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Journal Home Page: <https://tjphs.tu.edu.iq> -- Email: tjops@tu.edu.iq**The Relation of Various Durations of Type II Diabetes Mellitus and Endothelial Dysfunction**Mohammed Ali Ismael Al-Hameed^{1*}, Ausama Ayob Jaccob², Haider A. Alidrisi³¹ Basrah-directorate, Basrah-teaching hospital, Basrah, Iraq² University of Basrah, College of Pharmacy, Basrah, Iraq³ University of Basrah, College of Medicine, Basrah, Iraq**Keywords:**Type II diabetes,
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<http://doi.org/10.25130/tjphs.2024.18.1.2.12.21>**Abstract****Background:** Patients with long-term diabetes have a higher prevalence of endothelial dysfunction. Hypercholesterolemia strongly associates this impairment with atherosclerosis, while the duration of diabetes may relate to early macrovascular and microvascular injury.**Objectives:** The present study assessed the possible relation between various durations of type II diabetes and endothelial dysfunction.**Methods:** A case-control study of 182 participants included 20 healthy subjects, 30 newly diagnosed diabetes patients, and 132 with varying diabetes durations of type II diabetes. Those patients were divided according to their duration: 50 patients were (1-5 years), 32 patients were (5-10 years), and 50 patients were (more than ten years) in addition to the newly diagnosed group and the healthy control group. Lipid profile, glycemic parameters, and endothelium biomarkers: oxidized nitric oxide (NO), endoglin, intercellular adhesion molecule (ICAM-1), and glutathione were measured. Data was collected from November 20, 2022, to March 31, 2023.**Results:** Body mass index was significantly elevated in diabetic groups compared to the normal control group. All diabetic groups had highly significant differences in lipid profile and glycemic control parameters compared to the control group. Patients with more than ten years of diabetes had significantly higher (NO) levels than other groups, while the ICAM-1 level in these patients was significantly higher than all other groups.**Conclusions:** Various duration of type II diabetes was related to endothelial dysfunction that was strongly linked with factors like body mass index, systolic hypertension, hyperlipidemia, and poor control of glycemic index.

العلاقة بين المدد الزمنية المختلفة من مرض السكري من النوع الثاني والخلل وظيفي للبطانة الغشائية للاوعية الدموية

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الخلاصة

وجد ان الخلل الوظيفي للبطانة الغشائية للاوعية الدموية يكون أكثر انتشاراً بين المرضى الذين يعانون من مرض السكري على المدى الطويل. وبسبب ارتفاع مستوى الكوليسترول في الدم ، ارتبط هذا الخلل بالاصابة بتصلب الشرايين ، في حين أن مدة مرض السكري قد ترتبط بإصابة اغشية الأوعية الدموية الكبيرة واغشية الأوعية الدموية الدقيقة. **الأهداف:** بحثت هذه الدراسة في العلاقة المحتملة بين الخلل الوظيفي لبطانة الاوعية الدموية والمدة الزمنية للاصابة بمرض السكري من النوع الثاني. **الطريقة:** تضمنت هذه الدراسة 182 مشاركاً: 20 شخصاً اصحاء، 30 تم تشخيصهم حديثاً كمرضى السكري من النوع الثاني، و 132 مريضاً مشخصاً بمرض السكري من النوع الثاني مع مدد زمنية مختلفة من الاصابة بهذا المرض. قسم المرضى وفقاً لمدد الاصابة الزمنية للمرض: 50 مريضاً (1-5 سنوات) ، 32 كانوا (5-10 سنوات) ، و 50 مريض (اكبر من 10 سنوات)؛ تم قياس مستويات الدهون المختلفة، معلمات نسبة السكر في الدم ، والعلامات الحيوية للبطانة الاوعية الدموية: أكسيد النيتريك المؤكسد ، وإندوجلين ، و الجزيء اللاصق بين الخلايا-I والجلوتاثيون. جمعت البيانات من 20 نوفمبر 2022 إلى 31 مارس 2023. **النتائج:** ارتفع مؤشر كتلة الجسم بشكل ملحوظ في مجموعات مرضى السكري من النوع الثاني مقارنة بمجموعة الأشخاص الأصحاء. كان لدى جميع مجموعات مرضى السكري اختلافات كبيرة للغاية في مستويات الدهون ومعلمات التحكم في نسبة السكر في الدم مقارنة بمجموعة الأشخاص الأصحاء. المرضى الذين يعانون من مرض السكري من النوع الثاني أكثر من عشر سنوات كانت لديهم مستويات أكسيد النيتروجين أعلى بكثير من المجموعات الأخرى كما أن مستوى الجزيء اللاصق بين الخلايا-I في هؤلاء المرضى كان كبيراً للغاية من جميع المجموعات الأخرى. **الخلاصة:** ارتبطت مدد الاصابة المختلفة من مرض السكري من النوع الثاني بخلل وظيفي في بطانة الاوعية الدموية وارتبط هذا الخلل الوظيفي في البطانة الاوعية الدموية ارتباطاً وثيقاً بعوامل أخرى مثل مؤشر كتلة الجسم وارتفاع ضغط الدم الانقباضي وفرط دهون الدم وضعف التحكم في مؤشر نسبة السكر في الدم.

الكلمات الرئيسية: مرض السكري من النوع الثاني، مدة مرض السكري، الاختلالات البطانية للأوعية الدموية، المؤشرات الحيوية.

Introduction:

Diabetes mellitus, as defined by the World Health Organization, is a persistent metabolic disorder that is characterized by increased blood glucose levels. This condition gradually causes harm to various organs, such as the heart, blood vessels, eyes, kidneys, and nerves⁽¹⁾. The incidence of type II diabetes mellitus (T2DM) has escalated to epidemic levels. In addition, experts predict a persistent increase in the incidence of diabetes⁽²⁾. The development of (T2DM) is generally attributed to the combination of two key mechanisms: impaired insulin production by pancreatic beta cells (β -cells) and the diminished responsiveness of insulin-sensitive tissues to insulin⁽³⁾. The cardiovascular disease (CVD) rate is 2-3 times higher in adults with diabetes compared to adults without diabetes, which is the most significant leading cause of morbidity and mortality⁽⁴⁾. Additionally, the risk of macro- and microvascular diseases increases in diabetic patients by (11%) for every (1%) increase in glycated hemoglobin (HbA1c) levels over 6.5%. Other factors include elevated low-density lipoproteins (LDL), lower high-density lipoproteins (HDL), hypertension, and endothelium dysfunction. T2DM increases lipid abnormalities that cause atherosclerosis and increases CVD risk.⁽⁵⁾ Endothelial dysfunction is an early warning sign of atherosclerosis. It plays a crucial role in the development of atherosclerosis, leading to vascular disorders⁽⁶⁾. In normal vascular health, nitric oxide (NO) bioavailability is crucial because of its action on vasodilation⁽⁷⁾. Hyperglycemia and insulin resistance enhance oxidative stress. Consequently, reduced (NO) synthesis production leads to abnormalities in vascular endothelial cells' function⁽⁸⁾. Moreover, endoglin (ENG) is a transmembrane glycoprotein that plays a crucial role in the vascular endothelium functioning in several pathological states,

such as hyperglycemia, hyperlipidemia, and hypertension. Soluble endoglin can be a detectable biomarker in patients with these conditions⁽⁹⁾. Intercellular adhesion molecule-1 (ICAM) is the other factor that affects endothelial dysfunction in diabetes. Its primary function is facilitating circulating leukocyte attachment and trans-endothelial migration⁽¹⁰⁾. Increasing levels of (ICAM-1) promotes the initiation and progression of inflammation, which, in turn, increases the permeability of the endothelium and promotes the development of endothelial dysfunction⁽¹¹⁾. Diabetes causes an elevation of serum (ICAM-1), which is significantly associated with microvascular complications⁽¹²⁾. On the other hand, diabetes causes an increase in reactive oxygen species (ROS) production due to many factors. Hyperglycemia is directly involved in the onset of vascular complications in diabetes and oxidative stress due to increased (ROS) production⁽¹³⁾. Glutathione (GSH) is a prominent intracellular antioxidant that decreases the consequences of oxidative stress. The concentration of (GSH) is reduced in patients with (T2DM). The exact mechanism that causes glutathione deficiency in T2DM is unknown. Diabetes may cause permanent GSH consumption due to oxidative stress⁽¹⁴⁾. Long-term type II diabetic patients were more likely to have endothelial dysfunction. This impairment was highly associated with atherosclerosis due to hypercholesterolemia, and the duration of diabetes is related to early macro- and micro-vascular damage⁽¹⁵⁾. Therefore, it is helpful to determine the alteration of endogenous factors at various durations of diabetes to enhance the prevention and treatment of endothelial dysfunction caused by diabetes⁽¹⁶⁾. The aim of the present study was designed to investigate the possible relationship of the various

durations of type II diabetes with serum blood concentrations of oxidized (NO), (ENG), (ICAM-1), and (GSH) as a predicting biomarker for assessing endothelial dysfunction in patients with (T2DM).

Patients and methods

The case-control designed study was conducted at the Faiha Teaching Hospital in Basrah, Iraq. Data was collected from November 20, 2022, to March 31, 2023. The research ethics committee of the Basrah directorate and the University of Basrah College of Medicine authorized this study, (no. 030406/063/2023 on 19/12/2022). After being provided with the necessary information about this study, the control group participants and the patients were granted their signed written consent.

Inclusion and exclusion criteria

Patients were selected for a study based on meeting the following criteria: adult populations who had been either newly diagnosed with T2DM or previously diagnosed with various duration of their T2DM.

The exclusion criteria were those with Type I DM, currently pregnant or nursing, individuals with malignancies, and patients receiving biological or chemotherapeutic medications.

Methods

The present study followed the Strengthening of the reporting of observational studies in the epidemiology checklist. The study included 182 participants; 20 of them were healthy people with normal blood glucose levels, 30 patients were newly diagnosed with T2DM, and 132 were previously diagnosed with T2DM for different durations of disease. According to the duration of diabetes patients with T2DM who included in this study, patients were divided into four groups: 30 patients were newly diagnosed patients with T2DM; 50 patients had (1-5 years) of diagnosed diabetes; 32 patients had (5-10 years), and 50 patients had (more than 10 years) of diagnosed diabetes.

Investigations and methodology

Blood samples were collected from all participants. A total of six milliliters of venous blood were separated into serum. The serum derived from the biochemical tube, which included a clot activator separation gel, was subjected to clotting and then centrifuged at a speed of 3000 revolutions per minute for 10 minutes to measure the concentrations of total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), and random blood glucose (RBG). To facilitate subsequent measurements of oxidized nitric oxide (NO), endoglin (ENG), intercellular adhesion molecule (ICAM-1), and glutathione (GSH), we stored a volume of 1 milliliter of serum in a deep freezer at a temperature of -35 degrees Celsius. RBG, total cholesterol, TG, and LDL were measured using commercially available biochemical kits using Roche Cobas C 111® equipment (17). A technique based on ion-exchange high-performance liquid chromatography (BioRad D-10®) was used to quantify glycated hemoglobin (HbA1c) (18). Blood pressure was measured using a digital blood pressure monitor. Body mass index (BMI) was calculated using the height and weight of the participant from the formula ($BMI = Kg/M^2$).

The oxidized nitric oxide (NO) concentration was determined using a colorimetric test technique kit provided by Elabscience® using the ChemWell-T® spectrophotometer device. To quantify level concentrations of endoglin (ENG), intercellular adhesion molecule-1 (ICAM-1), and glutathione (GSH) used the enzyme-linked immunosorbent assay (ELISA) kit provided by Elabscience® using the (Thermo Scientific Multiskan™ FC Microplate Photometer) reader for measurement purposes.

Statistical data

The current study underwent statistical evaluation via the use of the Chi-square approach for both analysis and demographic assessment. This study's statistical approach was a one-way variance analysis (ANOVA) with Bartlett's post hoc analysis for multiple comparisons. GraphPad Prism for Windows (version 8.0) was applied for this purpose. The value of significance was set at (P-value 0.05)

Results

The present study included a total of 182 participants. The body mass index of the study's participant groups is shown in Figure 1. Our data demonstrated significant differences among groups regarding BMI compared to the normal control (Group 1).

The glycemic parameters are seen in Figure 2; all the groups differed in glycosylated hemoglobin (HbA1c) and random blood glucose (RBG). As anticipated, newly diagnosed T2DM (Group 2) and other diabetic groups with different disease durations had highly significant differences regarding RBG HbA1C compared to the normal control group.

The measurement of the participant's blood pressure is seen in Figure 3. As expected, newly diagnosed T2DM (Group 2) and other diabetic groups with different disease durations had highly significant differences in systolic blood pressure compared to the normal control group. However, there are no statistically significant differences in diastolic blood pressure across the groups, and the readings remain within or close to the normal range.

Regarding lipid profile, all diabetic groups exhibited highly significant increases in serum cholesterol, TG, and LDL concentrations compared to the normal control (Group 1). These data are well presented in Figure 4.

The present work indicates a significant difference in oxidized nitric oxide (NO) concentration levels among patients with more than ten years of diabetes duration (Group 5) compared to the normal control group. Our findings show no statistically significant differences in endoglin serum concentration measurements among all the groups.

Regarding the serum concentration of (ICAM-1), our result demonstrated that Group 5 of patients with a diabetes duration above ten years had significant differences compared to the control group, as illustrated in Figure 5.

According to the findings of our study, no statistically significant difference in (GSH) concentration was observed among all of the groups, as shown in Figure 6.

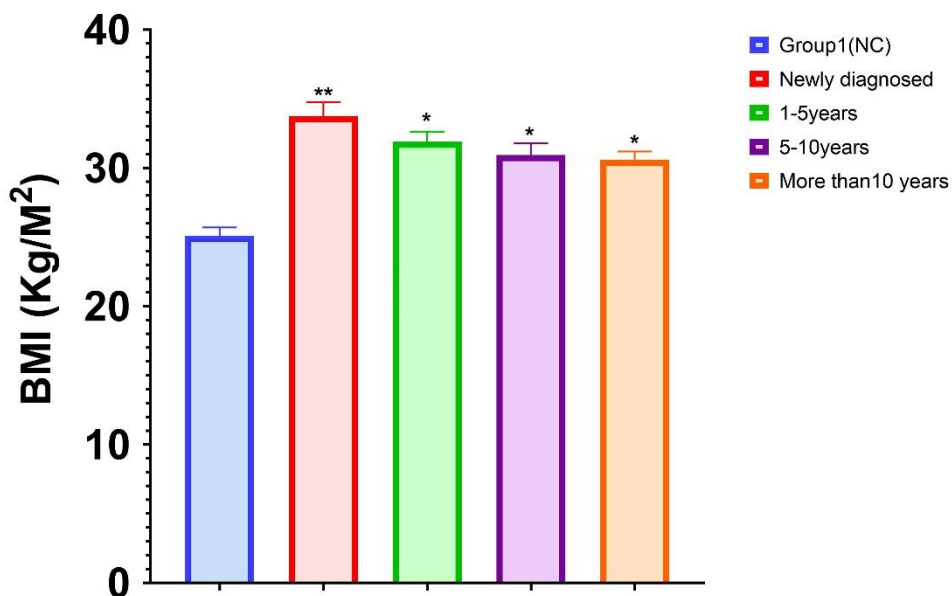


Figure 1. The BMI of the participants in the study.

BMI: body mass index; Group 1 NC: normal control; Group 2: newly diagnosed; Group 3:1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes. A one-way ANOVA with Bartlett's post hoc test was used to analyze the data. The data was labeled as * P < 0.05, representing a significant difference compared to a normal control group; ** P < 0.001.

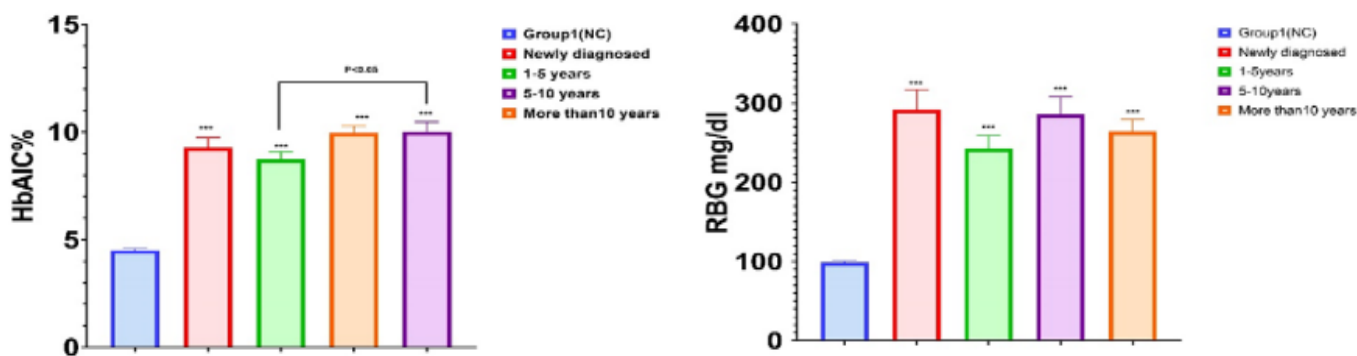


Figure 2. The glycemic parameters of the participants.

Group 1 NC: normal control; Group 2: newly diagnosed; Group 3:1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes. RBG: random blood glucose; HbA1c: glycated hemoglobin. A one-way ANOVA with Bartlett's post hoc test was used to analyze the data. The data was labeled as *** highly significant compared to the normal control group P < 0.0001.

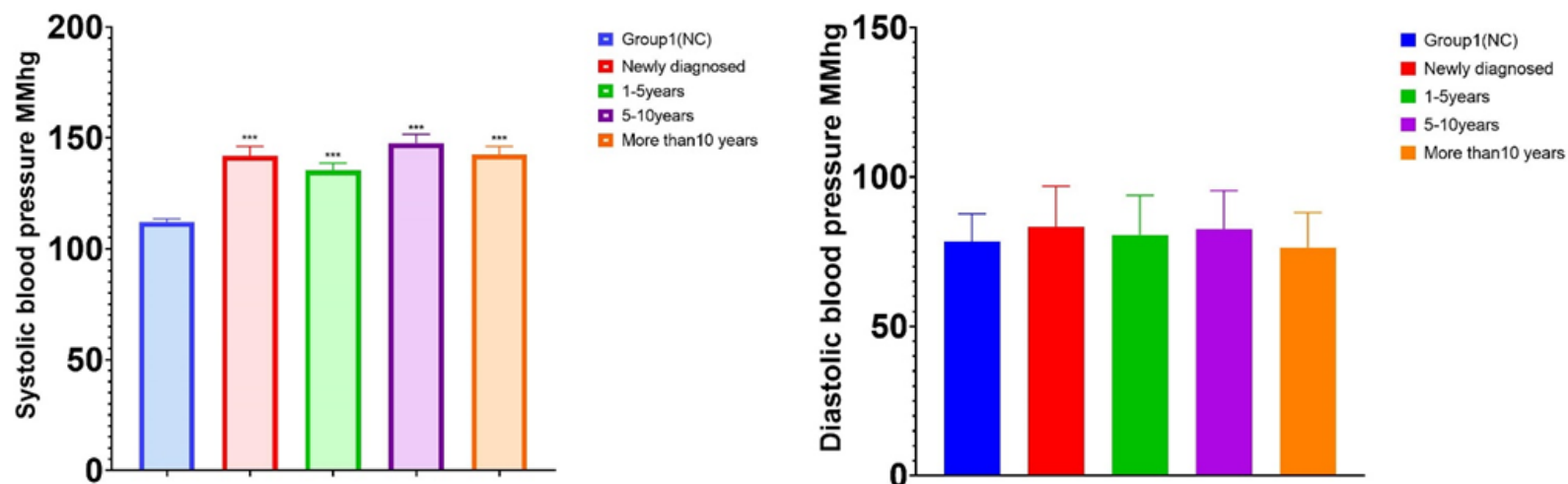


Figure 3. The blood pressure measurement of the participants.

Group 1 NC: normal control; Group 2: newly diagnosed; Group 3: 1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes. A one-way ANOVA with Bartlett's post hoc test was used to analyze the data. The data was labeled as *** highly significant compared to the normal control group $P < 0.0001$.

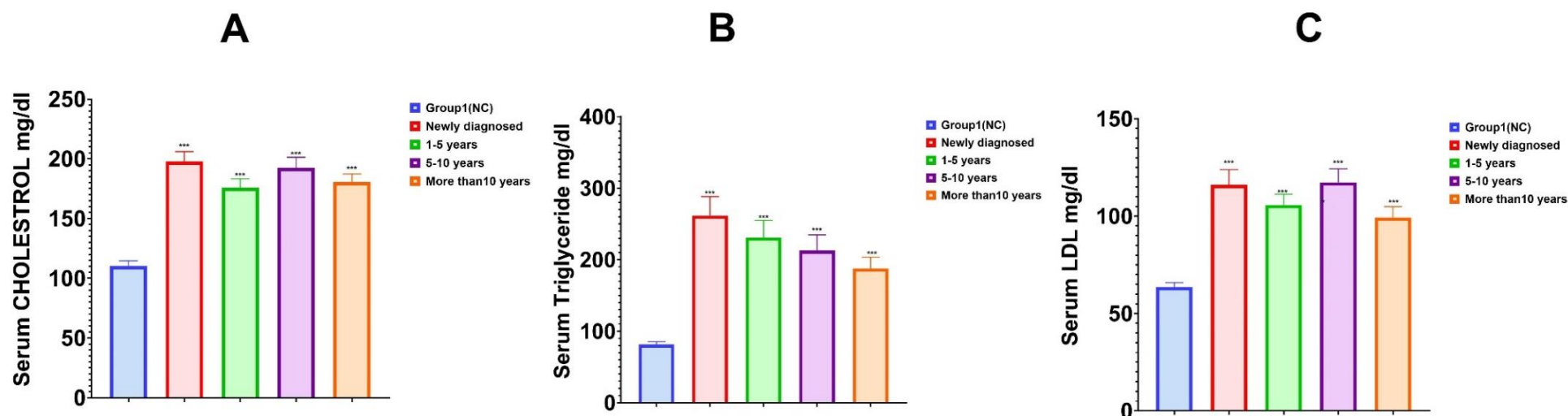


Figure 4. The serum concentration of total cholesterol (A), triglycerides (B), and low-density lipoprotein: LDL (C) in different durations of diabetes.

Group 1 NC: normal control; Group 2: newly diagnosed; Group 3: 1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes. A one-way ANOVA with Bartlett's post hoc test was used to analyze the data. The data was labeled as *** highly significant compared to the normal control group $P < 0.0001$.

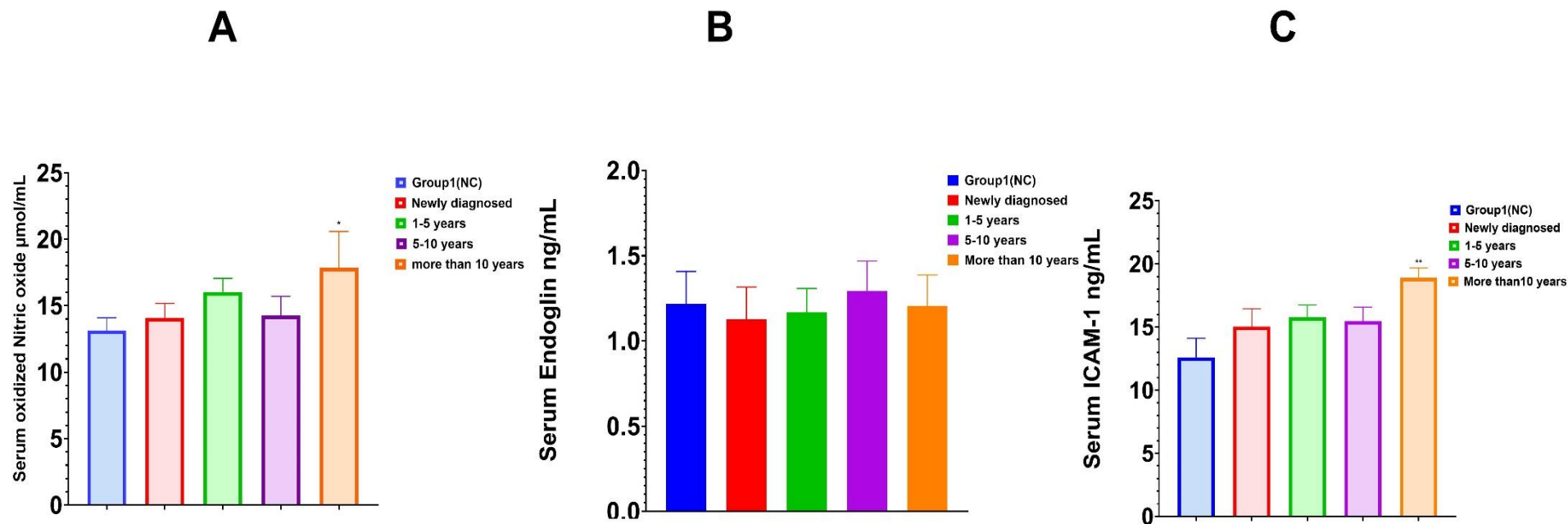


Figure 5. Serum concentration of the oxidized nitric oxide (A), endoglin (B), and ICAM-1 (C) of the participants.

Group 1 NC: normal control; Group 2: newly diagnosed; Group 3: 1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes; ICAM-1: Intercellular Adhesion Molecule-1. A one-way ANOVA with Bartlett's post hoc test was used to analyze the data. The data was labeled as * $P < 0.05$, representing a significant difference compared to a normal control group, ** $P < 0.001$

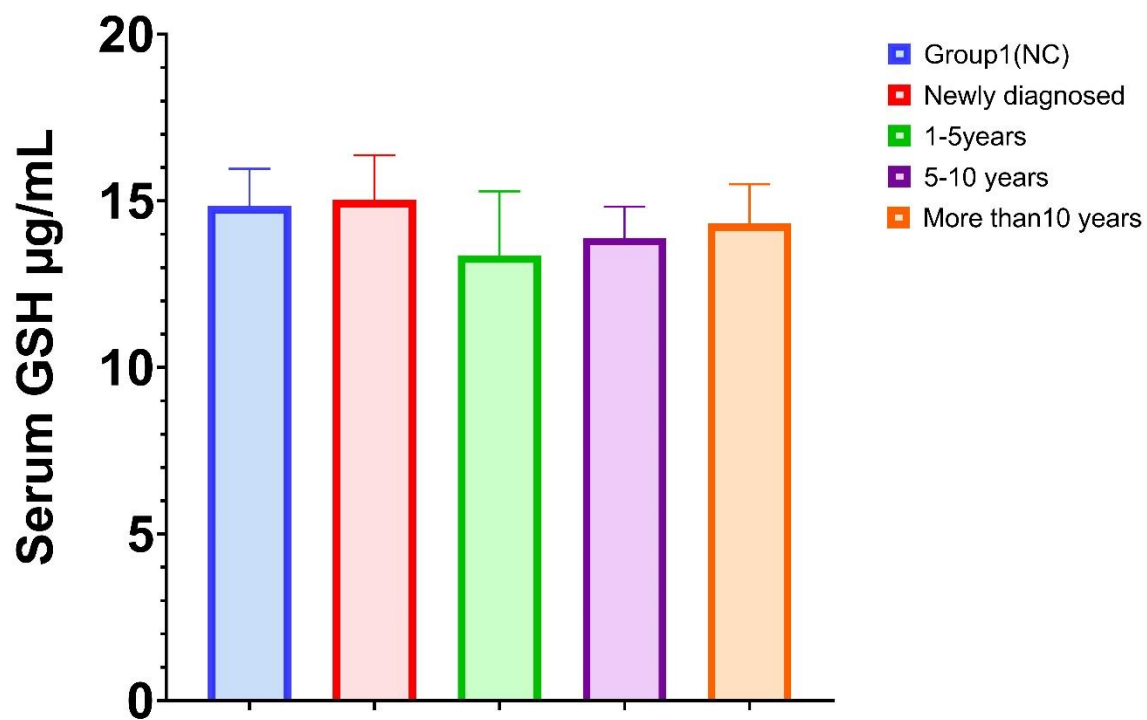


Figure 6. Serum concentration of the glutathione of the participants.

Group 1 NC: normal control; Group 2: newly diagnosed; Group 3: 1-5 years of diabetes; Group 4: 5-10 years of diabetes; Group 5: more than ten years of diabetes; GSH: glutathione.

Discussion

Endothelial dysfunction parameters have helped to predict diabetic vascular problems and identify treatment targets⁽⁸⁾. It is beneficial to assess the modification of endothelial function at different stages of the duration of diabetes to determine the effect of diabetes duration on the vascular endothelium⁽¹⁶⁾. The present study showed that all diabetic groups had a significantly higher body mass index when compared to the normal control group. This result agreed with the Rossi et al. and the Doghish et al.^(19,20) studies that showed increased BMI over time among diabetic patients and the control group correlated with a significant increase in endothelial dysfunction. Therefore, it is crucial to take into account the impact of obesity on the progression of vascular complications in individuals with diabetes⁽²¹⁾.

The present study found that Group 2 patients with newly diagnosed T2DM and other diabetic groups that had different durations of diabetes showed significantly higher RBG, HbA1C, and systolic blood pressure than the normal control group. This finding is consistent with the study by Li et al.⁽²²⁾, which reported that an increase in HbA1c level was related to diabetes duration and the highest risk of vascular complications in the most prolonged diabetes durations with poor control of HbA1c. Regarding systolic blood pressure, the study by Venuraju et al.⁽²³⁾ found that a significant increase in blood pressure was associated with a longer duration of diabetes and a higher risk of diabetic vascular complications.

Additionally, the present study also revealed that all groups of patients with different diabetic durations had statistically significant elevations in total cholesterol, TG, and LDL as compared to the normal control group. The study by Nakhjavani et al.⁽²⁴⁾ found that an increase in LDL is significantly associated with the duration of diabetes and its association with the development of macro- and microvascular complications, which is in good agreement with the results of the present study.

Studies have recognized nitric oxide as a significant modulator of endothelial function due to its vasodilation action⁽²⁵⁾. Regarding oxidized nitric oxide, our study showed that Group 5 patients with more than ten years of diabetes had significantly higher NO concentrations than other groups. The present finding also supports the Wiczór et al. study⁽²⁶⁾, which concluded that the increase in serum asymmetric dimethylarginine level, which is responsible for nitric oxide synthesis, is not directly proportional to the duration of diabetes. Instead, it may be associated with the metabolic management of diabetes.

(ICAM-1) was measured to detect adhesion molecules and the endothelium inflammation process. Our result showed that patients with diabetes for more than ten years had a highly significant difference from all other groups. The study result agrees with Siddiqui et al.⁽¹²⁾, which found that plasma (ICAM-1) levels had significantly increased throughout the follow-up period. It seems that patients with diabetes for an extended period of duration have elevated (ICAM-1) levels, which increase endothelium inflammation and

the risk of cardiovascular complications⁽²⁷⁾. In order to comprehend the factors that contribute to endothelial dysfunction, it is essential to examine the role of raised oxidative stress, which is caused by increased production of reactive oxygen species (ROS)⁽²⁸⁾. The serum concentration of glutathione (GSH) was assessed to predict the antioxidant status. Our finding indicated no statistically significant difference in (GSH) concentration among all the groups. This finding conflicts with the Ezekiel et al.⁽²⁹⁾ study, which found that the pre-diabetic and diabetic groups had significantly different GSH levels than the control group.

This study has some limitations, such as the number of participants in the healthy control group. The data cannot be generalizable since they were drawn from one center and do not represent the general community. Patients with more severe diseases were to be expected to attend consultant clinics, which might have caused sample bias.

Conclusion

Various durations of T2DM have been associated with a deterioration of endothelial dysfunction demonstrated by elevated levels of (ICAM-1) in diabetic patients with more than ten years duration of diabetes. Also, the body mass index, systolic hypertension, hyperlipidemia, and poor control glycemic index play a significant role in developing endothelial dysfunction in these patients.

Recommendation

Biomarkers measuring in diabetic patients, such as (ICAM-1) may provide additional information for developing strategies for managing diabetes vascular damage. Suggestion for future work is to study these biomarkers as predictors of the occurrence of vascular dysfunction in early diabetics for prevention of major causes of mortality and morbidity in these patients.

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