11 years-long nationwide genetic screening program. Pediatr Diabetes. 2018;19(1):53–8. https://doi.org/10.1111/pedi.12532.
13. Delvecchio M, Mozzillo E, Salzano G, Iafusco D, Frontino G, Patera PI, Rabbone I, Cherubini V, Grasso V, Tinto N, Giglio S, Contreas G, Di Paola R, Salina A, Cauvin V, Tumini S, d'Annunzio G, Iughetti L, Mantovani V, et al. Monogenic diabetes accounts for 6.3% of cases referred to 15 Italian Pediatric Diabetes Centers During 2007 to 2012. J Clin Endocrinol Metab. 2017;102(6): 1826–34. https://doi.org/10.1210/jc.2016-2490.
14. Taneera J, Mussa B, Saber-Ayad M, Dhaiban S, Aljaibeji H, Sulaiman N. Maturity-onset diabetes of the young: an overview with focus on the Middle East. Curr Mol Med. 2017;17(8):549–62. https://doi.org/10.2174/1566524018666180222121158.
15. Aarthy R, Aston-Mourney K, Mikocka-Walus A, Radha V, Amutha A, Anjana RM, Unnikrishnan R, Mohan V. Clinical features, complications and treatment of rarer forms of maturity-onset diabetes of the young (MODY) - a review. J Diabetes Complicat. 2021;35(1):107640. https://doi.org/10.1016/j.jdiacomp.2020.107640.
16. Kanthimathi S, Jahnavi S, Balamurugan K, Ranjani H, Sonya J, Goswami S, Chowdhury S, Mohan V, Radha V. Glucokinase gene mutations (MODY 2) in Asian Indians. Diabetes Technol Ther. 2014;16(3):180–5. https://doi.org/10.1089/dia.2013.0244.
17. Anuradha S, Radha V, Mohan V. Association of novel variants in the hepatocyte nuclear factor 4A gene with maturity onset diabetes of the young and early onset type 2 diabetes. Clin Genet. 2011;80(6):541–9. https://doi.org/10.1111/j.1399-0004.2010.01577.x.
18. Radha V, Ek J, Anuradha S, Hansen T, Pedersen O, Mohan V. Identification of novel variants in the hepatocyte nuclear factor-1alpha gene in South Indian patients with maturity onset diabetes of young. J Clin Endocrinol Metab. 2009;94(6):1959–65. https://doi.org/10.1210/jc.2008-2371.
19. Mohan V, Radha V, Nguyen TT, Stawiski EW, Pahuja KB, Goldstein LD, Tom J, Anjana RM, Kong-Beltran M, Bhangale T, Jahnavi S, Chandni R, Gayathri V, George P, Zhang N, Murugan S, Phalke S, Chaudhuri S, Gupta R, et al. Comprehensive genomic analysis identifies pathogenic variants in maturity-onset diabetes of the young (MODY) patients in South India. BMC Med Genet. 2018;19(1):22. https://doi.org/10.1186/s12881-018-0528-6.
20. Aykut A, Karaca E, Onay H, Goksen D, Cetinkalp S, Eren E, Ersoy B, Cakir EP, Buyukinan M, Kara C, Anik A, Kirel B, Ozen S, Atik T, Darcan S, Ozkinay F. Analysis of the GCK gene in 79 MODY type 2 patients: a multicenter Turkish study, mutation profile and description of twenty novel mutations. Gene. 2018;641:186–9. https://doi.org/10.1016/j.gene.2017.10.057.
21. Karaca E, Onay H, Cetinkalp S, Aykut A, Goksen D, Ozen S, Atik T, Darcan S, Tekin IM, Ozkinay F. The spectrum of HNF1A gene mutations in patients with MODY 3 phenotype and identification of three novel germline mutations in Turkish Population. Diabetes Metab Syndr. 2017;11(Suppl 1):S491–6. https://doi.org/10.1016/j.dsx.2017.03.042.
22. Ozsu E, Cizmecioglu FM, Yesiltepe Mutlu G, Yuksel AB, Caliskan M, Yesilyurt A, Hatun S. Maturity onset diabetes of the young due to glucokinase, HNF1-A, HNF1-B, and HNF4-A mutations in a cohort of Turkish children diagnosed as type 1 diabetes mellitus. Horm Res Paediatr. 2018;90(4):257–65. https://doi.org/10.1159/000494431.
23. Haliloglu B, Abali S, Bugrul F, Celik E, Bas S, Atay Z, Guran T, Turan S, Bereket A. The distribution of different types of diabetes in childhood: a single center experience. J Clin Res Pediatr Endocrinol. 2018;10(2):125–30. https://doi.org/10.4274/jcrpe.5204.
24. Beysel S, Eyerci N, Pinarli FA, Kizilgul M, Ozcelik O, Caliskan M, Cakal E. HNF1A gene p.I27L is associated with early-onset, maturity-onset diabetes of the young-like diabetes in Turkey. BMC Endocr Disord. 2019;19(1):51. https://doi.org/10.1186/s12902-019-0375-2.
25. Ateş EA, Üstay Ö, Polat H, Apaydın T, Elbasan O, Yıldırım Ö, Güney Aİ. Genetic and clinical characterization of patients with maturity-onset of diabetes of the young (MODY): identification of novel variations. Balkan Med J. 2021 Sep;38(5):272–7. https://doi.org/10.5152/balkanmedj.2021.20155. PMID: 34462253
26. Agladioglu SY, Aycan Z, Cetinkaya S, Bas VN, Onder A, Peltek Kendirci HN, Dogan H, Ceylaner S. Maturity onset diabetes of youth (MODY) in Turkish children: sequence analysis of 11 causative genes by next generation sequencing. J Pediatr Endocrinol Metab. 2016;29(4):487–96. https://doi.org/10.1515/jpem-2015-0039.
27. Yalçıntepe S, Özgüç Çömlek F, Gürkan H, Demir S, Atlı Eİ, Atlı E, Eker D, Tütüncüler Kökenli F. The Application of Next Generation Sequencing Maturity Onset Diabetes of the Young Gene Panel in Turkish Patients from Trakya Region. J Clin Res Pediatr Endocrinol. 2021 Aug 23;13(3):320–31. https://doi.org/10.4274/jcrpe.galenos.2021.2020.0285. Epub 2021 Feb 10. PMID: 33565752; PMCID: PMC8388052
28. Demirci DK, Darendeliler F, Poyrazoglu S, Al ADK, Gul N, Tutuncu Y, Gulfidan G, Arga KY, Cacina C, Ozturk O, Aydogan HY, Satman I. Monogenic childhood diabetes: dissecting clinical heterogeneity by next-generation sequencing in maturity-onset diabetes of the young. OMICS. 2021 Jul;25(7):431–49. https://doi.org/10.1089/omi.2021.0081. Epub 2021 Jun 25. PMID: 34171966

Affiliations

Asena Gökçay Canpolat[1] · Çağlar Keskin[1] · Berna İmge Aydoğan[1] · Sevim Güllü[1] · Özgür Demir[1] · Mustafa Şahin[1] · Rıfat Emral[1] · Murat Faik Erdoğan[1] · Vedia Tonyukuk Gedik[1] · Demet Çorapçıoğlu[1] · Gülay Ceylaner[2] · Serdar Ceylaner[2] · Nilgün Başkal[1]

✉ Asena Gökçay Canpolat
asena-gokcay@hotmail.com

Çağlar Keskin
caglaron@hotmail.com

Berna İmge Aydoğan
imgehalici@gmail.com

Sevim Güllü
Sevim.Gullu@medicine.ankara.edu.tr

Özgür Demir
dr.ozgurdemir@gmail.com

Mustafa Şahin
drsahinmustafa@yahoo.com

Rıfat Emral
rifatemral@gmail.com

Murat Faik Erdoğan
murat.erdogan@temd.org.tr

Vedia Tonyukuk Gedik
vediagedik@gmail.com

Demet Çorapçıoğlu
dcorapci@medicine.ankara.edu.tr

Gülay Ceylaner
gulayceylaner@intergen.com.tr

Serdar Ceylaner
serdarceylaner@intergen.com.tr

Nilgün Başkal
nbaskal@gmail.com

[1] Department of Endocrinology and Metabolism, Faculty of Medicine, Ankara University, Ankara, Turkey

[2] Intergen Genetic Diagnosis Center, Ankara, Turkey

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):252–257
https://doi.org/10.1007/s13410-022-01068-1

ORIGINAL ARTICLE

Effects of an isometric exercise training program on muscular strength, ankle mobility, and balance in patients with diabetic peripheral neuropathy in the lower legs in South Africa

Ronél du Plessis[1] · Nadia Dembskey[2] · Susan H. Bassett[1]

Received: 23 August 2021 / Accepted: 22 March 2022 / Published online: 5 April 2022

Abstract

Background Patients who suffer from diabetic peripheral neuropathy in the lower leg experience a greater risk of falls due to a decrease in strength of the lower extremities.

Methods Fourteen participants, diagnosed with diabetic peripheral neuropathy or nocturnal allodynia in either one or both extremities, volunteered to participate in this study. Participants were purposively selected from two private Podiatry practices based on their signs and symptoms, age, gender, and doctor's clearance to participate in any form of physical activity. Dependent variables included isometric muscle strength of the hip, knee and ankle, range of motion of the ankle in plantarflexion and dorsiflexion and an assessment of balance, which were measured pre- and post-intervention. The researcher developed a scientifically based exercise intervention program to target the entire kinetic chain, and to develop a standard isometric protocol for patients with DPN. The intervention program consisted of a combination of ankle, hip, and knee specific rehabilitation. The intervention took place 3 times a week for 45 min per session.

Results The Mann-Whitney test was used to evaluate the differences in dependent variables from pre- to post-intervention. The level of significance was set at $p<0.05$. Notable increases were observed in range of motion in ankle plantarflexion and in balance time in the intervention group, post-intervention.

Conclusions Although many of the changes noted were insignificant, the trends indicated an improvement in the intervention group over the 10-week intervention period. These improvements can be considered clinically important.

Keywords Diabetic autonomic neuropathy · Diabetic nerve pain · Distal polyneuropathy · Hyperglycemia · Peripheral neuropathy · Pressure air biofeedback system

Introduction

One of the main complications of diabetes mellitus (DM) is diabetic peripheral neuropathy (DPN) and can be classified as a decrease in sensation and proprioception in the distal extremities [1]. Diabetic neuropathy is a result of nerve damage caused by chronically uncontrolled high blood glucose levels and is a common complication of DM, affecting up to 50% of patients suffering from both types of diabetes [2]. Since DM frequently results in peripheral neuropathies, the result is associated with reduced muscle strength and balance, gait impairment, and decreased ankle stability [3, 4]. Hip alterations during walking occur due to a decrease in strength in the plantar flexion muscle group. Patients with DPN experience a decrease in movement during the late stance phase of gait. Due to the strength deficit, the patient will adapt a "hip strategy" [3], where the leg is pulled forward using the hip flexors instead of using the plantar flexor muscles to push forward (ankle strategy). This phenomenon is also known as the "slowness strategy" [3]. Diabetic sarcopenia is associated with systematic insulin resistance, which is related to mobility disorders and is associated with a decrease in muscle activation and a decrease in myofascial structures, leading to an increased fall risk in DM patients [5]. Approximately, 2.1% of

✉ Ronél du Plessis
duplessisbio@gmail.com

[1] Department of Sport, Recreation and Exercise Science, University of the Western Cape, Bellville, South Africa

[2] Private Practice, Dembskey Podiatry, 33 Jim Fouche Street, Verwoerd Park, 1449 Alberton, South Africa

the world's population suffer from DM [6], which currently affects over 420 million people worldwide [6, 7], with the prevalence of DM still increasing rapidly each year [8]. As a result, DM can have a major impact on one's quality of life due to high-risk complications that may be either chronic or acute [9–11].

In South Africa, the most common form of DM is type II, with less than 10% of reported cases being type I. In an audit conducted in 1997 of 300 people with DM in Cape Town, 27% had diabetic neuropathy [12], while in 2012, the SANHANES-1 report [13] indicated that, among individuals with diabetes, nearly half were unscreened (45.4%). An additional 14.7% were screened but undiagnosed, 2.3% were diagnosed but untreated, and 18.1% were treated but uncontrolled. With the current prevalence of DM in South Africa being unclear, the IDF estimated that, by 2025, 7.04% (3.5 million) of the total South African population will have DM and a further 1.5 million will be undiagnosed [14]. Thus, the prevalence of diabetes in South Africa appears to be increasing over time and it appears that a large proportion of individuals in South Africa may be susceptible to DPN and that more research needs to be conducted in this area.

The treatment of those diagnosed with DPN in South Africa, however, is inadequate and a course of action must be implemented to improve both its diagnosis and management [15]. It is well known that exercise training improves and increases muscle mass, decreases blood pressure, and improves glucose tolerance [16–18]. Early aerobic and resistance training intervention is of great benefit to DM patients, as it is an effective way to control blood glucose levels and enhance insulin action up to 72 h [2]. Improvements thereof lead to health-related quality of life. Further, a combination of lower limb muscle strengthening, balance, and proprioceptive rehabilitation improves range of motion, balance, muscular strength, and glycated hemoglobin in older patients with DPN [19–21]. Low-intensity exercise improves/enhances vascular and metabolic pathways, which decreases painful neuropathic symptoms and improves quality of life [22]. In other words, exercise is an excellent treatment modality for people with DPN. Therefore, the purpose of this research study was to evaluate the effectiveness of an exercise protocol that was developed for people with DPN and was different from the standard clinical protocol commonly used today.

Materials and methods

Design and sampling

The research design was a pre-test post-test study using quantitative methods. A single-blind approach was chosen as only the researcher knew which treatment the participants would receive, as this would have led to spurious results. The study was designed to evaluate muscular strength and flexibility of the ankle joint, as well as balance, to determine if an exercise intervention would be effective treatment for patients who suffer from DPN in the lower leg. Patient files were conveniently selected from two Podiatry practices to determine DM status. Those with a confirmed diagnosis of DPN were approached to be part of the study. Participants received an information leaflet and a consent form prior to the recruitment process of the study took place. Recruitment was strictly voluntary, and participants had the right to withdraw without penalty. Before signing the consent form, volunteers were assured that they had the right to withdraw from the study at any time without any penalties. The final study sample consisted of 14 participants, aged 18–80 years. The inclusion criteria for the study comprised DPN in one or both lower limbs, any age, male or female, any level on physical activity, and a pre-test health risk evaluation done by their doctor. Exclusion criteria included any current fracture(s) or any injury in the lower limb and any participant who suffered from foot ulcers.

Procedures

A total of 14 participants underwent pre- and post-intervention testing, which consisted of the following: range of motion of the ankle in plantar- and dorsiflexion using goniometry, a static balance test, using the stork stand test, and an isometric strength assessment of the muscles surrounding the hip (gluteus maximus, gluteus medius and gluteus minimus, piriformis, adductor magnus and the long head of the biceps femoris), knee (vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris) and ankle (the tibialis anterior, extensor hallicus longus, extensor digitorum longus and the peroneus tertius [ankle plantarflexion], and tibialis posterior, peroneus longus, peroneus brevis, flexor hallucis longus and the flexor digitorium longus [ankle dorsiflexion] muscles) and joints using a pressure air biofeedback system. All 14 participants were then randomly divided into an intervention group and a comparison group. The process of randomization was based on previous diagnosis of diabetic peripheral sensory neuropathy in one or both lower limbs, participants' symptom severity, and a clinical assessment and diagnosis conducted by a Registered Podiatrist. Assessment tools utilized to diagnose neuropathy included a Tip-Therm, 28Hz Tuning Fork, 10g Semmes Weinstien Monofilament, 2-point discriminator, and a patellar hammer. The intervention group received a 10-week training program specifically designed by a clinical exercise specialist (biokineticist) for people with diabetic neuropathy. At the end of the study, the intervention training program was offered to the comparison group, so that they too could benefit from the study. The participants from both groups received the intervention programs along with the necessary rehabilitation equipment. Each exercise was explained

and demonstrated in detail to all the participants. Due to the worldwide pandemic (COVID-19), the restrictions in place, and the severity of the disease, no long-term follow-ups could have been implemented, which restricted the time frame of the research and exercise intervention and follow-up.

Blood pressure

An aneroid blood pressure cuff and stethoscope were used to measure the participants' blood pressure before, during and after exercise to ensure the participant was not hypo- or hypertensive as these are both absolute indications to terminate any exercise training according to the ACSM [23–25]. The participant was asked to take a seat during the before and after exercise readings with their feet flat on the floor and legs uncrossed. The reading was recorded in millimeters of mercury (mmHg) and any false or abnormal measurements were noted and acted upon immediately.

Blood glucose

A Contour Plus® blood glucose analyzer was used to measure the participants' blood glucose levels. Blood glucose was measured before, during, and after exercise to monitor the participant's glycemic state. Blood glucose was measured 5 min before the exercise session took place, 5 min after the first set of exercises, and 5 min after the session was completed. The clinician strictly followed the universal health precautions for drawing blood [26] and always used medical gloves during the procedure. A single-use lancet was used to draw a sample of blood from the index finger, which was applied to the testing strip, according to the manufacturer's specifications. The reading was measured and recorded in millimoles per liter (mmol L^{-1}). The participant was informed about the results, which were explained and discussed immediately.

Ankle mobility

Ankle mobility (range of motion) was assessed in plantar and dorsiflexion. The participant was asked to sit upright on a plinth, with legs straight and both ankles slightly elevated over a rolled towel. For both plantarflexion and dorsiflexion, the researcher placed the fulcrum of the goniometer over the lateral malleolus with the proximal arm being placed along the fibula using the head of the fibula for reference.

Balance

The stork stand test was utilized to measure static balance. A static test was used as a dynamic test would have led to an increased risk of injury and/or pain and discomfort. The participants performed the stork stand test on both legs with their shoes removed and with their hands on their hips. The amount of time that the participant was able to stand on one leg was measured in seconds. The stork stand test is a valid and reliable test method to use to measure static balance for any age group.

Isometric muscular strength

The Pressure Air Biofeedback System® (PAB) was used to measure isometric muscle function. This device measures the force applied to an air bladder located inside the product. The device enables the clinician to test maximum isometric strength as well as fatigue performance patterns of the muscles. Assessing isometric muscular strength allows the clinician to evaluate the patient's maximum muscular strength within the patient's ROM capacity and the ability to produce sufficient strength in the various joints in particular movement patterns. Specific movements, such as hip extension (Fig. 1a), knee extension (Fig. 1b) and ankle-plantar (Fig. 1c), and dorsiflexion (Fig. 1d), were conducted.

Exercise intervention

For this study, the researcher developed an isometric exercise protocol for people with DPN, which was different from the standard isometric clinical protocol commonly used today, and designed to specifically target the entire kinetic chain, and to assist in developing an effective isometric protocol for patients with DPN that will help reduce fall risks. The program consisted of low intensity muscular strength training exercises, low intensity static and passive stretches, balance training and proprioceptive rehabilitation exercises to increase muscular strength, ROM and balance time and decrease fall risk. The 10-week intervention took place 3 times a week for 45 min per session and was divided in three categories: range of motion exercises, strengthening exercises, and balance and proprioception training exercises. All exercise sessions were conducted under strict supervision of the researcher. Progression of exercises was determined and adjusted according to each participant's individual progress. Where progression was needed, an increase in repetitions and intensity was made accordingly.

Results

A Mann-Whitney test was used to evaluate the differences in dependent variables from pre- to post-intervention. The level of significance was set at $p<0.05$. The only notable increases observed were for range of motion in the right ankle plantarflexion ($p=0.022$) and balance time ($p=0.018$) for the left and right leg in the intervention group after a 10-week follow-up assessment. However, a decrease in systolic (−9.09%) and diastolic blood pressure (−13.89%) and a

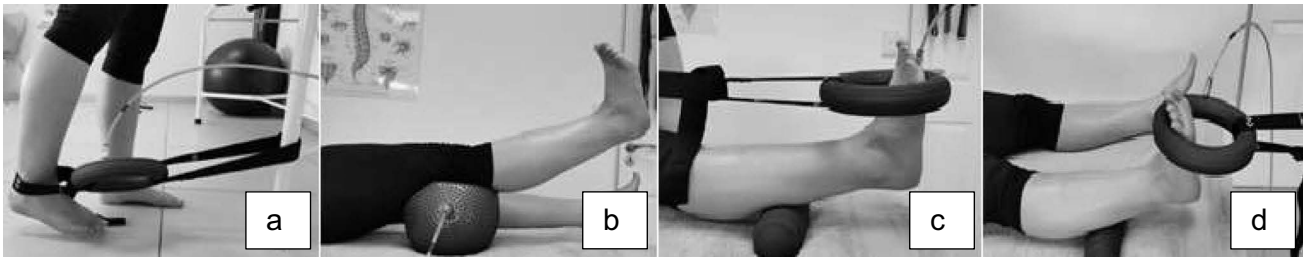

Fig. 1 Isometric muscle testing using the Pressure Air Biofeedback System. (**a**) Hip extension, (**b**) Knee extension, (**c**) Ankle-plantar flexion, (**d**) Ankle dorsiflexion

decrease in blood glucose levels (−17.89%) were observed post-intervention for the intervention group, which may be clinically important, as an increase in these variables was noted in the comparison group.An increase in plantarflexion, 8% (left) and 8% (right) and dorsiflexion 5.26% (left) and an 11.11% (right) increase in ROM for both left and right ankles, and balance time for both legs, 200% (left) and 159% (right) were observed in the intervention group post-intervention. The muscular strength variables showed a mix of an increase and decrease in strength post-intervention for the intervention group; however, they were insignificant. The strength test results observed in the comparison group post-intervention may also be clinically important. A summary of the expected and observed changes from pre- to post-intervention results is presented in Table 1.

Discussion

Diabetes mellitus is increasing rapidly worldwide each year and the prevalence of DPN is increasing along with the duration of DM [27]. It is predicted that in the year 2030, more

Table 1 A summary of the expected and observed changes from pre- to post-intervention

Variable	Expected change post-intervention (intervention group)	Observed change	
		Control group	Intervention group
Blood glucose (mmol L^{-1})	⬇	⬆ (37.84%)*	⬇ (−17.89%)
Systolic blood pressure (mmHg)	⬇	⬇ (−4.69%)*	⬇ (−9.09%)
Diastolic blood pressure (mmHg)	⬇	⬇ (−10.0%)	⬇ (−13.89%)
ROM plantarflexion (deg)			
Left ankle	⬆	⬇ (−4.62%)	⬆ (12.81%)
Right ankle	⬆	⬇ (−3.23%)	⬆ (8%)*
ROM dorsiflexion (deg)			
Left ankle	⬆	↔	⬆ (5.26%)
Right ankle	⬆	⬆ (4.17%)	⬆ (11.11%)
Stork stand (s)			
Left leg	⬆	⬆ (77.65%)	⬆ (200%)*
Right leg	⬆	⬆ (2.95%)	⬆ (159%)*
Strength in extension (kg)			
Left knee	⬆	⬆ (22.78%)	⬇ (−10.65%)
Right knee	⬆	⬆ (2.95%)	⬆ (179.63%)
Strength in plantarflexion (kg)			
Left ankle	⬆	⬆ (41.67%)	⬆ (27.45%)
Right ankle	⬆	⬇ (−27.47%)	⬆ (31. 37%)
Strength in dorsiflexion (kg)			
Left ankle	⬆	⬇ (−41.07%)	⬇ (−31.43%)
Right ankle	⬆	⬇ (−34.88%)	⬇ (−17.24%)
Strength in extension (kg)			
Left hip	⬆	⬇ (−36.04%)	⬇ (−10.53%)
Right hip	⬆	⬆ (27.78%)	⬆ (22.92%)

Key: ⬆ increased; ⬇ decreased; ↔ unchanged. *Significant at $p<0.05$

than 70% of people living with T2DM will reside in developing countries, and primary prevention of type II DM should be an urgent priority for such regions [28]. Proper assessments and intervention programs will guide clinicians to identify physical characteristics where improvements are required [29]. It is important for clinicians to understand the effect of DPN on muscular strength and balance, to be able to prescribe appropriate rehabilitation protocols to improve the quality of life of DPN patients [5]. Exercise training improves mitochondrial function, improving insulin sensitivity and glucose tolerance, and decreases blood pressure and increases muscle mass [17, 20]. Static range of motion/flexibility training is frequently used to increase joint mobility and flexibility and decrease the risk of any injury; balance and proprioceptive training decreases fall risk and improves postural stability in DPN patients [17, 20]. This pre- and post-intervention study was undertaken to evaluate the effectiveness of an isometric rehabilitation program for patients with DM, as there is a lack of currently researched protocols available for patients with DPN in South Africa. In this study, it was evident that a 10-week isometric rehabilitation program improved ankle range of motion (plantar- and dorsiflexion) and muscular strength of the surrounding musculature of the hip, knee, and ankle, and increased balance time. The researcher also found a non-significant decrease in blood pressure and blood glucose levels. These findings have important clinical implications for DPN patients as these improvements would improve quality of life of patients with DPN in South Africa. It has been shown that exercise positively affects blood glucose homeostasis, which improves due to structural remodeling of skeletal muscle as a result of the exercise intervention, and muscular strength/resistance training reduces systolic blood pressure in DPN patients [17, 20], which is supported by the findings in this study. Further, a combination of strengthening, balance, and proprioceptive rehabilitation will improve their gait patterns and decrease fall risk, leading to improved quality of life [20]. This study showed meaningful changes in objective measurements, and it appears to have been a clinically effective program for people with DPN in South Africa.

The total number of participants completing this study was affected by the following: COVID-19 restrictions, no clearance from a doctor to participate in any form of physical activity, noncompliance from participants and participants being medically booked off activities due to other chronic diseases/conditions. Therefore, a larger sample size would have increased the power of the study. Recommendations for future research include evaluations of isotonic muscle contractions of the entire kinetic chain, development and evaluation of isometric rehabilitation protocols based on the PAB® and to include the use of electromyography in the assessments of the distal musculature, to evaluate muscle function and to adapt specific rehabilitation exercises accordingly.

Conclusion

Individual, scientifically based rehabilitation protocols/interventions can be prescribed to DPN patients to improve muscular strength in the lower limbs and to improve overall muscular strength and endurance and improving functional capacity in the performance of daily living activities. The findings of this study indicate that an isometric evaluation and exercise program is effective in the treatment of DPN to evaluate and determine an effective treatment plan/intervention for patients with DPN, and to determine muscular strength deficits in both lower limbs in patients with DPN in South Africa.

Data availability Available

Code availability Not applicable

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval The study protocol was approved by the institution's Biomedical Research Ethics Committee (Ethics number: BM19/7/12).

Consent to participate Informed consent has been obtained from all the participants prior to the inclusion into the study.

Consent for publication The participant provided informed consent for publication of the images in Fig. 1a, b, c, and d.

References

1. Azhary H, Farooq MU, Bhanushali M, Majid A, Kassab MY. Peripheral neuropathy: differential diagnosis and management. Am Fam Physician. 2010;81(7):887–92.
2. Gangwar AK. Diabetic neuropathy: classification pathogenesis and treatment. Eur J Pharm Sci. 2015;2(4):1476–95.
3. El-Refay BH, Ali OI. Efficacy of exercise rehabilitation program in improving gait of diabetic neuropathy patients. Med J Cairo Uni. 2014;82(2):225–32.
4. Katoulis EC, Ebdon-Parry M, Lanshammar H, Vileikyte L, Kulkarni J, Boulton AJM. Gait abnormalities in diabetic neuropathy. Diabetes Care. 1997;20(12):1904–7.
5. Ozturk ZA, Turkbeyler IH, Demir Z, Bilici M, Kepekci Y. The effect of blood glucose regulation on sarcopenia parameters in obese and diabetic patients. Turk J Phys Med Rehabil. 2018;64(1):72–9.
6. Hingorani A, La Muraglia GM, Henke P, Meissner MH, Loretz L, Zinszen KM, Driver VR, Frykberg R, Carmen TL, Marston W, Mills JL, Murad MH. The management of diabetic foot: a clinical practice guideline by the society for vascular surgery in collaboration with the American podiatric medical association and the society for vascular medicine. J Vasc Surg. 2016;63(25):35–215.
7. Atlas D. International Diabetes Federation. IDF Diabetes Atlas. 7th ed. International Diabetes Federation: Brussels, Belgium; 2015.

8. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol. 2013;4(4):46–57.
9. Bruschi LKM, da Rocha DA, Filho ELG. de Moura Pancoti Barboza N, Frisanco PAB, Callegaro RM, de Sa LBPC, Arbex AK. Diabetes mellitus and diabetic neuropathy. OJEMD. 2017;7(1):12–21.
10. Sartor CD, Watari R, Passaro AC, Picon AP, Hasue RH, Sacco ICN. Effects of a combined strengthening, stretching and functional training program versus usual-care on gait biomechanics and foot function for diabetic neuropathy: a randomized controlled trail. BMC Musculoskelet Disord. 2012;13(36):1–10.
11. Singh R, Kishore L, Kaur N. Diabetic peripheral neuropathy: current perspective and future directions. Pharmacol Res. 2014;80:21–35.
12. Levitt NS, Bradshaw D, Swarenstein MF, Bawa AA, Maphumolo S. Audit of public sector primary diabetes care in Cape Town, South Africa: high prevalence of complications, uncontrolled hyperglycaemia, and hypertension. Diabet Med. 1997;14(12): 1073–7.
13. Stokes A, Berry KM, Mchiza Z, Parker WA, Labadarios D, Chola L, Hongoro C, Zuma K, Brennan AT, Rockers PC, Rosen S. Prevalence and unmet need for diabetes care across the care continuum in a national sample of South African adults: evidence from the SANHANES-1, 2011-2012. PLoS One. 2017;12(10): e0184264.
14. Jacovides A, Bogoshi M, Distiller LA, Mahgoub EY, MKA O, Tarek IA, Wajsbrot DB. An epidemiological study to assess the prevalence of diabetic peripheral neuropathic pain among adults with diabetes attending private and institutional outpatient clinics in South Africa. Int J Med Res. 2014;42(4):1018–28.
15. Coetsee C, Terblanche E. The effect of three different exercise training modalities on cognitive and physical function in a healthy older population. EURAPA. 2017;14(1):1–10.
16. Pan B, Ge L, Xun YQ, Chen YJ, Gao CY, Han X, Zuo LQ, Shan HQ, Yang KH, Ding GW, Tian JH. Exercise training modalities in patients *with type 2 diabetes* mellitus: a systematic review and network meta-analysis. IJBNPA. 2018;15(1):1–14.
17. Shu J, Matarese A, Santulli G. Diabetes, body fat, skeletal muscle and hypertension: the ominous chiasmus? J Clin Hypertens. 2019;21(2):239–42.
18. Chen SM, Shen FC, Chen JF, Chang WD, Chang NJ. Effects of resistance exercise on glycated hemoglobin and functional performance in older patients with comorbid diabetes mellitus and knee osteoarthritis: a randomized trial. Int J Env Res Pub He. 2020;17(1):224–37.
19. Jamshidpour B, Bahrpeyma F, Khatami MR. The effect of aerobic and resistance exercise training on the health-related quality of life, physical function, and muscle strength among hemodialysis patients with Type 2 diabetes. J Bodyw Mov Ther. 2020;24(2):98–103.
20. Pan X, Bai JJ. Balance training in the intervention of fall risk in elderly with diabetic peripheral neuropathy. Int J Nurs Sci. 2014;1: 441–5.
21. Johnson C, Takemoto JK. A review of beneficial low-intensity exercises in diabetic peripheral neuropathy patients. J Pharm Pharm Sci. 2019;22:22–7.
22. McCusker K, Gunaydin S. Research using qualitative, quantitative or mixed methods and choice based on research. SAGE Open MEd. 2015;30(7):537–42.
23. Pescatella LS, Arena R, Riebe D, Thompson PD. ACSM's guidelines for exercise testing and prescription. (Ninth Edition). Place of publication: Williams & Wilkins. 2013.
24. Sharman J, LaGerche A. Exercise blood pressure: clinical relevance and correct measurement. J Hum Hypertens. 2014;29(6):1–4.
25. Riebe D, Ehrman JK, Liguori G, Magal M, American College of Sports Medicine, editors. ACSM's guidelines for exercise testing and prescription. Philadelphia: Wolters Kluwer; 2018.
26. Broussard I, Kahwaji CI. Universal precautions. StatPearl Publishing [Online], 2019. http://www.ncbi.nlm.nih.gov/books/NBK470223/. Accessed 20 Feb 2020
27. Ganu D, Fletcher N, Caleb NK. Physical disability and functional impairments resulting from type 2 diabetes in sub-Saharan Africa: A systematic review. Afr J Diabetes Med. 2016;24(1):1–5.
28. Echouffo-Tcheugui JB, Dagogo-Jack S. Preventing diabetes mellitus in developing countries. Nat Rev Endocrinol. 2012;8(9): 557–62.
29. Williams VJ, Nagai T, Sell TC, Abt JP, Rowe RS, McGrail MA, Lephart SM. Prediction of dynamic postural stability during single-leg jump landing by ankle and knee flexibility and strength. J Sport Rehabil. 2016;25(3):266–72.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):258–266
https://doi.org/10.1007/s13410-022-01077-0

ORIGINAL ARTICLE

Dietary patterns and diabetes risk in Southern Chinese in Guangxi Zhuang autonomous region

Xiaojing Guo[1] · Shun Liu[2] · Xiaoyun Zeng[1] · Li Su[1] · Dongping Huang[3] · Xiaoyun Ma[1] · Bihu Liu[1] · Peng Tang[1] · Xiaoqiang Qiu[1]

Received: 5 October 2021 / Accepted: 17 April 2022 / Published online: 19 May 2022

Abstract

Background The association between dietary patterns (DPs) and diabetes risk remains inconsistent in terms of intake and frequency.

Objectives To explore the association between dietary patterns (DPs) and diabetes risk.

Methodology We recruited 13,587 volunteers from a cohort study in Guangxi. Principal component analysis was used to define DPs, and logistic regression was used to explore the associations.

Results In both model 1 (grouped by history of diabetes) and model 2 (grouped by current Glu), frequencies of dessert intake and fruit intake are significantly different between people with and without diabetes risk (all $p<0.01$). Uni- and multivariate logistic regression analyses all show that a limited frequency of dessert intake (less than *3–4 times per week*) significantly reduced diabetes risk before or after adjustment in both model 1 and model 2, and a more frequency of fruit intake (more than *3–4 times per week*) significantly decreased diabetes risk in model 1. After sub-analysis by age and sex, the associations between diabetes risk and frequency of dessert intake and fruit intake still exist.

Conclusion The DPs of Southern Chinese in Guangxi were related to food abundance and frequency of intake. Habitual fruit intake and moderate frequencies of sweets were associated with decreased diabetes risk.

Keywords Dietary patterns · Diabetes · Southern Chinese · Principal component analysis · Logistic regression

Introduction

Diabetes brings heavy burden to the global population, including macrovascular and microvascular complications [1]. The overall prevalence of diabetes in China had increased from 1980 to 2013 [2]. The latest nationwide cross-sectional survey in mainland China indicated that the estimated standardized prevalence of diabetes was 10.9% [3].

Up to 90% of diabetes cases worldwide are preventable if individuals follow a healthy diet and lifestyle [4]. In the UK, Conklin et al. indicated that the regular consumption of all five food groups (dairy products, fruits, vegetables, meat and alternatives, and grains) and a greater variety of dairy, fruit, and vegetable subtypes may be important to reduce the risk of diabetes [5]. In China, Sun et al. identified four dietary patterns (DPs), including healthy diet, high-salt diet, meat diet, and carbohydrate-rich diet, and suggested that high-salt diet and carbohydrate-rich diet are positively associated with diabetes risk compared with healthy diet [6]. Characteristic DPs, such as the Mediterranean diet (MeDiet), have been confirmed to reduce the incidence of cardiovascular disease by 30% [7]. Adherence to the MeDiet and Dietary Approaches to Stop Hypertension is associated with a remarkable reduction in the risk of incident diabetes [8]. However, DPs in the Chinese population may be difficult to derive from a

Xiaojing Guo and Shun Liu are contributed equally

✉ Xiaoqiang Qiu
xqqiu9999@163.com

[1] Department of Epidemiology and Health Statistics, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

[2] Department of Maternal, Child and Adolescent Health, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

[3] Department of Sanitary Chemistry, School of Public Health, Guangxi Medical University, Nanning, Guangxi, China

consistent standard because of the great difference in the eating habits among different ethnicities [9, 10].

There is a certain relationship existing between diet and diabetes, and regional differences in dietary behavior. To date, conclusions on the relationship between diet and diabetes risk are still inconsistent. A previous study in Guangxi evaluated the influence of dietary habit such as dietary laws, diet tastes, and cooking methods [11]. The present study is based on a Guangxi Zhuang prospective cohort and aims to explore the association between DPs and diabetes risk. As supplementary evidence, it could alert us to formulate a targeted disease management meal plan for populations.

Methods

Study population

The studied population is from the baseline survey data of local residents in Guangxi of the Prospective Cohort Study of Chronic Diseases in Natural Populations in South China from May 2, 2018, to November 6, 2019. Volunteers were aged 30–82 years. Included individuals were Guangxi permanent residents (validated by identity card or household register).

Diabetes diagnosis and assessment

History of diabetes was investigated, and blood glucose (Glu) of the study population was determined with morning fasting blood using the Roche® C702 biochemical analyzer. Model 1 is grouped by self-reported history of diabetes. Model 2 is grouped by Glu value. Diabetes risk assessment methods refer to the guidelines by the Chinese Diabetes Society [12].

Assessment of diet and covariates

A self-designed, simplified food frequency questionnaire (FFQ) was used during face-to-face interview. The FFQ used in this study was consistent with FFQ25, which was validated in populations all over the world previously [13]. The content of FFQ was shown in the Supplementary file. Participants provided standardized answers to the investigators, selecting from six food frequency categories, that is, *<1 time per month*, *≤3 times per month*, *1–2 times per week*, *3–4 times per week*, *5–6 times per week*, and *everyday*, which were assigned range from 0 to 5. In the logistic regression analysis, *<1 time per month* is always a reference. Trained physicians measured subjects' body composition using the TANITA BC-601 Body Fat Monitor. The subjects stood on the instrument with both hands clasping the handle. After the instrument automatically measured, it directly read the subject's body composition data, including height, weight, BMI, body fat percentage, body water, visceral fat, body age, and other indicators.

Statistical analysis

DPs were derived by PCA using R 3.6.3 software (visualize with RStudio). Packages *readxl* and *xlsx* were for importing and exporting data, package *dplyr* was for data cleaning, packages *Hmisc* and *psych* were for statistical description and PCA, the built-in function *glm()* was for uni- (ULR) and multi- (MLR) variate logistic regression, package *CATT* was for Cochran–Armitage trend test, and packages *forestplot* and *ggbiplot* was for plotting. *T* test or one-way ANOVA was used for measurement data, whereas chi-squared test was used for counting data. Nonparametric test was used when variance was not homogeneous. Two-tailed *p* value less than 0.05 was defined as significant. Data were described as mean ± standard deviation (SD) or number (*N*) with percentage (%).

Results

Demographics

The cohort included 13,587 participants with complete records of the questionnaire, physical examination, and laboratory data. After credibility evaluation of questionnaires and data cleaning, we enrolled participants with high reliability and complete information. Over 93.7% participants are of Zhuang ethnicity, and their eating habit is representative of the Zhuang. The flow chart of the participant screening process is shown in Figure S1. Demographic data are shown in Table 1.

DP evaluation

Dietary food items were divided into 14 categories (Table S1). The parallel testing suggested four principal components (PCs) to be involved, and cumulative proportion of the top three PCs (i.e., DPs) exceeded 0.8 (Fig. 1). DP1, DP2, and DP3 were separately named Balanced, Basic, and Insufficient. The DPs in models 1 and 2 are consistent with each other. DP1 contains a wide variety of nutrients, including pasta (0.57), grains (0.47), desserts (0.46), poultry (0.44), seafood (0.48), eggs (0.57), fruit (0.45), nuts (0.49), and milk (0.54). DP2 contains basic nutrients, including livestock (0.47), poultry (0.53), seafood (0.45), and vegetables (0.50), with a less grain intake frequency (−0.40). DP3 contains limited nutrients, only including livestock (0.50) and vegetables (0.43), with a less pickles intake frequency (−0.40). The PCA result also suggested that the population with DP2 has less grain intake and the population with DP3 has less pickle intake.

Table 1 Demographic data of the studied population

Items	Model 1 (*N*=10,972)		Model 2 (*N*=10,622)	
	N/mean±SD	*%*	*N/mean±SD*	*%*
Age	54.58±10.41		54.51±10.40	
<60	7,283	66.4%	7,075	66.6%
≥60	3,689	33.6%	3,547	33.4%
Sex				
Male	5,048	46.0%	4,868	45.8%
Female	5,924	54.0%	5,754	54.2%
Education				
No formal schooling	1,430	13.0%	1,371	12.9%
Primary school	4,293	39.1%	4,177	39.3%
Junior high school	3,206	29.2%	3,113	29.3%
High school	1,394	12.7%	1,337	12.6%
College	412	3.8%	396	3.7%
University	230	2.1%	221	2.1%
Postgraduate and above	7	0.1%	7	0.1%
Occupation				
Farmer	6,061	55.2%	5,896	55.5%
Worker	1,266	11.5%	1,218	11.5%
Administrative and management personnel	276	2.5%	262	2.5%
Professional and technical personnel	229	2.1%	220	2.1%
Sales and service staff	219	2.0%	217	2.0%
Private owner	220	2.0%	217	2.0%
Housewife/husband	618	5.6%	595	5.6%
Unemployed/laid-off	274	2.5%	271	2.6%
Others	493	4.5%	478	4.5%
Retired	1,316	12.0%	1,248	11.7%
Marital status				
Married	9,746	88.8%	9,441	88.9%
Widowed	907	8.3%	878	8.3%
Divorce	190	1.7%	181	1.7%
Unmarried	129	1.2%	122	1.1%
Income				
<￥10,000	2,105	19.2%	2,042	19.2%
￥10,000~29,900	3,899	35.5%	3,781	35.6%
￥30,000~59,900	2,589	23.6%	2,506	23.6%
￥60,000~99,900	1,375	12.5%	1,328	12.5%
￥100,000~149,900	794	7.2%	765	7.2%
￥150,000~299,900	187	1.7%	178	1.7%
>￥3000,000	23	0.2%	22	0.2%
Family history of NCD				
No	9,006	82.1%	8,726	82.2%
Yes	1,966	17.9%	1,896	17.8%
Diseases affecting bowel function				
No	10,299	93.9%	9,967	93.8%
Yes	673	6.1%	655	6.2%
BMI	25.53±3.54		23.50±3.52	
Healthy	2,641	24.1%	2,590	24.4%

Table 1 (continued)

Items	Model 1 (*N*=10,972)		Model 2 (*N*=10,622)	
	N/mean±SD	*%*	*N/mean±SD*	*%*
Warning	5,263	48.0%	5,097	48.0%
More	2,353	21.4%	2,265	21.3%
Overmuch	715	6.5%	670	6.3%
Glu	4.97±1.40		4.94±1.40	
With history of diabetes	10,618	96.8%	*N/A*	*N/A*
Without history of diabetes	354	3.2%		
Model 2				
3.8~6.1mmol/L	*N/A*	*N/A*	10,121	95.3%
≥ 7.0mmol/L			501	4.7%

Differences between Southern Chinese with or without diabetes risk

In model 1, the distribution of age, occupation, family history of non-communicable diseases (NCD), body mass index (BMI), visceral fat level, habit of drinking soup, Glu, intake frequencies of dessert, livestock, fruit, milk, and beverage are significantly different between people with and without history of diabetes (all $p < 0.05$). In model 2, the distribution of age, sex, education, occupation, smoking status, use of health care products, BMI, visceral fat level, alcohol use, habit of drinking soup, Glu, intake frequencies of rice, grain, dessert, seafood, and fruit ($p = 0.010$) are significantly different between people with and without diabetes risk defined by current Glu

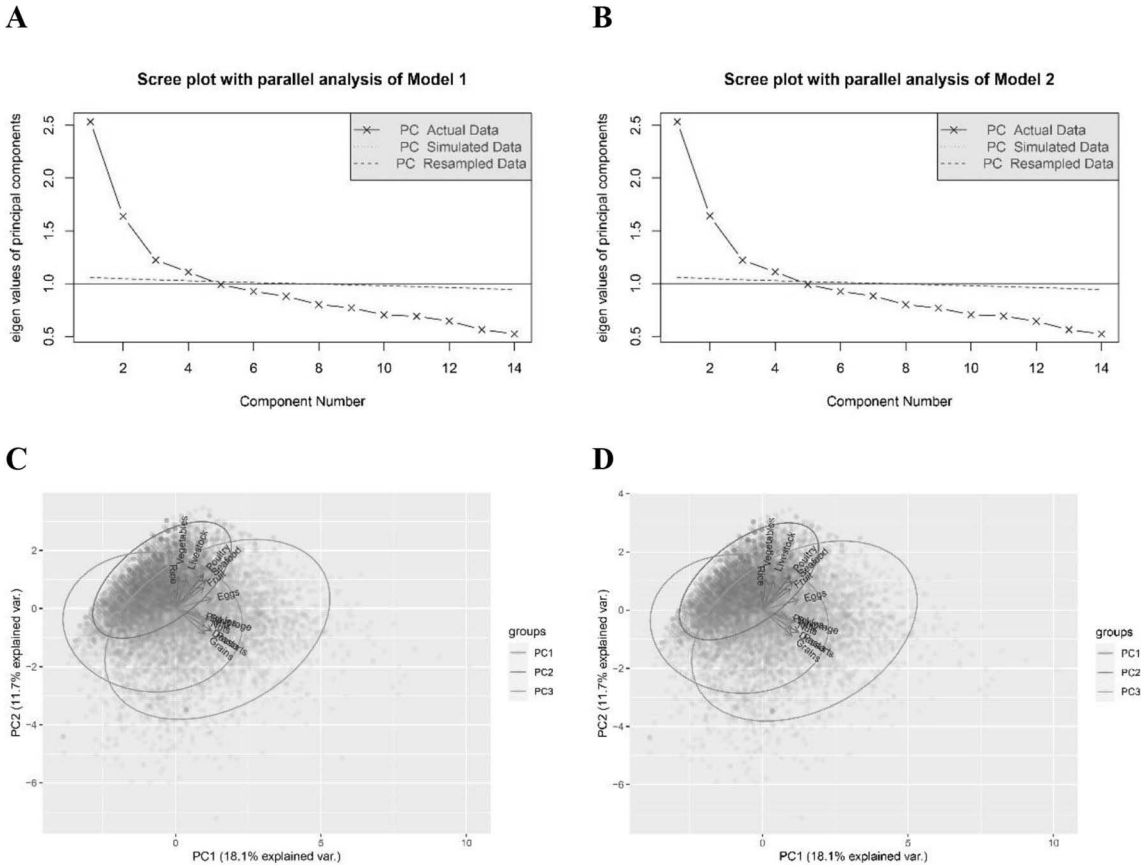

Fig. 1 Principal component analysis. Scree plots with parallel analysis of **a** model 1 and **b** model 2, respectively. PCA results of **c** model 1 and **d** model 2

(all $p < 0.05$). However, in both model 1 and model 2, there is no statistical difference in the distribution of DPs. Therefore, both model 1 and model 2 are sub-analyzed by age, and only model 2 is sub-analyzed by sex (Table S2).

Associations between diet and diabetes risk in Guangxi Southern Chinese

DPs had no significant association with diabetes risk

There was no significant correlation between DPs and diabetes risk in Guangxi residents. The results of the ULR analysis are shown in Figure S2. Dietary variables with $p < 0.05$ were included in MLR analysis (shown in Fig. 2, S3, and Table S3).

Increased pasta, moderate amounts of grains, seafood, and milk were positively associated with diabetes risk

In model 1, increased pasta intake contributed to diabetes risk (*≤3 times per month*: $\beta_{adj} = 0.364$, $OR_{adj} = 1.439$ [1.063–1.943], $p_{adj} = 0.018$; *1–2 times per week*: $\beta_{adj} = 0.565$, $OR_{adj} = 1.760$ [1.217–2.524], $p_{adj} = 0.002$; *3–4 times per week*: $\beta_{adj} = 0.494$, $OR_{adj} = 1.639$ [0.998–2.616], $p_{adj} = 0.044$; *5–6 times per week*: $\beta_{adj} = 0.923$, $OR_{adj} = 2.518$ [1.405–4.333], $p_{adj} = 0.001$; *everyday*: $\beta_{adj} = 1.049$, $OR_{adj} = 2.854$ [1.671–4.739], $p_{adj} < 0.001$). Milk intake of *5–6 times per week* was a risk for diabetes ($\beta_{adj} = 1.238$, $OR_{adj} = 3.449$ [1.505–7.113], $p_{adj} = 0.002$).

In model 2, grain intake of *3–4 times per week* ($\beta_{adj} = 0.538$, $OR_{adj} = 1.713$ [1.154–2.490], $p_{adj} = 0.006$) and seafood intake of *everyday* ($\beta_{adj} = 0.497$, $OR_{adj} = 1.643$ [1.036–2.552], $p_{adj} = 0.030$) were associated with increased diabetes risk.

Increased fruit, moderate amounts of rice, desserts, livestock, and beverage were inversely associated with diabetes risk

In model 1, increased fruit intake was inversely associated with diabetes risk (*1–2 times per week*: $\beta_{adj} = -0.630$, $OR_{adj} = 0.532$ [0.349–0.819], $p_{adj} = 0.004$; *3–4 times per week*: $\beta_{adj} = -0.889$, $OR_{adj} = 0.411$ [0.258–0.655], $p_{adj} < 0.001$; *5–6 times per week*: $\beta_{adj} = -0.637$, $OR_{adj} = 0.529$ [0.317–0.874], $p_{adj} = 0.013$; *everyday*: $\beta_{adj} = -0.701$, $OR_{adj} = 0.496$ [0.334–0.747], $p_{adj} = 0.001$). Moderate amounts of sweets may mitigate diabetes risk, including desserts (*≤3 times per month*: $\beta_{adj} = -0.797$, $OR_{adj} = 0.450$ [0.305–0.647], $p_{adj} < 0.001$; *3–4 times per week*: $\beta_{adj} = -1.884$, $OR_{adj} = 0.152$ [0.025–0.494], $p_{adj} = 0.009$), and beverage (*≤3 times per month*: $\beta_{adj} = -0.419$, $OR_{adj} = 0.658$ [0.447–0.945], $p_{adj} = 0.028$; *1–2 times per week*: $\beta_{adj} = -0.959$, $OR_{adj} = 0.383$ [0.175–0.739], $p_{adj} = 0.008$). Livestock intake of *5–6 times per week* was also negatively associated with diabetes risk ($\beta_{adj} = -0.701$, $OR_{adj} = 0.496$ [0.278–0.914], $p_{adj} = 0.020$).

In model 2, a more frequent rice intake was associated with decreased diabetes risk (*5–6 times per week*: $\beta_{adj} = -2.581$, $OR_{adj} = 0.076$ [0.004–0.482], $p_{adj} = 0.020$; *everyday*: $\beta_{adj} = -0.947$, $OR_{adj} = 0.388$ [0.166–1.068], $p_{adj} = 0.043$). Moderate desserts were also linked to lower diabetes risk (*≤3 times per month*: $\beta_{adj} = -0.333$, $OR_{adj} = 0.717$ [0.544–0.934], $p_{adj} = 0.016$; *5–6 times per week*: $\beta_{adj} = -0.991$, $OR_{adj} = 0.371$ [0.144–0.784], $p_{adj} = 0.020$).

Associations between diet and diabetes risk in adults under 60 years old

In model 1, desserts (*≤3 times per month*: $\beta_{adj} = -0.897$, $OR_{adj} = 0.408$ [0.233–0.671], $p_{adj} = 0.001$), livestock (*5–6 times per week*: $\beta_{adj} = -0.850$, $OR_{adj} = 0.427$ [0.192–1.026], $p_{adj} = 0.044$), fruit (*1–2 times per week*: $\beta_{adj} = -0.965$, $OR_{adj} = 0.381$ [0.207–0.718], $p_{adj} = 0.002$; *3–4 times per week*: $\beta_{adj} = -0.882$, $OR_{adj} = 0.414$ [0.222–0.788], $p_{adj} = 0.006$; *5–6 times per week*: $\beta_{adj} = -0.952$, $OR_{adj} = 0.386$ [0.185–0.794], $p_{adj} = 0.010$; *everyday*: $\beta_{adj} = -0.995$, $OR_{adj} = 0.370$ [0.211–0.670], $p_{adj} = 0.001$), and beverage (*≤3 times per month*: $\beta_{adj} = -0.570$, $OR_{adj} = 0.565$ [0.342–0.900], $p_{adj} = 0.021$; *1–2 times per week*: $\beta_{adj} = -0.993$, $OR_{adj} = 0.370$ [0.139–0.821], $p_{adj} = 0.026$) were inversely associated with diabetes risk. But milk (*1–2 times per week*: $\beta_{adj} = 0.745$, $OR_{adj} = 2.105$ [1.051–3.941], $p_{adj} = 0.021$; *5–6 times per week*: $\beta_{adj} = 1.606$, $OR_{adj} = 4.983$ [1.747–12.089], $p_{adj} = 0.001$) had positive associations with diabetes risk.

In model 2, dessert intake of *3–4 times per week* was negatively associated with diabetes risk ($\beta_{adj} = -1.118$, $OR_{adj} = 0.327$ [0.097–0.821], $p_{adj} = 0.035$), while grain intake of *3–4 times per week* was positively associated with diabetes risk ($\beta_{adj} = 0.687$, $OR_{adj} = 1.987$ [1.241–3.112], $p_{adj} = 0.003$).

Associations between diet and diabetes risk in adults aged 60 and older

In model 1, fruit (*3–4 times per week*: $\beta_{adj} = -1.001$, $OR_{adj} = 0.368$ [0.183–0.721], $p_{adj} = 0.004$) and pickles (*3–4 times per week*: $\beta_{adj} = -2.060$, $OR_{adj} = 0.127$ [0.007–0.621], $p_{adj} = 0.046$) both had associations with decreased diabetes risk, while pasta (*5–6 times per week*: $\beta_{adj} = 1.093$, $OR_{adj} = 2.984$ [1.271–6.347], $p_{adj} = 0.007$; *everyday*: $\beta_{adj} = 1.236$, $OR_{adj} = 3.441$ [1.659–6.658], $p_{adj} < 0.001$) had associations with increased diabetes risk.

In model 2, moderate amount of desserts (*≤3 times per week*: $\beta_{adj} = -0.589$, $OR_{adj} = 0.555$ [0.341–0.864], $p_{adj} = 0.021$) was related to decreased diabetes risk, while pasta intake of *everyday* ($\beta_{adj} = 0.873$, $OR_{adj} = 2.394$ [1.151–4.575], $p_{adj} = 0.012$) was related to increased diabetes risk.

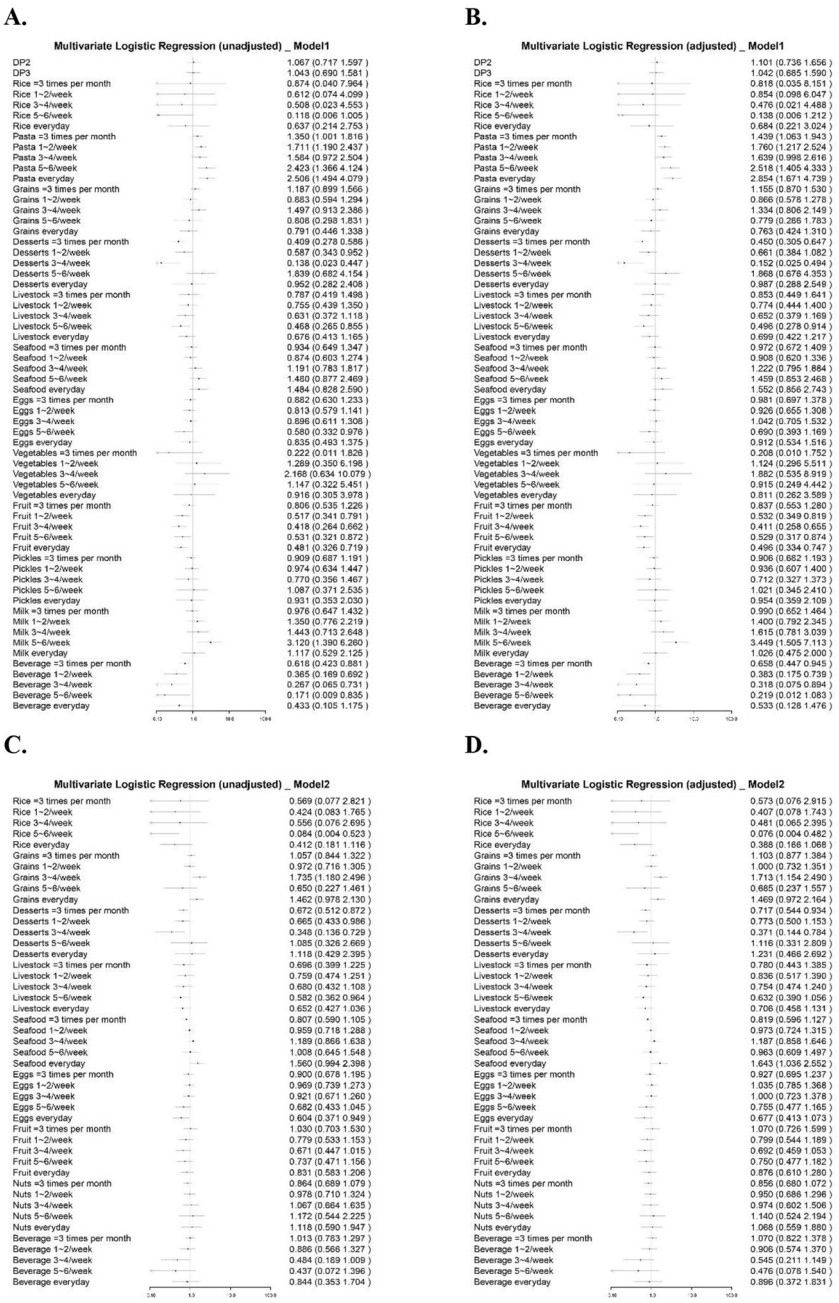

Fig. 2 Forest plots of multivariate logistic regression. **a** Model 1 without adjustment. **b** Model 1 after adjustment. **c** Model 2 without adjustment. **d** Model 2 after adjustment

Discussion

This study specific to the minority population in Southern China briefly reveals dietary habits of Guangxi Southern Chinese and the associations between food intake and diabetes risk, and provides new evidence for study in diabetes risk factors. Self-reported history of diabetes (model 1) could be referenced for research, and daily eating habit may be affected by the recognition of known disease states. The current Glu level (model 2) could contribute to diabetes discovery of persons with prediabetes, who retained the most common dietary habits. The baseline data could only provide blood Glu level without typical self-symptom description or retested Glu data; therefore, diabetes is difficult to diagnose [14]. However, random blood glucose is strongly associated with undiagnosed diabetes and a robust dose response [15].

Dietary control is necessary for diabetes prevention and treatment. The differences in the eating habits of various ethnicities make the division of DPs difficult [16–19]. People who adhere to *Balanced* (DP1) most probably obtain abundant and balanced nutrients. People with *Basic* (DP2) and *Insufficient* (DP3) may be prone to malnourished. In China, the intake of low-fat milk, fruit, vegetables, nuts, and seafood have increased moderately over time from 1991 to 2011, but

the average intake of these foods was still below optimal levels in 2011 [20]. The simplified FFQ is difficult to determine the quantity but only frequency of food intake. However, overnutrition is difficult to define and needs to be defined according to specific nutrients. Determining what to eat and how to eat remains very challenging for many individuals with diabetes, and meal planning should be individualized as individuals have different nutrient requirements [21]. Many DPs could help people from developing diabetes [22, 23], under which the total consumption of carbohydrates or fats is reduced, and specific food categories and quality are not strictly regulated [24].

Higher frequency of fruit intake is protective factor for diabetes, which is consistent with the evidence all over the world [25–27]. Vitamin C and total carotenoids are the most consistently responsive biomarkers from fruits and vegetables in plasma, which can reduce the risk of developing T2DM [27]. Regular consumption of berries is suggested for T2DM prevention; the biological function of the extract is related to glucose metabolism [28]. Interestingly, we discovered a more frequent dessert consumption ($p_{trend} \leq 0.001$) has a higher diabetes risk, but a limited frequency of dessert and beverage consumption may be more protective to diabetes than no consumption, which is consistent with the Shanghai cohort [29]. But why small sugar content from desserts or beverage can reduce diabetes risk? A randomized controlled trial (RCT) indicated that sweet sugar beverages can modulate sugar metabolism and lipid metabolism [30]. Accordingly, low frequency of sweet intake from desserts or beverage may promote the secretion of insulin or regulate the concentrations of glucose-related substances.

The dietary habits of Southern Chinese are rice-based, and the frequency of rice intake among Southern Chinese people with different DPs was not substantially different. Daily pasta consumption is related to increased diabetes risk, especially in population over 60 years old. Pasta consumption within the limits recommended for total carbohydrate intake is not associated with poor glycemic control [31]. A RCT indicated substantially lower peak glucose levels in higher protein pasta group and regular pasta group compared with the white rice group, suggesting different food types with similar macronutrient content produce considerably different postprandial glycemic responses in diabetes patients [32].

According to Dietary Guidelines for Chinese Residents [33], the recommended daily milk and milk product intake is 300 g. However, the milk intake of Chinese people is usually insufficient, and the awareness rate about nutrition related to milk is worrying [34]. We found that milk intake of *5–6 times per week* increased diabetes risk in males and people under 60 years old. It puzzled us and the result is not in line with previous studies [35]. This difference may be caused by the geographical difference of the participants (Southern Chinese in Guangxi vs. Singapore-based Chinese).

Strengths and limitations

The strengths are large samples and specific populations from the representative minority areas. However, the limitations of the cohort field investigation, such as the long duration and imperfect condition of sample storage, may be unavoidable, which may cause a certain difference between detected and actual values. Converting Glu into binary data may help reduce error or bias. Besides, DPs may not be consistent with the standards introduced in the guideline. The simplified FFQ may be subject to measurement error and recall bias [36]. Future work in the follow-up process will try to improve the self-report instrument and include larger samples.

Conclusions

The DPs of Southern Chinese in Guangxi were related to food abundance and frequency of intake, which had no significant association with diabetes risk. Increased pasta and moderate amounts of grains, seafood, and milk were positively associated with diabetes risk, while increased fruit and moderate amounts of rice, desserts., livestock, eggs, and beverage were inversely associated with diabetes risk. Habitual fruit intake and moderate sweetness may be beneficial in diabetes management.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-022-01077-0.

Acknowledgment We sincerely thank and acknowledge all participants and investigators contributed to this research, without whom the present study would not be possible.

Availability of data and material Not applicable

Code availability Not applicable

Author contribution Qiu X., Zeng X., Su L., Liu S., and Huang D.: designed the research; Ma X., Liu B., and Tang P.: collected the data and assisted with statistics; Guo X.: collected the data, analyzed the data and wrote the manuscript; Qiu X.: critically revised the manuscript; Liu S.: data management; Guo X. and Qiu X.: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Funding This study is supported by the National Key Technologies Research and Development (R&D) Program of China (2017YFC0907103).

Declarations

Ethics approval This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Ethics Committee of Guangxi Medical University (No. 20170201-1).

Consent to participate Written informed consent was obtained from all subjects after they were briefed on the study.

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

1. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019;62(1):3–16. https://doi.org/10.1007/s00125-018-4711-2.
2. Ma RCW. Epidemiology of diabetes and diabetic complications in China. Diabetologia. 2018;61(6):1249–60. https://doi.org/10.1007/s00125-018-4557-7.
3. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, Li Y, Zhao Z, Qin X, Jin D, Zhou M, Tang X, Hu Y, Wang L. Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. JAMA. 2017;317(24):2515–23. https://doi.org/10.1001/jama.2017.7596.
4. Ley SH, Ardisson Korat AV, Sun Q, Tobias DK, Zhang C, Qi L, Willett WC, Manson JAE, Hu FB. Contribution of the Nurses' Health Studies to Uncovering Risk Factors for Type 2 Diabetes: Diet, Lifestyle, Biomarkers, and Genetics. Am J Public Health. 2016;106(9):1624–30. https://doi.org/10.2105/AJPH.2016.303314.
5. Conklin AI, Monsivais P, Khaw KT, Wareham NJ, Forouhi NG. Dietary Diversity, Diet Cost, and Incidence of Type 2 Diabetes in the United Kingdom: A Prospective Cohort Study. PLoS Med. 2016;13(7):e1002085. https://doi.org/10.1371/journal.pmed.1002085.
6. Sun Q, Wang H, Qiao N, Zhang HX, Cui Y, Huang JJ, Wang T. Association of dietary patterns and diabetes risk level among adult residents in Datong city of Shanxi Province. Zhonghua Yu Fang Yi Xue Za Zhi. 2020;54(3):278–82. https://doi.org/10.3760/cma.j.issn.0253-9624.2020.03.007.
7. Schroder H, Salas-Salvado J, Martinez-Gonzalez MA, Fito M, Corella D, Estruch R, et al. Baseline adherence to the Mediterranean diet and major cardiovascular events: Prevencion con Dieta Mediterranea trial. JAMA Intern Med. 2014;174(10):1690–2. https://doi.org/10.1001/jamainternmed.2014.3463.
8. Jannasch F, Kroger J, Schulze MB. Dietary Patterns and Type 2 Diabetes: A Systematic Literature Review and Meta-Analysis of Prospective Studies. J Nutr. 2017;147(6):1174–82. https://doi.org/10.3945/jn.116.242552.
9. Cai J, Nuli R, Zhang Y, Zhang Y, Abudusemaiti M, Kadeer A, et al. Association of Dietary Patterns with Type 2 Diabetes Mellitus among Middle-Aged Adults in Uygur Population of Xinjiang Region. J Nutr Sci Vitaminol (Tokyo). 2019;65(4):362–74. https://doi.org/10.3177/jnsv.65.362.
10. Chiu THT, Pan WH, Lin MN, Lin CL. Vegetarian diet, change in dietary patterns, and diabetes risk: a prospective study. Nutr Diabetes. 2018;8(1):12. https://doi.org/10.1038/s41387-018-0022-4.
11. Zhong X, Dai X, Ma C, Huang Y, Huang S, Zhang Q, et al. Study on the dietary habits and the risk of type 2 diabetes among ZhuangZú residents in Nanning City, Guangxi Zhuang Autonomous Region. China Medical Herald. 2020;17(27):12–5.
12. Society CD. Chinese guideline for the prevention and treatment of type 2 diabetes mellitus(2017 edition). Chinese Journal of Diabetes Mellitus. 2018;10(1):4–67. https://doi.org/10.3760/cma.j.issn.1674-5809.2018.01.003.
13. Gao J, Fei JQ, Jiang LJ, Yao WQ, Lin B, Guo HW. Assessment of the reproducibility and validity of a simple food-frequency questionaire used in dietary patterns studies. Acta Nutrimenta Sinica. 2011;33(5):452–6. https://doi.org/10.13325/j.cnki.acta.nutr.sin.2011.05.012.
14. Jia W, Weng J, Zhu D, Ji L, Lu J, Zhou Z, Zou D, Guo L, Ji Q, Chen L, Chen L, Dou J, Guo X, Kuang H, Li L, Li Q, Li X, Liu J, Ran X, et al. Standards of medical care for type 2 diabetes in China 2019. Diabetes Metab Res Rev. 2019;35(6):e3158. https://doi.org/10.1002/dmrr.3158.
15. Bowen ME, Xuan L, Lingvay I, Halm EA. Random blood glucose: a robust risk factor for type 2 diabetes. J Clin Endocrinol Metab. 2015;100(4):1503–10. https://doi.org/10.1210/jc.2014-4116.
16. Valerino-Perea S, Lara-Castor L, Armstrong MEG, Papadaki A. Definition of the Traditional Mexican Diet and Its Role in Health: A Systematic Review. Nutrients. 2019;11(11). https://doi.org/10.3390/nu11112803.
17. Buisman ME, Jonkman J. Dietary trends from 1950 to 2010: a Dutch cookbook analysis. J Nutr Sci. 2019;8:e5. https://doi.org/10.1017/jns.2019.3.
18. Zhai FY, Du SF, Wang ZH, Zhang JG, Du WW, Popkin BM. Dynamics of the Chinese diet and the role of urbanicity, 1991-2011. Obes Rev. 2014;15(Suppl 1):16–26. https://doi.org/10.1111/obr.12124.
19. Suzuki N, Goto Y, Ota H, Kito K, Mano F, Joo E, et al. Characteristics of the Japanese Diet Described in Epidemiologic Publications: A Qualitative Systematic Review. J Nutr Sci Vitaminol (Tokyo). 2018;64(2):129–37. https://doi.org/10.3177/jnsv.64.129.
20. Li Y, Wang DD, Ley SH, Vasanti M, Howard AG, He Y, Hu FB. Time Trends of Dietary and Lifestyle Factors and Their Potential Impact on Diabetes Burden in China. Diabetes Care. 2017;40(12):1685–94. https://doi.org/10.2337/dc17-0571.
21. American DA. 5. Facilitating Behavior Change and Well-being to Improve Health Outcomes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44(Suppl 1):S53–72. https://doi.org/10.2337/dc21-S005.
22. Caprara G. Mediterranean-Type Dietary Pattern and Physical Activity: The Winning Combination to Counteract the Rising Burden of Non-Communicable Diseases (NCDs). Nutrients. 2021;13(2). https://doi.org/10.3390/nu13020429.
23. Suri S, Kumar V, Kumar S, Goyal A, Tanwar B, Kaur J, Kaur J. DASH Dietary Pattern: A Treatment for Non-communicable Diseases. Curr Hypertens Rev. 2020;16(2):108–14. https://doi.org/10.2174/1573402115666191007144608.
24. Papamichou D, Panagiotakos DB, Itsiopoulos C. Dietary patterns and management of type 2 diabetes: A systematic review of randomised clinical trials. Nutr Metab Cardiovasc Dis. 2019;29(6):531–43. https://doi.org/10.1016/j.numecd.2019.02.004.
25. Burch E, Ball L, Somerville M, Williams LT. Dietary intake by food group of individuals with type 2 diabetes mellitus: A systematic review. Diabetes Res Clin Pract. 2018;137:160–72. https://doi.org/10.1016/j.diabres.2017.12.016.
26. Du H, Li L, Bennett D, Guo Y, Turnbull I, Yang L, et al. Fresh fruit consumption in relation to incident diabetes and diabetic vascular complications: A 7-y prospective study of 0.5 million Chinese adults. PLoS Med. 2017;14(4):e1002279. https://doi.org/10.1371/journal.pmed.1002279.
27. Zheng JS, Sharp SJ, Imamura F, Chowdhury R, Gundersen TE, Steur M, Sluijs I, van der Schouw YT, Agudo A, Aune D, Barricarte A, Boeing H, Chirlaque MD, Dorronsoro M, Freisling H, el-Fatouhi D, Franks PW, Fagherazzi G, Grioni S, et al. Association of plasma biomarkers of fruit and vegetable intake with incident type 2 diabetes: EPIC-InterAct case-cohort study in eight

European countries. BMJ. 2020;370:m2194. https://doi.org/10.1136/bmj.m2194.
28. Castro-Acosta ML, Lenihan-Geels GN, Corpe CP, Hall WL. Berries and anthocyanins: promising functional food ingredients with postprandial glycaemia-lowering effects. Proc Nutr Soc. 2016;75(3):342–55. https://doi.org/10.1017/S0029665116000240.
29. Zhu WL, Guan Y, Xu CZ, Liu ZX, Zhao GM, Jiang YG, Wang WB. Influence of dietary patterns on type 2 diabetes mellitus in local residents aged 40 years and above in Songjiang district, Shanghai. Zhonghua Liu Xing Bing Xue Za Zhi. 2020;41(4):508–13. https://doi.org/10.3760/cma.j.cn112338-20190702-00486.
30. Varsamis P, Formosa MF, Larsen RN, Reddy-Luthmoodoo M, Jennings GL, Cohen ND, Grace M, Hawley JA, Devlin BL, Owen N, Dunstan DW, Dempsey PC, Kingwell BA. Between-meal sucrose-sweetened beverage consumption impairs glycaemia and lipid metabolism during prolonged sitting: A randomized controlled trial. Clin Nutr. 2019;38(4):1536–43. https://doi.org/10.1016/j.clnu.2018.08.021.
31. Vitale M, Masulli M, Rivellese AA, Bonora E, Babini AC, Sartore G, et al. Pasta Consumption and Connected Dietary Habits: Associations with Glucose Control, Adiposity Measures, and Cardiovascular Risk Factors in People with Type 2 Diabetes-TOSCA.IT Study. Nutrients. 2019;12(1). https://doi.org/10.3390/nu12010101.
32. Zavitsanou S, Massa J, Deshpande S, Pinsker JE, Church MM, Andre C, Doyle III FJ, Michelson A, Creason J, Dassau E, Eisenberg DM. The Effect of Two Types of Pasta Versus White Rice on Postprandial Blood Glucose Levels in Adults with Type 1 Diabetes: A Randomized Crossover Trial. Diabetes Technol Ther. 2019;21(9):485–92. https://doi.org/10.1089/dia.2019.0109.
33. Wang SS, Lay S, Yu HN, Shen SR. Dietary Guidelines for Chinese Residents (2016): comments and comparisons. J Zhejiang Univ Sci B. 2016;17(9):649–56. https://doi.org/10.1631/jzus.B1600341.
34. Huang F, Wang H, Wang Z, Zhang J, Su C, Du W, et al. Knowledge, behavior and consumption types of milk and dairy products among the Chinese aged 60 and above in 15 provinces (autonomous regions and municipalities) in 2015. Wei Sheng Yan Jiu. 2019;48(1):9–15.
35. Talaei M, Pan A, Yuan JM, Koh WP. Dairy intake and risk of type 2 diabetes. Clin Nutr. 2018;37(2):712–8. https://doi.org/10.1016/j.clnu.2017.02.022.
36. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. J Natl Cancer Inst. 2011;103(14):1086–92. https://doi.org/10.1093/jnci/djr189.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):267–273
https://doi.org/10.1007/s13410-022-01081-4

ORIGINAL ARTICLE

Type 2 diabetes mellitus increases the risk of penile inflammatory disorders in men aged between 30 and 49: a 5-year follow-up study

Chung-Cheng Wang[1,2] · Ivy Wang[3] · Yi-Horng Lai[4]

Received: 19 March 2022 / Accepted: 6 May 2022 / Published online: 20 May 2022

Abstract

Aims Diabetes can be an important cofactor in the development of penile inflammation. Here, we explored the association between penile inflammatory disorders and diabetes mellitus (DM), hypertension, and hyperlipidemia in the Taiwanese population, using a population-based data set.

Methods The research data in this study were obtained from Taiwan's National Health Insurance Research Database between January 1997 and December 2010. We identified 12,819 patients who had a diagnosis of DM, and randomly matched 38,457 subjects as controls. The data were analyzed with Poisson regression analysis and with Cox regression with a frailty model after propensity score matching.

Results DM (odd ratio (OR) = 1.43, $p < 0.01$), and age (OR = 0.71, $p < 0.01$), but not hyperlipidemia (OR = 0.96, p =0.36), or hypertension (OR = 1.09, $p = 0.10$) had an impact on the development of penile inflammatory disorders. DM was a risk factor associated with penile inflammatory disorder (HR = 1.42, 95% CI = 1.27~1.68, $p < 0.01$), whereas no statistical difference was noted between penile inflammation and hypertension (HR = 0.98, 95% CI = 0.89~1.09) and hyperlipidemia (HR = 1.03, 95% CI = 0.92~1.14).

Conclusions T2DM and younger age, but not hypertension or hyperlipidemia, were associated with an increased risk of penile inflammatory disorders in men between 30 and 49 years of age. Our findings suggest the need for preemptive circumcision for selective men with diabetes may prevent diabetes-associated penile inflammatory disorders.

Keywords Epidemiology · Penile inflammatory disorders · Balanitis · Diabetes mellitus · Cox regression with frailty model

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) and its associated complications have been rapidly rising worldwide over the past three decades, making it one of the most emergent global public health challenges [1, 2]. The age-adjusted prevalence of diagnosed diabetes was about 2.9% in 1990 and increased to 4.5% in 2000 and to 5.3% in 2005 in the USA [3]. Diabetes can affect many different organ systems and lead to many serious complications over time. Diabetic complications can be classified as microvascular (neuropathy, nephropathy, and retinopathy) and macrovascular (cardiovascular diseases, stroke, and peripheral vascular diseases). In addition, diabetes increases many complications of the genitourinary tract system, including urinary tract infection (UTI), genital organ infection, chronic renal disease, diabetic cystopathy, and erectile dysfunction [4, 5]

Penile inflammatory disorders mainly include balanitis, balanoposthitis, and balanitis xerotica obliterans (BXO), a severe manifestation of chronic balanitis. The onset of T2DM is

✉ Yi-Horng Lai
yhlai1108@gmail.com

Chung-Cheng Wang
ericwcc@ms27.hinet.net

Ivy Wang
ivywang0711@gmail.com

[1] Department of Urology, En Chu Kong Hospital, College of Medicine, National Taiwan University, New Taipei City 23702, Taiwan

[2] Department of Biomedical Engineering, Chung Yuan Christian University, Taoyuan 32023, Taiwan

[3] Department of Accountancy, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

[4] Department of Healthcare Administration, Asia Eastern University of Science and Technology, New Taipei City 22061, Taiwan

proposed as being linked to facilitating penile inflammatory disorders. In an outpatient clinical setting, it was estimated that uncircumcised men with diabetes have a high (35%) prevalence of symptomatic balanitis [6]. In a case control study, Huang et al. demonstrated that phimosis with preputial fissures is a specific sign of undiagnosed diabetes mellitus (DM) in young men who should undergo blood testing for diabetes [7]. In 2005, an epidemiological study showed that 12% of 1,173 newly diagnosed T2DM patients presented with acquired phimosis [8]. In 2008, a cross-sectional analysis of data collected by mail from respondents with and without T2DM, was conducted to ascertain prevalence, recurrence, and predisposing factors of UTIs and genital infections [9]. The prevalence rate of balanitis was 41.8 per 1,000 men for those with T2DM, and 19.2 per 1,000 men for those without diabetes ($p < 0.01$). The odds of having a balanitis infection were 2.27 times (1.46–3.53) higher among T2DM than non-DM men. In 2012, Hirji et al. reported the incidence of genital infection among patients with T2DM in the UK General Practice Research Database [10]. The incidence of balanitis among diabetes patients was 8.4/1000 PY (95% CI 7.8–9.1) with a relative risk of 2.85 (2.39–3.39) compared to patients without diabetes. These studies have provided some evidence of the association between diabetes and penile inflammatory disorders.

The triple H (hypertension, hyperlipidemia, and hyperglycemia) often coexists in patients with metabolic syndromes. However, the association between penile inflammatory disorder, hyperlipidemia, and hypertension is poorly understood. Thus, the aim of this study was to use a nationwide population-based dataset to investigate whether diabetes mellitus with/without hyperlipidemia and hypertension, increased the risk for a subsequent penile inflammatory disorder during a 5-year follow-up period.

Materials and methods

Research data

We conducted a nationwide cohort study of 1 million patients from Taiwan's National Health Insurance Research Database (NHIRD) between 1997 and 2010 [11]. To identify patients with newly diagnosed DM (ICD-9-CM code 250) between 1998 and 2005, this study excluded patients who had a DM diagnosis before 1998 or after 2005, and patients who had penile inflammatory disorders before a DM diagnosis. We also observed whether penile inflammatory disorders were diagnosed within five years of a new DM diagnosis. A total of 107,086 out of 1 million patients qualified for this study, and 12,819 were identified with newly diagnosed DM between 1998 and 2005. Given that the NHIRD consisted of de-identified secondary data released to the public for research purposes, this study was waived from full review by the Institutional Review Board of En Chu Kong Hospital (IRB: ECK 110008).

The primary outcome observed in this study was the occurrence of penile inflammatory disorders (ICD-9-CM code 607.1, 607.2, 607.81). The occurrence of these diseases during the follow-up period was defined as having three or more outpatient visits, with a diagnosis of penile inflammatory disorder. Comorbidities with hypertension (ICD-9CM codes 401–405) and hyperlipidemia (ICD-9CM code 272), were defined as having at least three outpatient visits or one inpatient admission. To enhance diagnostic validity, only patients who had at least three claims for penile inflammatory disorders filed by their physicians were qualified for this study. We presumed that it could be clinically significant if patients had at least three outpatient visits for penile inflammatory disorders. Patients with type 1 DM were excluded in this study.

After the above processes, the participants who met the inclusion and exclusion criteria were classified into the DM group. The other patients were classified into the non-DM group. A detailed schematic of the process is depicted in Fig. 1.

Data analysis

The research data were first analyzed with Poisson regression analysis and were then analyzed by Cox regression with a frailty model after propensity score matching. The results of these two different analyses were compared and all data analyses were performed using R 4.1.3 with the survival package [12].

Poisson regression analysis was used to determine the cumulative incidence of penile inflammatory disorder in both cohorts. The DM and non-DM cohorts were compared by using Poisson regression analysis with adjustments for comorbidity with hypertension, comorbidity with hyperlipidemia, and age.

Propensity score matching was used to reduce selection bias due to measurable confounding variables between the exposed and non-exposed groups and to make this study similar to a randomized trial [13]. Conducting a propensity score matching can make the two groups with similar probability to exposure of DM or not. A logistic regression and the previously mentioned covariates were used to calculate a propensity score for each patient. Then, patients in the two groups were matched by the propensity score through a one-to-one greedy matching process [14]. Finally, Cox regression with a frailty model was also used to re-assess the results.

Results

The demographic characteristics of the study participants in each group are presented in Table 1. A total of 107,086 patients were included in this study. Among them (Table 1),

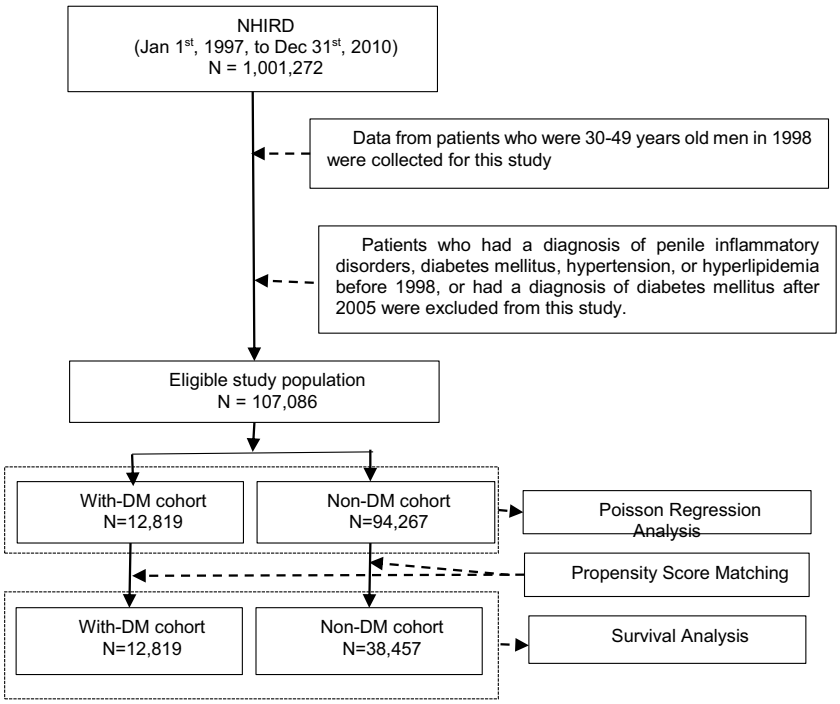

Fig. 1 Flowchart depicting the process of identifying the study participants and their classification into the diabetes mellitus (DM) and non-DM groups

3,071 (2.9%) had penile inflammatory disorders and 104,015 (97.1%) did not, during the follow-up period. A total of 12,819 patients (12.0%) had T2DM, 23,338 patients (21.8%) had comorbid hypertension, and 23,032 patients (21.5%) had comorbid hyperlipidemia.

Table 2 showed that among the 12,819 patients with DM during the follow-up period which ended in December 2010, 481 developed penile inflammatory disorders, 7,501 developed hypertension, and 9,015 developed hyperlipidemia. Compared with the non-DM cohort, the DM cohort exhibited higher prevalence rates of penile inflammatory disorders (3.8% vs. 2.7%, respectively), hypertension (58.5% vs. 16.8%, respectively), and hyperlipidemia (70.3% vs. 14.9%, respectively) (all p-value < 0.001).

Table 1 The demographics of the research data used in this study (N = 107,086)

Characteristics		Case number	%
Penile inflammation	Yes	3,071	2.9
	No	104,015	97.1
Diabetes mellitus	Yes	12,819	12.0
	No	94,267	88.0
Comorbidity-Hypertension	Yes	23,338	21.8
	No	83,748	78.2
Comorbidity-Hyperlipidemia	Yes	23,032	21.5
	No	84,054	78.5
Age in 1998, mean (SD)		38.3 (5.5)	

The Poisson regression analysis results

Table 3 shows the relationship between the development of penile inflammatory disorder and DM, hypertension, hyperlipidemia, and age. DM (Odds Ratio (OR) = 1.43, $p < 0.01$), and age in 1998 (OR = 0.71, $p < 0.01$) but not hyperlipidemia (OR = 0.96, $p = 0.36$) or hypertension (OR = 1.09, $p = 0.10$) had an impact on the development of penile inflammatory disorders.

The results of Cox regression with frailty model

Individual characteristics and the initial health status in the DM and non-DM groups are shown in Table 2. After using propensity score matching for DM and non-DM groups, comorbid hypertension, hyperlipidemia, and age in 1998, with a 1:3 ratio, there were 12,819 participants included in the DM-group and 38,457 participants included in the non-DM group (Table 4).

Table 5 shows the results of the univariable and multivariable Cox regression analysis with a frailty model. Patients with diabetes had a significantly increased risk of developing penile inflammatory disorders compared to non-DM patients (adjusted hazard ratio (HR) = 1.418, 95% CI = 1.266~1.588). The adjusted HR of penile inflammation decreased by 3.7% for every year of increase in age (adjusted HR = 0.963, 95% CI = 0.954~0.972). However, hypertension or hyperlipidemia were not correlated with the development of penile inflammatory disorders. These results were consistent with the results of the Poisson regression analysis.

Table 2 Comparing the development of penile inflammation disorders, hypertension, and hyperlipidemia in the diabetes mellitus (DM) and non-DM cohorts ($N = 107,086$)

		DM $N = 12,819$ n (%)	Non-DM $N = 94,267$ n (%)	p-value
Inflammatory disorders of penis	Yes	481 (3.8)	2,590 (2.7)	<0.001a
	No	12,338 (96.2)	91,677 (97.3)	
Comorbid with Hypertension	Yes	7,501 (58.5)	15,837 (16.8)	<0.001[a]
	No	5,318 (41.5)	78,430 (83.2)	
Comorbid with Hyperlipidemia	Yes	9,015 (70.3)	14,017 (14.9)	<0.001[a]
	No	3,804 (29.7)	80,250 (85.1)	
Age in 1998, mean (SD)		40.9 (5.4)	38.0 (5.4)	<0.001[b]

[a] Chi-Square Test

[b] Independent two sample t-test

The mean length of time for follow-ups were 165.5 ± 14.4 months for the DM cohort and 165.9 ± 14.4 months for the non-DM cohort. Figure 2 outlined the results of the Kaplan-Meier and log-rank test. The log-rank test showed that patients with DM had a significantly higher incidence of penile inflammatory disorders than those without DM ($p < 0.001$). The cumulative incidence of penile inflammatory disorders was 1.0% higher in the DM cohort (2.6%; 1–97.4%) than in the non-DM cohort (1.6%; 1–98.4%) at the 10-year of follow-up, and 1.20% higher in the DM cohort (3.8%; 1–96.2%) than in the non-DM cohort (2.6%; 1–97.4%) at the end of follow-up period.

Discussion

To our knowledge, this study is the first to investigate the risk of developing penile inflammatory disorders in a large cohort of Taiwanese men aged 30 to 49, with or without DM, hypertension, and hyperlipidemia during a 5-year follow-up period. Our study showed that T2DM and younger age were associated with penile inflammatory disorders. The findings from the study confirm those from a previous SHIELD study which revealed that genital infection is more common among individuals with T2DM [9]. However, hypertension or hyperlipidemia were not associated with the occurrence of penile inflammatory disorders.

Table 3 Poisson regression model for development of penile inflammatory disorders

Variables	Estimate	S. E.	z value	Pr (>|z|)	OR[a]
(Intercept)	-2.455	0.130	-18.851	<0.001	0.086
Diabetes Mellitus	0.360	0.058	6.240	<0.001	1.434
Hypertension	-0.046	0.050	-0.919	0.358	0.955
Hyperlipidemia	0.084	0.051	1.653	0.098	1.087
Age in 1998	-0.342	0.039	-8.713	<0.001	0.710

[a] Odds ratio

Diabetes-associated penile inflammatory disorders have a wide spectrum of clinical presentation from mild balanitis to severe BXO [15]. Patients may present with asymptomatic, pruritus, pain, discharge from the prepuce and glans, voiding difficulty, or being unable to retract the prepuce during intercourse. The genital discomfort or irritation usually prompts patients to seek medical evaluation and at that point, newly onset diabetes is diagnosed or poorly control diabetes is identified. Though definitive diagnosis is made by a histological exam, some experts have proposed specific macroscopic features of diabetes-associated penile inflammatory disorders. Ke et al. reported that a volcano-like appearance of prepuce might be a typical finding in diabetic balanitis [16]. Huang et al. showed that acquired phimosis and preputial fissures was a specific sign of undiagnosed diabetes in young men [7]. Thus, due to its insidious development and inconspicuous symptoms, physicians should attempt early detection or suspect undiagnosed T2DM in patients with these specific presentations of balanitis.

Multifactorial mechanisms are involved with the pathophysiology of diabetes-associated penile inflammatory disorders. First, patients with diabetes are immune compromised and vulnerable to pathogens [17]; Glycosuria promotes the growth of pathogenic bacteria. In addition, patients with diabetes suffer from more infections in the other genitourinary organs, kidney, urinary bladder, urethra, and prostate, which may influence local inflammation in the prepuce. Second, prolonged or uncircumcised prepuce provides an adequate growth environment for pathogens. The outer surface of the foreskin and penile shaft are covered by a keratinized stratified squamous epithelium that provides a protective barrier against pathogen invasion. In contrast, the mucosal lining of the prepuce is not keratinized and may be more vulnerable to pathogens [18]. Third, Candida albicans, Staphylococcus, and

Table 4 Demographic characteristics of patients after stratification by propensity score matching

		DM (n = 12,819)	Non-DM (n =38,457)	p-value
Penile Inflammatory disorders	Yes	481	1,005	<0.001[a]
	No	12,338	37,452	
Comorbidity with Hypertension	Yes	7,501	15,837	<0.001[a]
	No	5,318	22,620	
Comorbidity with Hyperlipidemia	Yes	9,015	14,017	<0.001[a]
	No	3,804	24,440	
Age in 1998, mean (SD)		40.88 (5.415)	41.18 (5.608)	<0.001[b]

DM, diabetes mellitus

[a] Chi-Square Test

[b] Independent two sample *t*-test

groups B and D Streptococci, were the most frequently isolated pathogens in diabetic balanitis [19]. Repeated infection, poor hygiene, and buildup of smegma result in different severities of diabetes-associated penile inflammatory disorders.

T2DM is frequently associated with hypertension and hyperlipidemia. Evidence suggests that the triple H or metabolic syndrome, are associated with the mortality and morbidity of cardiovascular diseases, malignant diseases, infectious diseases, and surgical complications [20–22]. However, little is known about the relationship between balanitis and hypertension/hyperlipidemia. The study demonstrated that only diabetes, but not hypertension or hyperlipidemia, was a risk factor for balanitis. The findings were similar to a previous study that revealed that men with balanoposthitis may have a higher future risk of T2DM, but balanoposthitis and hypertension, or balanoposthitis and hyperlipidemia, did not confer any significant additive interaction on T2DM risk [23]. In addition, men with T2DM have a higher risk for bladder dysfunction and erectile dysfunction (ED). The results of the study also confirm our previous findings that men with T2DM who were <45 years old, had fewer urinary tract symptoms (LUTS) and less ED [24]. However, metabolic syndrome did not aggravate the severity of LUTS, or ED in the early stages of DM. Taken together, we suggest that diabetes itself is the predominant contributor to the development of chronic penile inflammatory disorders.

One interesting finding was that age was inversely associated with penile inflammatory disorders in men aged between 30 and 49. We excluded men over 50 years old because confounding factors may increase with age. Previous studies

Table 5 The results of extended Cox regression with frailty model of inflammatory disorders of penis with type 2 diabetes mellitus associated covariates

Variables	Crude[b] HR[a]	(95% CI[d])	Adjusted[c] HR[a]	(95% CI[d])
DM	1.443	(1.294, 1.608)	1.418	(1.266, 1.588)
Comorbid Hypertension	0.983	(0.888, 1.089)	-	-
Comorbid Hyperlipidemia	1.202	(1.086, 1.331)	1.028	(0.923, 1.144)
Age in 1998	0.962	(0.954, 0.971)	0.963	(0.954, 0.972)

DM, diabetes mellitus

*p<0.05

[a] Hazard Ratio

[b] Crude HR, relative hazard ratio

[c] Adjusted HR: multivariable analysis including with/without DM, comorbidities of hypertension, hyperlipidemia, and age in 1998

[d] 95% confidence interval

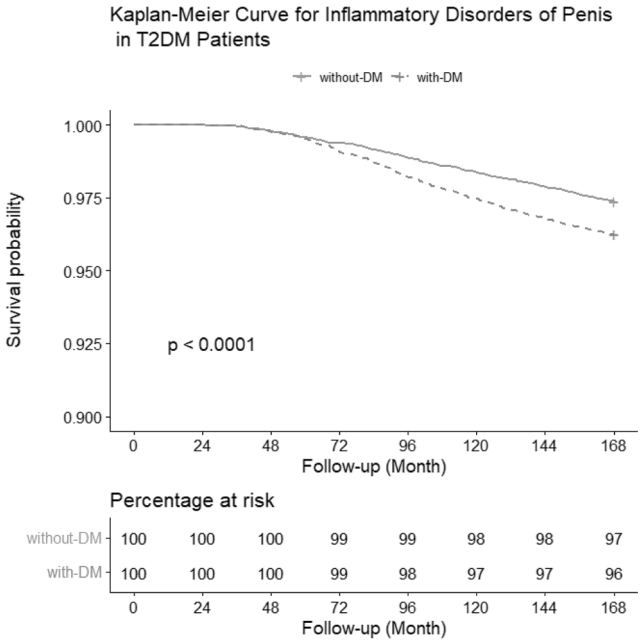

Fig. 2 Kaplan-Meier Curve for penile inflammatory disorders in men with and without diabetes, aged between 30 to 49 years. (log-rank test, $p < 0.0001$)

reported controversial results about the impact of age on penile inflammation. Vignera et al. showed that the risk of genital infections was significantly increased in men with diabetes versus those without diabetes, with a higher incidence in younger individuals (18–39 years old) and in uncircumcised patients [25]. However, Lisboa et al. found that being above 40 years old (OR: 2.27; 95% confidence interval [CI]: 1.01–4.50), having DM (OR: 19.39; 95% CI: 7.79–48.27), and more than ten candida colonies recovered by culture (OR: 9.586; 95% CI: 2.682–34.263), were risk factors for candida balanitis [26]. The discrepancy might be explained by the different age groups. The prevalence of female UTI increases with age, apart from a spike in young women due to sexual activity [26]. Thus, we assumed a similar condition might happen in young men with penile inflammatory disorders.

The strength of this study was the use of a nationwide population-based data set that provided a sufficient sample size and statistical power to explore the association between diabetes and penile inflammatory disorders. However, the findings of this study need to be interpreted in the context of the following limitations. First, the diagnosis of penile inflammatory disorders relies on administrative claims data and ICD codes, but not on pathological reports. In addition, the severity of penile inflammation did not correlate with the quality of diabetic control, which is a factor. Second, the influence of lifestyle risk factors, including diet, exercise, personal hygiene, body mass index, diabetic medication, sexual activity, and other clinical characteristics, was not evaluated. These variables might be important confounding factors for developing balanitis. Third, this database might not entirely represent patients with penile inflammatory disorders. Some patients with mild balanitis might not have been seeking healthcare services, because they thought the disease was embarrassing and might be improved by self-care. Finally, Taiwan ranks #1 in the health care index of Numbeo (https://www.numbeo.com/health-care/rankings_by_country.jsp). The study population mainly consisted of people of Taiwanese ethnicity, and it is unclear whether the results can be generalized to other ethnic populations or countries.

Conclusion

In conclusion, our study provides epidemiological evidence that T2DM, and younger age, but not hypertension or hyperlipidemia, are associated with an increased risk of developing penile inflammatory disorders in men between 30 and 49 years old. Diabetes can be considered an important cofactor in the development of penile inflammation, which may influence the risks of penile cancer as well as female cervical cancer. Thus, given the worldwide effect of these diseases on public health, preemptive circumcision for selective men with diabetes may be needed to prevent diabetes-associated penile inflammatory disorders.

Abbreviations BXO, balanitis xerotica obliterans; CI, confidence interval; DM, diabetes mellitus; ED, erectile dysfunction; HR, Hazard Ratio; NHIRD, National Health Insurance Research Database; OR, Odds RatioT2DM: type 2 diabetes mellitus.

Acknowledgments The authors thank En Chu Kong Hospital and Asia Eastern University of Science and Technology, for their assistance with this research project.

Author contribution CCW designed the study and wrote the study protocol. CCW, IW, and YHL were involved in the conception, design, analysis, and interpretation of the data, as well as the drafting and revision of the paper. All authors have approved the final version for publication and accept responsibility for all aspects of the work. CCW is the guarantor of this work.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to the license with Bureau of National Health Insurance, Taiwan (BNHI) and National Health Research Institutes, Taiwan, the administrators of National Health Insurance Research Database (NHIRD).

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol. 2012;8:228–36. https://doi.org/10.1038/nrendo.2011.183.

2. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocr Rev. 2016;37(3):278–316. https://doi.org/10.1210/er.2015-1137.
3. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther. 2008;88:1254–64. https://doi.org/10.2522/ptj.20080020.
4. Yoshimura N, Chancellor MB, Andersson KE, Christ GJ. Recent advances in understanding the biology of diabetes-associated bladder complications and novel therapy. BJU Int. 2005;95(6):733–8. https://doi.org/10.1111/j.1464-410X.2005.05392.x.
5. Gandhi J, Dagur G, Warren K, Smith NL, Khan SA. Genitourinary complications of diabetes mellitus: an overview of pathogenesis, evaluation, and management. Curr Diabetes Rev. 2017;13(5):498–518. https://doi.org/10.2174/1573399812666161019162747.
6. Fakjian N, Hunter S, Cole GW, et al. An argument for circumcision: prevention of Balanitis in the adult. Arch Dermatol. 1990;126:1046–7. https://doi.org/10.1001/archderm.1990.01670320070011.
7. Huang YC, Huang YK, Chen CS, Shindel AW, Wu CF, Lin JH, Chiu KH, Yang TH, Shi CS. Phimosis with preputial fissures as a predictor of undiagnosed type 2 diabetes in adults. Acta Derm Venereol. 2016;96:377–80. https://doi.org/10.2340/00015555-2244.
8. Drivsholm T, Olivarius NDF, Nielsen ABS, et al. Symptoms, signs and complications in newly diagnosed type 2 diabetic patients, and their relationship to glycaemia, blood pressure and weight. Diabetologia. 2005;48:210–4. https://doi.org/10.1007/s00125-004-1625-y.
9. Grandy S, Fox KM, Hardy E. Prevalence and recurrence of urinary tract and genital infections among adults with and without type 2 diabetes mellitus in the general population: a longitudinal cohort study. J Diabetes Res Clin Metab. 2013;2:8. https://doi.org/10.7243/2050-0866-2-5.
10. Hirji I, Andersson SW, Guo Z, Hammar N, Gomez-Caminero A. Incidence of genital infection among patients with type 2 diabetes in the UK General Practice Research Database. J Diabetes Complications. 2012;26:501–5. https://doi.org/10.1016/j.jdiacomp.2012.06.012.
11. National Health Insurance Research Database, Taiwan. http://nhird.nhri.org.tw/en/index.htm. Accessed 30 Nov 2020.
12. Therneau TM, Lumley T, Elizabeth A. The survival package. (2021). http://brieger.esalq.usp.br/CRAN/web/packages/survival/survival.pdf.
13. Ho DE, Imai K, King G, Stuart EA. Matching as nonparametric preprocessing for reducing model dependence in parametric causal Inference. Polit Anal. 2007;15:199–236. https://doi.org/10.1093/pan/mpl013.
14. Ho DE, Imai K, King G, et al. MatchIt: Nonparametric preprocessing for parametric causal inference. J Stat Softw. 2011;42(28). https://doi.org/10.18637/jss.v042.i08.
15. Depasquale I, Park AJ, Bracka A. The treatment of balanitis xerotica obliterans. BJU Int. 2000;86:459–65. https://doi.org/10.1046/j.1464-410x.2000.00772.x.
16. Ke CC, Chen CH, Chen JJ, et al. Balanoposthitis with a volcano-like appearance may be the first clinical presentation of undiagnosed diabetes mellitus. Incont Pelvic Floor Dysfunct. 2011;5:120-121. 10.6259%2fIPFD.2011.5.4.120.
17. Tandogdu Z, Cai T, Koves B, Wagenlehner F, Bjerklund-Johansen TE. Urinary tract infections in immunocompromised patients with diabetes, chronic kidney disease, and kidney transplant. Eur Urol Focus. 2016;2:394–9. https://doi.org/10.1016/j.euf.2016.08.006.
18. Hussain LA, Lehner T. Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia. Immunology. 1995;85:475–84 https://www.ncbi.nlm.nih.gov/pubmed/7558138.
19. Lisboa C, Ferreira A, Resende C, Rodrigues AG. Infectious balanoposthitis: management, clinical and laboratory features. Int J Dermatol. 2009;48:121–4. https://doi.org/10.1111/j.1365-4632.2009.03966.x.
20. Isomaa BO, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care. 2001;24:683–9. https://doi.org/10.2337/diacare.24.4.683.
21. Shariq OA., Hanson KT, McKenna, et al. Does metabolic syndrome increase the risk of postoperative complications in patients undergoing colorectal cancer surgery?. Dis Colon Rectum. 2019;62:849-858. https://journals.lww.com/dcrjournal/toc/2019/07000.
22. De Haas EC., Oosting SF, Lefrandt JD, et al The metabolic syndrome in cancer survivors. Lancet Oncol. 2010;11:193-203. 10.1016/S1470-2045(09)70287-6.
23. Lien CS, Lee SW, Chang CH, Chung CJ, Lin CL, Huang CP. Adult balanoposthitis patients have a higher risk of Type 2 diabetes mellitus: A nationwide population-based cohort study. Urol Sci. 2018;29:55–60. https://doi.org/10.1016/j.urols.2017.07.002.
24. Wang CC, Chancellor MB, Lin JM, Hsieh JH, Yu HJ. Type 2 diabetes but not metabolic syndrome is associated with an increased risk of lower urinary tract symptoms and erectile dysfunction in men aged< 45 years. BJU Int. 2010;105:1136–40. https://doi.org/10.1111/j.1464-410X.2009.08913.x.
25. La Vignera S, Condorelli RA, Cannarella R, et al. Urogenital infections in patients with diabetes mellitus: Beyond the conventional aspects. Int J Immunopathol Pharmacol. 2019;33:2058738419866582. https://doi.org/10.1177/2058738419866582.
26. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol. 2019;11:1756287219832172. https://doi.org/10.1177/1756287219832172.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):274–280
https://doi.org/10.1007/s13410-022-01055-6

ORIGINAL ARTICLE

Evaluation of parotid gland function in type 2 diabetes patients using diffusion-weighted imaging before and after acid stimulation

Xuemei Wang[1] · Mingjia Niu[2] · Yuan Fang[3] · Shuang Wu[4]

Received: 13 July 2021 / Accepted: 10 February 2022 / Published online: 20 May 2022

Abstract

Purpose To investigate the performance of diffusion-weighted magnetic resonance imaging (DW-MRI) as a noninvasive tool for assessing parotid gland function in type 2 diabetes mellitus (T2DM) patients.

Methods T2DM patients (n = 40, male, age 40–60 years) and healthy controls (n = 40) were examined. Salivary function was assessed using 1.5D echo-planar diffusion-weighted imaging. The examinations were performed before and every 3 min until 10 times after acid stimulation with a 500-mg vitamin C pill. The maximum apparent diffusion coefficient (ADC) in the parotid glands (pADCmax) and time to peak ADC in the parotid glands (pTmax) during stimulation were obtained. ADC values at every time point and the peak value of ADC were compared between the two groups and statistically analyzed.

Results The ADC values in the parotid gland in the resting state in the patient group were slightly lower than those in the healthy controls ($(1.02 \pm 0.08) \times 10^{-}3$ mm^2/s vs. $(1.11 \pm 0.09) \times 10^{-3}$ mm^2/s, $p > 0.05$). The ADCs in the healthy controls increased after stimulation until they gradually reached their peak. The ADCs in the patient group first decreased after stimulation and then gradually increased until they peaked. The pADCmax in the patient group was significantly lower than that in the healthy controls ($(1.45 \pm 0.08) \times 10^{-3}$ mm^2/s vs. $(1.7 \pm 0.06) \times 10^{-3}$ mm^2/s, $p < 0.05$). The peak ADCs in the parotid gland and relative signal intensity after acid stimulation were significantly correlated ($r = 0.666$, $p < 0.05$).

Conclusion DW-MRI before and after acid stimulation is potentially useful for noninvasive prediction of diabetes-induced xerostomia severity. ADC value is a sensitive indicator of parotid gland dysfunction and may indirectly reflect the degree of this dysfunction caused by fat deposition.

Keywords Acid stimulation · Diabetes · Diffusion-weighted imaging · Parotid gland · Xerostomia

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome characterized by hyperglycemia and carbohydrate and lipid metabolism [1]. In 2010, China had 90 million people with diabetes, making it the country with the largest population with diabetes in the world [2]. Dry mouth is one of the most common complications of T2DM, and clinical investigations have found that oral lesions in diabetes patients reach 100%, mainly manifesting as gingivitis, periodontitis, and dental caries, which causes difficulties, even in joint activities such as eating and speaking, and lowers the quality of life [3–5]. These are usually associated with non-inflammatory and non-neoplastic diseases of the salivary glands, known as sialopathy, which refers to insufficient salivary secretion, reduced oral lubrication, and increased susceptibility to infection [4]. Some studies have focused on the structure and function of salivary glands in diabetes patients to understand the oral complications in diabetes [6, 7].

Numerous methods are available to evaluate dry mouth. The various scoring systems used to judge functional screening are subjective [8]. Objective methods, such as saliva flow measurement, are most commonly used; however, their low repeatability may lead to inconsistent results and limit their

✉ Mingjia Niu
fyynmj@sohu.com

✉ Yuan Fang
nmjyfy@sohu.com

[1] Physical Examination Center, The First Hospital of Qinhuangdao, Qinhuangdao, Hebei Province, People's Republic of China

[2] Department of Endocrinology, The First Hospital of Qinhuangdao, Qinhuangdao, Hebei Province, People's Republic of China

[3] Medical Imaging Center, Chongqing Yubei District People's Hospital, Chongqing, People's Republic of China

[4] Medical Imaging Center, The First Hospital of Qinhuangdao, Qinhuangdao, Hebei Province, People's Republic of China

usefulness [9]. Biopsy is another method to measure dry mouth, but it is invasive [10]. As a clinically important detection method, diagnostic imaging of salivary gland disease may be useful for evaluating the structure and function of the salivary gland [11]. For instance, salivary gland scintigraphy has been extensively used to evaluate salivary gland function. Nevertheless, it relies on the use of ionizing radiation and its spatial resolution is not satisfactory [12].

Magnetic resonance imaging (MRI) has become the favorite technology used for noninvasive diagnosis of soft tissue diseases since it not only provides high soft tissue contrast but also does not use ionizing radiation. Reda et al. have clearly demonstrated the potential and advantages of MRI for diagnosis in dental clinical practice [13]. Diffusion-weighted MRI (DW-MRI) is an imaging technique that measures the diffusion of water molecules in tissues. This diffusion can be quantified by the apparent diffusion coefficient (ADC) to exclude the influence of blood flow. This technique plays an important role in the evaluation of normal salivary glands, salivary tumors, and non-neoplastic diseases affecting salivary glands [14–17]. In particular, DW-MRI has been used in many studies to irradiate salivary glands [15–17]. However, the application of DW-MRI in diabetes-induced parotid glands dysfunction has rarely been reported. In addition, the correlation between ADC values and salivary dysfunction remains unclear.

In this study, we aimed to explore the feasibility of quantitative evaluation of parotid function in diabetes patients using DW-MRI before and after acid stimulation, and analyze the relationship between ADC and parotid dysfunction caused by fat deposition.

Methods

Study design and participants

Between November 2017 and August 2018, 45 male patients with T2DM, inpatients and outpatients, participated in this prospective study. Five patients were excluded from the study, and 40 were included in the analysis. The inclusion criteria were as follows: (1) male patients aged 40–60 years; (2) the clinical diagnosis was in accordance with the diagnostic criteria for T2DM and without complications such as diabetic foot, diabetic retinopathy, and autonomic neuropathy. We excluded patients with solid lesions of the oral mucosa; parotid diseases, such as tumors and inflammation; history of smoking and alcoholism; recent administration of drugs that affect parotid secretion function; received head and neck radiotherapy; endocrine and other disorders related to nutrition and hormones that cause parotid enlargement; anemia; and large fixed metal dental implants that affect image quality. Forty healthy individuals of the same age as diabetes patients were enrolled in the control group, including routine medical examiners or medical family members.

MRI examinations

Image acquisition was performed using a 1.5-T MRI scanner (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) with a 12-channel head coil. All participants were required to fast for at least 1 h before the experiment and avoid swallowing during examination. Initially, a T1-weighted TSE sequence (repetition time [TR]: 2000 ms; echo time [TE]: 7.8 ms; slice thickness: 5 mm; slice gap: 1 mm; field of view [FOV]: 230 mm × 230 mm; matrix: 256 × 256; number of signal averaged: 2), T2-weighted TSE sequence with fat saturation (TR: 4780 ms; TE: 107 ms; slice thickness: 5 mm; slice gap: 1 mm; FOV: 230 mm × 230 mm; matrix: 256 × 256), and 3D T1-weighted volumetric interpolated breath-hold examination (VIBE) sequence (TR: 8.74 ms; TE: 2.38 ms; slice thickness: 1 mm; FOV: 200 mm × 200 mm; matrix: 287 × 287; voxel size: 0.70 mm × 0.70 mm × 1.00 mm; no interval) were performed for anatomical localization and morphological evaluation of the parotid gland on the transverse axis. The range of images was from the skull base to the lower boundary of the parotid gland, including the whole parotid gland.

Subsequently, a transverse EPI-DWI sequence (*b* values: 0 s/mm^2; 400 s/mm^2; 800 s/mm^2; TR: 4000 ms; TE: 70 ms; slice thickness: 5 mm; slice gap: 1 mm; FOV: 230 mm × 230 mm; matrix: 256 × 256; number of signals averaged: 4) was used. The DWI sequence was performed in the resting and stimulated states. After scanning for the rest period, the patient was instructed to take five tablets of vitamin C orally and let the tablets melt in the mouth without chewing. Each imaging sequence consisted of 20 layers covering the entire parotid gland with a total scanning time of 2 min and 8 s. The DWI order was repeated 11 times with a continuous interval of 52 s and a total scanning time of 33 min.

Image post-processing and quantitative analysis

The measurements were conducted using ITK-SNAP software (https://itk.org/). All images were independently measured by two experienced and well-trained radiologists, who were blinded to the participants' clinical information. To evaluate reproducibility within the observer, one of them measured and recorded the ADC values again within 2 weeks. In the amplification mode, the parotid gland volume was delineated layer-by-layer on the ADC map and T1-weighted VIBE image. The standard for delineating the parotid gland was to include all substances in the gland, except for the external carotid artery and the posterior maxillofacial vein. To improve delineation

precision, T1-weighted image sequences were used to identify anatomical structures and guide the delineation of the glands (Fig. 1). After the entire parotid gland volume was demarcated, the delineated volume was saved as a template for the measurement of other stimulated states.

The average value for each gland was calculated for subsequent data analysis and statistics. For ADC analysis, the change trend, peak value, peak time (Tmax), and rate of ADC change (an absolute value of the difference between peak ADCs after acid stimulation and resting ADCs) divided by the resting ADC value before and after acid stimulation between the two groups were calculated. Furthermore, a circular region was selected as the region of interest (ROI) at the same level as the brainstem center to measure its ADC value [18]. For quantification of fat content, a T1-weighted image was used, and the signal intensity of the parotid and the pons was measured. Fat content was evaluated according to the following formula:

$$\mathrm{RSI} = \mathrm{SI}_{\mathrm{parotid}}/\mathrm{SI}_{\mathrm{pons}}$$

where RSI is the relative signal intensity and SI is the signal intensity.

Moreover, local fat deposition was identified as having higher signal intensity on T1-weighted images of the parotid gland and decreased signal intensity on T2-weighted images of fat suppression, while both were shown as high signal $T1_{\text{in-phase}}$ and $T1_{\text{opposed-phase}}$ MRI images.

Statistical analysis

All statistical analyses were conducted using SPSS 17 (SPSS 17.0; SPSS, Chicago, IL, USA). Numerical data are expressed as absolute numbers and mean ± standard deviation. The differences between the two groups regarding the resting ADCs of the parotid gland, the peak ADC values after acid stimulation, and the rate of change and peak time (Tmax) were compared using an independent samples *t*-test. The correlation between the RSI in the parotid gland and the resting ADC values in the parotid gland and the peak ADCs after acid stimulation was analyzed. All data confidence intervals were 95%. Associations between the dependent and independent variables were analyzed using linear regression analysis. Statistical significance was set at $p < 0.05$.

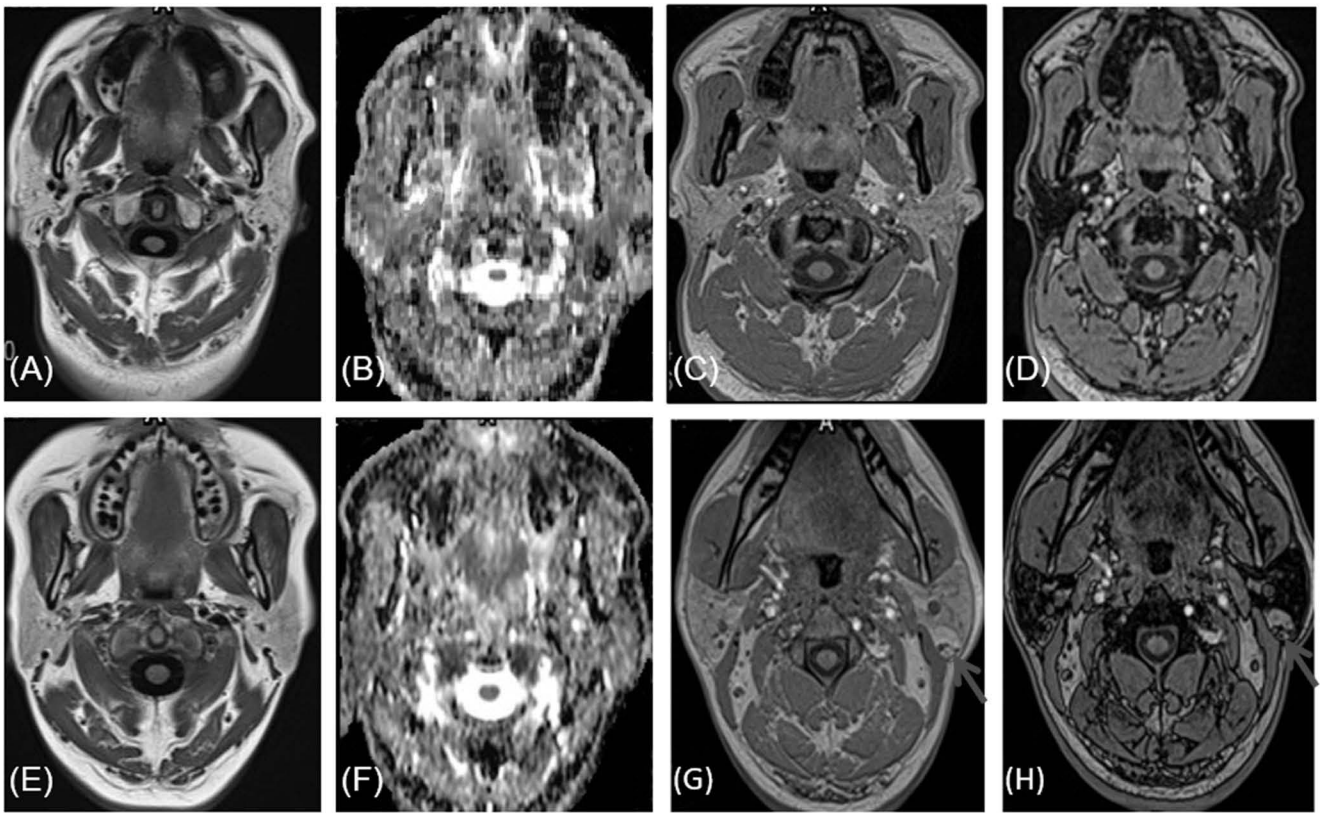

Fig. 1 (**A**) T1WI image of the parotid gland in the control group. (**B**) ADC image of the parotid gland in the control group. (**C**, **D**) $T1_{\text{in-phase}}$ and $T1_{\text{opposed-phase}}$ MRI images of the parotid gland in the control group. (**E**) T1WI image of the parotid gland in the case group. (**F**) ADC images of the parotid gland in the case group and (**G**, **H**) $T1_{\text{in-phase}}$ and $T1_{\text{opposed-phase}}$ MRI images of the parotid gland in the case group, in which the red arrows refer to the local fat deposition. ADC, apparent diffusion coefficient

Results

Image morphological evaluation and the parotid gland fat content

There was no image distortion even at high *b* values, and no obvious artifacts interfered with the measurement of ADC values in the parotid region. High-quality images were obtained in all patients before and after acid stimulation (Fig. 1).

The parotid T1WI-RSI (28.65 ± 9.72) was higher in the patients than in the control group (22.54 ± 7.38), and the difference was statistically significant ($t = -3.501$, $p = 0.001$).

In addition, the parotid glands in 40 T2DM patients showed diffuse lipid deposition (hyperintensity on T1WI and hypointensity on FS-T2WI) on conventional MRI and local mature fat deposition in the parotid gland in 22 cases (Fig. 1).

Comparison of parotid gland ADC value between patients and the control group before and after acid stimulation

Reproducibility of the measured ADC values was satisfactory. The inter-observer intraclass correlation coefficient (ICC) calculated based on the measurement results of the two radiologists was 0.93 ($p < 0.001$), and according to the two measurements of observer 1, the intra-observer ICC was 0.96 ($p < 0.001$). Hence, the first measurement data of measurer 1 were used as the final result.

The resting ADCs in the parotid gland in the patient group were slightly lower than those in the control group, but the difference was not statistically significant ($p > 0.05$) (Table 1).

After the participants took vitamin C, the ADC values in the control group increased significantly in the first phase (after 3 min), then increased gradually to the peak value (at 19.65 ± 1.71 min), and then gradually decreased. In contrast, the ADC values in the patient group decreased significantly in the first stage, and then increased gradually to a peak (at 25.83 ± 2.00 min) on average, and then gradually declined. The peak ADC value ($(1.45 \pm 0.08) \times 10^{-3}$ mm^2/s) was significantly lower in the patients than in the control group ($(1.7 \pm 0.06) \times 10^{-3}$ mm^2/s) ($p < 0.05$), and the change rate (0.42 ± 0.017) was lower than that in controls (0.53 ± 0.017) ($p < 0.05$) (Fig. 2).

During the entire study period, no significant changes were found in the ADC values in the pons as a reference tissue. Furthermore, there was no significant correlation between the RSI in the parotid fat area and resting ADC values, but a high correlation with the peak ADC values after acid stimulation ($r = 0.666$, $p < 0.05$) (Fig. 3).

Discussion

Changes in parotid gland function in diabetes patients were evaluated using DWI. To our knowledge, this is the first study to use functional parameters defined by DWI to estimate the degree of diabetes-induced dry mouth. The results showed that the ADC values in the parotid gland in diabetes patients were decreased, while ADC values after acid stimulation were different from those in normal individuals, showing a low rate of change. Therefore, the prediction of xerostomia based on DWI could be used to identify patients who need more active investigation of salivary function to enable early prevention or treatment intervention.

Recent studies have shown that, in addition to the disturbance of body fluids and electrolytes caused by the disturbance of the antidiuretic hormone regulation center [19], diabetes xerostomia is related to the decrease in salivary gland secretory function [6, 20]. DWI-MRI was widely used in head and neck diseases. Particularly, the ADC could reflect the microstructure and pathophysiology of tissues to a certain extent, even in the early stage of the disease [21]. Previous histopathological studies on the parotid gland in diabetes patients have confirmed that there is a large amount of fat infiltration in the stroma of the parotid gland, more lipid vacuoles in the cytoplasm, and a relatively reduced structural volume of the acini [22–24]. Gupta et al. demonstrated a positive relationship between parotid gland enlargement in T2DM patients using ultrasound [25] and found that the ultrasonic features of parotid glands depended on dominant histological changes (acinar atrophy and fatty infiltration) [26]. We speculate that the increase in

Table 1 ADC values in the parotid gland between patients and healthy controls before and after acid stimulation

	Patient group $N = 40$	Control group $N = 40$	T	p
Rest ADC (mm^2/s)	$(1.02 \pm 0.08) \times 10^{-3}$	$(1.11 \pm 0.09) \times 10^{-3}$	0.742	0.460
Peak ADC (mm^2/s)	$(1.45 \pm 0.08) \times 10^{-3}$	$(1.7 \pm 0.06) \times 10^{-3}$	2.469	0.015
ADC change rate	0.42 ± 0.017	0.53 ± 0.017	4.472	0.001
Time to peak (min)	25.83 ± 2.00	19.65 ± 1.71	2.439	0.021

ADC, apparent diffusion coefficient

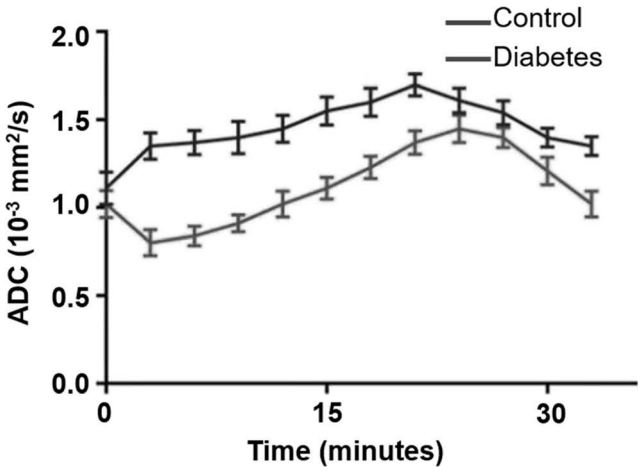

Fig. 2 ADC changes in the parotid gland before and after acid stimulation. ADC, apparent diffusion coefficient

fat and interstitial fat in the parotid cells of diabetes patients limits the diffusion of water molecules, which may affect ADC values. Previous studies found that the ADCs in the parotid gland were changed (increased or decreased in different studies) after taste stimulation [27, 28]. To confirm whether the changes in ADC in the parotid gland of diabetes patients were related to xerostomia, a DWI sequence was used to evaluate changes in parotid gland function in diabetes patients.

On comparison of the resting ADCs between the two groups, the resting ADCs in the parotid gland in patients were slightly lower than those in the controls, but the difference was not statistically significant. The decrease in extracellular space limits the diffusion of water molecules, which may explain the decrease in the ADC values. However, saliva secreted by the parotid gland in the resting state accounted for only 10%; therefore, there was no significant difference in parotid gland function between the healthy controls and patients in the resting state.

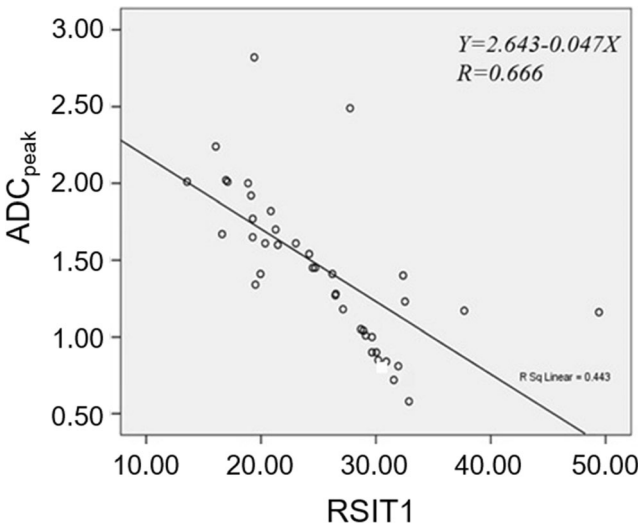

Fig. 3 Correlation analysis between peak ADC and the ratio of signal intensity in the parotid gland ($SI_{parotid}/SI_{pons}$) using T1-weighted sequence after acid stimulation. ADC, apparent diffusion coefficient

Unlike in resting states, the parotid glands secrete approximately 60% of saliva in response to acid or food stimulation. The resting ADC value reflects basic parotid gland function, whereas the dynamic ADC value after stimulation measurement shows its reserve function. Hence, DWI after taste stimulation can better reflect the secretory activity of the parotid gland. Indeed, previous studies have used acid-stimulated DWI to evaluate parotid gland function [12, 14, 29].

Our results showed that the ADC value in the parotid gland in healthy controls increased significantly in the short term (the first phase, 2.1 min) after acid stimulation and then it gradually increased. Previous studies have arrived at the same conclusion [14, 30]. The ADC values in the normal parotid gland were increased significantly after stimulation, which may be the result of the parotid gland activity caused by acid stimulation and the rapid synthesis of large amounts of saliva. However, the salivary component of the parotid gland is mainly serous, with low protein content; therefore, the free water in the extracellular space increases. After reaching its peak, it gradually decreases. A possible explanation could be that the extracellular free water was decreased, and the ADC value was decreased as the saliva was released from the gland. Habermann et al. found significantly increased ADC values in the parotid gland, which was the result of the instantaneous increase in extracellular free water (saliva secretion) after acid stimulation [12]. This finding is consistent with the conclusions of the present study.

However, Thoeny et al. found that the ADC value in the parotid gland showed a two-phase response during acid stimulation, and that in the parotid gland in pre-5 min after acid stimulation was decreased, then slowly increased to a high peak, and the median time of high peak value was 17 min after acid stimulation [31]. We believe that the different types, doses, and dosage forms of acid stimulators may be the main factors leading to the different results observed in these studies. In addition, different levels of taste receptor stimulation in the oral mucosa directly affect salivary secretion. Although the methods and results were different, the ADC in the parotid gland changed with time, which indicated that the ADC value before and after acid stimulation could reflect parotid gland function.

In T2DM patients, the trend in ADC values after acid stimulation was significantly different from that in healthy controls. First, the ADC values did not increase, but decreased in a short period of time, because the saliva stored in the gland was emptied rapidly under acid stimulation, and the salivary synthesis function of the gland was insufficient. Thus, both the extracellular free water and the ADC value decreased. The saliva accumulated in the gland was gradually increased, the extracellular free water increased, the ADC value increased, and the peak time in the patient group occurred significantly

later than that in the healthy controls. The peak value in patients was significantly lower than that in the control group, and the change rate was lower than that in the normal control group. This showed that parotid gland synthesis was reduced in diabetes patients. In this study, the ADC changes in the parotid gland could reflect the decline in parotid gland function in diabetes patients.

We found that the ADC value in the parotid gland was decreased gradually after reaching its peak in both the patient and control groups, and the difference between the two was much smaller than that after acid administration ($p < 0.05$). We estimated that the extracellular free water in the parotid gland decreased sharply because a large amount of saliva was secreted after the peak of ADC, and then, it gradually reached the resting state (Fig. 2).

The RSI of fat and ADCs in the parotid gland were correlated ($r = 0.666$; $p < 0.05$) (Fig. 3). This demonstrated that the higher the fat deposition in the parotid gland, the lower the ADC value, and there was a significant correlation between the two. In a previous study, we observed the morphology and microstructure of the parotid gland in diabetes patients, and found that lipid deposition may be the pathogenesis of sialadenosis in diabetes, including ectopic deposition of mature or diffuse fat (Fig. 1), which destroyed the normal glandular structure and weakened the secretion of salivary glands [32]. Subsequently, the saliva was reduced, leading to dry mouth [6]. In this study, the changes in ADC values before and after acid stimulation were used to detect the synthesis and secretion of the parotid gland, which indicated that the essence of the parotid gland lesions induced by diabetes was lipid deposition. Parotid gland hypofunction, especially in the stimulative state, is closely related to fat deposition in diabetes patients.

There were some limitations to this study. First, this was the first study to use DWI to assess parotid gland function in diabetes patients; therefore, there were no applicable rules and guidelines. Second, due to the long scanning duration of the DWI sequence, we only evaluated the secretory function of the parotid gland under stimulated conditions, which accounted for 60–70% of the total saliva volume. However, we did not use DWI to evaluate changes in the submandibular gland caused by diabetes. In addition, the age range of the selected participants was relatively narrow, and the sample size was insufficient. Finally, this study was not controlled using clinical dry mouth evaluation, which could not fully reflect parotid gland function in diabetes patients. Future studies should be more closely related to clinical practice, expand the sample size, and explore a new method for quantitative evaluation of parotid gland function in diabetes patients using DWI before and after acid stimulation.

In conclusion, our results suggest that DW-MRI may be a noninvasive tool for accurate detection of parotid gland function in diabetes patients using ADC. Second, the correlation analysis of RSI and parotid gland function in diabetes patients proved that lipid deposition may be an important cause of parotid gland dysfunction.

Declarations

Ethics approval The study was approved by the institutional review committee of Qinhuangdao Municipal No. 1 Hospital (Hebei Province, P.R. China).

Consent to participate Written informed consent was obtained from all participants before study commencement.

Competing interests The authors declare no competing interests.

References

1. Bakianian Vaziri P, Vahedi M, Mortazavi H, Abdollahzadeh S, Hajilooi M. Evaluation of salivary glucose, IgA and flow rate in diabetic patients: a case-control study. J Dent (Tehran). 2010;7:13–8.
2. Wang B, Du J, Zhu Z, Ma Z, Wang S, Shan Z. Evaluation of parotid salivary glucose level for clinical diagnosis and monitoring type 2 diabetes mellitus patients. Biomed Res Int. 2017;2017:2569707.
3. Huang DL, Chan KC, Young BA. Poor oral health and quality of life in older U.S. adults with diabetes mellitus. J Am Geriatr Soc. 2013;61:1782–8.
4. Lilliu MA, Loy F, Cossu M, Solinas P, Isola R, Isola M. Morphometric study of diabetes related alterations in human parotid gland and comparison with submandibular gland. Anat Rec (Hoboken). 2015;298:1911–8.
5. Molania T, Alimohammadi M, Akha O, Mousavi J, Razvini R, Salehi M. The effect of xerostomia and hyposalivation on the quality of life of patients with type II diabetes mellitus. Electron Physician. 2017;9:5814–9.
6. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal. 2006;11:E309–14.
7. Maciejczyk M, Matczuk J, Żendzian-Piotrowska M, Niklińska W, Fejfer K, Szarmach I, Ładny J, Zieniewska I, Zalewska A. Eight-week consumption of high-sucrose diet has a pro-oxidant effect and alters the function of the salivary glands of rats. Nutrients. 2018;10:1530.
8. Little M, Schipper M, Feng FY, Vineberg K, Cornwall C, Murdoch-Kinch CA, Eisbruch A. Reducing xerostomia after chemo-IMRT for head-and-neck cancer: beyond sparing the parotid glands. Int J Radiat Oncol Biol Phys. 2012;83:1007–14.
9. Eisbruch A, Rhodus N, Rosenthal D, et al. How should we measure and report radiotherapy-induced xerostomia? Semin Radiat Oncol. 2003;13:226–34.
10. Feen Rønjom M. Radiation-induced hypothyroidism after treatment of head and neck cancer. Dan Med J. 2016;63:B5213.
11. Riyaz MA. Diagnostic salivary gland imaging - a review. Integr J Med Sci. 2021;8:296.
12. Habermann CR, Gossrau P, Kooijman H, Graessner J, Cramer MC, Kaul MG, Reitmeier F, Jaehne M, Adam G. Monitoring of gustatory stimulation of salivary glands by diffusion-weighted MR imaging: comparison of 1.5T and 3T. AJNR Am J Neuroradiol. 2007;28:1547–51.

13. Reda R, Zanza A, Mazzoni A, Cicconetti A, Testarelli L, Di Nardo D. An update of the possible applications of magnetic resonance imaging (MRI) in dentistry: a literature review. J Imaging. 2021;7:75.
14. Kato H, Kanematsu M, Toida M, Kawaguchi T, Shibata T, Kajita K, Hoshi H. Salivary gland function evaluated by diffusion-weighted MR imaging with gustatory stimulation: preliminary results. J Magn Reson Imaging. 2011;34:904–9.
15. Zhang Y, Ou D, Gu Y, He X, Peng W. Evaluation of salivary gland function using diffusion-weighted magnetic resonance imaging for follow-up of radiation-induced xerostomia. Korean J Radiol. 2018;19:758–66.
16. Zhou N, Chu C, Dou X, Li M, Liu S, Zhu L, Liu B, Guo T, Chen W, He J, Yan J, Zhou Z, Yang X, Liu T. Early evaluation of irradiated parotid glands with intravoxel incoherent motion MR imaging: correlation with dynamic contrast-enhanced MR imaging. BMC Cancer. 2016;16:865.
17. Chao KS, Deasy JO, Markman J, et al. A prospective study of salivary function sparing in patients with head-and-neck cancers receiving intensity-modulated or three-dimensional radiation therapy: initial results. Int J Radiat Oncol Biol Phys. 2001;49:907–16.
18. Habermann CR, Cramer MC, Graessner J, Gossrau P, Reitmeier F, Fiehler J, Schoder V, Jaehne M, Adam G. Functional imaging of parotid glands: diffusion-weighted echo-planar MRI before and after stimulation. Rofo. 2004;176:1385–9.
19. Lima-Aragão MV, de Oliveira-Junior JJ, Maciel MC, Silva LA, do Nascimento FR, Guerra RN. Salivary profile in diabetic patients: biochemical and immunological evaluation. BMC Res Notes. 2016;9:103.
20. Sashikumar R, Kannan R. Salivary glucose levels and oral candidal carriage in type II diabetics. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109:706–11.
21. Zhang Q, Wei YM, Qi YG, Li BS. Early changes in apparent diffusion coefficient for salivary glands during radiotherapy for nasopharyngeal carcinoma associated with xerostomia. Korean J Radiol. 2018;19:328–33.
22. Merlo C, Bohl L, Carda C, Gómez de Ferraris ME, Carranza M. Parotid sialosis: morphometrical analysis of the glandular parenchyme and stroma among diabetic and alcoholic patients. J Oral Pathol Med. 2010;39:10–5.
23. Aitken-Saavedra J, Rojas-Alcayaga G, Maturana-Ramírez A, Escobar-Álvarez A, Cortes-Coloma A, Reyes-Rojas M, Viera-Sapiain V, Villablanca-Martínez C, Morales-Bozo I. Salivary gland dysfunction markers in type 2 diabetes mellitus patients. J Clin Exp Dent. 2015;7:e501–5.
24. Carda C, Carranza M, Arriaga A, Díaz A, Peydró A, Gomez de Ferraris ME. Structural differences between alcoholic and diabetic parotid sialosis. Med Oral Patol Oral Cir Bucal. 2005;10:309–14.
25. Gupta A, Ramachandra VK, Khan M, Jha KS, Vedaraju KS, Channaiah NA. A cross-sectional study on ultrasonographic measurements of parotid glands in type 2 diabetes mellitus. Int J Dent. 2021;2021:5583412.
26. Chen S, Paul BC, Myssiorek D. An algorithm approach to diagnosing bilateral parotid enlargement. Otolaryngol Head Neck Surg. 2013;148:732–9.
27. Thoeny HC, De Keyzer F, Boesch C, Hermans R. Diffusion-weighted imaging of the parotid gland: influence of the choice of b-values on the apparent diffusion coefficient value. J Magn Reson Imaging. 2004;20:786–90.
28. Zhang Y, Ou D, Gu Y, He X, Peng W, Mao J, Yue L, Shen X. Diffusion-weighted MR imaging of salivary glands with gustatory stimulation: comparison before and after radiotherapy. Acta Radiol. 2013;54:928–33.
29. Ries T, Arndt C, Regier M, Graessner J, Cramer MC, Reitmeier F, Jaehne M, Adam G, Habermann CR. Value of apparent diffusion coefficient calculation before and after gustatory stimulation in the diagnosis of acute or chronic parotitis. Eur Radiol. 2008;18:2251–7.
30. Loimu V, Seppälä T, Kapanen M, Tuomikoski L, Nurmi H, Mäkitie A, Tenhunen M, Saarilahti K. Diffusion-weighted magnetic resonance imaging for evaluation of salivary gland function in head and neck cancer patients treated with intensity-modulated radiotherapy. Radiother Oncol. 2017;122:178–84.
31. Thoeny HC, De Keyzer F, Claus FG, Sunaert S, Hermans R. Gustatory stimulation changes the apparent diffusion coefficient of salivary glands: initial experience. Radiology. 2005;235:629–34.
32. Ren YD, Li XR, Zhang J, Long LL, Li WX, Han YQ. Conventional MRI techniques combined with MR sialography on T2-3D-DRIVE in Sjögren syndrome. Int J Clin Exp Med. 2015;8:3974–82.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):281–288
https://doi.org/10.1007/s13410-022-01085-0

ORIGINAL ARTICLE

The effects of parental monitoring on the quality of life and diet quality of adolescents with type 1 diabetes

Rukiye Bozbulut[1] · Zekiye Küpçü[1] · Esra Döğer[1] · M. Orhun Çamurdan[1] · Aysun Bideci[1]

Received: 27 December 2021 / Accepted: 17 May 2022 / Published online: 4 June 2022

Abstract

Background The burdens of type 1 diabetes management in adolescence adversely affect their quality of life, reduce adherence to diet, and worsen metabolic control. Parental monitoring can play a protective role in adolescent diabetes management.

Aim This study was conducted to determine the effects of parental monitoring on quality of life and diet quality in adolescents in diabetes care.

Material and methods 100 adolescents (52 males, 48 females) with type 1 diabetes aged 13–18 years and their parents participated in the study. The parental monitoring of diabetes care scale (PMDC) in adolescents with type 1 diabetes was used to evaluate parental monitoring. PedsQL^{TM} (Pediatric Quality of Life Inventory) Diabetes Module Version 3.0 was used to assess quality of life. Three-day retrospective food intake records were collected from the adolescents with type 1 diabetes, and diet quality was evaluated with the Healthy Eating Index-2010 (HEI-2010) calculated from data obtained from food intake records.

Results Statistically significant and one-sided correlations were identified between the PedsQL total scale and subscale scores of adolescents with type 1 diabetes and the scores of the parental monitoring of diabetes care scale ($r = 0.437$ and $p < 0.001$). Statistically significant and one-sided correlations were found between some subcomponents of diet quality, i.e., dark leafy greens and dried legumes, whole grains, dairy products, fatty acids, and total HEI levels, and parental monitoring of diabetes scale scores ($r = 0.321$ and $p < 0.001$). When HbA1c levels remained constant in adolescents with type 1 diabetes, each 10-point increase in the parental monitoring of diabetes care scale increased the total HEI level by 0.68 points (95% CI: 0.20–1.16).

Conclusions As parental monitoring increases in adolescents with type 1 diabetes, the diet quality and quality of life of adolescents with diabetes also increase.

Keywords Type 1 diabetes · Adolescent · Parental monitoring · Quality of life · Diet quality

Introduction

Adolescence is the transitional period from childhood to adulthood, where growth and development accelerate and changes are experienced. In the presence of a diagnosis of diabetes, which is a chronic disease, it may be difficult for the adolescent to fulfill their responsibilities concerning the disease in addition to their developmental tasks, affecting the psychological and emotional well-being of both the adolescent and their family [1]. Studies have shown that glycemic control worsens during adolescence compared to preadolescence [2, 3]. Parental monitoring is parental behavior that includes communication between the adolescent and the parent, and paying attention to the tracking of the child's whereabouts, activities, and disease compliance [4]. Monitoring behavior, which is a dimension of parenting practices, is defined as the parent's knowledge about the activities of adolescents outside the home. It is reported that parental monitoring of adolescents is very important and it significantly impacts the negativities in the adolescent's life [5]. Studies have shown that as parental monitoring increases, diabetes management and metabolic control will improve [1–3, 6].

Many children and adolescents with diabetes cannot comply with diabetes management due to various reasons, such as the desire to be independent, avoiding the responsibilities regarding diabetes management, and complexity of diabetes management. As a result, their quality of life is adversely

✉ Rukiye Bozbulut
dyt_rukiye@hotmail.com

[1] Faculty of Medicine, Department of Pediatric Endocrinology, Gazi University, Beşevler, Ankara, Turkey

affected [7]. Improving the quality of life in the treatment of diabetes is very important [8]. Quality of life in type 1 diabetes is an important indicator of the course of the disease and shows the well-being of the child. As well as metabolic control and prevention of complications, the child's well-being and quality of life play a major role in treatment. Measuring quality of life in adolescents with diabetes provides an opportunity to evaluate the impact of treatment and care practices on all aspects of adolescent life. It is reported that objective assessments are also important in the evaluation of quality of life, and therefore, parental evaluations are also valuable [9].

Medical nutrition therapy is of great importance in type 1 diabetes, both for preventing complications and for providing metabolic control [10]. Diet quality is an integral part of medical nutrition therapy in type 1 diabetes. With adequate and balanced nutrition, the diet quality of children and adolescents increases, while metabolic control is ensured and the risk of complications related to type 1 diabetes decreases [10]. In general, the consumption of fruit, vegetables, and whole grains in the adolescent group is less than the recommended amounts [11–13] while the intake of saturated fat and refined grains is above recommended limits, resulting in poor diet quality [13–15]. Therefore, close monitoring of children and adolescents with type 1 diabetes and frequent repetition of nutrition education are very important [10, 11].

Although the importance of parental monitoring in adolescents with diabetes is emphasized, there are no studies in Turkey to evaluate the effect of parental monitoring on the quality of life and diet quality in adolescents with type 1 diabetes. The aim of this study is to determine the effect of parental monitoring on the quality of life and diet quality of adolescents with type 1 diabetes.

Material and methods

Study site, time, and sample selection

This study was carried out on 100 adolescents aged 13–18 years with type 1 diabetes (diagnosed at least 1 year ago, receiving multiple-dose insulin, or insulin pump therapy) and their parents, who presented to Gazi University, Pediatric Endocrinology Outpatient Clinic. Those who had neurological or metabolic disorders, chronic diseases that may accompany diabetes, used any medication that may affect depression, and had mental retardation that makes it difficult to communicate were not included in the study.

General plan of the study

Information about all individuals participating in the study was obtained by directly asking them and/or their parents. After anthropometric measurements of the adolescent with type 1 diabetes were taken and clinical evaluations were made, a questionnaire was applied. As an indicator of metabolic control, the last measured HbA1c values (the day of anthropometric measurements and food intake record) were recorded from the patient files.

Data collection and evaluation

Anthropometric measurements

All anthropometric measurements [body weight (kg), height (cm)] were taken according to standard methods. Body mass index (BMI) was calculated with the data obtained, and these values were evaluated according to the growth references published by the World Health Organization (WHO) in 2007 for the 5–19 age group [16].

Questionnaire form

The questionnaire form included questions about sociodemographic characteristics such as the age, gender, level of income, place of residence, level of education of the adolescent and their parent, and about the adolescent's diabetes (HbA1c level, duration of diabetes, total insulin dose, etc.) and nutritional status. In order to evaluate the nutritional status of the participants, a 3-day retrospective food intake record was collected and portion sizes were determined using a photographic food catalog. The daily intake of energy and nutrients were calculated using the Nutrition Information System (BeBiS version 7.2) computer program [17].

Measures

Parental monitoring of diabetes care scale (PMDC) in adolescents with type 1 diabetes

The parental monitoring of diabetes care scale consists of 27 items. The scale was published by Ellis et al. [18] in 2008, and revised again by Ellis et al. [4] in 2012. The scale is a 5-point Likert-type scale, where "at least once a week" is scored as "1" and "more than once a day" is scored as "5," and the score increases with increased parental monitoring. This scale was translated into Turkish by Türk et al. (2016) and a validity and reliability study was performed [19]. Cronbach's alpha internal consistency coefficient of the PMDC was 0.91.

Quality of life

The PedsQL™ (Pediatric Quality of Life Inventory) Diabetes Module Version 3.0 adolescent (13–18 years) report and the PedsQL™ Diabetes Module Version 3.0 parent proxy-report for adolescents aged 13–18 years were used to assess the quality of life. The PedsQL 3.0 Diabetes Module is designed to measure health-related quality of life specific to type 1 diabetes. The Turkish validity and reliability study of the PedsQL 3.0 Diabetes Module, adolescent report, and parent proxy-report [20], which was developed by Varni et al., was carried out by Özçelik-Çövener and Aktaş (2015), and the Cronbach's alpha coefficient was 0.92 for the adolescent report and 0.88 for the parent proxy-report [9]. The 28-item multidimensional PedsQL™ 3.0 Diabetes Module consists of five subgroups: diabetes symptoms (11 items), treatment barriers (4 items), adherence to treatment (7 items), worry (3 items), and communication (3 items). All of the items are reverse scored between 0 and 100 (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0). When calculating the total score from the scale and the scores of the subgroups, the total score is divided by the number of items. There is no cut-off point, and a high score indicates good quality of life.

Diet quality

The Healthy Eating Index-2010 (HEI-2010) was used to assess diet quality. The Healthy Eating Index-2010, which will be calculated from the data obtained from the food intake records of the individuals, includes a total of 12 components. The first 9 of the 12 components determine the adequacy of the diet, and the last 3 determine foods that should be consumed in a limited way. Each of the qualification components has its own standard. With the increase in intake, the scores increase proportionally. With moderation components, low intake causes the score to increase. The Healthy Eating Index-2010 total score is expressed over 100 points by adding together the components of adequacy and moderation. Component scores range from 0 to 5, 0 to 10, or 0 to 20, with a total score of 100% meaning that the recommended amounts have been met or exceeded. When the diet quality of individuals is categorized according to their total HEI score, scores of 50 and less than 50 are defined as "poor diet quality," 51 to 80 as "diet quality needing improvement," and above 80 as "good diet quality" [21].

Statistical methods

Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). Kolmogorov-Smirnov test was used to investigate whether the normal distribution assumption was met. Categorical data were expressed as percentage (%) while quantitative data were given as mean ± standard deviation (SD). The mean differences in subdomain and overall PedsQL scores between the adolescents with type I diabetes mellitus and their parents were analyzed using the paired samples *t*-test. Degrees of association between continuous variables were evaluated with Spearman's rank correlation analyses. The differences between groups in terms of quantitative data (i.e., the PedsQL total scores of adolescents with type 1 diabetes, total PedsQL parent proxy-report scores for adolescents and HEI total scores) were evaluated by Student's t test or One-Way ANOVA according to the number of independent groups. To determine the best independent predictors which, effect on dependent variables (i.e., the PedsQL total scores of adolescents with type 1 diabetes, total PedsQL parent proxy-report scores for adolescents and HEI total scores) were investigated multiple linear regression analyses. Any variable whose univariable test had a p-value < 0.10 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Coefficient of regression and 95% confidence intervals for each independent variable were also calculated. A p-value less than 0.05 was considered statistically significant.

Results

52.0% of the adolescents were male with a mean age of 14.5 ± 1.6 years, a diabetes age of 5.5 years, and a mean HbA1c of 8.1%. The mean age of their parents was 41.6 ± 6.1 years, 78% of which were women and 57.0% of which consisted of nuclear families. The parental monitoring of diabetes care scale score was 97.2 ± 15.2, and the total HEI score was 52.9 ± 3.6, 80.0% of which are in the diet quality classification that need improvement (Table 1).

The PedsQL adolescent total score was 69.6 ± 14.9 while the total PedsQL parent proxy-report score for adolescents was 65.3 ± 12.8. Compared to adolescents reports, the PedsQL parental proxy-report for adolescents subscale scores (excluding the communication subscale) and total scale scores were found to be statistically significantly lower ($p < 0.05$) (Table 2).

Statistically significant and one-sided correlations were found between the PedsQL subscale and total scale scores of adolescents with type 1 diabetes, and the parental monitoring of diabetes care scale scores ($p < 0.05$). Statistically significant and one-sided correlations were found between parent proxy-report PedsQL subscale scores (excluding the worry subscale) and total scale scores for adolescents, and parental monitoring of diabetes care scale scores ($p < 0.05$) (Table 3).

Statistically significant and one-sided correlations were found between some subcomponents of diet quality, i.e., dark leafy greens and dried legumes, whole grains, dairy products, fatty acids, and total HEI levels, and parental monitoring of diabetes scale scores ($p < 0.05$). No statistically significant correlation was determined between other subcomponents of diet quality and parental monitoring of diabetes scale scores ($p > 0.05$) (Table 3).

Table 1 Demographic and clinical characteristics of the adolescents

	$n = 100$
Age (years)	14.5 ± 1.6
Gender	
Female	48.0%
Male	52.0%
Parent age (years)	41.6 ± 6.1
Parent gender	
Female	78.0%
Male	22.0%
Mother's level of education	
Primary school	35.0%
Secondary school	41.0%
Bachelor's degree	18.0%
Postgraduate degree	6.0%
Father's level of education	
Primary school	21.0%
Secondary school	42.0%
Bachelor's degree	34.0%
Postgraduate degree	3.0%
Family's income level	
Low	18.0%
Middle	72.0%
High	10.0%
Type of family structure	
Nuclear	57.0%
Extended	35.0%
Divorced	8.0%
Diabetes age (years)	5.5 (3.0–8.0)
Insulin treatment method	
Insulin pump therapy	21.0%
Multiple-dose injection therapy	79.0%
Insulin dose (unit)	50.0 (40.0–66.0)
HbA1c	8.1 (7.0–9.2)
Body mass index (kg/m^2)	21.0 ± 2.7
Parental monitoring scale	97.2 ± 15.2
Total HEI	52.9 ± 3.6
Total HEI classification	
Diet quality needing improvement	80.0%
Poor diet quality	20.0%

Table 3 Correlation coefficients and significance levels between parental monitoring of diabetes scale scores, and PedsQL and diet quality subcomponents and total scores in adolescents with type 1 diabetes

	Correlation coefficient	p-value†
PedsQL adolescent report		
Diabetes symptoms	0.221	**0.027**
Treatment barriers	0.355	**< 0.001**
Adherence to treatment	0.326	**< 0.001**
Worry	0.261	**0.009**
Communication	0.444	**< 0.001**
Total	0.437	**< 0.001**
PedsQL parent proxy-report for adolescents		
Diabetes symptoms	0.207	**0.039**
Treatment barriers	0.292	**0.003**
Adherence to treatment	0.401	**< 0.001**
Worry	0.130	0.196
Communication	0.316	**< 0.001**
Total	0.435	**< 0.001**
Diet quality		
Total fruit	0.039	0.698
Whole fruit	0.184	0.068
Total vegetables	−0.055	0.589
Dark leafy greens and dried legumes	0.345	**< 0.001**
Whole grains	0.321	**< 0.001**
Dairy	0.207	**0.038**
Total protein	0.192	0.055
Seafood	0.118	0.242
Fatty acids	0.246	**0.014**
Refined grains	−0.141	0.162
Sodium	0.015	0.881
Added sugar	0.095	0.349
Saturated fat	−0.175	0.081
Total HEI	0.321	**< 0.001**

†Spearman's rank order correlation test

As the family's monthly income level increased, the total PedsQL score of adolescents with type 1 diabetes ($r = 0.249$ and $p = 0.013$) and the total PedsQL parent proxy-report score for adolescents ($r = 0.224$ and $p = 0.025$) also increased

Table 2 Adolescent and parent proxy-report for adolescents PedsQL subscale scores and total scale scores

	PedsQL adolescent report	PedsQL parent proxy-report for adolescents	p-value†
Diabetes symptoms	67.0 ± 17.0	63.9 ± 16.3	**0.015**
Treatment barriers	67.9 ± 18.9	60.2 ± 17.9	**< 0.001**
Adherence to treatment	72.6 ± 17.6	66.8 ± 17.0	**< 0.001**
Worry	69.7 ± 21.1	64.5 ± 21.4	**0.025**
Communication	70.9 ± 24.1	70.8 ± 21.8	0.984
Total	69.6 ± 14.9	65.3 ± 12.8	**< 0.001**

statistically significantly. As HbA1c level increased, total PedsQL score (r = −0.199 and p = 0.047) and total HEI level (r = −0.215 and p = 0.032) of adolescents with type 1 diabetes decreased statistically significantly (Table 4).

The PedsQL total scores of adolescents with type 1 diabetes, total PedsQL parent proxy-report scores for adolescents, and HEI total scores displayed no statistically significant difference based on the gender of the adolescent, the gender of the parent, the level of education of the parents, type of family structure, and insulin treatment method, respectively (p > 0.05) (data not shown).

When adjusted for all other possible factors, the parental monitoring of diabetes scale score was an independent indicator in predicting the change in total PedsQL scores of adolescents with type 1 diabetes (B = 0.355, 95% CI: 0.154–0.556, and p < 0.001). Similarly, the parental monitoring of diabetes scale score was an independent factor in predicting the change in total PedsQL parental proxy-report scores for adolescents with type 1 diabetes, irrespective of other factors (B = 0.278, 95% CI: 0.096–0.459, and p = 0.003). In addition, when the HbA1c levels of the adolescents remained constant, each 10-point increase in the parental monitoring of diabetes scale score increased the total HEI level by 0.68 points (95% CI: 0.20–1.16) (p = 0.006) (Table 5).

Discussion

Adolescence is a period when type 1 diabetes management and metabolic control is the most difficult, and adolescents and parents experience the most intense problems, and this reduces the adherence of adolescents to diabetes and adversely affects glycemic control and quality of life [22]. It has been reported that increasing the quality of life and well-being of children with diabetes is as important as metabolic control in preventing secondary morbidity. Therefore, the primary goal of modern diabetes care in children and adolescents has evolved from a purely medical approach to one aimed at optimal glycemic control, normal psychological development, and maximum quality of life [23]. This is the first study in Turkey to evaluate the quality of life in type 1 diabetes from the perspective of both adolescents with type 1 diabetes and their parents, and examine the effects of parental monitoring on the quality of life and diet of adolescents with diabetes. In our study, the mean quality of life score of adolescents with type 1 diabetes was 69.6 ± 14.9. Compared to other studies, the quality of life of adolescents with type 1 diabetes in Turkey was found to be lower than that of adolescents with diabetes in many countries [19, 24–26]. Similar to other studies, the diabetes symptoms score, which is one of the quality of life subscale scores, was found to be lower in our study. A low diabetes symptoms subscale score is associated with poor

Table 4 Correlation coefficients and significance levels between total PedsQL and HEI scale scores and some demographic and clinical characteristics

	PedsQL adolescent report	PedsQL parent proxy-report for adolescents	Total HEI
Age			
Correlation coefficient	0.059	0.101	0.070
p-value†	0.562	0.315	0.491
Parent age			
Correlation coefficient	0.025	0.075	−0.027
p-value†	0.804	0.459	0.793
Diabetes age			
Correlation coefficient	0.037	−0.078	−0.105
p-value†	0.717	0.438	0.300
Family's monthly income			
Correlation coefficient	0.249	0.224	0.063
p-value†	**0.013**	**0.025**	0.534
Insulin dose			
Correlation coefficient	0.041	0.023	0.075
p-value†	0.633	0.822	0.459
HbA1c			
Correlation coefficient	−0.199	−0.178	−0.215
p-value†	**0.047**	0.077	**0.032**
Body mass index			
Correlation coefficient	0.135	0.170	0.081
p-value†	0.180	0.091	0.422

Table 5 Examination of the combined effects of factors considered most effective in predicting the change in PedsQL and HEI total scale scores – results of the multivariate linear regression analysis

	B	95% CI for *B*		*p*-value
		LL	UL	
PedsQL adolescent report				
Monthly income	1.862	−1.357	5.082	0.254
HbA1c	0.163	−1.253	1.578	0.820
Insulin dose	0.107	−0.041	0.255	0.153
Parental monitoring	0.355	0.154	0.556	**< 0.001**
PedsQL parent proxy-report for adolescents				
Body mass index	−0.502	−1.642	0.639	0.385
Monthly income	2.414	−0.474	5.301	0.100
HbA1c	0.484	−0.780	1.748	0.449
Insulin dose	0.100	−0.061	0.260	0.222
Parental monitoring scale	0.278	0.096	0.459	**0.003**
Total HEI				
HbA1c	−0.150	−0.491	0.192	0.387
Parental monitoring scale	0.068	0.020	0.116	**0.006**

B coefficient of regression, *CI* confidence interval, *LL* lower limits of 95% CIs, *UL* upper limits of 95% CIs

glycemic control. In adolescents with diabetes, metabolic control deteriorates due to the lifestyle changes necessary to manage the disease often being difficult as well as hormonal and psychosocial changes that occur during this transitional period [8]. In this study, the HbA1c levels of the adolescents were suboptimal, with an average of 8.1%. In our study, the total scores and sub-scores of the quality of life scale in parent proxy-reports were lower than in adolescent reports (except for the communication sub-score). In other studies, parents also often do not agree with the perspectives of their children regarding diabetes, and similar to our study, they have reported lower quality of life scores about their children [20, 23, 27]. This may be caused by parents' concern for the long- and short-term complications of diabetes, and the parents' perception of their child's difficulties with lifestyle changes. It is also thought that this difference may be caused by differences in the cognitive development of adolescents and parents. Because the brain areas responsible for executive functions, including long-term planning, decision-making, and the ability to understand the consequences of decisions, prioritize, and assess risk, develop in the 20s [8].

In our study, close parental supervision and parental monitoring of the completion of the adolescent's diabetes care also increased the quality of life of adolescents with type 1 diabetes and facilitated the management of diabetes. As parental monitoring scores increased, both the total quality of life scale scores and the subscale (diabetes symptoms, treatment barriers, adherence to treatment, worry, and communication) scores of adolescents with type 1 diabetes increased significantly (Table 3). Similarly, in this study, as parental monitoring increased, a positive increase was observed in the views of the parents on the quality of life of the adolescents. It has been reported that parental involvement in diabetes management in adolescents has positive effects on diabetes outcomes. It has been reported that parental monitoring plays a protective role in healthy adolescents as well as in adolescents with type 1 diabetes, and that increased parental support facilitates adaptation to diabetes in adolescents, and they make fewer mistakes regarding their self-care [6, 28]. In our study, as parental monitoring increased, the adolescent's adherence to diabetes treatment increased, they picked up the symptoms of diabetes better, and coped with the conditions that prevented treatment more easily. Poor glycemic control in adolescence increases the possibility of complications, anxiety, and diabetes-related stress, and decreases the quality of life [8]. In this study, parental support increased the psychological belief of coping with diabetes in adolescents, decreased anxiety levels, and thus, increased the quality of life. Similarly, Ellis et al. found that adolescents whose parents questioned whether they completed their diabetes care or who were with them more often during the completion of diabetes care increased their adherence to diabetes treatment and their metabolic controls improved [6]. In studies, diabetes-specific parental monitoring has been associated with better diabetes management in individuals aged 10–15 years [29] and better metabolic control in individuals aged 12–16 years [30]. In another study carried out with adolescents with type 1 diabetes, diabetic ketoacidosis was found to be more common in those who had negative perceptions towards their parents, had little communication with their parents, and had a negative family relationship, and it was recommended to implement practices aimed at solving problems and strengthening communication between parents and adolescents for good diabetes management [3].

Dietary compliance generally decreases during adolescence [11]. It has been reported that the dietary compliance of adolescents with type 1 diabetes varies between 21 and 95% [31]. Although a healthy diet is one of the most important components of type 1 diabetes management in children and adolescents, studies have shown that the diet quality of those with type 1 diabetes is inadequate [11, 32]. In this study, the mean total HEI was 52.9 ± 3.6. Total diet quality scores were in the "poor-need improvement" group according to the HEI-2010 classification, which is consistent with the literature [32]. Western-style diets, which are frequently consumed by adolescents today, have low dietary quality, containing high amounts of saturated fat, low amounts of omega-3 fatty acids, excess sodium, and high amounts of refined sugar [11]. In general, the consumption of fruit, vegetables, and whole grains in this age group is less than the recommended amounts while the intake of saturated fat is above recommended limits,

resulting in poor diet quality [11–15]. In a study conducted by Nansel et al. [13], adolescents with type 1 diabetes consumed less than half of the recommended amount of fruit, vegetables, and whole grain products; only 3–12% of adolescents could meet the recommended amounts; and adolescents with good dietary quality preferred fruits, whole grain products, and vegetables rather than processed grains, fats, and sugars [13].

Studies in adolescents with type 1 diabetes have reported that an increase in diet quality reduces HbA1c levels, provides optimal growth and development, and better metabolic control [10, 32]. In this study, as parental monitoring increased, total diet quality scores of adolescents with type 1 diabetes and intake of dark leafy greens and dried legumes, whole grains, dairy, and fatty acids (the ratio of mono- and polyunsaturated fatty acids to saturated fatty acids), which are among the dietary quality components, increased significantly (Table 3). In a study, adolescents had the lowest diet quality score from the intake of fruit, vegetables, and dairy products [33]. Lipsky et al. (2012) found a positive correlation between diet quality and consumption of fruit, vegetables, and whole grain products, and a negative correlation with the consumption of refined grains [34]. With adequate and balanced nutrition, the diet quality of children and adolescents increases, while metabolic control is ensured and the risk of complications related to type 1 diabetes decreases [10]. In this study, it is clear that parental monitoring is one of the factors that determines diet quality, increases adherence to treatment, and improves diet quality with healthy food choices. When the HbA1c levels of the adolescents remained constant, each 10-point increase in the parental monitoring of diabetes scale score increased the total HEI level by 0.68 points. Cardiovascular diseases are more common in individuals with type 1 diabetes, occur earlier, and are the main cause of premature death [35]. Good diet quality is associated with a lower cardiovascular disease risk profile. Increasing the consumption of whole grain products, dried legumes, fruit and vegetables, and the composition of the diet's fat pattern with unsaturated fatty acids provide beneficial effects on cardiovascular and other chronic disease risk indicators [11]. In our study, the increase in the consumption of dark leafy greens and dried legumes, whole grains, dairy, and healthy fatty acids, which are components of good diet quality, both explain the high diet quality scores and give an idea about low cardiovascular risk.

The strengths of the study were the evaluation of parental monitoring and quality of life of the adolescent with type 1 diabetes with valid scales, and the evaluation of the quality of life of the adolescent with type 1 diabetes from both family and adolescent perspectives.

The limitations of the study were that it was a single-center study and a generalization could not be made for Turkey in terms of results. There is a need for comprehensive multicenter studies in this area.

Conclusions

In our study, parental monitoring was found to be an independent indicator of both quality of life and diet quality of adolescents with type 1 diabetes. The increase in parental monitoring scores resulted in an increase in both the quality of life scores and diet quality scores of the adolescents with type 1 diabetes, and therefore, it was one of the main factors determining the quality of life and diet quality of the diabetic adolescent.

Positive family environment and relationships and parental support will reduce the burden of the adolescent and increase the self-management and adherence of the diabetic adolescent to the therapeutic regimen. Therefore, a bio-psychosocial and family-centered care approach is very important in the diabetes management of adolescents with type 1 diabetes.

Declarations

Ethics approval Ethics committee approval was obtained from the Gazi University Ethics Committee (with the approval number: 43.11.01.2021), and the adolescents and parents that participated in the study were informed verbally and in writing about the study, and an informed consent form was signed.

Conflict of interest The authors declare no competing interests.

References

1. Almeida AC, Leandro ME, Pereira MG. Adherence and glycemic control in adolescents with type 1 diabetes: the moderating role of age, gender, and family support. J Clin Psychol Med. 2020;27(2):247–55.
2. Wagner VM, Muller-Godeffroy E, von Sengbusch S, Hager S, Thyen U. Age, metabolic control and type of insulin regime influences health-related quality of life in children and adolescents with type 1 diabetes mellitus. Eur J Pediatr. 2005;164:491–6.
3. Geffken GR, Heather L, Walker KN, Storch EA, Heidgerken AD, Lewin A, et al. Family functioning processes and diabetic ketoacidosis in youths with type I diabetes. Rehabil Psychol. 2008;53:231–7.
4. Ellis DA, Templin TN, Moltz K, Naar-King S, Dekelbab B, Carcone AI. Psychometric properties of the revised parental monitoring of diabetes care questionnaire in adolescents with type 1 diabetes. J Adolesc Health. 2012;50:289–95.
5. Smetana JG, Daddis C. Domain-specific antecedents of parental psychological control and monitoring: the role of parenting beliefs and practices. Child Dev. 2002;73(2):563–80.
6. Ellis DA, Podolski CL, Frey M, Naar-King S, Wang B, Moltz K. The role of parental monitoring in adolescent health outcomes: impact on regimen adherence in youth with type 1 diabetes. J Pediatr Psychol. 2007;32(8):907–17.
7. Baş VN, Bideci A, Yeşilkaya E, Soysal AŞ, Çamurdan O, Cinaz P. Evaluation of factors affecting quality of life in children with type 1 diabetes mellitus. J Diabetes Metab. 2011;2(8):154–8.
8. AlBuhairan F, Nasim M, Al Otaibi A, Shaheen NA, Al Jaser S, Al AI. Health related quality of life and family impact of type 1 diabetes among adolescents in Saudi Arabia. Diabetes Res Clin Pract. 2016;114:173–9.
9. Özçelik ÇÇ, Aktaş E. The reliability and validity of the pediatrics quality of life inventory™ (PedsQL) diabetes module 3.0 for

Turkish adolescents with type 1 diabetes. Int J Diabetes Dev Ctries. 2015;35(2):109–16.
10. Smart CE, Annan F, Bruno LPC, Higgins LA, Acerini CL. Nutritional management in children and adolescents with diabetes. Pediatr Diabetes. 2014;15(20):135–53.
11. Nansel TR, Laffel LM, Haynie DL, Mehta SN, Lipsky LM, Volkening LK, et al. Improving dietary quality in youth with type 1 diabetes: randomized clinical trial of a family-based behavioral intervention. Int J Behav Nutr Phys Act. 2015;12(1):1–11.
12. Mayer-Davis EJ, Liese NM, Bell RA, Dabelea DM, Johansen JM, Pihoker C, et al. Dietary intake among youth with diabetes: the search for diabetes in youth study. J Am Diet Assoc. 2006;106(5): 689–97.
13. Nansel TR, Haynie DL, Lipsky LM, Laffel LMB, Mehta SN. Multiple indicators of poor diet quality in children and adolescents with type 1 diabetes are associated with higher body mass index percentile but not glycemic control. J Acad Nutr Diet. 2012;112: 1728–35.
14. Katz ML, Mehta S, Nansel T, Quinn H, Lipsky LM, Laffel LM. Associations of nutrient intake with glycemic control in youth with type 1 diabetes: differences by insulin regimen. Diabetes Tech Therapeutics. 2014;16(8):512–8.
15. Helgeson VS, Viccaro L, Becker D, Escobar O, Siminerio L. Diet of adolescents with and without diabetes: trading candy for potato chips? Diabetes Care. 2006;29(5):982–7.
16. World Health Organization. Growth reference 5-19 years. 2007. Available at: http://www.who.int/growthref/tools/en/. Accessed 23 Dec 2019.
17. BeBiS. Nutrition Data Base Software Data Base. The German Food Code and Nutrient Data Base (BLS II.3, 1999) with additions from UDSA-sr and other sources. Istanbul, 2004.
18. Ellis D, Templin T, Podolski C, Naar-King S, Moltz K. The parental monitoring of diabetes care scale: development, reliability and validity of a scale to evaluate parental supervision of adolescent illness management. J Adolesc Health. 2008;42(2):146–53.
19. Türk Ç, Karataş H, Bektaş M. Tip 1 Diyabetli Adölesanlarda Diyabet Bakımında Ebeveyn İzlemi Ölçeğinin Geçerlik ve Güvenirlik Çalışması. J Pediat Res. 2016;3(1):35–40.
20. Varni JW, Burwinkle TM, Jenifer R, Jacobs JR, Gottschalk M, Kaufman F, et al. The PedsQL™ in type 1 and type 2 diabetes reliability and validity of the Pediatric Quality of Life Inventory™ generic core scales and type 1 diabetes module. Diabetes Care. 2003;26:631–7.
21. Guenther PM, Kirkpatrick SI, Reedy J, Krebs-Smith SM, Buckman DW, Dodd KW, Casavale KO, Carroll RJ. The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for Americans. J Nutr. 2014;144: 399–407.
22. Moore SM, Hackworth NJ, Hamilton VE, Northam EP, Cameron FJ. Adolescents with type 1 diabetes: parental perceptions of child health and family functioning and their relationship to adolescent metabolic control. Health Qual Life Outcomes, 2013;11(1):1-8.
23. Abdul-Rasoul M, AlOtaibi F, Abdulla A, Rahme Z, AlShawaf F. Quality of life of children and adolescents with type 1 diabetes in Kuwait. Med Princ Pract. 2013;22(4):379–84.
24. Sand P, Kljajić M, Schaller J, Forsander G. The reliability of the health related quality of life questionnaire PedsQL 3.0 Diabetes ModuleTM for Swedish children with type 1 diabetes. Acta Paediatr. 2012;101(8):e344–9.
25. Lukacs A, Varga B, Barótfi S,Kiss-Toth E, Barkai L.Health related quality of life of youths with type 1 diabetes: reliability and validity of the Hungarian version of the PedsQL 3.0 diabetes module. J Diabetes Metab. 2012;3(4):2–6.
26. d'Annunzio G, Gialetti S, Carducci C, Rabbone I, Lo Presti D, Toni S, Zito E, Bolloli S, Lorini R, Della Casa Alberighi O. Italian translation, cultural adaptation and validation of the PedsQL™ 3.0 diabetes module questionnaire in children with type 1 diabetes and their parents. Health Qual Life Outcomes. 2014;12:115.
27. Kalyva E, Malakonaki E, Eiser C, Mamoulakis D. Health related quality of life (HRQoL) of children with type 1 diabetes mellitus (T1DM): self and parental perceptions. Pediatr Diabetes. 2011;12(1):34–40.
28. Jaser SS. Family interaction in pediatric diabetes. Curr Diab. 2011;11(6):480–5.
29. Anderson B, Ho J, Brackett J, Finkelstein D, Laffel L. Parental involvement in diabetes management tasks: relationships to blood glucose monitoring adherence and metabolic control in young adolescents with insulin-dependent diabetes mellitus. J Pediatr. 1997;130:257–65.
30. Gowers SG, Jones JC, Kiana S, North CD, Price DA. Family functioning: a correlate of diabetic control? J Child Psychol Psychiatry. 1995;36:993–1001.
31. Patton SR. Adherence to diet in youth with type 1 diabetes. J Am Diet Assoc. 2011;111(4):550–5.
32. Nansel TR, Lipsky LM, Liu A. Greater diet quality is associated with more optimal glycemic control in a longitudinal study of youth with type 1 diabetes. Am J Clin Nutr. 2016;104(1):81–7.
33. Rodrigues PRM, Luiz RR, Monteiro LS, Ferreira MG, GoncalvesSilva RMV, Pereira RA. Adolescents' unhealthy eating habits are associated with meal skipping. Nutrition. 2017;42:114-20.e1.
34. Lipsky LM, Nansel TR, Haynie DL, Mehta SN, Laffel LMB. Associations of food preferences and household food availability with dietary intake and quality in youth with type 1 diabetes. Appetite. 2012;59(2):218–23.
35. de Ferranti SD, de Boar IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Circulation. 2014;130:1110–30.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):289–290
https://doi.org/10.1007/s13410-022-01137-5

LETTER TO THE EDITOR

Hyperinsulinemia, obesity, and diabetes mellitus

Prakash SS[1]

Received: 12 May 2021 / Accepted: 24 September 2022 / Published online: 7 October 2022

Insulin clearance is an important piece of the puzzle that needs to be incorporated to gain an understanding of obesity and type 2 diabetes mellitus (T2DM). Recently, Gastaldelli and colleagues provided evidence by investigating the role of insulin clearance in a large study to understand the differences between obese and nonobese individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and T2DM [1]. Insulin clearance has been estimated both during euglycemic clamp and oral glucose tolerance test (OGTT). Hyperinsulinemia is associated with both obesity and insulin resistance [2]. Hyperinsulinemia can result from increased insulin secretory capacity and/or decreased insulin clearance. Hyperinsulinemia, especially the levels of insulin at 30 min during the OGTT, has been found to be causal to obesity [3]. In the study by Gastaldelli and colleagues hyperinsulinemia (insulin response during OGTT) is much higher in obese compared to nonobese individuals [1]. There are differences at baseline (including the conditions of NGT and IGT) between obese and nonobese individuals with respect to insulin secretory capacity and insulin clearance. Insulin clearance, especially during OGTT, is specifically concerned with the liver. One of the main manifestations of hepatic insulin resistance could be its inability to decrease insulin clearance, and this can be the case in nonobese individuals [4], whereas in obese individuals, it is the insulin resistance in adipose tissue that results in lesser demand for insulin, leading to a decreased secretory capacity [5].

Progression from NGT to IGT to diabetes is characterized by a diminished secretion of insulin due to reduced β-cell function, due to glucotoxicity and lipotoxicity apart from other mechanisms [5]. The disposition index, which is a measure of the β-cell function, is related to both hyperinsulinemia as well as insulin sensitivity [6]. Obese individuals are characterized by insulin resistance in the adipose tissue that results in increased lipolysis and lipotoxicity in other tissues, including muscle and liver [7, 8]. On the other hand, the progression of disease in lean individuals could be driven by insulin resistance in the liver, leading to inefficient insulin clearance and muscle insulin resistance. The difference between insulin resistance in liver/muscles and adipose tissue could be related to the increased 'capacity' of liver and muscle tissues to handle glucose and the increased 'capability' of adipose tissue in the efficient storage of glucose as fat in the context of the whole-body glucose metabolism [8].

Based on these observations, it can be surmised that hyperinsulinemia in obese individuals could be due to high insulin secretory capacity in the face of insulin resistance in adipose tissue to compensate but ultimately leads to β-cell failure and decreased insulin secretion, whereas in nonobese individuals it could be due to low insulin clearance because of insulin resistance in the liver [1, 4, 5, 7]. It can be envisaged that insulin secretory capacity has wider scope to increase than the scope by which insulin clearance can decrease in obese and nonobese individuals, respectively. This may partly explain why there is hyperinsulinemia in obese compared to nonobese individuals [5, 6]. Further extending this argument, in obese T2DM individuals, the main pathogenic mechanism could be the decrease in insulin secretory capacity, whereas in nonobese individuals, a decrease in insulin clearance leading to hyperinsulinemia is likely to have a major role in the pathogenesis of T2DM.

The onset of adipose insulin resistance in obese individuals and hepatic insulin resistance in nonobese individuals could be the critical events in the progression of NGT and IGT to T2DM. Longitudinal studies that mechanistically analyze obese and nonobese subjects with or without T2DM are required to clarify these hypotheses.

Declarations

Conflict of interest The author declares no competing interests.

✉ Prakash SS
sspkmc2k@yahoo.com; sspcmc@cmcvellore.ac.in

[1] Department of Biochemistry, Christian Medical College, Vellore 632002, India

References

1. Gastaldelli A, Ghani MA, DeFronzo RA. Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. Diabetes. 2021;70:377–85.
2. Prakash SS. Models that explain the cause of obesity. Indian J Endocrinol Metab. 2018;22:569–70.
3. Astley CM, Todd JN, Salem RM, Vedantam S, Ebbeling CB, Huang PL, et al. Genetic evidence that carbohydrate-stimulated insulin secretion leads to obesity. Clin Chem. 2018;64:192–200.
4. Bojsen-Møller KN, Lundsgaard AM, Madsbad S, Kiens B, Holst JJ. Hepatic insulin clearance in regulation of systemic insulin concentrations—role of carbohydrate and energy availability. Diabetes. 2018;67:2129–36.
5. Gastaldelli A, Gaggini M, DeFronzo RA. Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the San Antonio Metabolism Study. Diabetes. 2017;66:815–22.
6. Defronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009;58:773–95.
7. Smith U, Kahn BB. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med. 2016;280:465–75.
8. Fazakerley DJ, Krycer JR, Kearney AL, Hocking SL, James DE. Muscle and adipose tissue insulin resistance: malady without mechanism? J Lipid Res. 2019;60:1720–32.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):291–297
https://doi.org/10.1007/s13410-022-01064-5

ORIGINAL ARTICLE

Serum asymmetric dimethylarginine (ADMA) level and cognitive dysfunction in diabetic patients

Ali Arab[1] · Anahita Zakeri[2] · Mehriar Nadermohammadi[3] · Sara Mostafalou[1]

Received: 29 September 2021 / Accepted: 11 February 2022 / Published online: 18 March 2022

Abstract

Background Diabetes is a chronic disease with lots of health complications and has been shown to reduce memory function and cognitive abilities. It has been suggested that diabetic microvascular complications may be involved in the pathophysiology of mild cognitive dysfunction. Raised levels of asymmetric dimethylarginine (ADMA) in the blood have been linked to the risk of vascular disorders.

Objective This study was conducted to evaluate the serum level of ADMA in diabetic patients and its association with cognitive function and metabolic parameters.

Methods In this cross-sectional study, 169 diabetic patients were enrolled. Metabolic parameters including body mass index, fasting blood sugar, fasting blood insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and ADMA were measured. Cognitive function was evaluated using the Wechsler Adult Intelligence Scale-Revised (WAIS-R).

Results Positive correlations were found between all performed subtests of WAIS-R including information, vocabulary, digit-span, picture completion, and block design subtest scores. The subtests of WAIS-R were associated negatively with age and positively with educational status. Serum ADMA was negatively associated with picture completion score after adjustment by age and educational status, significantly (β=−0.191, p<0.049).

Conclusion The involvement of ADMA in the pathogenesis of diabetic cognitive dysfunction is plausible and further studies are required to clarify the mechanism underlying the link of diabetic complications with cognitive decline.

Keywords Asymmetric dimethylarginine · Cognitive impairment · Diabetes · Insulin resistance · Wechsler Adult Intelligence Scale

Introduction

Diabetes-related complications have been traditionally known to originate from vascular damage [1]. Therefore, cardiovascular disorders which are a major cause of disability among diabetic patients occur earlier compared to nondiabetics [2]. There is growing evidence on the involvement of vascular disorders in the development of cognitive impairment, dementia, Alzheimer's disease, and brain structural changes [3]. In this regard, various neuropsychological studies have shown that diabetes may reduce memory function and cognitive abilities including learning, processing speed, and attention [4]. It is believed that vascular disorders are strongly associated with oxidative stress [5]. Oxidative stress gives rise to endothelial dysfunction which is an early step of atherosclerosis. Key molecular mechanisms which lead to the promotion of atherosclerosis are inhibited by nitric oxide (NO) [6].

NO is an endothelium-derived vasodilating factor that is released from normal endothelium cells to keep a balance between vasodilation and vasoconstriction [7]. Two other main protective mechanisms of NO in the cardiovascular system are inhibition of platelet aggregation and suppression of smooth muscle proliferation [8]. In addition to the role of NO deficiency in the pathogenesis of the cardiovascular disease, NO has a well-documented role in cognitive decline [3, 9]. Compromised NO activity has been related to cognitive

✉ Sara Mostafalou
s.mostafalou@gmail.com; s.mostafalou@arums.ac.ir

[1] Department of Pharmacology and Toxicology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil 5618953141, Iran

[2] Department of Internal Medicine, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

[3] Department of Psychology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

impairment due to reduced cerebral blood flow, reduced oxygen supply of the brain, and disruption of neurovascular function [10]. A possible mechanism for NO deficiency is the reduction of NO synthesis by elevating asymmetric dimethylarginine (ADMA) serum levels. ADMA is a derivative of L-arginine and a by-product of protein modifications [11, 12]. ADMA is an endogenous, competitive inhibitor of NO synthase (NOS) [13]. Raised plasma ADMA level has been linked to the vascular pathophysiology of atherosclerosis and has also been suggested as a predictor of cardiovascular mortality [14]. Thus far, most studies have focused on the association of elevation in ADMA levels with cardiovascular risk factors such as hypertension, obesity, and diabetes but only a few studies have linked ADMA levels to cognitive functions [15].

In the present cross-sectional study, we primarily aimed at understanding the association of serum level of ADMA in diabetic patients with their cognitive functions. Our secondary aim was to evaluate the relationship of ADMA levels with various demographic factors and medicines.

Materials/patients and method

Study area and population

Diabetic patients were recruited from the Endocrinology Clinic, Imam Khomeini Hospital, Ardabil, Iran, from December 2019 to August 2020 by a convenience sampling method. All participants were Iranian and lived in the province of Ardabil in the northwest of Iran.

To be included, individuals had to be 18 years old and over with adequate vision and hearing and previously diagnosed with diabetes type I or II by an internist specialist. Patients with clinical signs or self-report of familial mental retardation, major systemic disorder, current psychiatric disorder, stroke, brain trauma or surgery, encephalitis, and/or ongoing anemia were excluded. The eligible population was informed about the project and assured that the whole procedure will be free. A total of 169 patients accepted to participate in the study and completed the predetermined subtests of WAIS-R but 41 of them did not consent (either not replied or refused) to take a blood test to provide a blood sample.

Ethical considerations

All procedures described in the study were approved by the ethics committee of Ardabil University of Medical Sciences and received the ethics approval code IR.ARUMS.REC.1398.575. Written consent was signed by all of the participants prior to the beginning of the study and the identity of patients was not mentioned. In this study, no cost was imposed on the patient and all costs were borne by the council.

Assessment of cognitive function

Cognitive function was evaluated using the Persian version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R). WAIS-R measures various abilities which may be lowered by brain damage. Five subtests of WAIS-R, namely information, vocabulary, digit-span, picture completion, and block design, were recommended to assess cognitive functioning (Table 1). Information and vocabulary are subtests of verbal comprehension index, digit-span is a subtest of working memory index, and picture completion and block design are subtests of perceptual organization index of WAIS-R [16]. Each participant completed at least four subtests. More than four subtests were performed only in patients who wished to continue. The assessment was completed by three trained psychologists.

Blood sample collection and laboratory techniques

Peripheral venous blood was collected from each fasted individual via venipuncture. The serum from each sample was extracted after centrifugation of the blood samples. Measurement of glucose and insulin concentration was performed on the fresh sera and the remained samples were stored at −70°C until the time of the other biochemical analyses.

Measurement of fasting serum glucose

Fasting serum glucose was determined directly by an enzymatic colorimetric method, glucose oxidase-phenol, and 4 aminophenazone (GOD-PAP), using commercially available kits (Pars Azman Co., Karaj, Iran) with an assay range of 5 to 400 mg/dL (Cat. No: 117500).

Measurement of serum insulin

Fasting serum insulin concentrations were measured by a solid-phase enzyme-linked immunosorbent assay (ELISA) human insulin kit (Demeditec Diagnostics GmbH, Germany), according to the manufacturer's instructions (Cat. No: DE2935).

Assessment of insulin resistance

As a marker of insulin resistance, homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by using the following formula: insulin (mU/mL) × glucose (mg/dL) / 405 [17].

Measurement of serum ADMA

Serum levels of ADMA were assessed using a quantitative ELISA kit (Hangzhou Eastbiopharm Co., Ltd, China), with

Table 1 WAIS-R subtests' outcome measures and neurologic functions administered to diabetic patients

Subtests	Outcome measures	Neurologic functions
Information	• The general fund of knowledge	• Long-term memory functions
	• The ability to access and express this information	• Working memory
Vocabulary	• Word knowledge	• Long-term memory
	• The ability to access and to effectively communicate that knowledge	• Working memory
	• Expressive and receptive language skills	
Digit-span	• Mental manipulation	• Memory span
	• Attention	• Short-term verbal memory
Picture completion	• Ability to recognize familiar items	• Visual memory
	• Ability to identify missing parts	
	• Conceptual reasoning skills	
	• Visual scanning	
Block design	• Ability to construct a design to a model	• Visual-spatial processing and integration

an assay range of 200 to 60,000 ng/L and a sensitivity of 100.21 ng/L (Cat. No: CK-E11310).

Other variables

The relevant sociodemographic information, including age, sex, occupation, weight, height, education, duration of diabetes, and medications, were collected through a personal interview unless patients' medical history was available. Background cardiovascular disorders were ascertained based on participants' medical history and/or self-report of medication use.

Statistical analysis

Participants' sociodemographic and medical information were analyzed using frequency, mean, and standard deviation (SD). The Pearson correlation analysis was used to investigate the possible associations between covariates, WAIS-R subtests, and ADMA levels. The Spearman rank-order analysis was assessed to find out the associations of WAIS-R subtest scores with educational status. The association between serum ADMA levels and WAIS-R subtests was also assessed by multiple linear regression and adjusted for potential confounders. The Shapiro-Wilk test was used for evaluating the normal distribution of the variables. The Mann-Whitney *U* test was used to compare non-normally distributed variables between gender, medicine, and background disease groups. The p values lower than 0.05 were considered statistically significant. All data analyses were carried out using SPSS 26.0.

Results

The characteristics of the study population are presented in Table 2. The mean age of the study population was 57.3 years and the mean duration of diabetes was about 9.7 years. Over half of the subjects were women (57.8%) and almost all of the patients were married (98.4%). Half of the subjects were uneducated and two-third of them were unoccupied. More than 64% of the population had at least one first-degree family member with diabetes. Nearly to half of the patients had cardiovascular diseases or dyslipidemia, and 10% of the patients had thyroid dysfunction as well. The frequency of use of medicines in diabetic patients of our study was as follows: metformin 75.8%, losartan 37.5%, aspirin 28.9%, atorvastatin 40.6%, and insulin 21.9%. The results of WAIS-R tests showed that the mean values of Full-Scale Intelligence Quotient (FSIQ), Performance Intelligence Quotient (PIQ), and Verbal Intelligence Quotient (VIQ) of the patients were 81.12, 77.62, and 88.53, respectively. Based on the FSIQ grade, it became clear that 77.3% of the patients had different stages of cognitive impairment while only 22.7% of the patients were diagnosed to have normal cognitive functions. The mean values of body mass index (BMI) and HOMA-IR in the patients were estimated to be respectively 29.3 and 4.8, both of which are considered higher than the normal range. The mean concentration of ADMA in the serum of the patients was also estimated to be 11.791 ng/mL (Table 2).

Fast blood sugar (FBS), fast blood insulin (FBI), FSIQ, and ADMA levels were not normally distributed. The results of the comparison of the mean variables (by the Mann-Whitney *U* test) for gender groups indicated that only FSIQ was significantly higher in men than in women. It is worth mentioning that further analysis has shown that in this study population men were more educated than women (chi-score=15.14,

Table 2 Characteristics of the study population (type 2 diabetic patients enrolled from Endocrinology Clinic, Imam Khomeini Hospital, Ardabil, Iran, from December 2019 to August 2020)

Characteristics	*n* (%)	Mean ± SD
Gender		
Male	54 (42.2)	-
Female	74 (57.8)	-
Marital status		
Single	2 (1.6)	-
Married	126 (98.4)	-
Occupational status		
Occupied	43 (33.6)	-
Unoccupied	85 (66.4)	-
Educational status		
Illiterate	64 (50)	-
Undergraduate	41 (32)	-
College education	23 (18)	-
Family history of diabetes		
At least one first-degree	82 (64.1)	-
No first-degree	46 (35.9)	-
DCD (based on FSIQ)		
Severe	29 (22.7)	-
Moderate	40 (31.5)	-
Mild	29 (22.7)	-
No	29 (22.7)	-
Background disease		
Cardiovascular disorders	66 (51.6)	-
Dyslipidemia	63 (49.2)	-
Thyroid dysfunction	14 (10.9)	-
Medicine		
Metformin	97 (75.8)	-
Losartan	48 (37.5)	-
Aspirin	37 (28.9)	-
Atorvastatin	52 (40.6)	-
Insulin	28 (21.9)	-
Age (years)	-	57.2±10.8
Duration of diabetes (years)	-	9.7±6.9
FSIQ	-	81.12±13.24
VIQ	-	77.62±13.20
PIQ	-	88.53±16.57
BMI (kg/m^2)	-	29.3±5.1
FBS (mg/dL)	-	173.6±66.6
FBI (mU/mL)	-	11.62±7.97
HOMA-IR	-	4.8±3.3
ADMA (ng/mL)	-	11.791±8.432

ADMA asymmetric dimethylarginine, *BMI* body mass index, *DCD* diabetic cognitive dysfunction, *FBI* fasting blood insulin, *FBS* fasting blood sugar, *FSIQ* Full-Scale Intelligence Quotient, *HOMA-IR* homeostatic model assessment-insulin resistance, *PIQ* Performance Intelligence Quotient, *SD* standard deviation, *VIQ* Verbal Intelligence Quotient

$p<0.0005$). The serum level of ADMA in men was shown to be higher than in women but this difference was not statistically significant (Table 3).

Among sociodemographic information, age of participants had a weak negative correlation with duration of diabetes ($r=-0.248$, $p<0.005$), and HOMA-IR was only significantly correlated to BMI ($r=0.281$, $p<0.05$) (data were not shown).

Educational status had been correlated negatively with age (rho=−0.401, $p<0.001$) and positively with information (rho=0.336, $p<0.001$), vocabulary (rho=0.471, $p<0.001$), digit-span (rho=0.436, $p<0.002$), picture completion (rho=0.299, $p<0.001$), and block design (rho=0.217, $p<0.016$) subtest scores (Table 4). Age of participants had a weak negative correlation with information, picture completion, and block design ($r=-0.223$ to $r=-0.297$, $p<0.05$), and a stronger negative correlation with vocabulary subtests ($r=-0.427$, $p<0.001$). The potential correlation between serum levels of ADMA and subtests of WAIS-R was evaluated by performing multiple linear regression analysis. Results of the multiple linear regression analysis are presented for crude and adjusted analyses, separately. Among the five most performed subtests of WAIS-R in this study, namely information, vocabulary, digit-span, picture completion, and block design, serum levels of ADMA were only negatively associated with picture completion score after adjustment of results by age and educational status, significantly ($\beta=-0.191$, $p<0.049$) (Table 4).

Comparison of serum ADMA level in the patients with and without some health problems and use of medicines was also done. The serum ADMA levels of participants with and without cardiovascular disease, dyslipidemia, and thyroid dysfunction were compared by the same test separately and no significant results have been found. Although, mean serum ADMA

Table 3 Comparison of mean of variable by the Mann-Whitney *U* test for gender groups of the study population (type 2 diabetic patients enrolled from Endocrinology Clinic, Imam Khomeini Hospital, Ardabil, Iran, from December 2019 to August 2020, *n*=128)

Variable	Gender	Mean	SD	*p* value
FBS (mg/dL)	Male	171.51	67.04	0.760
	Female	175.17	66.75	
FBI (mU/mL)	Male	11.11	5.98	0.761
	Female	11.99	9.17	
FSIQ	Male	84.57	15.61	0.012*
	Female	78.65	10.69	
ADMA (ng/mL)	Male	13.47	9.30	0.064
	Female	10.57	7.57	

ADMA asymmetric dimethylarginine, *FBS* fasting blood sugar, *FBI* fasting blood insulin, *FSIQ* Full-Scale Intelligence Quotient, *SD* standard deviation

*p value<0.05

Table 4 Association of the main five WAIS-R subtests' scores with age, educational status, and serum ADMA level of the study population (type 2 diabetic patients enrolled from Endocrinology Clinic, Imam Khomeini Hospital, Ardabil, Iran, from December 2019 to August 2020, *n*=128)

		Information (*n*=128)	Vocabulary (*n*=82)	Digit-span (*n*=73)	Picture completion (*n*=128)	Block design (*n*=124)
Age (Pearson corr.)	*r*	−0.223*	−0.427**	−0.182	−0.285**	−0.297**
	p value	0.012	0.000	0.124	0.001	0.001
Education (Spearman corr.)	rho	0.366**	0.471**	0.436**	0.299**	0.217*
	p value	0.000	0.000	0.000	0.001	0.016
ADMA unadjusted	*β*	−0.071	−0.074	0.088	−0.150	−0.91
	p value	0.445	0.528	0.477	0.107	0.337
ADMA adjusted[a]	*β*	−0.129	−0.064	−0.016	−0.191*	−0.097
	p value	0.188	0.646	0.902	0.049	0.319

ADMA asymmetric dimethylarginine

[a] Adjusted by age (years) and education status (illiterate, undergraduate, and college education)

**p* value<0.05, ***p* value<0.005

levels were higher in losartan, aspirin, atorvastatin, and insulin users compared to non-users, the Mann-Whitney *U* test result has shown no significant difference in any of these groups (data were not shown).

Discussion

The association of cardiovascular disorders with cognitive impairment is well accepted but researches on related biomarkers are still ongoing. Prior studies have suggested significant links between an increase in ADMA levels and Alzheimer's disease, reduction of verbal memory score, subjective memory impairment, and declined cognitive performance, but none of them evaluated cognitive functions of diabetic patients [11, 18]. To our knowledge, this is the first study on the relationship between serum ADMA levels and cognitive functions in diabetic patients. The results have shown a significant negative correlation between ADMA levels and picture completion score which is a subtest of performance IQ of WAIS-R and mostly related to conceptual relevancy judgment, visual recognition, perceptual organization, and long-term memory [19].

Evidences have shown that ADMA levels can be influenced by supplements and drug interventions [20]. As ADMA is a competitive inhibitor of NOS, an increase in the level of L-arginine and reduction in serum level of ADMA lead to an increase in the ratio of L-arginine/ADMA. Various studies have shown that L-arginine consumption may reduce the risk of cardiovascular disorders [21]. Therefore, there may be possible links between L-arginine consumption and the prevention of cognitive decline. Synthesis of a selective drug by means of lowering ADMA levels has also been demonstrated in the Maas et al. (2005) study [22]. In our study, a few medicines which are frequently used by diabetic patients have been evaluated based on their effects on ADMA levels. However, the results of comparisons of serum ADMA levels in users and non-users of specific medicines and also in patients with and without health conditions were not statistically significant and the results were mostly consistent with previous studies. For example, the Nishiyma et al. (2011) study on 56 statin users including atorvastatin users and 58 non-users has shown that ADMA levels are lower in statin users [23]. Our study has shown the same result. About losartan, the Ito et al. (2001) study showed 8 weeks of drug therapy by losartan leads to reduction in ADMA levels [24]. The losartan users in our study also had a lower mean of ADMA levels. The Mehmetoğlu et al. (2011) study proved that only high doses of aspirin affect ADMA levels [25]. In our study, the mean ADMA levels were only slightly different in users and non-users of aspirin. Contrary to the results of Asagami et al. and Hsu et al. studies, our findings have shown that mean ADMA levels were lower in insulin users and higher in metformin users [26, 27]. This observation can be explained by a lower risk of cardiovascular disorders in type I diabetic patients (insulin users) comparing to type II diabetic patients (metformin users) [28].

However, the researches provide evidence to support the hypothesis of increasing serum ADMA levels in both cardiovascular disorders and dyslipidemia [29]. But about thyroid dysfunction, unlike the Arikan et al. (2007) study, our findings have shown lower levels of ADMA in patients with thyroid dysfunction [30]. This difference might be due to the methods used for diagnostic criteria. Diagnosis of thyroid dysfunction in our study was based on the medical history of the patients and their medication while the Arikan et al. (2007) study included recently diagnosed patients with laboratory examinations.

However, our study had some limitations, among which its cross-sectional nature must be mentioned. As ADMA and L-arginine compete with each other on NOS, it is better to assess L-arginine levels at the same time to calculate L-arginine/ADMA ratio as well. Moreover, serum ADMA levels of patients were not influenced by a single drug, and it is not clear that these drugs lower ADMA levels by ameliorating patient's health conditions or by a specific mechanism related to ADMA levels. In this way, future research focusing on the mechanistic role of ADMA pathway in the cognitive problems may give a clear perspective on the ways through which metabolic disorders and environmental factors can lead to the neurological problems in the suffering patients.

Conclusion

The results of the present study indicate the association of increased serum levels of ADMA and reduced cognitive functions. Therefore, the involvement of ADMA in the pathogenesis of cognitive decline in diabetic patients is likely. Further prospective studies are required to clarify the mentioned mechanism and assess the impact of reduction of serum ADMA levels on a decrease in diabetic complications including microvascular complications and also cognitive decline.

Author contribution Conceptualization: Sara Mostafalou; Sample collection: Anahita Zakeri, Ali Arab; WAIS-R examination: Mehriar Nadermohammadi; Biochemical analyses: Sara Mostafalou, Ali Arab; Statistical analyses: Ali Arab; Writing of manuscript: Sara Mostafalou, Ali Arab

Funding This work was supported by the Council of Research at Ardabil University of Medical Sciences, Ardabil, Iran (Approval code: 2843).

Data availability The authors confirm that all data and materials as well as software application or custom code support their published claims and comply with field standards.

Declarations

Ethics approval This work was approved by the Regional Research Ethics Committee at Ardabil University of Medical Sciences, Ardabil, Iran (Ethics code: IR.ARUMS.REC.1398.575).

Consent to participate All patients signed a written consent to participate to this work.

Consent to publication All authors declare their consent for publication of this work.

Conflict of interest The authors declare no competing interests.

References

1. Mauricio D, Alonso N, Gratacòs M. Chronic diabetes complications: the need to move beyond classical concepts. Trends Endocrinol Metab. 2020;31(4):287–95.
2. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019;62(1):3–16.
3. Stephan BCM, Harrison SL, Keage HAD, Babateen A, Robinson L, Siervo M. Cardiovascular disease, the nitric oxide pathway and risk of cognitive impairment and dementia. Curr Cardiol Rep. 2017;19(9):87.
4. Palta P, Schneider AL, Biessels GJ, Touradji P, Hill-Briggs F. Magnitude of cognitive dysfunction in adults with type 2 diabetes: a meta-analysis of six cognitive domains and the most frequently reported neuropsychological tests within domains. J Int Neuropsychol Soc. 2014;20(3):278–91.
5. Steven S, Frenis K, Oelze M, Kalinovic S, Kuntic M, Bayo Jimenez MT, Vujacic-Mirski K, Helmstädter J, Kröller-Schön S, Münzel T, Daiber A. Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. Oxidative Med Cell Longev. 2019;2019:7092151–26.
6. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res. 2017;120(4):713–35.
7. Vinik A, Flemmer M. Diabetes and macrovascular disease. J Diabetes Complicat. 2002;16(3):235–45.
8. Lei J, Vodovotz Y, Tzeng E, Billiar TR. Nitric oxide, a protective molecule in the cardiovascular system. Nitric Oxide. 2013;35:175–85.
9. Ryan CM, Williams TM. Effects of insulin-dependent diabetes on learning and memory efficiency in adults. J Clin Exp Neuropsychol. 1993;15(5):685–700.
10. Toda N, Ayajiki K, Okamura T. Cerebral blood flow regulation by nitric oxide in neurological disorders. Can J Physiol Pharmacol. 2009;87(8):581–94.
11. Selley MJN. Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. J Diabetes Complicat. 2003;24(7):903–7.
12. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation. 2002;106(8):987–92.
13. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PAM. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Anal Biochem. 2002;303(2):131–7.
14. Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E, Chowdhury R. Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. J Am Heart Assoc. 2015;4(6):e001833.
15. Dowsett L, Higgins E, Alanazi S, Alshuwayer NA, Leiper FC, Leiper J. ADMA: a key player in the relationship between vascular dysfunction and inflammation in atherosclerosis. J Clin Med. 2020;9(9):3026.
16. Franzen MD. The Wechsler Adult Intelligence Scale-Revised and Wechsler Adult Intelligence Scale-III. In. Reliability and validity in neuropsychological assessment. Boston, MA: Springer US; 2002. p. 55-70.

17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
18. Malden DE, Mangoni AA, Woodman RJ, Thies F, McNeil C, Murray AD, Soiza RL. Circulating asymmetric dimethylarginine and cognitive decline: a 4-year follow-up study of the 1936 Aberdeen Birth Cohort. Int J Geriatr Psychiatry. 2020;35(10): 1181–8.
19. van Ommen C. Putting the PC in IQ: images in the Wechsler Adult Intelligence Scale - Third edition (WAIS III). S Afr J Psychol. 2005;35:532–54.
20. Be[3]towski J, Kêdra A. Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy. Pharmacol Rep 2006;58(159):159-178.
21. Rodrigues-Krause J, Krause M, Rocha IMG, Umpierre D, Fayh APT. Association of L-arginine supplementation with markers of endothelial function in patients with cardiovascular or metabolic disorders: a systematic review and meta-analysis. Nutrients. 2019;11(1):15.
22. Maas RJVM. Pharmacotherapies and their influence on asymmetric dimethylarginine (ADMA). Vasc Med. 2005;10(1_suppl):S49–57.
23. Nishiyama Y, Ueda M, Otsuka T, Katsura K-i, Abe A, Nagayama H, Katayama Y. Statin treatment decreased serum asymmetric dimethylarginine (ADMA) levels in ischemic stroke patients. J Atheroscler Thromb. 2011;18(2):131–7.
24. Ito A, Egashira K, Narishige T, Muramatsu K, Takeshita A. Renin-angiotensin system is involved in the mechanism of increased serum asymmetric dimethylarginine in essential hypertension. Jpn Circ J. 2001;65(9):775–8.
25. Mehmetoğlu İ, Kurban S. Effects of two different doses of acetylsalicylic acid on serum nitric oxide, asymmetric dimethylarginine, and homocysteine levels in healthy volunteers. Turk J Med Sci. 2012;42(2):269–74.
26. Asagami T, Abbasi F, Stuelinger M, Lamendola C, McLaughlin T, Cooke JP, Reaven G, Tsao P. Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. Metabolism. 2002;51(7):843–6.
27. Hsu C-P, Hsu P-F, Chung M-Y, Lin S-J, Lu T-M. Asymmetric dimethylarginine and long-term adverse cardiovascular events in patients with type 2 diabetes: relation with the glycemic control. Cardiovasc Diabetol. 2014;13(1):156.
28. De Ferranti SD, De Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Circulation. 2014;130(13):1110–30.
29. Böger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from prospective clinical trials. Vasc Med. 2005;10(1_suppl):S19–25.
30. Arikan E, Karadag CH, Guldiken S. Asymmetric dimethylarginine levels in thyroid diseases. J Endocrinol Investig. 2007;30(3):186–91.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):298–303
https://doi.org/10.1007/s13410-022-01066-3

ORIGINAL ARTICLE

Association of serum level of chemerin with visceral fat obesity in type 2 diabetic patient

Shaheena Qazi[1] · Iftikhar Ahmed Siddiqui[1] · Muhammad Saeed[1] · Kahkashan Perveen[1] · Kulsoom Baqa[2] · Asher Fawwad[1,2]

Received: 14 February 2020 / Accepted: 22 March 2022 / Published online: 12 April 2022

Abstract

Aim and objective This study aimed to investigate whether circulating chemerin levels might be associated with visceral obesity and other metabolic parameters in subject with newly diagnosed type 2 diabetes.

Methodology The study was conducted at Baqai Institute of Diabetology and Endocrinology (BIDE), Baqai Medical University (BMU), Karachi. The study was carried out after ethical approval from ethics committee of BMU. Type 2 diabetic subjects ≥ 20 years of age and without any complication were included whereas subjects with type I diabetes mellitus, gestational diabetes, and acute or chronic illness were excluded. A total of 75 subjects participated in the study and were divided into three groups, (n=25) normal glucose tolerance (NGT), (n=25) type 2 diabetes (T2DM), and (n=25) impaired glucose tolerance (IGT) according to oral glucose tolerance test (OGTT) [1].

Results A total of 75 subjects participated in the study, out of which 25 subjects were newly diagnosed with type 2 diabetes (T2DM) and 25 subjects had impaired glucose tolerance (IGT) while 25 individuals had normal glucose tolerance (NGT). Subjects with NGT had significantly lower FBS and RBS level than subjects with IGT and T2DM. HbA1c was significantly higher in subjects with T2DM whereas chemerin level was significantly lower in T2DM as compared to subjects with NGT and IGT. Among subjects with T2DM, it was observed that only HbA1c had positively strong correlation with chemerin level (r=0.645, p=0.001) while FBS had positively moderate correlation with chemerin level (r=0.471, p=0.017). Correlation of chemerin with diastolic blood pressure, RBS, and HDL was also moderate but non-significant.

Conclusion Newly diagnosed diabetic subjects had low serum chemerin levels with obesity compared to healthy individuals, which could indicate a possibility of lifestyle modification practice in T2DM subjects.

Keywords Chemerin · Type 2 diabetes · Visceral obesity · Impaired glucose tolerance

Introduction

Incidence of diabetes mellitus is rising rapidly worldwide as a result of modifications in lifestyle, such as unhealthy diet and physical inactivity. Sedentary lifestyle and obesity have been considered the main lifestyle-associated risk factors for causing diabetes. It has been proved that diabetes can be prevented through lifestyle intervention, including

✉ Asher Fawwad
asherfawwad@bide.edu.pk; asherfawwad@baqai.edu.pk

Shaheena Qazi
drshaheena.qazi@gmail.com

Iftikhar Ahmed Siddiqui
dr.iftikhar@baqai.edu.pk

Muhammad Saeed
drmuhammadsaeedk@gmail.com

Kahkashan Perveen
kahkashanadib@yahoo.com

Kulsoom Baqa
kbaqa@bide.edu.pk

[1] Department of Biochemistry, Baqai Medical University, Karachi, Pakistan

[2] Research Department, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Plot No. 1-2, II-B, Nazimabad No2, Karachi 74600, Pakistan

weight loss and increasing physical activity [2]. According to the National Diabetes Survey (NDSP 2016-17), prevalence of generalized obesity in Pakistan is 57.9% (42% in males and 58% in females) and central obesity is 73.1% (37.3% in males and 62.7% in females) as per WHO Asia pacific cutoffs [3]. Furthermore, the population of Pakistan is 207.77 million, out of which 48.21 million are hypertensive and 27.4 million are suffering from type 2 diabetes (T2DM) [3].

Adipose tissue synthesizes many kinds of adipokines such as chemerin, leptin, adipocyte fatty acid-binding protein (A-FABP), omentin, retinol resisting binding protein-4 (RBP-4), and adiponectin. Excessive secretion of adipokines causes chronic inflammation and has a key role in the development of insulin resistance in subjects with T2DM [4]. Chemerin also plays an important role in monitoring the metabolism of adipocytes and controlling the rate of adipogenesis. These effects of chemerin can be augmented through novel therapeutic methods for obesity treatment [5, 6]. Serum chemerin is positively associated with several indicators of metabolic syndrome such as fasting serum insulin (FSI), serum triglyceride (TG), fasting serum glucose (FPG), low-density lipoprotein (LDL), body mass index (BMI), and homeostasis model assessment for insulin resistance (HOMA-IR) [7].

The relationship between serum chemerin level and visceral fat amount in subjects with newly diagnosed type 2 diabetes has not been well studied yet, and this relationship may be different in individuals with or without treatment [8]. Therefore, this study aimed to investigate whether circulating chemerin levels are associated with visceral obesity and other metabolic parameters in subject with newly diagnosed type 2 diabetes.

Methodology

The study was conducted at Baqai Institute of Diabetology and Endocrinology (BIDE), Baqai Medical University (BMU), Karachi. Type 2 diabetic subjects ≥ 20 years of age and without any complication were included whereas subjects with type I diabetes mellitus, gestational diabetes, and acute or chronic illness were excluded. A total of 75 subjects participated in the study and were divided into three groups, (n=25) with normal glucose tolerance (NGT), (n=25) with type 2 diabetes (T2DM), and (n=25) with impaired glucose tolerance (IGT) according to oral glucose tolerance test (OGTT) [1]. A self-structured questionnaire was developed and details of the person's demographic, medical, socioeconomic history, and physical examination were recorded.

Anthropometric measurements

Physical parameters included weight, height, body mass index (BMI), and waist and hip circumference and BMI was calculated as follows.

$$\text{Body mass index (BMI)} = \text{weight in kilograms}/\text{height in meters square}$$

Weight and height were measured at the same time when the individual was standing on a balance beam scale with light clothing, and without shoes. Height was determined nearest to 0.1 cm and weight closest to 0.1 kg by means of a stadiometer. Waist and hip circumference was measured from the mid-point between the center point of the lower margin of the ribs and iliac crest [9].

Blood pressure was measured with a mercury sphygmomanometer. To minimize the chances of error, participants had taken a 10-min break in a resting position before the recording of blood pressure and mean of the two readings was taken [9].

Biochemical parameters

Biochemical analysis of HbA1c, fasting and random blood sugar, serum chemerin, serum triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low-density lipoproteins (VLDL), and total cholesterol was performed. Fasting and random blood sugar levels of the study participants were estimated by the GOD-PAP method [10]. HbA1C was estimated by the HPLC method on Bio-Rad D10 analyzer. Blood sample of 2 ml volume was drawn in a vacutainer containing ethylenediaminetetraacetic acid (EDTA). The D-10 analyzer calibrated automatically with the calibrator loaded [10]. HDL cholesterol was estimated by Immuno FS, a homogeneous method for HDL cholesterol measurement. Antibodies against human lipoproteins were used to form antigen-antibody complexes with LDL (low-density lipoproteins), VLDL (very low-density lipoproteins), and chylomicrons in a way that only HDL cholesterol is selectively determined by an enzymatic cholesterol measurement [11]. Serum chemerin levels were measured with an enzyme immunoassay kit (Creative Diagnostics, USA), with a sensitivity of less than 3 pg/ml, using ELISA plate reader equalizer ER 2005 (Eqiupar, Italy) [12].

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Version 20. Normally distributed continuous variables were presented as mean ± SD whereas continuous variables that were not distributed normally were expressed as median (interquartile range) while categorical variables presented as n (%). Student's t-test, Mann–Whitney U test, and chi-square test were used as applicable. Pearson's correlation

Table 1 Comparison of baseline anthropometric and clinical characteristics between subjects with NGT, IGT, and T2DM

Parameters		NGT	IGT	T2DM
n		25	25	25
Age (years)		44.92±12.81	45.32±13.24	52±9.7[ab]
Height (cm)		160.68±9.51	160±11.59	165.6±9.23
Weight (kg)		67.76±14.42	70.16±12.38	77.08±15.95[ab]
BMI (kg/m^2)		26.16±4.52	27.76±5.55	28.24±6.59
Gender	Male	12(48%)	15(60%)	11(44%)
	Female	13(52%)	10(40%)	14(56%)
Marital status	Single	2(8%)	3(12%)	0(0%)
	Married	23(92%)	22(88%)	25(100%)
Waist circumference (cm)		92.56±11.7	91.68±12.02	165.6±9.23[ab]
Hip circumference (cm)		100.32±10.37	101.68±18.95	77.08±15.95a
Systolic blood pressure (mmHg)		119.08±15.69	125.2±18.74	100.4±14.32a
Diastolic blood pressure (mmHg)		82.2±10.87	81.6±9.87	106.76±13.32

Data presented as mean ± SD or *n* (%)

[a] Significantly different to NGT

[b] Significantly different to IGT

analysis was used to examine the relationship of serum chemerin level with other variables. To determine predictable factors of serum chemerin level, a multiple linear regression was performed. Variables which showed a potential association (p-value<0.20) with serum chemerin level in the Pearson's correlation were selected for multiple linear regression. Variables that were not normally distributed such as chemerin level, fasting blood sugar, random blood sugar, cholesterol, and triglyceride were log transformed for better approximate normal distribution (in correlation and regression analysis). Results were considered to be statistically significant at p-value <0.05.

Result

A total of 75 subjects participated in the study, out of which 25 subjects were newly diagnosed type 2 diabetes subjects (T2DM), 25 had impaired glucose tolerance (IGT), and 25 were normal glucose tolerant (NGT) individuals. Table 1 presents comparison of the baseline anthropometric and clinical characteristics between subjects with NGT, IGT, and T2DM. Subjects with T2DM were significantly older than subjects with NGT and IGT with significantly elevated weight and waist circumference (p-value <0.05), whereas hip circumference and systolic blood pressure were significantly lower in subjects with T2DM than subjects with NGT.

Table 2 presents comparison of biochemical variables between subjects with NGT, IGT, and T2DM. Subjects with NGT had significantly lower FBS and RBS level than subjects with IGT and T2DM. HbA1c was significantly higher in subjects with T2DM whereas chemerin level was significantly lower in T2DM as compared to subjects with NGT and IGT. However, no significant difference was observed in lipid profile.

Table 2 Comparison of biochemical variables between subjects with NGT, IGT, and T2DM

Parameters	NGT	IGT	T2DM
n	25	25	25
Fasting blood sugar (mg/dl)	88 (82.5–95.5)	113 (102.5–115)a	137 (126.5–178)[ab]
Random blood sugar (mg/dl)	101 (84.5–121.5)	147 (124–173)a	196.5 (127.5–232.7)[ab]
Cholesterol (mg/dl)	209 (184.5–273.5)	250 (188.5–291.5)	245 (203–304)
Triglyceride (mg/dl)	165 (96.5–219.5)	183 (129.5–243)	184 (145.5–234.5)
HDL (mg/dl)	39.36±9.36	37.24±5.93	38.84±6.68
LDL (mg/dl)	111.6±20.47	110.24±33.62	113.56±32.94
HbA1c (mg/dl)	5.48±0.51	5.52±0.71	6.4±1.66[ab]
Chemerin (pg/ml)	40 (36–363.5)	44 (34.5–433.5)	35 (33.5–40.5)[ab]

Data presented as mean ± SD or *n* (%)

[a] Significantly different to NGT

[b] Significantly different to IGT

Table 3 Association of chemerin level with obesity in subjects with NGT, IGT, and T2DM

	Chemerin level (pg/ml)	*p*-value
NGT		
Non-obese (BMI< 25 kg/m^2)	122.5 (38–679.5)	0.134
Obese (BMI≥ 25 kg/m^2)	37 (34–301)	
Normal waist circumference (male <90 cm, female < 80 cm)	38 (36–1176)	0.544
High waist circumference (male ≥90 cm, female ≥ 80 cm)	41 (35.5–315.75)	
Normal waist to hip ratio (< 0.9 in males, < 0.85 in females)	40 (37.5–1259)	0.444
High waist to hip ratio (≥ 0.9 in males, ≥ 0.85 in females)	40 (34–360)	
IGT		
Non-obese (BMI< 25 kg/m^2)	87 (38–1282)	0.289
Obese (BMI≥ 25 kg/m^2)	42 (34–240)	
Normal waist circumference (male <90 cm, female < 80 cm)	65.5 (40.75–730.75)	0.503
High waist circumference (male ≥90 cm, female ≥ 80 cm)	42 (34–320)	
Normal waist to hip ratio (< 0.9 in males, < 0.85 in females)	547 (60.5–1073)	0.038
High waist to hip ratio (≥ 0.9 in males, ≥ 0.85 in females)	40 (34.25–117.25)	
T2DM		
Non-obese (BMI< 25 kg/m^2)	34 (31.5–34.3)	0.021
Obese (BMI≥ 25 kg/m^2)	36 (34–46)	
Normal waist circumference (male <90 cm, female < 80 cm)	33 (30.5–34.75)	0.044
High waist circumference (male ≥90 cm, female ≥ 80 cm)	36 (34–44)	
Normal waist to hip ratio (< 0.9 in males, < 0.85 in females)	35 (33.5–60)	0.864
High waist to hip ratio (≥ 0.9 in males, ≥ 0.85 in females)	35.5 (33.25–41.25)	

Data presented as median (IQR); *p*-value < 0.05 considered to be statistically significant

Table 3 shows the association of chemerin level with obesity in subjects with NGT, IGT, and T2DM. Among subjects with NGT, no significant difference was found in any category of obesity in terms of chemerin level ($p>0.05$).

Among subjects with IGT, it was observed that subjects with normal waist to hip ratio had significantly higher chemerin level as compared to subjects with high waist to hip ratio ($p<0.05$).

Among subjects with T2DM, chemerin level in subjects with normal BMI and normal waist circumference was significantly lower than subjects with obesity and high waist circumference, while no significant difference in chemerin level was found between subjects with normal and high waist to hip ratio.

Table 4 presents relationship of serum chemerin levels with various parameters among subjects with NGT, IGT, and T2DM. Among subjects with NGT, chemerin level showed

Table 4 Relationship of serum chemerin level with various parameters in subjects with NGT, IGT, and T2DM

Parameters	NGT		IGT		T2DM	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Age	−0.274	0.184	−0.28	0.175	−0.093	0.657
BMI	−0.349	0.088	−0.013	0.951	−0.037	0.86
Waist circumference	−0.281	0.173	−0.274	0.185	0.001	0.995
Hip circumference	−0.253	0.222	0.085	0.688	0.034	0.872
Waist to hip ratio	−0.148	0.48	−0.407	0.044	−0.036	0.864
Systolic blood pressure	−0.179	0.391	−0.313	0.127	0.092	0.663
Diastolic blood pressure	−0.054	0.796	−0.33	0.108	−0.228	0.274
Fasting blood sugar	−0.429	0.032	−0.185	0.376	0.471	0.017
Random blood sugar	−0.017	0.937	0.054	0.797	0.301	0.173
Cholesterol	−0.127	0.545	−0.464	0.019	−0.09	0.668
Triglyceride	−0.3	0.145	−0.33	0.107	−0.196	0.348
HDL	0.145	0.488	0.012	0.954	0.205	0.325
LDL	−0.164	0.434	−0.15	0.473	−0.082	0.696
HbA1c	−0.179	0.393	−0.293	0.155	0.645	0.001

p-value < 0.05 considered to be statistically significant

significantly moderate negative correlation with fasting blood sugar (r=−0.429, p<0.05), while age, BMI, triglyceride, and waist and hip circumference showed weak negative non-significant correlation with chemerin level.

Among subjects with IGT, a significantly moderate negative correlation of chemerin level with waist to hip ratio (r=−0.407, p=0.044) and cholesterol (r= −0.464, p=0.019) was observed. Moreover, age, waist circumference, systolic blood pressure, diastolic blood pressure, triglyceride, and HbA1c showed weak negative non-significant correlation with chemerin level, while no correlation of chemerin level was found with BMI, hip circumference, FBS, RBS, HDL, and LDL.

Among subjects with T2DM, it was observed that only HbA1c had positively strong correlation with chemerin level (r=0.645, p=0.001) and FBS had positively moderate correlation with chemerin level (r=0.471, p=0.017). Correlation of chemerin with diastolic blood pressure, RBS, and HDL was also moderate but non-significant, while no correlation of chemerin level was found with age, BMI, waist circumference, hip circumference, waist to hip ratio, systolic blood pressure, cholesterol, and LDL in subjects with type 2 diabetes.

Table 5 presents multiple linear regression of variables associated with serum chemerin levels in subjects with type 2 diabetes. It was noted that only fasting blood sugar and triglycerides were significantly associated with serum chemerin level among subjects with type 2 diabetes.

Discussion

In the present study, serum chemerin level was found to be lower in subjects with newly diagnosed type 2 diabetes as compared with other groups. Study by Bozaoglu et al. did not find any difference in chemerin level between subjects with T2DM and controls that can acknowledge our findings [13]. As in general, the most common method used to check abdominal adiposity is waist circumference [14]. Corresponding with waist circumference and obesity in our study in subjects with newly diagnosed type 2 diabetes revealed low serum chemerin levels. This might be due to relatively small sample size and lifestyle intervention in T2DM subjects [15].

In our study, HbA1c and FBS showed positive correlation with chemerin and no correlation was seen with blood pressure in subjects with newly diagnosed type 2 diabetes. Evidence from the past has revealed that systolic blood pressure was also significantly higher in subjects with type 2 diabetes [16]. In accordance with the present study, chemerin was not higher in type 2 diabetic subjects with hypertension and no correlation of blood pressure with chemerin was identified [17]. Many studies in the past determined a weak positive correlation between blood pressure and chemerin in T2DM subjects with coronary artery disease, renal dysfunction, and hypertension [18]. In previous studies, serum chemerin levels were positively correlated with FBS and HbA1c that is consistent with current findings [19]. This consistency supports the proposed theory that chemerin is entailed in the pathophysiology of insulin resistance.

As earlier studies had reported, multiple factors like BMI, waist-to-stature ratio (WSR), 2-h plasma glucose (2hPG), HbA1c, and HDL-c were independently influencing plasma chemerin levels [18]. Multiple linear regression analysis revealed that fasting blood glucose and triglycerides were independent predictors of serum chemerin level in this study in subjects with newly diagnosed type 2 diabetes. Thus, circulating chemerin showed a strong and independent association with key markers of metabolic syndrome, especially obesity and insulin resistance. Moreover, it raises the possibility that chemerin may be of value as a biomarker for these disorders.

The cross-sectional nature of the study, small sample size, and unavailability of subcutaneous fat data are the main limitations of our study. The strength of this study is to provide important data considering the relationship between visceral adiposity and chemerin in T2DM subjects. Further longitudinal studies with a large sample size and genetic factors analysis are required.

Conclusion

Newly diagnosed diabetes subjects having obesity expressed low elevated serum chemerin levels as compared to healthy subjects that may attribute to intensive lifestyle intervention practices. Circulating levels of adipokines are known to change with increased adiposity and are therefore recognized as contributing factors in the metabolic changes that are seen in obesity and lead to the development of T2DM. These findings suggested that chemerin may be a marker that reflects central and general obesity in response. Further large-scale studies are required to validate our findings.

Table 5 Multiple linear regression analysis expressing independent predictors of serum chemerin levels in subjects with type 2 DM

Factors	Standardized coefficients (β)	p-value
Fasting blood sugar	0.682	0.001
Triglyceride	−0.489	0.013

Stepwise backward elimination method was used

p-value < 0.05 considered to be statistically significant

Author contribution S Qazi: concept and design, interpretation of data and wrote the manuscript
IA Siddiqui: concept and design, edited and approved the manuscript
M Saeed: concept and design, edited and approved the manuscript
K Perveen: interpretation of data, wrote, edited and reviewed the manuscript
K Baqa: interpretation of data, wrote and reviewed the manuscript
A Fawwad: concept and design, edited and approved the manuscript

Data availability No further data is available.

Declarations

Ethics approval The study was carried out after ethical approval from ethics committee of BMU.

Consent to participate Written informed consent was obtained from study subjects.

Conflict of interest The authors declare no competing interests.

References

1. American diabetes association. Available at: https://www.diabetes.org/a1c/diagnosis. Last assessed at: 1 November 2020
2. Qiao Q, Nyamdorj R. Is the association of type II diabetes with waist circumference or waist-to-hip ratio stronger than that with body mass index? Eur J Clin Nutr. 2010;64(1):30–4. https://doi.org/10.1038/ejcn.2009.93.
3. Basit A, Askari S, Zafar J, Riaz M, Fawwad A, Members ND. NDSP 06: Prevalence and risk factors for obesity in urban and rural areas of Pakistan: a study from second National Diabetes Survey of Pakistan (NDSP), 2016–2017. Obes Res Cli Pract. 2020.
4. Stefanov T, Blüher M, Vekova A, Bonova I, Tzvetkov S, Kurktschiev D, Temelkova-Kurktschiev T. Circulating chemerin decreases in response to a combined strength and endurance training. Endocrine. 2014;45:382–91. https://doi.org/10.1007/s12020-013-0003-2.
5. Zhang M, Chen P, Chen S, Sun Q, Zeng QC, Chen JY, Liu YX, Cao XH, Ren M, Wang JK. The association of new inflammatory markers with type 2 diabetes mellitus and macrovascular complications: a preliminary study. Eur Rev Med Pharmacol Sci. 2014;18:1567–72.
6. Gu P, Jiang W, Lu B, Shi Z. Chemerin is associated with inflammatory markers and metabolic syndrome phenotypes in hypertension subjectss. Clin Exp Hypertens. 2014;36(5):326–32. https://doi.org/10.3109/10641963.2013.827697.
7. Aksu F, Caliskan M, Keles N, Toprak EA, Uzuncakmak KT, Kostek O, et al. Chemerin as a marker of subclinical cardiac involvement in psoriatic subjects. Cardiol J. 2017;24(3):276–83. https://doi.org/10.5603/CJ.a2017.0031.
8. Cheon DY, Kang JG, Lee SJ, Ihm SH, Lee EJ, Choi MG, Yoo HJ, Kim CS. Serum chemerin levels are associated with visceral adiposity, independent of waist circumference, in newly diagnosed type 2 diabetic subjects. Yonsei Med J. 2017;58(2):319–25. https://doi.org/10.3349/ymj.2017.58.2.319.
9. Basit A, Fawwad A, Qureshi H, NDSP Members, et al. Prevalence of diabetes, pre-diabetes and associated risk factors: second National Diabetes Survey of Pakistan (NDSP), 2016–2017. BMJ Open. 2018;8:e020961.
10. Sharma SP, Anjankar AP, Kale A. Comparison of glucose levels using glucometer and GOD-POD method in diabetic subjects. Indian J Clin Biochem Res. 2017;4(1):6–10. https://doi.org/10.18231/2394-6377.2017.0002.
11. Fawwad A, Sabir R, Riaz M, Moin H, Basit A. Measured versus calculated LDL-cholesterol in subjects with type 2 diabetes. Pak J Med Sci. 2016;32(4):955–60. https://doi.org/10.12669/pjms.324.989.
12. Fatima SS, Butt Z, Bader N, Pathan AZ, Hussain S, Iqbal NT. Role of multifunctional chemerin in obesity and preclinical diabetes. Obes Res Clin Pract. 2015;9(5):507–12. https://doi.org/10.1016/j.orcp.2015.01.004.
13. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, Walder K, Segal D. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology. 2007;148(10):4687–94. https://doi.org/10.1210/en.2007-0175.
14. Fang H, Berg E, Cheng X, Shen W. How to best assess abdominal obesity. Curr Opin Clin Nutr Metab Care. 2018;21(5):360–5. https://doi.org/10.1097/MCO.0000000000000485.
15. Han J, Kim SH, Suh YJ, Lim HA, Shin H, Cho SG, Kim CW, Lee SY, Lee DH, Hong S, Kim YS, Nam MS. Serum chemerin levels are associated with abdominal visceral fat in type 2 diabetes. J Korean Med Sci. 2016;31(6):924–31. https://doi.org/10.3346/jkms.2016.31.6.924.
16. Bigna JJ, Bahebeck J, Sobngwi E, Mbanya JC. Metabolic syndrome for sub-Saharan Africans diabetes with peripheral arterial disease: a case-control study. BMC Rese Notes. 2014;7(1):104. https://doi.org/10.1186/1756-0500-7-104.
17. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and non-alcoholic fatty liver disease. Int J Mol Sci. 2014;15(4):6184–223. https://doi.org/10.3390/ijms15046184.
18. Yang M, Yang G, Dong J, Liu Y, Zong H, Liu H, Boden G, Li L. Elevated plasma levels of chemerin in newly diagnosed type 2 diabetes mellitus with hypertension. J Investig Med. 2010;58(7):883–6. https://doi.org/10.2310/JIM.0b013e3181ec5db2.
19. Yang M, Zhou X, Xu J, Yang B, Yu J, Gong Q, et al. Association of serum chemerin and inflammatory factors with type 2 diabetes macroangiopathy and waist-to-stature ratio. Bosn J Basic Med Sci. 2019;19(4):328–35. https://doi.org/10.17305/bjbms.2019.4002.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):304–308
https://doi.org/10.1007/s13410-022-01078-z

ORIGINAL ARTICLE

Interaction of PTPRD (rs17584499) polymorphism with passive smoking in Chinese women with susceptibility to type 2 diabetes

Haixia Zeng[1] · Gui Pan[2] · Juan Wu[1] · Yanting Huang[3] · Xiaojun Zhou[4] · Xiaoyang Lai[1] · Jianping Liu[1]

Received: 19 October 2021 / Accepted: 17 April 2022 / Published online: 27 April 2022

Abstract

Objective Passive smoking is associated with an increased risk of type 2 diabetes (T2DM). We tested whether the protein tyrosine phosphatase receptor type D (PTPRD) gene rs17584499 single-nucleotide polymorphism is associated with (T2DM) and whether its interaction with passive smoking affects women's susceptibility to T2DM.

Methods The subjects were from a community-based survey conducted in Nanchang, China, between May 2017 and June 2018. The study included 160 women with 79 T2DM patients as the T2DM group and 81 healthy volunteers without diabetes as the control group. Peripheral blood genomic DNA was extracted to detect the genotype of the target site of the PTPRD gene. Logistic regression analysis was performed to study the association between the rs17584499 SNP and the risk of T2DM and to analyse the influence of the interaction between genes and passive smoking on susceptibility to T2DM.

Results After adjusting for age, LDL-C, HDL-C, TC, TG, FINS, IL-18, hs-CRP, and cotinine, binary logistic regression analysis did not find a significant association between rs17584499 and susceptibility to diabetes. After stratifying according to passive smoking, we found that passive smoking women with the CT/TT genotype had a higher risk of T2DM compared with the CC genotype (OR = 3.50, 95 % CI = 1.18~10.44, $p = 0.02$), but no significant difference was found in non-passive smoking.

Conclusions This study shows that the T allele of rs17584499 may be a risk factor for female T2DM and that there is a synergistic effect between passive smoking and PTPRD rs17584499 on the susceptibility to T2DM among females.

Keywords PTPRD · Passive smoking · Type 2 diabetes

Introduction

Type 2 diabetes (T2DM) has become a public health problem in the world, especially in developing countries. An epidemiological survey conducted by the Endocrinology Branch of the Chinese Medical Association in 31 provinces in China from 2015 to 2017 showed that the prevalence of diabetes in people aged 18 and over was 11.2% [1]. T2DM is caused by the interaction of a variety of genetic variants and environmental factors. Increasing evidence shows that passive smoking is an independent risk factor for T2DM [2]. PTPRD is a member of the receptor IIA (R2A) subfamily of protein tyrosine phosphatases (PTPs) and participates in the insulin signal transduction pathway. Studies have indicated that the role of PTPRD in the pathogenesis of T2DM may be caused by DNA hypermethylation, resulting in PTPRD silencing and reducing insulin signal transduction [3]. Large-scale genome-wide association studies have confirmed that the PTPRD rs17584499 SNP is closely related to the susceptibility of the Han population to T2DM [4]. However, there are differences in research in different populations. Studies in the Chinese population have shown that the rs17584499 site of the PTPRD gene can affect the response of cells to insulin to produce insulin resistance, which is a susceptibility site for T2DM [5]. The T allele frequencies of the PTPRD rs17584499 polymorphism in Chinese health controls and T2DM patients were 9.4% and 12.9%, respectively [6]. However, in the Japanese population, a negative result was

✉ Jianping Liu
liujpnfm@163.com

[1] Department of Endocrinology, Second Affiliated Hospital of Nanchang University, 1 Minde Road, Nanchang PO:330006, China

[2] Department of Endocrinology, Second People's Hospital of Jingdezhen City, Jingdezhen, China

[3] Department of Endocrinology, Xiangyang First People's Hospital, Xiangyang, China

[4] School of Public Health, Nanchang University, Nanchang, China

obtained, which may be related to the small sample size of the study, which makes the statistical power of the conclusions poor [7]. Currently, there are few reports about the relationship between tobacco smoke and the dysregulated expression of PTPRD. Whether passive smoking plays a role in the epigenetics of the PTPRD gene, and whether passive smoking and the occurrence of diabetes are related to diabetes susceptibility genes are still unclear, and more relevant studies are needed to prove it. Therefore, we explored the influence of passive smoking and PTPRD rs17584499 gene polymorphism on female T2DM.

Materials and methods

Participants

The subjects were from a community-based survey conducted in Nanchang, China, between May 2017 and June 2018. A total of 249 people were surveyed, 89 people who had been diagnosed with diabetes before the survey or who had omitted important data were excluded, and the remaining 160 people were finally included in the analysis of this study (age 25~95 years, mean age: 56.61 ± 11.20 years). A three-stage sampling method was used to select representative samples. In the first stage, three administrative regions of Nanchang City were randomly selected. In the second stage, one street was randomly selected from the three sampled administrative regions. In the third stage, two residential communities were randomly selected from the randomly selected streets. Inclusion criteria: ≥18 years old, non-smoking, female. Exclusion criteria: Type 1 diabetes, malignant tumors, subjects with active inflammation, hypothalamic diseases, pituitary diseases, adrenal gland diseases, pregnant and lactating women, and those with incomplete information collection. Through a questionnaire survey, we screened out patients exposed to passive cigarette smoke earlier than the onset of diabetes. Ultimately, 79 type 2 diabetes patients and 81 healthy controls were included, including 96 passive smokers and 64 nonpassive smokers. All subjects signed an informed consent form, and the study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Nanchang University.

Diagnostic criteria for T2DM

T2DM was defined as a self-reported history of T2DM (confirmed by the use of insulin or oral hypoglycemic agents) plus newly diagnosed T2DM using WHO diagnostic criteria for diabetes (fasting glucose ≥ 7.0 mmol/l or 2 h postprandial glucose ≥ 11.1 mmol/l) [8].

Diagnostic criteria for passive smoking

The passive smoking group in this study was defined as individuals with indoor exposed to cigarette smoke for ≥ 15 min/day, 3 days/week, for > 1 year. The nonpassive smoking group reported zero exposure to cigarette smoke or exposure to cigarette smoke for < 5 min/day.

Questionnaire

We designed the questionnaire used in this survey, including five parts: general items, family history, past diagnosis and treatment of major diseases, lifestyle, physical examination, and laboratory examination. Among them, general items include basic information and general conditions; past diagnosis and treatment of major diseases include the time of onset of diabetes, family history of diabetes, history of hypertension, history of coronary heart disease, history of stroke and drug use; lifestyle mainly include smoking, drinking, physical labor, and the time to start exposure to passive cigarette smoke. The questionnaires were filled out by endocrinologists and postgraduates of the Second Affiliated Hospital of Nanchang University, after unified training and one-to-one method.

Laboratory assays

Trained medical students or doctors conducted face-to-face interviews using structured questionnaires. The main content included age, occupation, education, passive smoking, and chronic diseases such as diabetes, hypertension, coronary heart disease, family history, and medication history. Blood was collected on an empty stomach (fasting for at least 8 h) and 2 h after OGTT (timing from the first intake of glucose). Fasting blood glucose (FPG) and 2-h blood glucose (2hPG) after OGTT were detected by the glucose oxidase method, fasting insulin (FINS) was detected by electrochemiluminescence immunoassay, and an automatic biochemical analyser was used to determine glycosylated haemoglobin (HbA1c) and total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Human interleukin-18 (IL-18) was detected by the double-antibody sandwich ELISA method. For the determination, the serum high-sensitivity C-reactive protein (hs-CRP) level was determined by the immuno-rate turbidimetry method, and the serum cotinine concentration was determined by the calorimetric method.

Genomic DNA extraction and genotyping

PCR amplification was performed on the PTPRD rs17584499 site, and a 2% agarose gel was used for detection. The amplified fragments were sequenced by

the Sanger DNA sequencing method. The sequencing results were analysed by Jellyfish software to determine the SNP type. The forward sequence of the PCR primer at the rs17584499 site was (5′-3′) TCAGTCCT ACACCTCACCCAAG, and the reverse primer sequence was (5′-3′) CCAGGATAACAGGAACAATGAAATAG C. The amplification conditions were as follows: denature at 95 °C for 5 min, then each temperature (94 °C/ 60 °C/72 °C) for 30 s each for 32 cycles, then extend at 75 °C for 5 min, and finally hold at a constant temperature of 4 °C.

Statistical analysis

SPSS 23.0 software was used for statistical analysis. Continuous variables conforming to a normal distribution are expressed as the mean ± standard deviation, and an independent sample *t* test was used for analysis. Data with a nonnormal distribution are represented by the median (IQR), and nonparametric tests of two independent samples were used. Categorical variables are expressed as frequencies or percentages, and the χ^2 test was used for statistical analysis. Genotype distribution and allele frequency were analysed using the χ^2 test. A goodness of fit χ^2 test was used to evaluate whether the genotypic distribution of the PTPRD rs17584499 polymorphism was in accordance with the Hardy–Weinberg equilibrium in the T2DM and control groups. Logistic regression was used to analyse the effects of PTPRD rs17584499 and passive smoking on T2DM. A bilateral p value <0.05 indicates a significant difference.

Results

Table 1 shows the clinical baseline data of the T2DM group and the control group. There were no significant differences in age, HDL, LDL, TC, CRP, IL-18, cotinine solubility, and passive smoking between the two groups ($p > 0.05$). The distribution of TG, FPG, 2hPBG, HbA1c, and FINS levels in the T2DM group were significantly higher than those in the control group ($p < 0.05$).

Table 2 shows the distribution frequency of PTPRD rs17584499 polymorphism genotype. The genotype distribution of rs17584499 in the control group accorded with the Hardy-Weinberg equilibrium distribution ($p > 0.05$). In the current female subjects, we have adjusted for age, LDL-C, HDL-C, TC, TG, FINS, IL-18, hs-CRP, and cotinine, and the susceptibility of PTPRD rs17584499 to T2DM was not statistically different (OR = 1.96, 95% CI = 0.78~4.95, $p = 0.15$).

Table 3 shows the effect of the interaction between the PTPRD rs17584499 genotype and passive smoking on T2DM. The average cotinine solubility level of the passive smoking group was significantly higher than that of the non-passive smoking group. After stratifying according to passive smoking, we found that passive smoking women with the CT/ TT genotype had a higher risk of T2DM compared with the CC genotype (OR = 3.50, 95 % CI = 1.18~10.44, $p = 0.02$), but no significant difference was found in non-passive smoking.

Discussion

In this study, the correlation between PTPRDrs17584499 polymorphism and T2DM was not statistically different,

Table 1 Characteristics of the study participants

Variables	T2DM (n = 79)	Control (n = 81)	p value
Age, y	57.70 ± 10.90	55.31 ± 11.32	0.85
LDL-C (mmol/L)	3.02 ± 0.88	2.80 ± 0.77	0.17
TC (mmol/L)	4.95 ± 0.95	4.63 ± 0.89	0.26
HDL-C (mmol/L)	1.43 ± 0.37	1.48 ± 0.26	0.31
TG (mmol/L)	1.78 ± 01.30	1.23 ± 0.60	<0.05
FPG (mmol/L)	5.78 ± 1.76	4.81 ± 0.59	<0.05
2hPG (mmol/L)	10.17 ± 3.88	5.98 ± 1.29	<0.05
HbA1c (%)	6.05 ± 1.16	5.51 ± 0.39	<0.05
FINS (μU/mL)	11.97 ± 12.72	6.13 ± 4.36	<0.05
IL-18 (ng/L)	157.20 (132.68~180.68)	149.01 (134.52~177.39)	0.35
Hs-CRP (mg/L)	2.22 (1.62~3.23)	1.84 (1.50~3.17)	0.11
Cotinine (pg/mL)	1600.39 (1376.08~1828.77)	1653.40 (1307.84~1919.78)	0.82
Passive smoking	41 (51.90 %)	55 (67.90%)	0.06

Data are presented as mean (SD) or median (IQR) or *n* (%). *LDL-C* low-density lipoprotein cholesterol, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglycerides, *FPG* fasting blood glucose, *2hPG* 2-h blood glucose after OGTT, *HbA1c* glycosylated haemoglobin, *FINS* fasting insulin, *IL-18* human interleukin-18, *Hs-CRP* high-sensitivity C-reactive protein

Table 2 PTPRD rs17584499 genotype frequency in the T2DM and control groups

SNP	T2DM/ Control	OR (95%CI)	*p* value
CC	67/61	1	
CT/TT	12/20	1.96(0.78~4.95)	0.15

Adjusted for age, LDL-C, HDL-C, TC, TG, FINS, IL-18, hs-CRP, cotinine. *CI* confidence interval, *OR* odds ratio

and we adjusted for factors influencing the risk of diabetes (age, LDL-C, HDL-C, TC, TG, FINS, IL-18, hs-CRP, and cotinine), the results were still insignificant, but there was a correlation trend, which may be related to the small sample size of the study and the poor statistical power of the conclusions obtained. However, after dividing passive smoking into groups, the effect of passive smoking and PTPRD polymorphism was significant. Compared with passive smoking women with CC genotype, CT/TT genotype had a higher risk of T2DM. Another study involving individuals of Chinese Han nationality showed that the rs17584499 TT mutation of PTPRD is a risk factor for diabetes, and the level of insulin resistance in TT gene carriers is elevated [5]. However, two studies conducted in Mexican Americans and Japanese populations did not find that the PTPRD gene is associated with diabetes [7, 9]. A recent prospective study in China found that mutations in the PTPRD gene are associated with the incidence of T2DM in the rural Han population, and confirmed that the risk allele T of rs17584499 in PTPRD is a risk factor for T2DM [10].

Some previous studies have focused on the association between PTPRD gene polymorphisms and diabetes, but the results of this association are inconsistent [5, 11, 12]. Increasing attention is being paid to the comprehensive influence of genes and environmental factors on T2DM. In this study, we did not observe that the polymorphism of PTPRD rs17584499 is associated with the susceptibility of Han women to T2DM. However, after stratifying the subjects according to whether passive smoking or not, it was found that the interaction between passive smoking and rs17584499 CT/TT genotype was associated with T2DM. The association between passive smoking and diabetes risk has been previously reported and confirmed [13, 14]. Cigarette smoke can increase oxidative stress, amplify inflammation, and cause endothelial dysfunction, all of which are related to insulin resistance [15, 16]. In addition, nicotine in cigarettes can cause progressive pancreatic mitochondrial damage and β-cell dysfunction [17]. The PTPRD protein is mainly distributed in insulin-sensitive tissues and cells. It is a subunit of the leukocyte antigen-related subfamily of protein tyrosine phosphatases (LAR-PTPs). LAR-PTP is an important subfamily of amino acid phosphatases, and protein tyrosine phosphatase is a key enzyme in insulin signal transduction, has an important impact on the occurrence and development of T2DM, and participates in regulating the speed and time of insulin receptor signal transduction. A study of 1138 Chinese Han participants also concluded that the risk-giving T allele of rs17584499 is related to insulin resistance [5]. Although the mechanism of the interaction between passive smoking and the PTPRDrs17584499 polymorphism in T2DM is unknown, and there have been few studies on the subject, it has been shown that both passive smoking and PTPRD can mediate insulin resistance. This study has certain limitations. First, the sample size is insufficient and more SNPs should be included, not just rs17584499. Second, to study the interaction between genes and the environment, more ways should be considered, such as lifestyle, exercise, and drinking. Therefore, large-scale studies are needed to further confirm.

In summary, the T allele of rs17584499 of PTPRD and passive smoking are related to the susceptibility of T2DM, which may be mediated by insulin resistance. However, passive smoking is very common in women, but most women do not know that passive smoking will seriously harm their health, as active smoking does. This study confirmed the influence of passive smoking and PTPRD gene on diabetes susceptibility, indicated by the appearance of diabetes. Genetics provides support. Thus, it is recommended that passive smoking education for diabetic patients be strengthened to prevent diabetes and other chronic diseases.

Table 3 Odds ratios for the combined effect of PTPRD rs17584499 and passive smoking on T2DM

Smoking status	Genotype	T2DM	Control	OR (95%CI)	*p* value
Non-passive smoking	CC	31(81.6)	24(92.3)	1	
	CT/TT	7(18.4)	2(7.7)	0.37 (0.07~1.94)	0.24
Passive smoking	CC	36(87.8)	37(67.3)	1	
	CT/TT	5(12.2)	18(32.7)	3.50 (1.18~10.44)	0.02

CI confidence interval, *OR* odds ratio

Acknowledgment We are grateful to The Second Affiliated Hospital of Nanchang University for approval for this study. We also thank all participants for their willingness to participate in this study.

Author contribution Haixia Zeng, Gui Pan, Juan Wu, and Yanting Huang collected the data. Xiaojun Zhou, Xiaoyang Lai, and Jianping Liu applied for research grants, designed the study, interpreted the data, and revised the manuscript. Haixia Zeng wrote the manuscript. All the authors reviewed the manuscript.

Funding National Natural Science Foundation of China (NO. 81560554); Natural Science Foundation of Jiangxi Province (NO. 20144BAB2050007)

Data availability The data are available from the corresponding authors.

Declarations

Ethics approval Approval for the study was obtained from the Clinical Ethics Committee of The Second Affiliated Hospital of Nanchang University. All of the patients and their guardians have given their written informed consent.

Conflict of interest The authors declare no competing interests.

References

1. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, Shi B, Sun H, Ba J, Chen B, du J, He L, Lai X, Li Y, Chi H, Liao E, Liu C, Liu L, Tang X, et al. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study[J]. Bmj. 2020;369:m997.
2. Hayashino Y, Fukuhara S, Okamura T, Yamato H, Tanaka H, Tanaka T, Kadowaki T, Ueshima H, for the HIPOP-OHP Research Group. A prospective study of passive smoking and risk of diabetes in a cohort of workers: the High-Risk and Population Strategy for Occupational Health Promotion (HIPOP-OHP) study[J]. Diabetes Care. 2008;31(4):732–4.
3. Chen YT, Lin WD, Liao WL, Lin YJ, Chang JG, Tsai FJ. PTPRD silencing by DNA hypermethylation decreases insulin receptor signaling and leads to type 2 diabetes[J]. Oncotarget. 2015;6(15): 12997–3005.
4. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, Wang TY, Chen RH, Shiu CF, Liu YM, Chang CC, Chen P, Chen CH, Fann CSJ, Chen YT, Wu JY. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese[J]. PLoS Genet. 2010;6(2):e1000847.
5. Chang YC, Chiu YF, Liu PH, Shih KC, Lin MW, Sheu WHH, Quertermous T, Curb JD, Hsiung CA, Lee WJ, Lee PC, Chen YT, Chuang LM. Replication of genome-wide association signals of type 2 diabetes in Han Chinese in a prospective cohort[J]. Clin Endocrinol (Oxf). 2012;76(3):365–72.
6. Dong M. Association analysis of SRR and PTPRD genetic polymorphisms with the development of T2DM and effects of both polymorphisms on the therapeutic efficacy of metformin in Chinese T2DM patients [D]. Hunan: Central South University; 2011.
7. Imamura M, Iwata M, Maegawa H, Watada H, Hirose H, Tanaka Y, Tobe K, Kaku K, Kashiwagi A, Kadowaki T, Kawamori R, Maeda S. Replication study for the association of rs391300 in SRR and rs17584499 in PTPRD with susceptibility to type 2 diabetes in a Japanese population[J]. J Diabetes Investig. 2013;4(2): 168–73.
8. Wang H, Qiu Q, Tan LL, Liu T, Deng XQ, Chen YM, Chen W, Yu XQ, Hu BJ, Chen WQ. Prevalence and determinants of diabetes and impaired fasting glucose among urban community-dwelling adults in Guangzhou, China[J]. Diabetes Metab. 2009;35(5):378–84.
9. Parra EJ, Below JE, Krithika S, et al. Genome-wide association study of type 2 diabetes in a sample from Mexico City and a meta-analysis of a Mexican-American sample from Starr County, Texas[J]. Diabetologia. 2011;54(8):2038–46.
10. Chen Y, Chen XY, Dong XL, Wang YZ, Wang N, Zhu JF, Chen Y, Jiang QW, Fu CW. Investigation of the association between 45 Tag SNPs and type 2 diabetes mellitus in Han Chinese adults: a prospective cohort study[J]. Public Health Genomics. 2021;24(3-4): 123–30.
11. Liu L, Chen L, Li Z, Li L, Wang M, Qu J, Xue J. Association of genetic variants in TOMM7 gene and gene environment interaction with type 2 diabetes in Chinese Dong population[J]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2015;40(1):31–8.
12. Wood AR, Jonsson A, Jackson AU, Wang N, van Leewen N, Palmer ND, Kobes S, Deelen J, Boquete-Vilarino L, Paananen J, Stančáková A, Boomsma DI, de Geus EJC, Eekhoff EMW, Fritsche A, Kramer M, Nijpels G, Simonis-Bik A, van Haeften TW, et al. A genome-wide association study of IVGTT-based measures of first-phase insulin secretion refines the underlying physiology of type 2 diabetes variants[J]. Diabetes. 2017;66(8):2296–309.
13. Ko KP, Min H, Ahn Y, Park SJ, Kim CS, Park JK, Kim SS. A prospective study investigating the association between environmental tobacco smoke exposure and the incidence of type 2 diabetes in never smokers[J]. Ann Epidemiol. 2011;21(1):42–7.
14. Lajous M, Tondeur L, Fagherazzi G, de Lauzon-Guillain B, Boutron-Ruault MC, Clavel-Chapelon F. Childhood and adult secondhand smoke and type 2 diabetes in women[J]. Diabetes Care. 2013;36(9):2720–5.
15. Bernard A, Ku JM, Vlahos R, Miller AA. Cigarette smoke extract exacerbates hyperpermeability of cerebral endothelial cells after oxygen glucose deprivation and reoxygenation[J]. Sci Rep. 2019;9(1):15573.
16. Pei C, Wang X, Lin Y, Fang L, Meng S. Inhibition of Galectin-3 alleviates cigarette smoke extract-induced autophagy and dysfunction in endothelial progenitor cells[J]. Oxid Med Cell Longev. 2019;2019:7252943–13.
17. Bruin JE, Petre MA, Raha S, Morrison KM, Gerstein HC, Holloway AC. Fetal and neonatal nicotine exposure in Wistar rats causes progressive pancreatic mitochondrial damage and beta cell dysfunction[J]. PLoS One. 2008;3(10):e3371.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):309–316
https://doi.org/10.1007/s13410-022-01073-4

ORIGINAL ARTICLE

The effect of aerobic, resistance, and concurrent training on the expression and protein levels of RBP4 visceral and subcutaneous adipose tissue in diabetic rats with STZ

Ahmad Abdi[1]

Received: 30 August 2020 / Accepted: 18 April 2022 / Published online: 2 May 2022

Abstract

Background Retinol-binding protein 4 (RBP4) is considered as an adipocaine which plays an important role in regulating insulin function and glucose metabolism.

Objectives The present study aimed to investigate the effect of a period of endurance, resistance, and concurrent training on the expression and protein levels of RBP4 in visceral adipose tissue (VAT) and subcutaneous fat tissue (SAT) in diabetic rats with STZ.

Method Thirty-six male Wistar rats (4 to 8 weeks old) became diabetic through nicotinamide and Streptozocin and were randomly divided into control (CON), aerobic exercise (AE), resistance exercise (RE), and concurrent training (CT) groups. AE with an intensity of 60 to 75% VO_2max was performed for 10–40 min on a treadmill (0% slope), and RE included 10 ascents of a 1-m ladder or a weight 30–30% of the rat weight. In addition, CT included a combined AE and RE. The exercises were performed for 8 weeks and 5 days a week.

Results All three types of exercise reduced the expression and RBP4 protein of VAT and SAT in diabetic rat. However, a significant decrease was observed in the expression and RBP4 protein of SAT in the CT group compared to that of RT. Further, homeostatic model assessment for insulin resistance (HOMA-IR) had a significant decrease compared to CON in the experimental groups.

Conclusion Three types of exercise improve insulin resistance in diabetic rats by reducing the expression and levels of RBP4 protein in VAT and SAT. However, the effect of CT is greater than RT.

Keywords Exercise · RBP4 · Insulin resistance · Diabetic rat

Introduction

Type 2 diabetes mellitus (T2DM) is still considered as one of the main causes of death worldwide despite some advances in medicine [1]. The studies indicated that chronic inflammation and insulin resistance, which are the features of T2DM, are closely related to inflammatory factors and abnormal production of adipokines [2]. Adipose tissue (AT) secretes many adipokines as an endocrine gland which modulates insulin action in other tissues [3]. Retinol 4–binding protein (RBP4) is a 21 kDa adipokine synthesized in liver tissue and carries retinol. It was found that RBP4 is released by adipocytes and macrophages [4]. Serum RBP4 levels are positively correlated with RBP4 mRNA in AT and abdominal adipose mass [5]. In addition, STZ-induced diabetes increases the expression of RBP4 in the visceral adipose tissue, liver, and muscle [6]. Increased expression of RBP4 in AT and serum levels of RBP4 is related to insulin resistance; however, some conflicting results were obtained by some studies [7, 8]. In diabetic rats, an increase in RBP4 is related to an increase in insulin resistance, and a decrease in RBP4 decreases insulin resistance [6]. In addition, RBP4 increases inflammation in mice through activating innate and acquired immune responses [9]. Since RBP4 is directly related to visceral fat and increase AT inflammation [5], some measures aimed at reducing fat reduces RBP4 secretion and insulin resistance. Exercise is considered as one of the most important ways to prevent and treat diabetes. Since physical exercise has multiple effects and low

✉ Ahmad Abdi
a.abdi58@gmail.com

[1] Department of Exercise Physiology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

cost, it was considered as a leading strategy for T2DM management [10]. Accordingly, exercise has the same effect, although it is not more effective than some drugs in reducing T2DM [1, 11]. Some indicated that AE [6] and RE [6] improve diabetes through reducing RBP4 levels. Most studies indicated that moderate-intensity AE is performed to control diabetes. Therefore, the American College of Sports Medicine (ACSM) and the American Diabetes Association (ADA) jointly recommended 150 min of moderate/severe AE to prevent or delay diabetes for T2DM patients [12]. However, RE improved metabolic function and the factors affecting T2DM, although some differences were observed [13]. On the other hand, combining AE and RE, called CT, has a more desirable effect on diabetic patients due to the principle of exercise specificity, and is recommended for such patients [14]. However, limited information on the effect of AE, RE, and CT on RBP4 changes in VAT and SAT of diabetic mice is available. The changes in this adipokine according to the type of exercise activity provide a suitable and important perspective for choosing the type of exercise activity to control and treat diabetes. Therefore, the present study aimed to compare three training methods on the changes in RBP4 of VAT and SAT of diabetic mice with STZ.

Materials and methods

Animals

Thirty-six male Wistar rats (4–8 weeks old, 125–135 g weight) were acquired and maintained (for a week) in the Animal House of the Faculty of Physical Education and Sports Science of Tehran. Five rats were housed per cage (46 L) with a 12:12-h light/dark cycle. Temperature and humidity were maintained at 22 °C ± 1.4 °C and 50% ± 5%, respectively. Diets (pellet form) and water were provided ad libitum. After transferring to the laboratory, rats underwent induction of diabetes and were allocated to a control group (CON; *n*=9), aerobic exercise (AE; *n*=9), resistance training (RT; *n*=9), and concurrent training (CT; *n*=9) using a randomized block design with stratification by body mass (_250 g or >250 g). Those in the T groups underwent a familiarization session with the exercise.

Generating diabetic animal

Rats became diabetic following administration of nicotinamide and streptozotocin [15]. First, nicotinamide (95 mg/kg body weight and dissolved in saline) was injected intraperitoneally. After 15 min, streptozotocin (55 mg/kg dissolved in 0.1M citrate buffer solution at 4.5 pH) was injected intraperitoneally. Diagnosis of diabetes in rats was determined 5 days after injections through a sampling of blood from the tail. Blood glucose levels as determined by glucometer between 126 and 400 mg/dl of blood were indicative of diabetes [16].

Biopsy

Forty-eight hours after the last training session, rats were anesthetized with intraperitoneal administration of a mixture of ketamine (30–50 mg/kg body weight) and xylazine (3–5 mg/kg body weight). VAT and SAT were removed and washed with saline, then underwent freeze clamping. The supernatant was separated and placed into special tubes which were cooled with liquid nitrogen and preserved in a freezer at a temperature of −80 °C until the time of measuring.

Sampling measurement and data analysis

Serum RBP4 concentration in VAT and SAT was measured by the ELISA method through a special kit (ZellBio GmbH, Germany) which among its components included the Anti-RBP4 Antibody Coated Plate (EKU07106).

Insulin and glucose

Plasma insulin and glucose were measured by the ELISA method, using a special kit (ZellBio GmbH- Germany). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as fasting blood glucose fasting insulin/22.5 [17].

RBP4 RNA isolation, cDNA synthesis, and real-time PCR

The total RNA was extracted from 20mg of VAT using RNA purification kits (QIAGEN Rneasy protects mini kit). Complementary DNA (cDNA), utilizing an extended cDNA synthesis kit (One Step SYBR TAKARA), was measured based on the standard manufacturer's protocol. Expected fragment size and gene bank accession numbers are listed in Table 1. PCR was carried out on the Rotor-Gene 6000 Real-time PCR system (Corbett Life Science) with cycler conditions listed in Table 2.

The melting curve was analyzed at the end of the PCR cycle to determine the validity of the expected PCR product. The thermal cyclic protocol used by the Rotor-Gene device in Real-Time PCR was 42° for 20 min, 95° for 2 min and 40 cycles at 94° for 10 s and 60° for 40 s. After the PCR steps, temperatures 50 to 99 °C were used to prepare the melting curve and analyze the primer properties. RNA polymerase was used as a control gene to determine the expression of RBP4 (Fig. 1). Reaction cycle thresholds were extracted and

recorded using Rotor-Gene 6000 Real-Time PCR software. A comparative DDCT method was used to quantify the expression of TCFmRNA. The relative levels of mRNA were analyzed by the 2–DDCt method.

Training protocol

Training group was performed 5 days/week over a total of 8 weeks. AE was performed with intensity set at an estimated 60–75% of VO_2max and each session lasting from 10 to 40 min duration. RT was performed consisting of 10 climbs on a 1-m ladder set at an incline of 85°; a weighted resistance of 30–100% of body mass (resistance exercise) was utilized during the climbs. There was a 90-s rest interval between climbs. In addition, CT included a combined AE (performed in the morning) and RT (performed in the afternoon). The interval between bouts of AE and RT was 8 h. The CT protocol was adapted from previous literature in rats [18], following common procedures to carry out animal exercise training [19].

Statistical analysis

The Shapiro–Wilk test was used to evaluate the normal distribution of the findings and one-way ANOVA along with Tukey's post hoc test was used to analyze the findings ($p<0.05$).

Results

Tables 3 and 4 show the mean changes in weight, insulin, and glucose in different groups.

Exercise decreases RBP4 mRNA levels in VAT of diabetic rats

Based on the data analysis, a significant difference was observed in the rate of changes in RBP4 expression in VAT among different groups ($p = 0.007$, $F = 4.762$). The results of Tukey post hoc test indicated a significant decrease in the rate of changes in RBP4 expression of VAT in AE ($p = 0.018$), RT ($p = 0.037$), and CT ($p = 0.014$) groups compared to CON group (Fig. 2).

Table 2 Real-time cycler conditions

Steps	Time	Temperature
PCR initial activation step	20 min	42 °C
Denaturation	2 min	95 °C
40 cycle Combined annealing/extension	10s	94 °C
Melting curve	5 min	50 to 99°C
Cooling	End	40 °C

Exercise decreases RBP4 mRNA levels in SAT of diabetic rats

In addition, a significant difference was reported in the rate of changes in RBP4 expression in SAT between different groups ($p = 0.000$, $F = 18.801$). Further, a significant decrease occurred in the rate of changes in RBP4 expression of SAT in AE ($p = 0.000$), RT ($p = 0.001$), and CT ($p = 0.000$) groups compared to that of CON group. The decrease was significant in the CT group ($p = 0.022$) compared to that of RT (Fig. 3).

Exercise decreases RBP4 protein levels in VAT of diabetic rats

Additionally, a significant difference occurred in RBP4 protein changes in VAT among the groups ($p = 0.000$, $F = 12.214$). Furthermore, there was a significant decrease in the amount of RBP4 protein in VAT of AE ($p = 0.000$), RT ($p = 0.004$), and CT ($p = 0.000$) groups compared to that of CON group (Fig. 4). The statistical analysis indicated a significant difference in the amount of RBP4 protein changes in SAT among different groups ($p = 0.000$, $F = 21.035$).

Exercise decreases RBP4 protein levels in SAT of diabetic rats

Moreover, a significant decrease occurred in the amount of RBP4 protein in SAT of AE ($p = 0.001$), RT ($p = 0.022$), and CT ($p = 0.000$) groups compared to that of the CON group. The decrease was significant in the CT group ($p = 0.046$) compared to that of RT (Fig. 5).

Table 1 Oligonucleotide primer sequences and real-time PCR amplification parameters

Genes	Forward and reverse primer sequences	Scale (OD)	Purification
F RBP4: (5' → 3')	TGCAGGGTGAGCAGCTTCAG	2	BioRP
R RBP4: (5' → 3')	CACTTCCCAGTTGCTCAGAAG	2	BioRP
F GAPDH: (5' → 3')	AAGTTCAACGGCACAGTCAAGG	2	BioRP
R GAPDH: (5' → 3')	CATACTCAGCACCAGCATCACC	2	BioRP

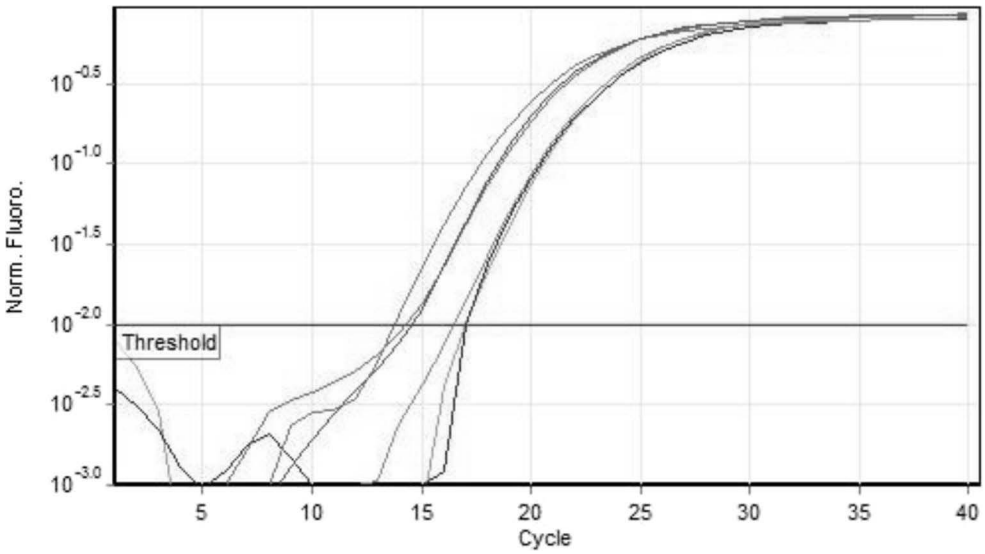

Fig. 1 RBP4 gene replication curve

Exercise decreases insulin resistance in diabetic rats

Statistical analysis indicated that a significant difference in the amount of HOMA-IR protein changes among different groups (p = 0.000, F = 11.370). The results of Tukey post hoc test indicated a significant decrease in HOMA-IR in AE (p = 0.008), RT (p = 0.000), and CT (p = 0.012) groups compared to that of the CON group (Fig. 6).

Discussion

Based on the results, all three types of exercises significantly reduced the expression and level of RBP4 protein in the tissues of VAT and SAT of diabetic rats with STZ. Increasing the RBP4 levels and inflammation is considered as one of the consequences of diabetes and exercise reduced the levels of such factors. Physical activity and exercise play a role in controlling chronic inflammatory diseases such as diabetes through reducing the release of pro-inflammatory cytokines and creating an anti-inflammatory environment. The decrease in RBP4 after exercise in the current study is in line with the result of previous reports indicating that the aerobic exercise is related to decreased levels of RBP4, insulin sensitivity, and GLUT4 expression in rat epididymal fat [20]. However, endurance training did not affect the expression and amount of liver RBP4 protein in diabetic rats [6].

Ramezani et al. found that 8-week RT was related to decreased levels of RBP4, serum glucose, and insulin resistance in diabetic rats [21]. The evidence suggested that inducting diabetes is related to increased RBP4 expression at the level of translation and transcription in the liver, muscle, and adipose tissue, and these changes are related to increased insulin resistance [6]. Since exercise increases the expression and protein levels of GLUT4 in adipocytes, the increase results in decreasing the concentration of RBP4 [22]. Increased GLUT4 in AT is considered as the main reason why RBP4 decreases as a result of aerobic activity in the present study. Reducing the size and amount of body fat mass is considered as another mechanism by which exercise reduces the expression of

Table 3 Body mass (grams) characteristics between groups

	Baseline	1week	4 weeks	8 weeks
CON (n=9)	234.3 ± 13.8	226.4 ± 13.6	252.7 ± 11.7$^{\pm}$	294.1 ± 19.4$^{\pm}$
AE (n=9)	233 ± 13.1	224.1 ± 12.8	257.6 ± 14.2$^{\pm}$	287.2 ± 9.6$^{*\ \#\pm}$
RT (n=9)	230.8 ± 13.1	224.4 ± 16.6	249.1 ± 20.8$^{\pm}$	259.2 ± 17.7$^{\#\pm}$
CT (n=9)	236.5 ± 13	228.6 ± 13.9$^{\#}$	245.7 ±13.4$^{*\#\pm}$	279.3 ± 19.7$^{*\#\pm}$

Data are presented as mean ± SD. *CON*, control; *AE*, aerobic exercise; *RT*, resistance training; *CT*, concurrent training

± Significantly different from baseline at p<0.05

\# Significantly different from CON at p<0.05

* Significantly different from CON at p<0.05

Table 4 Comparison of biological parameters between groups

	CON (n=9)	AE (n=9)	RT (n=9)	CT (n=9)
Insulin (μU/ml)	6.67 ± 0.66	5.95 ± 0.83	5.5 ± 1.06#	6.06 ± 0.78
Glucose (mg/dl)	324.89 ± 16.04	276.89 ± 27.59#	234.89 ± 66.9#	276.22 ± 30.5#

Data are presented as mean ± SD. *CON*, control; *AE*, aerobic exercise; *RT*, resistance training; *CT*, concurrent training

Significantly different from CON at $p<0.05$

RBP4 adipose tissue. Mansouri et al. showed that AE reduces RBP4 levels by reducing visceral and abdominal fat [6]. It was also shown that the decrease in BMI and body fat percentage following circuit RE [23] and CT [24] is associated with a significant decrease in plasma RBP-4 levels. Although body composition and AT size were not measured, the amount of weight compared to the CON group in all three exercise groups indicated a significant decrease. The findings indicated that weight loss and exercise result in reducing the fat size, which is considered as an important factor in the secretion of several adipokines [25]. In addition, decreasing RBP4 levels in AT may be related to the effects of exercise on reducing AT inflammation [6]. In addition, it leads to the reduction of RBP4 expression and protein levels in VAT. However, no decrease in RBP4 protein was observed in the study performed by Mansouri et al. despite reduced RBP4 expression of VAT [6].

In the current study, a decrease in the RBP4 fat tissue was observed along with a decrease in insulin resistance due to various exercise activities. Accordingly, Marchner et al. indicated that 10 weeks of AE significantly reduced RBP4 and increased insulin sensitivity in the rodents [20]. The studies indicated an inverse correlation between RBP4 levels with insulin resistance and VAT weight [20]. Decreased levels of RBP4 expression in VAT as a result of exercise reduce the serum levels of RBP4, which, in turn, decreases insulin resistance in various ways. The finding is supported by previous studies showing that exercise reduces RBP4 and improves insulin resistance [6, 26]. In addition, RBP4 has a negative effect on β cell function [6]. The improvement in insulin resistance in diabetic rats in the present study can be related to decreased serum RBP4 levels, although serum RBP4 concentrations were not measured in the present study. Moreover, the exercise can alter other adipokines which may affect insulin resistance which was not measured in the present study and is regarded as a limitation of the present study.

The results indicated that the decrease in expression and protein levels of RBP4 in the SAT of the CT group was more than RT. However, no change was observed in other variables and groups. Limited studies compared the effects of exercise type on RBP4 and the factors affecting insulin resistance. Smotok et al. found that both AE and RT exercises improved glucose and insulin and failed to cause any change in weight although the percentage of fat in the AE group decreased slightly [27]. In addition, RT has greater benefits versus AE

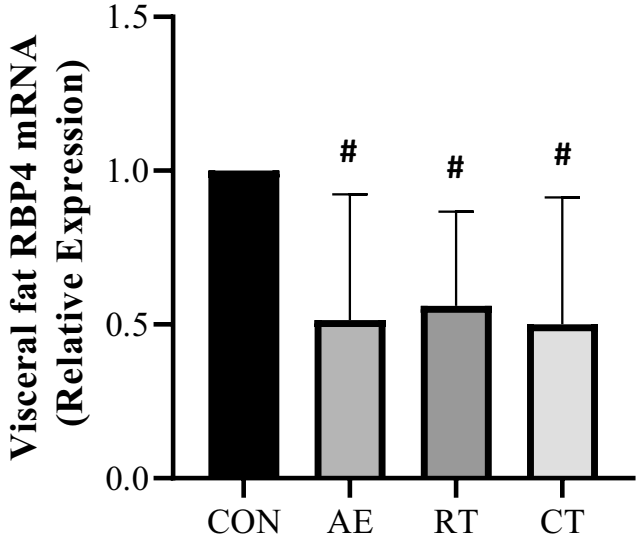

Fig. 2 Visceral fat RBP4 gene expression levels in four groups of study. #$p\leq0.01$ significant decrease compared to CON group. CON: control (n=9); AE: aerobic exercise (n=9); RT: resistance training (n=9); CT: concurrent training (n=9)

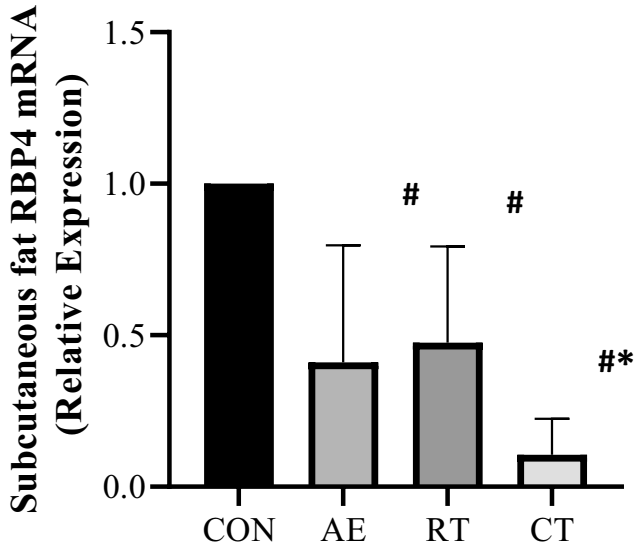

Fig. 3 Subcutaneous fat RBP4 gene expression levels in four groups of study. #$p\leq0.001$ significant decrease compared to CON group. *$p\leq0.001$ significant decrease compared to RT group. CON: control (n=9); AE: aerobic exercise (n=9); RT: resistance training (n=9); CT: concurrent training (n=9)

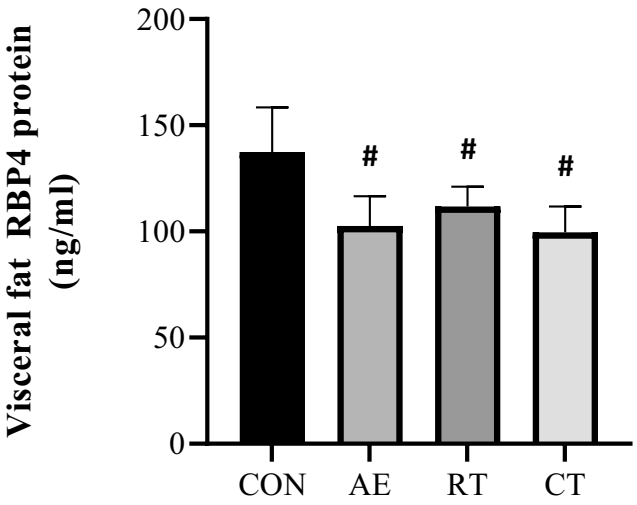

Fig. 4 Visceral fat RBP4 levels in four groups of study. #$p \leq 0.001$ significant decrease compared to CON group. CON: control (n=9); AE: aerobic exercise (n=9); RT: resistance training (n=9); CT: concurrent training (n=9)

exercises in controlling blood sugar in type 2 diabetic patients [28]. Further, CT (not AE) exercise improved glucose homeostasis better than RT exercise [29]. The difference between the two groups is related to the fact that the CT group used AE and RT at the same time, and the simultaneous effects of these two types of exercise increased energy expenditure and fat metabolism, thereby reducing the expression and RBP4 protein levels of SAT tissue. Also, since RBP4 levels are affected by inflammatory factors, perhaps the greater effect of CT on inflammatory markers [30] significantly reduced the expression and protein levels of RBP4 in the SAT in the CT group compared to RT. Accordingly, a meta-analysis examining the

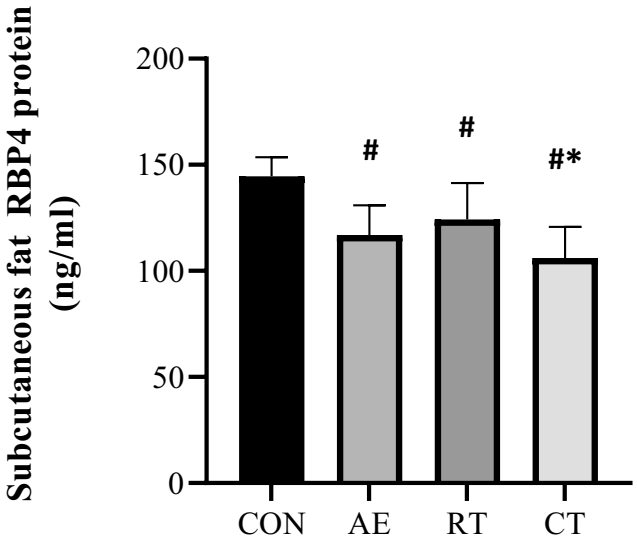

Fig. 5 Subcutaneous fat RBP4 levels in four groups of study. #$p \leq 0.001$ significant decrease compared to CON group. *$p \leq 0.001$ significant decrease compared to RT group. CON: control (n=9); AE: aerobic exercise (n=9); RT: resistance training (n=9); CT: concurrent training (n=9)

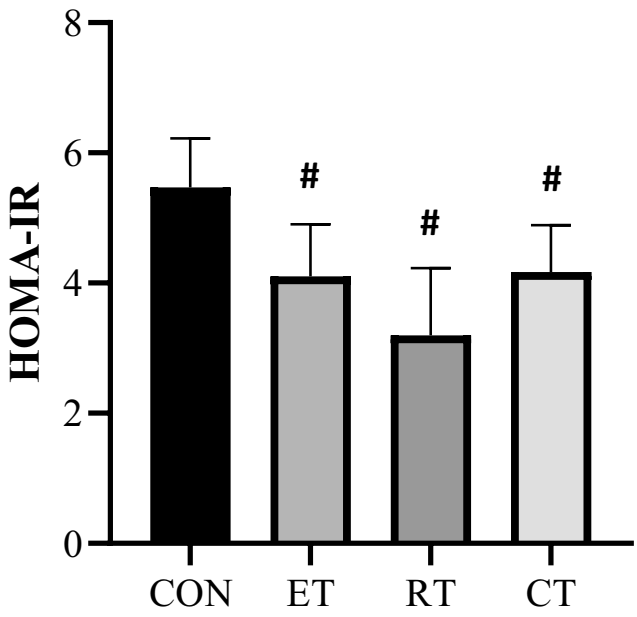

Fig. 6 Homeostasis model assessment of insulin resistance (HOMA-IR) levels in four groups of study. #$p \leq 0.001$ significant decrease compared to CON group. CON: control (n=9); AE: aerobic exercise (n=9); RT: resistance training (n=9); CT: concurrent training (n=9)

effect of different forms of exercise (AE, RE, or CT) on the risk factors associated with diabetes complications showed that CT was generally superior to AE or RE alone [31]. Slentz et al. also showed that CT reduced the insulin resistance index relative to RT, although the results of CT and AE were the same [32]. The length of the research period is considered as one of the limitations of the current study. Performing the intervention for 8 weeks may not be able to show the effect and difference of the type of exercise on adipokines in adipose tissue. Therefore, it is recommended to use longer protocols. Further, accurate measurement of AT changes by the methods such as MRI provides a better understanding of the effect of different types of exercise on AT changes followed by RBP4.

Conclusion

In this study, all three types of exercise reduced the expression and levels of RBP4 protein in the VAT and SAT of diabetic rats. However, the effect of CT on reduced expression and RBP4 protein levels of SAT was higher than that of the RT group.

Code availability Not applicable

Declarations All experiments were conducted based on the Iranian convention for the protection of vertebrate animal's policy; the Ethics Committee of the Sciences, Tehran University of Medical Sciences, approved the protocol.

Ethics approval This study was approved by the Ethics Committee of Tehran University.

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88–98. https://doi.org/10.1038/nrendo.2017.151.
2. Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. Endocrinology. 2003;144(6):2195-200. PMID: 12746274 Review.
3. Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab. 2002;13(1):18-23. PMID: 11750858 Review.
4. Broch M, Ramirez R, Auguet MT, Alcaide MJ, Aguilar C, Garcia-Espana A, et al. Macrophages are novel sites of expression and regulation of retinol binding protein-4 (RBP4). Physiol Res. 2010;59(2):299–303.
5. Klöting N, Graham TE, Berndt J, Kralisch S, Kovacs P, Wason CJ, Fasshauer M, Schön MR, Stumvoll M, Blüher M, Kahn BB. Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell metab. 2007;6(1):79–87. https://doi.org/10.1016/j.cmet.2007.06.002.
6. Mansouri M, Nikooie R, Keshtkar A, Larijani B, Omidfar K. Effect of endurance training on retinol-binding protein 4 gene expression and its protein level in adipose tissue and the liver in diabetic rats induced by a high-fat diet and streptozotocin. J Diabetes Investig. 2014;5(5):484–91. https://doi.org/10.1111/jdi.12186.
7. Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med. 2006;354(24):2552–63.
8. Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. Eur J Endocrinol. 2011;165(5):703–11. https://doi.org/10.1530/EJE-11-0431.
9. Moraes-Vieira PM, Castoldi A, Aryal P, Wellenstein K, Peroni OD, Kahn BB. Antigen presentation and T-cell activation are critical for RBP4-induced insulin resistance. Diabetes. 2016;65(5):1317–27. https://doi.org/10.2337/db15-1696.
10. Fletcher GF, Landolfo C, Niebauer J, Ozemek C, Arena R, Lavie CJ. Promoting physical activity and exercise: JACC health promotion series. J Am Coll Cardiol. 2018;72(14):1622–39. https://doi.org/10.1016/j.jacc.2018.08.2141.
11. Fiuza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. Physiology. 2013;28(5):330-58. PMID: 23997192 Review.
12. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B, American College of Sports Medicine, American Diabetes Association. 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(12):2692-2696. https://doi.org/10.2337/dc10-1548.
13. Acosta-Manzano P, Rodriguez-Ayllon M, Acosta FM, Niederseer D, Niebauer J. Beyond general resistance training. Hypertrophy versus muscular endurance training as therapeutic interventions in adults with type 2 diabetes mellitus: a systematic review and meta-analysis. Obes Rev. 2020. https://doi.org/10.1111/obr.13007.
14. Jorge MLMP, de Oliveira VN, Resende NM, Paraiso LF, Calixto A, Diniz ALD, Resende ES, Ropelle ER, Carvalheira JB, Espindola FS, Jorge PT, Geloneze B. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. Metabolism. 2011;60(9):1244–52. https://doi.org/10.1016/j.metabol.2011.01.006.
15. Pierre W, Gildas AJH, Ulrich MC, Modeste W-N, azTélesphore Benoît N, Albert K. Hypoglycemic and hypolipidemic effects of Bersama engleriana leaves in nicotinamide/streptozotocin-induced type 2 diabetic rats. BMC Complement Altern Med. 2012;12(1):264. https://doi.org/10.1186/1472-6882-12-264.
16. Skovsø S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. J Diabetes Investig. 2014;5(4):349–58.
17. Vogeser M, König D, Frey I, Predel H-G, Parhofer KG, Berg A. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. Clin Biochem. 2007;40(13-14):964–8. https://doi.org/10.1016/j.clinbiochem.2007.05.009.
18. Rodrigues B, Figueroa DM, Mostarda CT, Heeren MV, Irigoyen M-C, De Angelis K. Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. Cardiovasc Diabetol. 2007;6(1):38. https://doi.org/10.1186/1475-2840-6-38.
19. Feng R, Wang L, Li Z, Yang R, Liang Y, Sun Y, Yu Q, Gharteykwansah G, Sun Y, Wu Y, Zhang W, Zhou X, Xu M, Bryant J, Yan G, Isaacs W, Ma J, Xu X. A systematic comparison of exercise training protocols on animal models of cardiovascular capacity. Life Sci. 2019;217:128–40. https://doi.org/10.1016/j.lfs.2018.12.001.
20. Marschner RA, Pinto G, Borges J, Markoski MM, Schaan BD, Lehnen AM. Short-term detraining does not change insulin sensitivity and RBP4 in rodents previously submitted to aerobic exercise. Horm Metab Res. 2017;49(01):58–63. https://doi.org/10.1055/s-0042-115176.
21. Ramezani N, Gaiini A, CHoobineh S, Kordi M, Baesi K. The effect of resistance training on serum levels of RBP-4 and insulin resistance index in type 2 diabetic male rats. J North Khorasan Uni Med Sci. 2017;9(2):147-57. https://doi.org/10.18869/acadpub.jnkums.9.2.147.
22. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005;436(7049):356–62. https://doi.org/10.1038/nature03711.
23. Tayebi SM, Saeidi A, Fashi M, Pouya S, Khosravi A, Shirvani H, Ahmadian M, Abderrahman AB, Hackney AC, Zouhal H. Plasma retinol-binding protein-4 and tumor necrosis factor-α are reduced in postmenopausal women after combination of different intensities of circuit resistance training and Zataria supplementation. Sport Sci Health. 2019;15(3):551–8. https://doi.org/10.1007/s11332-019-00544-2.
24. Annibalini G, Lucertini F, Agostini D, Vallorani L, Gioacchini A, Barbieri E, Guescini M, Casadei L, Passalia A, del Sal M, Piccoli G, Andreani M, Federici A, Stocchi V. Concurrent aerobic and resistance training has anti-inflammatory effects and increases both plasma and leukocyte levels of IGF-1 in late middle-aged type 2 diabetic patients. Oxid Med Cell Longev. 2017;2017:3937842. https://doi.org/10.1155/2017/3937842.
25. Ibañez J, Izquierdo M, Argüelles I, Forga L, Larrión JL, García-Unciti M, et al. Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. Diabetes care. 2005;28(3):662–7. https://doi.org/10.2337/diacare.28.3.662.

26. Takebayashi K, Aso Y, Inukai T. Role of retinol-binding protein 4 in the pathogenesis of type 2 diabetes. Expert Rev Endocrinol Metab. 2008;3(2):161–73. https://doi.org/10.1586/17446651.3.2.161.
27. Smutok M, Reece C, Kokkinos P, Farmer C, Dawson P, Shulman R, et al. Aerobic versus strength training for risk factor intervention in middle-aged men at high risk for coronary heart disease. Metabolism. 1993;42(2):177–84. https://doi.org/10.1016/0026-0495(93)90032-j.
28. Cauza E, Hanusch-Enserer U, Strasser B, Kostner K, Dunky A, Haber P. Strength and endurance training lead to different post exercise glucose profiles in diabetic participants using a continuous subcutaneous glucose monitoring system. Eur J Clin Invest. 2005;35(12):745-51.
29. Davidson LE, Hudson R, Kilpatrick K, Kuk JL, McMillan K, Janiszewski PM, Lee SJ, Lam M, Ross R. Effects of exercise modality on insulin resistance and functional limitation in older adults: a randomized controlled trial. Arch Intern Med. 2009;169(2):122–31. https://doi.org/10.1111/j.1365-2362.2005.01573.x.
30. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, Fallucca S, Alessi E, Letizia C, Jimenez A, Fallucca F, Pugliese G. Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. N Nutr Metab Cardiovasc Dis. 2010;20(8):608–17. https://doi.org/10.1016/j.numecd.2009.04.015.
31. Snowling NJ, Hopkins WG. Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: a meta-analysis. Diabetes Care. 2006;29(11):2518–27. https://doi.org/10.2337/dc06-1317.
32. Slentz CA, Bateman LA, Willis LH, Shields AT, Tanner CJ, Piner LW, Hawk VH, Muehlbauer MJ, Samsa GP, Nelson RC, Huffman KM, Bales CW, Houmard JA, Kraus WE. Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by HOMA in overweight adults from STRRIDE AT/RT. Am J Physiol Endocrinol Metab. 2011;301(5):E1033–E9. https://doi.org/10.1152/ajpendo.00291.2011.

International Journal of Diabetes in Developing Countries (March–April 2023) 43(2):317–327
https://doi.org/10.1007/s13410-022-01080-5

ORIGINAL ARTICLE

Polysaccharides and flavonoids from cyclocarya paliurus modulate gut microbiota and attenuate hepatic steatosis, hyperglycemia, and hyperlipidemia in nonalcoholic fatty liver disease rats with type 2 diabetes mellitus

Lu Zhong[1] · Xiaojuan Peng[2] · Chutian Wu[1] · Qing Li[3] · Yanfang Chen[1] · Min Wang[1] · Yuting Li[1] · Kaiyin He[1] · Ying Shi[1] · Caiqun Bie[4] · Shaohui Tang[1]

Received: 12 October 2021 / Accepted: 20 April 2022 / Published online: 10 May 2022

Abstract

Objective The prevalence of nonalcoholic fatty liver disease (NAFLD) with type 2 diabetes mellitus (T2DM) is increasing, which causes greater harm to human health. *Cyclocarya paliurus* (CP) has antihyperglycemic and antihyperlipidemic effects. Here, we investigated the effects of polysaccharides (CPP) and flavonoids (CPF) from CP on gut microbiota, hepatic steatosis, and metabolic parameters in high-fat diet (HFD)/streptozotocin (STZ)-induced NAFLD rats with T2DM.

Methods NAFLD/T2DM rats, which were induced by high-fat diet (HFD) for 8 weeks and a low dose of 25 mg/kg STZ, were treated with CPP (8 g/kg/d) or CPF (6 g/kg/d) for 12 weeks. The alterations to gut microbiota, hepatic steatosis, and metabolic parameters were measured.

Results Treatment of both CPP and CPF could improve liver steatosis, NAFLD activity score (NAS), hyperglycemia, and hyperlipidemia. Importantly, administration with both CPP and CPF led to the significant reversion of increased abundance of the pathogenic bacteria *Escherichia-Shigella* in NAFLD/T2DM rats; moreover, CPP supplement also dramatically increased the beneficial bacteria *Akkermansia* abundance, while CPF treatment significantly elevated the abundances of the beneficial bacteria *Romboutsia* and *Weissella*.

Conclusion Both CPP and CPF as prebiotics have the significant therapeutic effects on hepatic steatosis and metabolic abnormalities induced by HFD and STZ in rats at least partially by modulating gut microbiota.

Keywords Nonalcoholic fatty liver disease · Type 2 diabetes mellitus · Cyclocarya paliurus polysaccharides · Cyclocarya paliurus flavonoids · Metabolism · Gut microbiota

Introduction

The emergence of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) as a global epidemic is one of the major challenges to human health in the twenty-first century. NAFLD is now recognized as the most prevalent chronic liver disease worldwide, with a prevalence as high as 30% in the general population [1]. NAFLD includes a

Lu Zhong, Xiaojuan Peng, Chutian Wu, and Qing Li contributed equally to this work.

✉ Shaohui Tang
tangshaohui206@163.com

[1] Department of Gastroenterology, The First Affiliated Hospital, Jinan University, Guangzhou, Guangdong 510630, People's Republic of China

[2] Department of Endocrinology, Liuzhou People's Hospital, Liuzhou, Guangxi 545006, People's Republic of China

[3] Department of Interventional vascular surgery, Affiliated Hospital (Clinical College) of Xiangnan University, Chenzhou, Hunan 423000, People's Republic of China

[4] Department of Gastroenterology, The Affiliated Shenzhen Shajing Hospital, Guangzhou Medical University, Shenzhen, Guangdong 518104, People's Republic of China

series of diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), and to advanced cirrhosis and hepatocellular carcinoma [2]. It has been well-recognized that obesity, hyperlipidemia, diabetes mellitus, metabolic syndrome, and insulin resistance (IR) are considered as risk factors of NAFLD [3, 4]. T2DM is a complex metabolic disorder characterized by hyperglycemia, low-grade inflammation, IR, and β-cell failure and mainly affects glucose, lipid, and protein metabolism [5, 6]. It is apparent that NAFLD and T2DM share common risk factors, such as obesity, IR. The prevalence of NAFLD in the population of T2DM is increasing year by year, and has been found to be higher compared with that without T2DM [7, 8]. It should be noted that NAFLD with T2DM causes greater harm to health. People with NAFLD and T2DM are more likely to suffer from cardiovascular disease, chronic kidney disease, and carcinoma [3, 9].

Recent studies have shown that gut microbiota dysbiosis is associated with several non-communicable diseases such as obesity, diabetes, cardiovascular diseases, and NAFLD [10–12]. A direct involvement of gut microbiota in the development of NAFLD is suggested by the finding that NAFLD can be delivered to germ-free mice by fecal microbiota transplantation [13]. Imbalances in the structure of gut microbiota are related to gut barrier dysfunctions and cause insulin resistance and endotoxemia, which may finally lead to obesity and T2DM [14–18]. These results suggest that regulating gut microbiota could treat NAFLD and T2DM. At present, many of the anti-diabetic drugs currently in clinical use, though effectively treating symptoms, have several side effects including hepatic and renal lesions [19]. On the other hand, there is still no effective drugs for the treatment of NAFLD except for lifestyle changes, including healthy diet, weight loss, and exercise [20]. In addition, there is no consensus or therapeutic strategies for the management of NAFLD patients with T2DM.

Cyclocarya paliurus (CP) (Batal.) Iljinsk (family Cyclocaryaceae) is a plant with edible and medicinal value, which is grown in mountainous regions of Southern China. The leaves of CP have long been used as a dietary food and a traditional herbal medicine for the prevention or treatment of diabetes mellitus, hypertension, hyperliposis [21–23]. CP leaves contain a variety of biologically chemical components, including polysaccharides, flavonoids, coumarins, amino acids, sterols, and triterpenes [24], in which polysaccharides and flavonoids are recognized as the main bioactive components in CP [22, 25, 26]. It has been reported that polysaccharides and flavonoids from CP possess many bioactivities, such as anti-inflammatory, anti-hyperlipidemic, and anti-diabetic activities [23, 25–28].

However, to the best of our knowledge, no investigation has been performed to explore the protective effects of polysaccharides (CPP) and flavonoids (CPF) from CP on metabolic abnormalities and liver damage in NAFLD rats with T2DM (NAFLD/T2DM rats) induced by high-fat diet and streptozotocin (STZ). In the present study, we explored the effects of CPP and CPF on gut microbiota, hepatic steatosis, and metabolic parameters in NAFLD/T2DM rats.

Materials and methods

Preparation of CPP and CPF

The extraction of CPP was performed according to the method described previously [29]. Briefly, the air-dried and powdered leaves were soaked with 95% (v/v) ethanol for 12 h, and the mixture was filtered. The residues were dried in air and were boiled with distilled water at 95°C for 2 h (1:20, mg/mL). The above operation was repeated twice. Then, the mixture was filtered, and the filter liquor was retained. The filter liquor was concentrated by rotary evaporation at 60°C and then allowed to put overnight at 80% (v/v) ethanol concentration. The protein in the obtained CPP was removed with Savag method, and the CPP was dried under vacuum at −40°C. The CPP content was 79.6%, which was determined by phenol-sulfuric acid method [30] (Fig. 1).

Preparation of CPF was conducted according to the method described by Cheng et al. [31]. Briefly, CP leaves were pulverized and extracted with distilled water at 95◦C for 40min. After extraction, the extract was centrifuged at 4500×g for 15 min, and the above operation was repeated. The supernatants were combined and concentrated using a rotary evaporator. The residue was dissolved with deionized water, filtered by a 0.45-μm microfiltration membrane, and applied to a column (30×1.6 cm) of AB-8 resin. Finally, the effluent of ethanol solution was collected and concentrated, leading to the CPF extract. The CPF content was 84.3%, which was determined by aluminum chloride method [32] (Fig. 1).

Animal model and experiment design

A total of 60 male Sprague Dawley rats (weight, 180±20 g; age, 6 weeks) were purchased from the medical laboratory animal center of Guangdong (Guangzhou, China). All the rats were acclimatized under a temperature of 24±2°C, a relative humidity of 55±10%, and a 12 h light/dark cycle for 10 days before commencement of the animal experiment. All animal experiments were approved by the experimental animal ethics committee of Jinan University and was performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Jinan University.

After acclimation, these rats were randomly divided into the normal control group (NC, n=10) and the model group with both NAFLD and diabetes (NAFLD/T2DM Model, *n*=50). NAFLD/T2DM Model rats were induced by high-fat diet (HFD) (containing 34% fat, 2% cholesterol, 26%

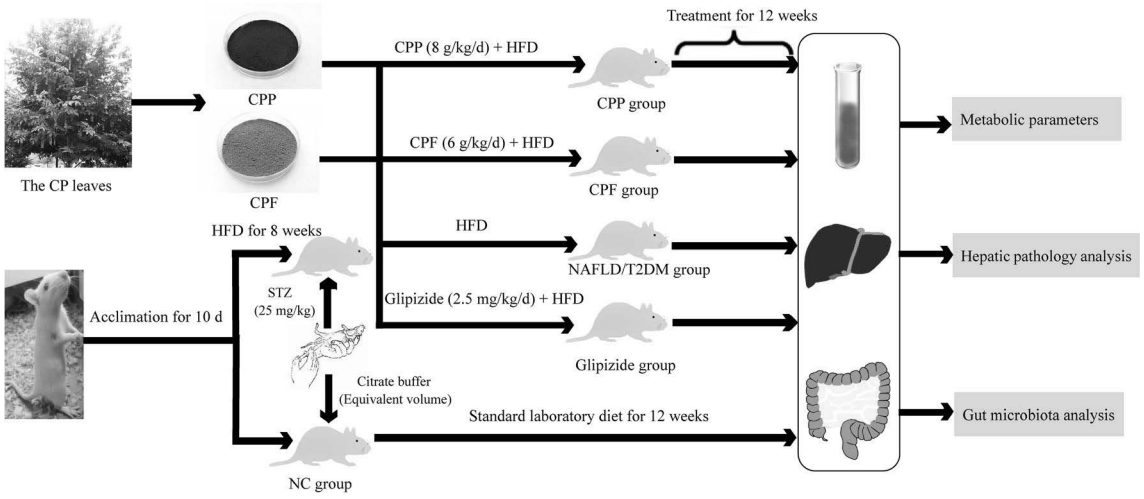

Fig. 1 CPP and CPF content

carbohydrate, 26% protein, and 12% basic feed (w/w)) for 8 weeks, followed by an intraperitoneal injection with streptozotocin (STZ) (25 mg/kg in citrate buffer). The rats with fasting glucose level higher than 11 mmol/L were considered as NAFLD/T2DM Model rats, and the NAFLD/T2DM Model rats with consecutive 10-day hyperglycemia (11 mmol/L or greater) were used for the experiment. Finally, 48 NAFLD/T2DM Model rats met the above experimental standard. In parallel, NC rats, which were fed a standard laboratory diet, were injected with an equal volume of citrate buffer solution.

The 48 NAFLD/T2DM Model rats were randomly divided into four groups: NAFLD/T2DM group (n=12), CPP group (n=12), CPF group (n=12), and Glipizide group (n=12), which were continuously fed with the HFD, and administrated by oral gavage once per day with distilled water, CPP (8 g/kg/d), CPF (6 g/kg/d), and Glipizide (2.5 mg/kg/d) (Glipizide extended release tablets**,** Glucotrol XL), respectively for the next 12 weeks. The NC group rats were fed with the standard laboratory diet for the next 12 weeks.

Observations on the general condition of the rats

The general condition of the rats was monitored daily, including physical activity, fur condition, water intake, food intake, urine output and survival condition. Body mass and food intake were determined weekly.

Analysis of metabolic parameters

At the end of the experiment, overnight-fasted rats were anesthetized by 1% pentobarbital solution (40mg/kg), and the blood samples were collected from abdominal aortic. The fasting blood glucose (FBG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), interleukin (IL)-6, and tumor necrosis factor (TNF)-α were analyzed using commercial assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturers' instructions. The fasting insulin (FINS) was measured with rat insulin kits (R&D Corporation, USA). The insulin resistant index (HOMA-IR) was also calculated by using the following formula:

$$\mathrm{HOMA-IR} = \mathrm{FBG\ (mmol/l)} \times \mathrm{FINS(\mu IU/ml)}/22.5$$

Analysis of hepatic pathology

At the end of the study, the rats were sacrificed to determine liver mass and liver mass index by using the following formula:

$$\text{live mass index} = \text{liver mass}/\text{body mass} \times 100\%$$

Then, the liver samples were immersed in 10% formalin neutral buffer solution for 48h, then processed routinely, embedded in paraffin, sectioned to 5 μm thickness and stained with hematoxylin and eosin (H&E). Then, we used Image-Pro Plus 6.0 software (*Media Cybernetics,* Rockville, MD, USA*)* to quantitatively analyze fat in liver [13]. The evaluation standard of NAFLD activity score (NAS) is shown in Table S1 [33, 34].

Gut microbiota analysis

At the end of the experiment, feces of rats were collected in sterilized plastic tubes and stored in a -80 °C until use. Total DNA of the samples was extracted using a HiPure Stool DNA Kits (Guangzhou Meiji biotechnology co. LTD, Guangzhou, China) following manufacturer's recommendations. The V3-V4 region of bacterial 16S rDNA gene was amplified using primers 341F (5'- CCT ACG GGN GGC WGC AG -3') and 806R (5'-GGA CTA CHV GGG TAT CTA AT-3').

Sequencing libraries were generated using two-step PCR amplification method. The second round of amplification products was purified using AMPure XP Beads (Beckman Coulter, USA) and quantified using a QuantiFluorTM fluoromete (Promega, USA). At last, the library was sequenced on IlluminaHiSeq 2500 platform (Illumina, USA).

Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (IBM Corp., USA). Quantitative data were analyzed by independent-Samples *t* Test or one-way analysis of variance (ANOVA) followed by Mann-Whitney *U* test or Kruskal-Wallis H test. Correlation was evaluated by Pearson correlation co-efficient analysis. A difference with $p<.05$ was considered significant.

Results

Effects of CPP and CPF on general condition

The rats in the NAFLD/T2DM group showed sluggish action, irritability, polydipsia, *bulimia,* and polyuria, while those in the NC group did not. The above general condition was significantly improved in the CPP group, CPF group, and Glipizide group rats compared with the NAFLD/T2DM group rats.

A significant decrease in the body mass was observed in the NAFLD/T2DM group compared with the NC group. After treatment of both CPP and CPF, the rats showed a significant increase in the body mass compared with the NAFLD/T2DM group rat (Table 1). A significant increase in the body mass was also found in the Glipizide group compared with the NAFLD/T2DM group.

Effects of CPP and CPF on metabolic parameters

A significant increase in serum liver enzymes (ALT, AST, and GGT), serum lipid profile (TC, TG, LDL-C), glucose metabolism indices (FBG, FINS, and HOMA-IR), serum proinflammatory cytokines (TNF-α and IL-6) was observed in the NAFLD/T2DM group compared with the NC group. After treatment of both CPP and CPF, a remarkable decrease in the above metabolic parameters was found compared with the NAFLD/T2DM group. In addition, Glipizide treatment significantly lowered serum TG, glucose metabolism indices (FBG, FINS, and HOMA-IR), serum proinflammatory cytokines (TNF-α and IL-6), but had no effect on serum liver enzymes (ALT, AST, and GGT), and serum TC and LDL-C compared with the NAFLD/T2DM group (Table 1).

Effects of CPP and CPF on hepatic pathological changes

The rats in the NAFLD/T2DM group showed a significant increase in liver mass, liver mass index, liver fat content, and NAS compared with the NC rats, whereas both CPP and CPF intervention significantly decreased the above hepatic pathological parameters. Glipizide treatment also lowered liver mass index, but had no significant influence on liver mass, liver fat content, and NAS compared with the NAFLD/T2DM group (Table 1 and Fig. 2).

Effects of CPP and CPF on gut microbiota composition

The structural changes of gut microbiota are crucial in the pathogenesis of obesity and some other metabolic diseases. To assess the changes of gut microbiota composition after treatment of both CPP and CPF in NAFLD/T2DM rats, 16S rDNA gene sequences from bacterial populations of intestinal contents were analyzed. At the phylum level, the 5 most abundant microbiota in all groups included *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* (Fig. 3A). The relative abundances of *Firmicutes* (51.1%) and *Bacteroidetes* (0.3%) decreased significantly, while the relative abundance of *Proteobacteria* (29.3%) increased significantly in the NAFLD/T2DM group compared with the NC group (Fig. 3B–D). CPP treatment significantly decreased the relative abundances of *Firmicutes* (24.0%) and *Proteobacteria* (1.8%) (Fig. 3B and D), and increased the relative abundance of *Verrucomicrobia* (69.4%) (Fig. 3E), whereas CPF treatment remarkably increased the abundances of *Firmicutes* (73.4%) and *Bacteroidetes* (1.9%) compared with the NAFLD/T2DM group (Fig. 3B and C). In addition, the ratio of *Firmicutes* to *Bacteroidetes* increased significantly in the NAFLD/T2DM group compared with the NC group, whereas treatment of both CPP and CPF led to the reduction of this ratio but this reduction was not significant compared with the NAFLD/T2DM group (Fig. 3F). In the Glipizide group, the variation of the gut microbiota abundance was similar to the CPF group (Fig. 3B and Table S2).

At the genus level, the composition of the bacteria was substantially changed (Fig. 4A). The relative abundances of *Ruminococcaceae_UCG-005* (0.6%), *Lactobacillus* (4.3%), *Weissella* (1.0%), *Romboutsia* (4.1%) decreased significantly (Fig. 4B–E), while the relative abundances of *Escherichia-Shigella* (22.0%), *Collinsella* (6.0%), and *Blautia* (6.6%) increased markedly in the NAFLD/T2DM group compared with the NC group (Fig. 4–H). CPP treatment decreased the relative abundance of *Escherichia-Shigella* (1.0%) and increased the relative abundance of *Akkermansia* (45.3%) significantly (Fig. 4F and I), while CPF treatment decreased the relative abundance of *Escherichia-Shigella* (4.6%) (Fig. 4F) and increased the relative abundances of *Romboutsia* (10.9%) (Fig. 4E) and

Table 1 Metabolic characteristics and hepatic pathological changes in laboratory rats

Items	NC (*n*=9)	NAFLD/T2DM (*n*=10)	CPP (*n*=9)	CPF (*n*=9)	Glipizide (*n*=9)
Body mass (g)					
Mean ± SD	589.1±70.2	429.4±55.9**	500.1±62.4#	498.3±25.5 ##	509.8±67.0 #
Median (IQR)	551.0 (532.0-643.0)	433.0 (372.4-479.3) **	505.0 (438.5-552.0) #	510.0 (472.5-520.0) ##	513.0 (439.5-571.0) #
Serum liver enzymes (U/L)					
ALT					
Mean ± SD	46.0±7.6	270.3±107.6 **	157.5±54.3 #	66.5±23.6 ##	210.8±30.3
Median (IQR)	43.3 (38.9-53.5)	248.8 (176.9-339.9) **	156.3 (128.6-174.4) ##	75.2 (40.6-88.6) ##	208.1 (183.6-228.2)
AST					
Mean ± SD	106.3±18.7	542.6±179.9**	196.4±78.3##	173.6±63.0##	564.7±92.6
Median (IQR)	109.9(89.4-125.8)	566.1(335.9-723.1) **	162.1(139.7-266.0) ##	156.5(137.3-200.0) ##	540.0(490.0-618.9)
GGT					
Mean ± SD	36.0±11.1	160.7±57.1**	86.3±17.8##	67.0±18.0##	154.4±44.1
Median (IQR)	33.2 (27.9-44.0)	142.9 (114.0-221.5) **	81.2 (71.8-97.5) ##	63.7 (56.1-82.6) ##	159.4 (115.3-189.6)
Serum lipid profile (mmol/L)					
TC					
Mean ± SD	1.6±0.6	7.1±4.0 **	2.7±0.9 ##	2.5±0.5 ##	6.8±2.7
Median (IQR)	1.6(1.0-2.1)	6.6(3.5-9.9) **	2.3(2.2-3.0) ##	2.3(2.2-3.0) ##	5.9(4.4-9.0)
TG					
Mean ± SD	0.8±0.6	7.6±2.2 **	1.0±0.8 ##	1.0±0.4 ##	4.3±1.2 ##
Median (IQR)	0.6(0.4-1.3)	7.7(5.7-9.5) **	0.6(0.6-1.0) ##	1.1(0.6-1.3) ##	4.0(3.2-5.3) ##
LDL-C					
Mean ± SD	0.7±0.4	2.5±1.5 **	1.2±0.2 #	1.2±0.5 #	2.2±1.5
Median (IQR)	0.6(0.4-1.1)	2.2(1.3-3.5) **	1.2(0.9-1.3) ##	1.1(0.8-1.7) #	2.0(0.9-3.2)
Glucose metabolism indices					
FBG (mmol/L)					
Mean ± SD	4.6±1.2	18.5±1.9 **	11.9±1.2 ##	13.8±2.1 ##	13.5±1.9 ##
Median (IQR)	4.3 (3.7-5.5)	18.5 (17.1-19.0) **	11.9 (11.0-13.0) ##	14.0 (12.4-15.7) ##	13.1 (12.3-15.6) ##
FINS (μIU/ml)					
Mean ± SD	15.9±2.9	49.7±4.2 **	21.3±3.8 ##	25.5±4.1##	25.3±3.2 ##
Median (IQR)	15.3 (13.2-18.7)	49.7 (46.1-53.3) **	19.7 (18.4-25.3) ##	25.3 (21.5-29.0) ##	25.8 (22.9-27.0) ##
HOMA-IR					
Mean ± SD	3.3±1.0	40.9±5.4 **	11.3±2.2 ##	15.7±3.7 ##	15.1±2.2 ##
Median (IQR)	3.1 (2.4-4.1)	38.7 (37.5-45.4) **	11.5 (9.1-13.5) ##	15.5 (14.8-16.5) ##	14.7 (13.4-17.0) ##
Serum proinflammatory cytokines (pg/ml)					
TNF-α					
Mean ± SD	38.0±15.5	128.5±18.8 **	60.5±13.5 ##	66.3±18.0 ##	93.3±10.8 ##
Median (IQR)	35.5 (24.1-52.6)	124.4 (119.0-136.5) **	63.0 (48.5-70.0) ##	59.5 (54.8-85.9) ##	91.8 (84.7-105.0) ##
IL-6					
Mean ± SD	61.1±22.7	840.3±119.2 **	538.2±157.5 ##	125.5±42.8 ##	317.2±45.9 ##
Median (IQR)	50.4 (45.7-85.0)	822.4 (769.1-965.4) **	501.8 (390.0-684.7) ##	106.5 (93.9-148.3) ##	319.8 (285,9-355.4) ##
Hepatic pathological indices					
Liver mass (g)					
Mean ± SD	12.6±1.8	29.2±4.5 **	18.1±3.0 ##	15.6±1.9 ##	27.8±2.5
Median (IQR)	13.2 (10.5-14.3)	28.4 (26.3-33.6) **	17.8 (15.9-20.6) ##	16.1 (13.8-16.9) ##	28.5 (25.4-30.0)
Liver mass index (%)					
Mean ± SD	2.2±0.4	6.8±1.0 **	3.7±0.9 ##	3.1±0.4 ##	5.5±0.6 ##
Median (IQR)	2.0 (1.9-2.5)	6.8 (5.9-7.8) **	3.8 (2.8-4.2) ##	3.2 (2.8-3.5) ##	5.3 (5.0-5.9) ##

Table 1 (continued)

Items	NC (*n*=9)	NAFLD/T2DM (*n*=10)	CPP (*n*=9)	CPF (*n*=9)	Glipizide (*n*=9)
Liver fat content (%)					
Mean ± SD	1.0±1.3	66.4±9.9 **	22.5±7.0 ##	26.8±8.2 ##	59.0±9.1
Median (IQR)	0.0 (0.0-2.0)	64.1 (58.4-76.5) **	23.8 (17.9-28.2) ##	27.6 (21.1-34.6) ##	58.7 (53.4-67.1)
NAS					
Mean ± SD	0.1±0.1	6.5±0.3 **	3.1±0.4 ##	2.0±0.5 ##	6.1±0.7
Median (IQR)	0.1 (0.0-0.1)	6.5 (6.2-6.9) **	3.2 (2.8-3.4) ##	2.1 (1.5-2.4) ##	6.0(5.6-6.7)

*p<0.05, **p<0.01 vs NC group; #p<0.05, ##p<0.01 vs NAFLD+T2DM group. The data are shown as mean and median (interquartile range, IQR). *NC* normal control, *NAFLD/T2DM* NAFLD with T2DM, *CPP* cyclocarya paliurus polysaccharides, *CPF* cyclocarya paliurus flavonoids, *SD* standard deviation, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* γ-glutamyl transpeptidase, *TC* total cholesterol, *TG* triglyceride, *LDL-C* low-density lipoprotein-cholesterol, *FBG* fasting blood glucose, *FINS* fasting insulin, *HOMA-IR* = (fasting glucose × fasting insulin)/22.5, *TNF-α* tumor necrosis factor α, IL-6 interleukin-6, NAS NALFD activity score

Weissella (4.2%) (Fig. 4D) significantly compared with the NAFLD/T2DM group. In the Glipizide group, the relative abundance of *Escherichia-Shigella* (2.5%) decreased markedly (Fig. 4F) and the relative abundances of *Lactobacillus* (10.4%) and *Romboutsia* (6.4%) and *Dubosiella* (8.1%) (Fig. 4C, E, and J) increased significantly (Table S3).

Correlation between improvements of metabolic indices as well as hepatic pathology and the changes in gut bacterial genera induced by CPP and CPF

We examined whether the improvements of metabolic indices and hepatic pathology were correlated with the alternations of bacterial genera induced by CPP and CPF. Pearson correlation analysis showed that the alternation of Akkermansia level was negatively correlated with the main metabolic and hepatic pathological indices, whereas Escherichia-Shigella level was positively correlated with most of the above results after CPP treatment. Moreover, other CPP-modulated genera, such as *Blautia, Lactobacillus, Dubosiella, Collinsell, Romboutsia, Weissella, Aerococcus, and Ruminococcaceae_UCG-005* also contributed to the changes in a few indices of the above results (Fig. 5A). On the other hand, the level of Romboutsia was negatively correlated with most of the metabolic and hepatic pathological parameters, whereas Escherichia-Shigella, Collinsell, Dubosiella, and Aerococcus were positively correlated with the partial above indices after CPF treatment. In addition, *Blautia, Lactobacillus, Akkermansia, Weissella,* and *Ruminococcaceae_UCG-005* enriched by CPF were also negatively associated with some serum liver enzymes and lipid profile (Fig. 5B).

Discussion

In the present study, a rat model of NAFLD/T2DM was induced by HFD for 8 weeks and a low dose of 25 mg/kg STZ,

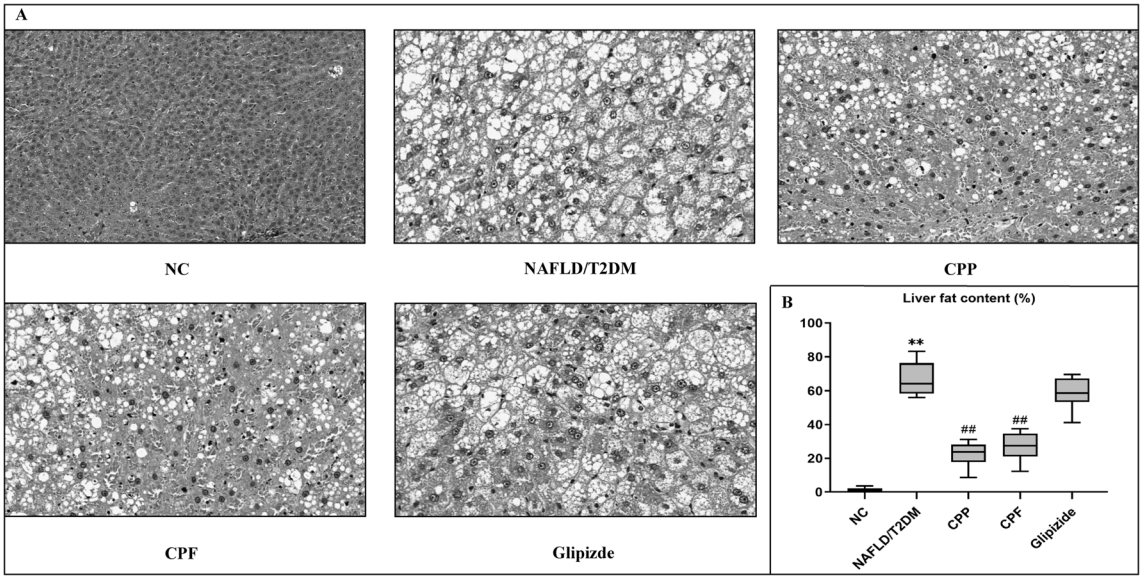

Fig. 2 Glipizide treatment also lowered liver mass index

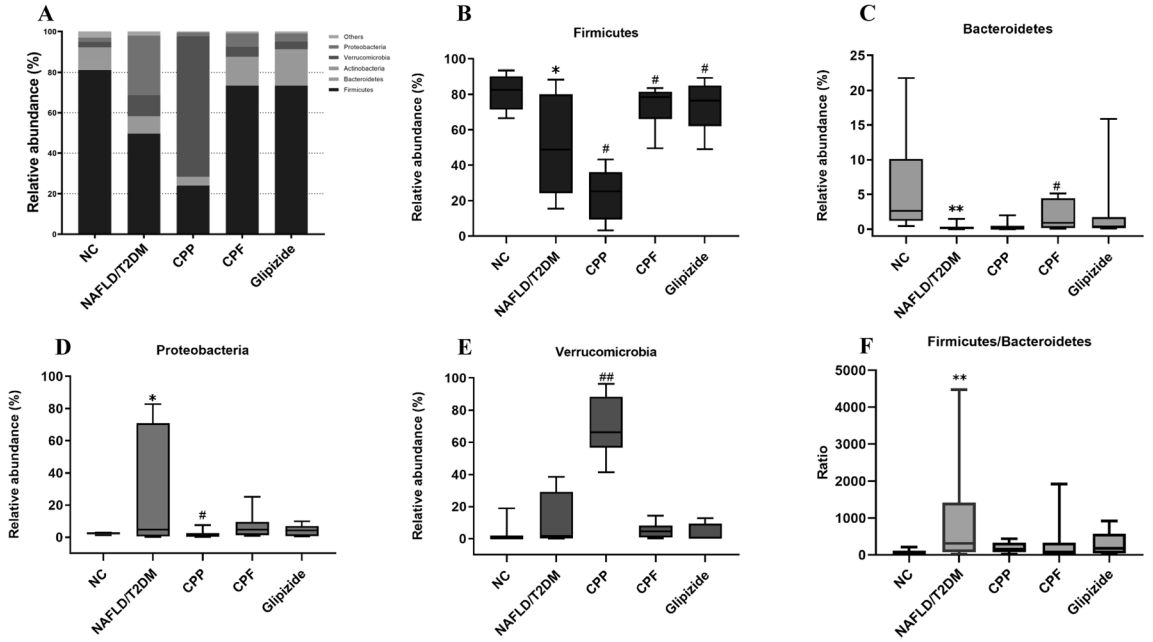

Fig. 3 The 5 most abundant microbiota in all groups

which reveals similar metabolic characteristics and liver damage of NAFLD with T2DM in humans [35, 36]. Treatment with both CPP and CPF significantly improved body mass, liver enzymes (ALT, AST, and GGT), blood lipids (TC, TG, and LDL-C), glucose metabolism parameters (FBG, FINS, and HOMA-IR), proinflammatory cytokines (TNF-α and IL-6), and hepatic pathological parameters (liver mass, liver mass index, liver fat content, and NAS), while Glipizide treatment elevated body mass and only reduced glucose metabolism parameters (FBG, FINS, and HOMA-IR) and proinflammatory cytokines levels (TNF-α and IL-6) in the NAFLD/T2DM rats. These results suggest that the protective effects of both CPP and CPF on NAFLD with T2DM in rat models are superior to Glipizide. Similar to our results, Lin et al. found

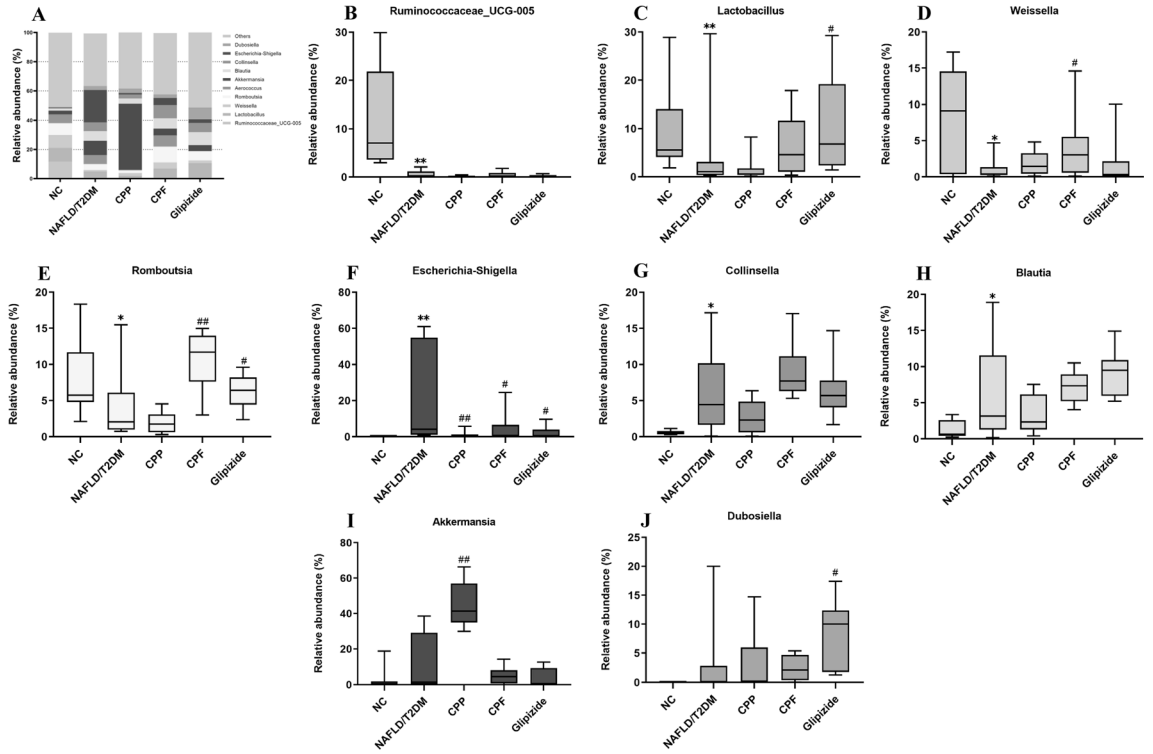

Fig. 4 The composition of the bacteria

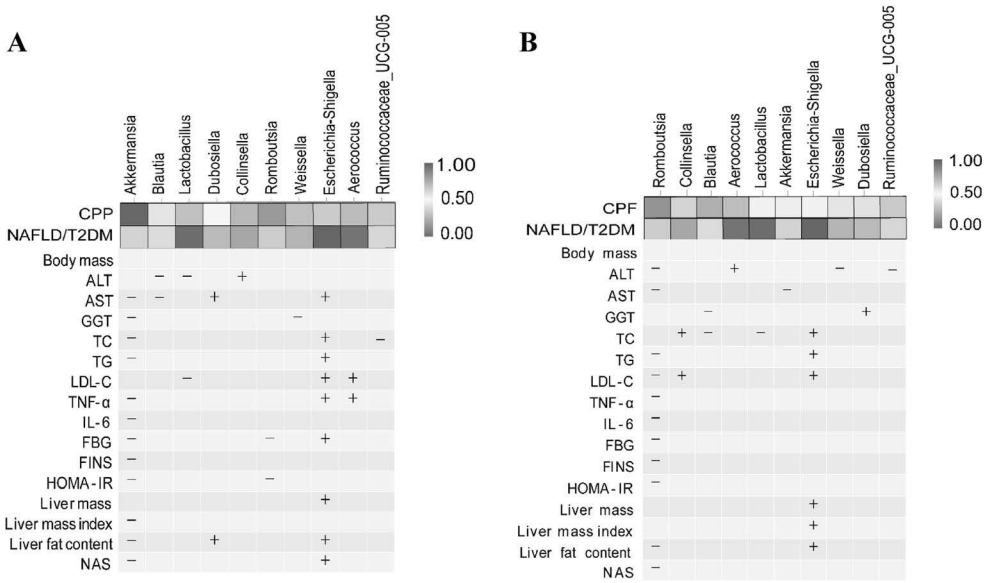

Fig. 5 CPP-modulated genera

that chloroform extract of CP markedly decreased the levels of serum liver enzymes (ALT, AST, and ALP), blood lipids (TC and TG) and liver lipids (TC and TG), and serum and liver TNF- α in NAFLD rats [37]. These effects were partially through decreasing serum NEFAs which might lead to a decrease in the amount of liver lipid intake, as well as suppressing hepatic lipid de novo synthesis. CPP and CPF also ameliorated HFD-induced hepatic oxidative stress and inflammation, leading to block the development of NAFLD.

The mechanisms responsible for the protective effect of CP on NAFLD and T2DM have attracted much attention. Accumulating evidence has indicated that gut microbiota dysbiosis is correlated with the pathogenesis of NAFLD, T2DM and obesity [23, 38–40], thus speculating that the therapeutic role of CP in metabolic diseases might be partially attributed to the alteration of gut microbes. It has been shown that a higher ratio of Firmicutes to Bacteroidetes is observed in obese individuals than lean individuals [40], and this ratio is significantly reduced after CPF treatment in a high-fat diet-induced obesity mouse model [31].

In the current study, at the phyla level, oral administration of CPP dramatically elevated the abundance of *Verrucomicrobia* and lowered the abundances of *Proteobacteria* and *Firmicutes* in the NAFLD/T2DM rats, and the ratio of *Firmicutes* to *Bacteroidetes* also showed a decrease trend after CPP treatment; on the other hand, CPF treatment significantly recovered dysbiosis of *Firmicutes* and *Bacteroidetes* and reduced the ratio of *Firmicutes* to *Bacteroidetes* (although there was no significant difference in this ratio) in the NAFLD/T2DM rats. At the genus level, oral administration with both CPP and CPF led to the significant reversion of increased abundance of *Escherichia-Shigella* induced by HFD and STZ in rats; in addition, CPP supplement dramatically increased the abundance of *Akkermansia*, while CPF treatment led to a significant increase in the abundances of *Romboutsia* and *Weissella*. Further pearson linear correlation analysis showed that the significant increase in *Akkermansia* abundance and the significant decrease in *Escherichia-Shigella* abundance induced by CPP treatment were associated with the improvements of the main metabolic and hepatic pathological indices; on the other hand, elevated *Romboutsia* level and reduced *Escherichia-Shigella* level after CPF treatment were correlated with the alleviation of most abnormal metabolic and hepatic pathological parameters in NAFLD/T2DM rats. Besides, Glipizide treatment led to the increased abundances of *Lactobacillus, Romboutsia*, and *Dubosiella* and the decreased abundance of *Escherichia-Shigella*.

Consistent with our results, the report by Yao et al. indicated that CPP treatment alleviates blood glucose, blood lipid, and HOMA-IR index by increasing the short-chain fatty acids (SCFAs)-producing gut bacteria in rats with T2DM [41]; Li et al. showed that CPP treatment increased the beneficial bacteria genus *Ruminococcaceae UCG-005* abundance, which in turn attenuated FBG and HOMA-IR in type 2 diabetic rats [38]; Bai et al. showed that treatment of flavonoids of Quzhou Fructus Aurantii extract significantly reduced obesity, inflammation and liver steatosis by the reduction of *Firmicutes* to *Bacteroidetes* ratio, the increase in genera *Akkermansia* and *Alistipes*, and the decrease in genera *Dubosiella*, *Faecalibaculum*, and *Lactobacillus* in HFD-induced obesity mouse model [42]; the report by Li et al. revealed that Silybin administration showed protective effects against high-fat diet-induced obesity, insulin resistance, and liver steatosis in mice, which was associated with lowering the *Firmicutes* to *Bacteroidetes* ratio and increasing the abundances of SCFA-producing bacteria (*Blautia, Bacteriodes, and Akkermensia*) [43].

It has been shown that *Escherichia-Shigella, Aerococcus, Collinsella,* and *Dubosiella* may contribute to the development of metabolic diseases such as obesity, NAFLD, and T2DM [42, 44–49]. On the other hand, it has been accepted that decreased abundances of some beneficial bacteria in gut, such as *Ruminococcaceae_UCG-005, Lactobacillus, Akkermansia, and Blautia,* are associated with obesity, NAFLD, and T2DM [42, 43, 50]. These beneficial bacteria can produce SCFAs by metabolizing polysaccharides, and in turn maintain the integrity of the intestinal mucosal barrier [38, 51, 52]. SCFAs have a variety of physiological functions, including shaping the gut environment, influencing the physiology of the colon, being utilized as energy sources by host cells and intestinal microbiota, and participating in different host-signaling mechanisms [53, 54]. *Akkermansia* can also degrade mucin, thereby protecting the intestinal mucosal barrier and reducing protein deposition [55]. *Weissella* species, which are Gram-positive coccobacilli, are potential probiotics [56, 57]. The genus *Romboutsia*, which also are Gram-positive organisms, are in the gut of healthy humans and rats [58–60].

Taken together, our findings suggest that both CPP and CPF as prebiotics could partially recover the gut microbiota equilibrium, especially with CPP inhibiting the growth of *Escherichia-Shigella* and dramatically increasing *Akkermansia* population, and with CPF restraining the growth of *Escherichia-Shigella* and enhancing the abundances of *Romboutsia* and *Weissella* in NAFLD/T2DM rat model. Such changes in the composition of the gut microbiota in turn improve liver steatosis, hyperglycemia, hyperlipidemia, insulin resistance, and inflammation induced by HFD and STZ in rats.

In conclusion, to the best of our knowledge, this study is the first report that demonstrated administration with both CPP and CPF had the significant therapeutic effects on liver steatosis and metabolic abnormalities induced by HFD and STZ in rats. The mechanism responsible for the effects may be at least partially correlated with modulating gut microbiota composition, as indicated by inhibiting the growth of pathogenic bacteria *Escherichia-Shigella* and discrepantly expanding the abundances of beneficial bacteria *Akkermansia*, or *Romboutsia* and *Weissella* by CPP and CPF treatment, respectively.

Abbreviations NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; CP, Cyclocarya paliurus; CPF, Cyclocarya paliurus polysaccharides; CPP, Cyclocarya paliurus flavonoids; HFD, High-fat diet; STZ, Streptozotocin.; NAS, NALFD activity score; NASH, Nonalcoholic steatohepatitis; IR, Insulin resistance; FBG, The fasting blood glucose; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; TC, Total cholesterol; TG, Triglyceride; LDL-C, Low-density lipoprotein cholesterol; IL, Interleukin; TNF, Tumor necrosis factor; FINS, The fasting insulin; HOMA-IR, The insulin resistant index

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-022-01080-5.

Acknowledgements The authors appreciate study investigators and staff who participated in this study.

Author contribution Lu Zhong, Xiaojuan Peng, Chutian Wu, and Qing Li contributed equally to this paper. Lu Zhong, Xiaojuan Peng, Chutian Wu, and Qing Li helped design the study, analyzed the study data, helped draft the manuscript, made critical revisions of the manuscript, and provided final approval of the version. Lu Zhong, Yanfang Chen, Min Wang, Yuting Li, Kaiyin He, and Ying Shi performed the experiment, analyzed the study data, helped draft the manuscript, and provided final approval of the version. Shaohui Tang designed the study, analyzed study data, drafted the manuscript, made critical revisions of the manuscript, and provided final approval of the version.

Funding This study was supported by the following funding source: XJ. Peng: Health Commission Funding Project of Hunan province, China (No. B2019145); XJ. Peng: Science and Technology Funding Project of Chenzhou, Hunan province, China (No. zdyf201848).

Declarations

Ethics approval All animal experiments were approved by the experimental animal ethics committee of Jinan University and was performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Jinan University (2019021101).

Conflict of interest The authors declare no competing interests.

References

1. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15(1):11–20.
2. Ong JP, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. Clin Liver Dis. 2007;11(1):1–16.
3. 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.
4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84.
5. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005;365(9467):1333–46.
6. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88–98.
7. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol. 2019;70(3):531–44.
8. Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X, Lai Z. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: A meta-analysis. Medicine (Baltimore). 2017;96(39):e8179.
9. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. Nat Rev Gastroenterol Hepatol. 2017;14(1):32–42.

10. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
11. Loscalzo J. Lipid metabolism by gut microbes and atherosclerosis. Circ Res. 2011;109(2):127–9.
12. Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013;58(1):120–7.
13. Le Roy T, Llopis M, Lepage P, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 2013;62(12):1787–94.
14. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091–103.
15. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101(44):15718–23.
16. Brugman S, Klatter FA, Visser JT, et al. Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? Diabetologia. 2006;49(9):2105–8.
17. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget Á, Delmée E, Cousin Ḃ, Sulpice T, Chamontin B, Ferrières J, Tanti JḞ, Gibson GR, Casteilla L, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761–72.
18. Cani PD, Bibiloni R, Knauf C, Waget Á, Neyrinck AM, Delzenne NM, Burcelin Ŕ. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008;57(6):1470–81.
19. Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs. 2005;65(3):385–411.
20. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol. 2018;24(30):3361–73.
21. Kurihara H, Fukami H, Kusumoto A, et al. Hypoglycemic action of Cyclocarya paliurus (Batal.) Iljinskaja in normal and diabetic mice. Biosci Biotechnol Biochem. 2003;67(4):877–80.
22. Xie JH, Dong CJ, Nie SP, Li F, Wang ZJ, Shen MY, Xie MY. Extraction, chemical composition and antioxidant activity of flavonoids from Cyclocarya paliurus (Batal.) Iljinskaja leaves. Food Chem. 2015;186:97–105.
23. Ma Y, Jiang C, Yao N, Li Y, Wang Q, Fang S, Shang X, Zhao M, Che C, Ni Y, Zhang J, Yin Z. Antihyperlipidemic effect of Cyclocarya paliurus (Batal.) Iljinskaja extract and inhibition of apolipoprotein B48 overproduction in hyperlipidemic mice. J Ethnopharmacol. 2015;166:286–96.
24. Wang YR, Cui BS, Han SW, Li S. New dammarane triterpenoid saponins from the leaves of Cyclocarya paliurus. J Asian Nat Prod Res. 2018;20(11):1019–27.
25. Yang Z, Wang J, Li J, Xiong L, Chen H, Liu X, Wang N, Ouyang K, Wang W. Antihyperlipidemic and hepatoprotective activities of polysaccharide fraction from Cyclocarya paliurus in high-fat emulsion-induced hyperlipidaemic mice. Carbohydr Polym. 2018;183:11–20.
26. Xiong L, Ouyang KH, Jiang Y, et al. Chemical composition of Cyclocarya paliurus polysaccharide and inflammatory effects in lipopolysaccharide-stimulated RAW264.7 macrophage. Int J Biol Macromol. 2018;107(Pt B):1898–907.
27. Wu Q, Wang M, Simon JE. Determination of isoflavones in red clover and related species by high-performance liquid chromatography combined with ultraviolet and mass spectrometric detection. J Chromatogr A. 2003;1016(2):195–209.
28. Zhang X, Duan X, Liu X. The effect of Cyclocarya paliurus polysaccharide (CP) on blood glucose and histomorphology of pancreas in diabetic mice (in Chinese). Acta Med Sin. 2010;23:15–7.
29. Xie JH, Liu X, Shen MY, Nie SP, Zhang H, Li C, Gong DM, Xie MY. Purification, physicochemical characterisation and anticancer activity of a polysaccharide from Cyclocarya paliurus leaves. Food Chem. 2013;136(3-4):1453–60.
30. Xie JH, Shen MY, Xie MY, Nie SP, Chen Y, Li C, Huang DF, Wang YX. Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of Cyclocarya paliurus (Batal.) Iljinskaja polysaccharides. Carbohydr Polym. 2012;89(1):177–84.
31. Cheng L, Chen Y, Zhang X, Zheng X, Cao J, Wu Z, Qin W, Cheng K. A metagenomic analysis of the modulatory effect of Cyclocarya paliurus flavonoids on the intestinal microbiome in a high-fat diet-induced obesity mouse model. J Sci Food Agric. 2019;99(8):3967–75.
32. Feng Z, Wu C, Fang S. Technology optimization of total flavonoids extraction from Cyclocarya paliurus leaves by ultrasonic assistance (in Chinese). T Chin Soc Agric March. 2009;40:130–4.
33. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–21.
34. Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. Semin Liver Dis. 2012;32(1):3–13.
35. Fan Y, Xiong W, Li J, Hu A, He Z, Zhang J, Zhou G, Yin Q. Mechanism of TangGanJian on nonalcoholic fatty liver disease with type 2 diabetes mellitus. Pharm Biol. 2018;56(1):567–72.
36. Ma H, Cui F, Dong JJ, You GP, Yang XJ, Lu HD, Huang YL. Therapeutic effects of globular adiponectin in diabetic rats with nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(40):14950–7.
37. Lin Z, Wu ZF, Jiang CH, Zhang QW, Ouyang S, Che CT, Zhang J, Yin ZQ. The chloroform extract of Cyclocarya paliurus attenuates high-fat diet induced non-alcoholic hepatic steatosis in Sprague Dawley rats. Phytomedicine. 2016;23(12):1475–83.
38. Li Q, Hu J, Nie Q, et al. Hypoglycemic mechanism of polysaccharide from Cyclocarya paliurus leaves in type 2 diabetic rats by gut microbiota and host metabolism alteration. Sci China Life Sci. 2020;64:117–32.
39. Liu Y, Qian C, Ding S, Shang X, Yang W, Fang S. Effect of light regime and provenance on leaf characteristics, growth and flavonoid accumulation in Cyclocarya paliurus (Batal) Iljinskaja coppices. Bot Stud. 2016;57(1):28.
40. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022–3.
41. Yao Y, Yan L, Chen H, Wu N, Wang W, Wang D. Cyclocarya paliurus polysaccharides alleviate type 2 diabetic symptoms by modulating gut microbiota and short-chain fatty acids. Phytomedicine. 2020;77:153268.
42. Bai YF, Wang SW, Wang XX, Weng YY, Fan XY, Sheng H, Zhu XT, Lou LJ, Zhang F. The flavonoid-rich Quzhou Fructus Aurantii extract modulates gut microbiota and prevents obesity in high-fat diet-fed mice. Nutr Diabetes. 2019;9(1):30.
43. Li X, Wang Y, Xing Y, Xing R, Liu Y, Xu Y. Changes of gut microbiota during silybin-mediated treatment of high-fat diet-induced non-alcoholic fatty liver disease in mice. Hepatol Res. 2020;50(1):5–14.
44. Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. Hepatobiliary Pancreat Dis Int. 2017;16(4):375–81.
45. Yin X, Peng J, Zhao L, Yu Y, Zhang X, Liu P, Feng Q, Hu Y, Pang X. Structural changes of gut microbiota in a rat non-alcoholic fatty liver disease model treated with a Chinese herbal formula. Syst Appl Microbiol. 2013;36(3):188–96.

46. Xiao S, Liu C, Chen M, Zou J, Zhang Z, Cui X, Jiang S, Shang E, Qian D, Duan J. Scutellariae radix and coptidis rhizoma ameliorate glycolipid metabolism of type 2 diabetic rats by modulating gut microbiota and its metabolites. Appl Microbiol Biotechnol. 2020;104(1):303–17.
47. Chen M, Liao Z, Lu B, Wang M, Lin L, Zhang S, Li Y, Liu D, Liao Q, Xie Z. Huang-Lian-Jie-Du-Decoction Ameliorates Hyperglycemia and Insulin Resistant in Association With Gut Microbiota Modulation. Front Microbiol. 2018;9:2380.
48. Astbury S, Atallah E, Vijay A, Aithal GP, Grove JI, Valdes AM. Lower gut microbiome diversity and higher abundance of proinflammatory genus Collinsella are associated with biopsy-proven nonalcoholic steatohepatitis. Gut Microbes. 2020;11(3):569–80.
49. Lambeth SM, Carson T, Lowe J, Ramaraj T, Leff JW, Luo L, Bell CJ, Shah VO. Composition, Diversity and Abundance of Gut Microbiome in Prediabetes and Type 2 Diabetes. J Diabetes Obes. 2015;2(3):1–7.
50. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. 2016;7(3):189–200.
51. Cui HX, Zhang LS, Luo Y, Yuan K, Huang ZY, Guo Y. A Purified Anthraquinone-Glycoside Preparation From Rhubarb Ameliorates Type 2 Diabetes Mellitus by Modulating the Gut Microbiota and Reducing Inflammation. Front Microbiol. 2019;10:1423.
52. Zhang DY, Zhu L, Liu HN, Tseng YJ, Weng SQ, Liu TT, Dong L, Shen XZ. The protective effect and mechanism of the FXR agonist obeticholic acid via targeting gut microbiota in non-alcoholic fatty liver disease. Drug Des Devel Ther. 2019;13:2249–70.
53. Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. Int J Obes (Lond). 2015;39(9):1331–8.
54. Rios-Covian D, Ruas-Madiedo P, Margolles A, et al. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. Front Microbiol. 2016;7:185.
55. Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol. 2007;73(23):7767–70.
56. Fusco V, Quero GM, Cho GS, Kabisch J, Meske D, Neve H, Bockelmann W, Franz CMAP. The genus Weissella: taxonomy, ecology and biotechnological potential. Front Microbiol. 2015;6:155.
57. Kot W, Neve H, Heller KJ, Vogensen FK. Bacteriophages of leuconostoc, oenococcus, and weissella. Front Microbiol. 2014;5:186.
58. Russell JT, Roesch L, Ordberg M, et al. Genetic risk for autoimmunity is associated with distinct changes in the human gut microbiome. Nat Commun. 2019;10(1):3621.
59. Song YL, Liu CX, McTeague M, Summanen P, Finegold SM. Clostridium bartlettii sp. nov., isolated from human faeces. Anaerobe. 2004;10(3):179–84.
60. Gerritsen J, Fuentes S, Grievink W, van Niftrik L, Tindall BJ, Timmerman HM, Rijkers GT, Smidt H. Characterization of Romboutsia ilealis gen. nov., sp. nov., isolated from the gastrointestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus Clostridium into the genera Romboutsia gen. nov., Intestinibacter gen. nov., Terrisporobacter gen. nov. and Asaccharospora gen. nov. Int J Syst Evol Microbiol. 2014;64(Pt 5):1600–16.

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 2022-2023

Patrons of RSSDI

Dr. H.B. Chandalia, Mumbai
Dr. C. Munichhoodappa, Bengaluru
Dr. Ashok K. Das, Puducherry
Dr. Siddarth Das, Cuttack
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. Brij Makkar, Delhi

President Elect

Dr. Rakesh Sahay, Hyderabad

Immediate Past President

Dr. Vasanth Kumar, Hyderabad

Secretary-General

Dr. Sanjay Agarwal, Pune

Vice-President

Dr. Sujoy Ghosh, Kolkata

Vice-President

Dr. L. Sreenivasamurthy, Bengaluru

Joint Secretary

Dr. Pratap Jethwani, Rajkot

Treasurer

Dr. J.K. Sharma, Delhi

Executive Committee

Dr. J Aravinda, Bengaluru
Dr. Manoj Chawla, Mumbai
Dr. N.K. Singh, Dhanbad
Dr. M. Shunmugavelu, Trichy
Dr. Amit Gupta, Greater Noida
Dr. Jothydev Kesavadev, Kerala
Dr. Rakesh Parikh, Jaipur
Dr. Anil Virmani, Jamshedpur

Co-opted

Dr.Vijay Viswanathan, Chennai
Dr. Anuj Maheshwari, Lucknow
Dr. Sunil Gupta, Nagpur

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?
Upload your Research proposals on the RSSDI Online Research Grant Platform.

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
20% of the grant amount will be disbursed initially. 30% of payment after receiving your project status report and utilisation of sanctioned amount, 25% on further completion and pending 25% on final submission of your project. All reports must be uploaded on the RSSDI Online Research Grant Platform.

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 conference 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
Upload your Research proposals on the RSSDI Online Research Grant Platform.

When to apply
Proposals will be accepted every quarter of a year. The first month will be for the proposal submission, the second month for the scrutiny of the submitted proposals and the third month for the grant disbursement. This cycle will repeat for each quarter.

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 Brahmachari 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
23.	SL Raheja Hospital	Mumbai, Maharashtra

carefully looked into all aspects of this course & has accredited & recognized 23 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 screening interview which will be conducted by the centre coordinator of all respective accredited centers. The results will be declared in a week's time. A maximum of 50 marks will be scored for this assessment. Those who have scored at least 50%, will be initially considered based on their merit.

NOTE : Post MD (Internal Medicine) will be given preference.

FEES FOR APPLICATION FORM: RS 1500/-

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)

Applications are taken twice a year - June and December

Check the RSSDI website for the dates

Click on the link to apply: https://rssdi.in/rssdi-accd/

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)

INCENTIVES FOR REVIEWERS AND SUBEDITORS OF THE IJDDC (the official journal of RSSDI)

Based on the annual reviewer performance data, top 10 reviewers of IJDDC, selected by the journal's editorial team, shall be honored at the RSSDI annual conference with certificate/awards. Reviewers submitting thorough critical evaluation comments of assigned articles would also have the leverage of coming onboard as part of the core editorial team of IJDDC.

The best three subeditors of IJDDC, displaying outstanding contribution and consistent support to the associate editors, shall be conferred with certificate/awards at the RSSDI annual conference and will also have the leverage of joining the main editorial team of IJDDC.

TRANSITION OF IJDDC FROM PRINT TO ONLINE

IJDDC, the journal of the society, will now be available in full-text pdf format on the RSSDI website for all RSSDI Members for easy access.

Print copies will also be available. However, members keen to move from print to online only may submit their request by visiting the RSSDI website https://www.rssdi.in and following instructions on the RSSDI homepage

Those who wish to continue receiving the print copy may also submit their request choosing the appropriate option through the same link on the RSSDI website https://www.rssdi.in

The deadline for submitting request for print copy will be 1st July, 2023.

RSSDI 2023- 51st Annual Conference

16th-19th November, 2023
Jio World Convention Centre, BKC, Mumbai
Connect for details: www.rssdi2023.com;
info@rssdi2023.com

Registered with Registrar of News Papers, India vide no. RNI-43019/85

International Journal of Diabetes in Developing Countries

Volume 43 | Issue 2 | March - April 2023

Springer