Pharmaceutical Sciences Asia

Pharm Sci Asia 2024; 51(1), 43-51

DOI:10.29090/psa.2024.01.23.838

Research Article

The impact of various hypoglycemic modalities on endothelium dysfunction biomarkers in patients with T2DM

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ABSTRACT

Major contributors to morbidity and mortality in diabetic patients are diabetic vascular complications. The impairment of endothelial function seems to be a constant observation in people with diabetes. The objective of the current study was to investigate the impact of various treatment modalities on the endothelial biomarker in T2DM. This study involved 182 participants, divided into seven groups: 20 healthy subjects as a control, 35 newly diagnosed patients with diabetes without treatment, and 127 patients already on different antidiabetic medications for three months. Levels of cholesterol, triglycerides (TG), low-density lipoprotein (LDL), glycated hemoglobin (HbA1c), random blood sugar (RBS), oxidized nitric oxide (NO_x), endoglin (ENG), intercellular adhesion molecule (ICAM-1), and glutathione (GSH) are measured for all participants compared with the healthy control. All the other groups significantly increased their lipid profile, HbA1c, RBS, and blood pressure, with a high significance observed in the untreated group. In contrast, a significant increase in NO_x levels in the pioglitazone-treated group and ENG levels seems close to normal control, while a significant elevation of ICAM-1 and reduction in GSH levels. Although SGLT2 therapy caused a significant decrease in cholesterol and LDL, the level of ENG was significantly higher, and the level of GSH was considerably lower than in the other groups. In conclusion, the pioglitazone-treated group had the lowest HbA1c and blood pressure and was equivalent to the normal control group. The pioglitazone-treated group had the greatest amount of NO_x, which prevents endothelial dysfunction, whereas the SGLT2 group had the lowest impact on these biomarkers.

Keywords:
Type 2 diabetes mellitus, Endothelial dysfunction, Biomarkers, Hypoglycemic drugs

1. INTRODUCTION

Diabetes mellitus (DM) is the most prevalent metabolic disorder in the world, characterized by chronic hyperglycemia resulting from inadequate insulin production or insulin resistance, as well as multiple micro- and macrovascular complications[1-2]. In people with diabetes, vascular problems are a leading cause of death and disability. Neuropathy, nephropathy, and retinopathy are all examples of microvascular consequences that contribute substantially to a decline in life quality. Among the primary causes of mortality are macrovascular complications, such as coronary heart disease, cerebrovascular disease, and peripheral artery disease[3]. Persistent complications of diabetes, including structural injury to the vascular endothelium that can lead to dysfunction or even failure of various organs and tissues and which are linked to increased morbidity, mortality, and disability among diabetic patients, are characterized by these complications[4].

Patients with type 2 diabetes mellitus (T2DM) typically have abnormal lipid levels[5]. Actually, in addition to being an early sign of atherosclerosis, endothelial dysfunction also plays a critical role in atherosclerosis progression and hence contributes to the development of vascular problems[6]. It seems that endothelial dysfunction is often seen in DM patients[7]. Endothelium that is at rest releases

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endothelin-1 and nitric oxide to control vasoconstriction, vasodilation, inflammation, oxidation, and the growth of vascular smooth muscle cells[8]. In fact, there is widespread consensus that chronic hyperglycemia and DM impair nitric oxide (NO) production and activity, shifting the balance toward endothelial dysfunction[4]. Nitric oxide is a gaseous, transient free radical that is secreted by the endothelium. It modulates vascular tone and remodeling, inhibits leukocytes and platelet aggregation and adhesion, and causes vasodilation, among other anti-atherosclerotic actions[9]. These effects of NO indicate that the amount of NO produced by endothelium plays a significant role in vascular disease control[10]. A strong relationship between diabetes and its chronic complications has been linked to changes in NO bioavailability and a rise in the risk of developing hypertension, the advancement of atherosclerosis, hypercholesterolemia, thrombosis, and stroke[11]. Moreover, endoglin (ENG) is a type I transmembrane glycoprotein with possible functions in hematopoiesis, cardiovascular development, and angiogenesis. It is predominantly found on proliferating endothelial cells, activated macrophages, smooth muscle cells, fibroblasts, and increased ENG expression in vessels during numerous pathological conditions such as hypoxia or damage[12]. ENG also appears to be a regulator of vascular tone, as the administration of ENG to mice results in an increase in arterial pressure via an increase in vascular resistance[13]. The other factor influencing the various micro- and macrovascular complications of DM is oxidative stress and reactive oxygen species (ROS), which have been linked to insulin resistance, β-cell dysfunction, impaired glucose tolerance, and the progression of DM[14-15]. ROS induces the release of pro-inflammatory molecules, NO, and intercellular adhesion molecule-1 (ICAM-1), leading to alterations in vessels and extravascular space[16-17]. The oral glucose tolerance test increased ICAM-1, which has been linked to endothelial dysfunction[18]. Adhesion molecules are proteins that control the interaction between the endothelium and leukocytes. ICAM-1 is associated with the occurrence of coronary heart disease later on. The levels of ICAM-1 are elevated many years prior to the onset of the first myocardial infarction, and it is a reliable alarm marker and is strongly associated with conventional cardiovascular risk factors[19]. Particularly, it has been observed that endothelial dysfunction overexpresses ICAM-1, and upregulation of adhesion molecules has been identified in diabetic patients, and their increased plasma concentration has been linked to hyperglycemia and increased oxidative stress[20]. On the other hand, the imbalance between the antioxidant system and ROS that favors ROS is referred to as oxidative stress. When the antioxidant system fails, the harmful consequences of ROS, such as signal transduction pathway blockage and alteration of normal cellular lipids and proteins, become evident. ROS are generated by various enzymes in human cells[17]. Chronic hyperglycemia increases endothelial cell permeability through increased ROS[21]. Elevated ROS levels result in oxidative stress, which plays a critical role in endothelial dysfunction. Multiple metabolic pathways are known to promote the development of oxidative stress in hyperglycemia. These pathways include the glycolytic pathway, increased production of advanced glycation end products, activation of protein kinase C, and the polyol pathway. In the polyol pathway, hyperglycemia triggers the activation of aldose reductase, resulting in an elevation in sorbitol levels. Subsequently, sorbitol dehydrogenase facilitates the conversion of sorbitol to fructose. Elevated concentrations of fructose have been shown to result in the buildup of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which subsequently leads to oxidative stress as a consequence of methylglyoxal production and activation of protein kinase C. Furthermore, heightened expression of aldose reductase results in a reduction in nicotinamide adenine dinucleotide phosphate, consequently leading to a decline in glutathione levels[22]. Glutathione (GSH), a non-enzymatic antioxidant defense, maintains ROS levels within normal ranges. GSH eliminates free radicals or reduces the level of hydrogen peroxide during oxidative stress. Diabetes has been associated with decreased GSH levels and impaired GSH metabolism. Because diminished GSH is the major antioxidant found inside the cell, there is an increase in oxidative stress within the cell, leading to endothelial dysfunction[23-24]. These circulating biomarkers have played a significant role in predicting the development of diabetes and its complications and identifying therapeutic targets[25]. Therefore, to prevent diabetic vascular complications, it is important to choose the diabetic treatment modalities that improve or augment endothelial function. In the present study, we investigate the effects of different diabetic treatment modalities on the vascular endothelium by measuring (oxidized nitric oxide, endoglin, ICAM-1, and glutathione) as a predicting biomarker for endothelium dysfunction.

2. PATIENTS AND METHODS

The study was conducted at the Faiha Teaching Hospital in Basrah, Iraq. The data was collected for around five months, from November 20, 2022 to March 31, 2023.

The research ethics committee of the Basrah directorate and the University of Basrah College of Medicine approved this study. After receiving appropriate information about this study, the control individuals and the patients gave their written permission.

2.1. Subjects in a Study

The study only included patients who met all three of the following criteria:

(1) adult patients previously diagnosed with T2DM;

(2) patients on treatment modalities with insulin plus metformin or oral hypoglycemic drugs for three months duration; and (3) patients on single different modalities of oral hypoglycemic drugs plus metformin for three months.

Exclusion criteria were Type 1 DM, T2DM patients on different treatment modalities, T2DM patients on treatment less than three months, current pregnancy, breastfeeding, malignancy, renal dysfunction, acute illness, and patients using corticosteroid, antihyperlipidemic drugs, biological, or chemotherapeutic drugs and other medication could affect endothelium function.

2.2. Procedures in a study

The current study was carried out on a total of 182 subjects (20 healthy individuals with normal blood glucose levels and 162 patients diagnosed with T2DM. A cross-sectional study design has been employed for the purpose of achieving study objectives. A questionnaire form was used to acquire information from the patients. According to the treatment modalities on patients with T2DM who arrived at the clinic, patients were divided into three groups; 35 were diabetes mellitus control (DMC) newly diagnosed patients who had not used treatment for DM and were admitted to specialist doctors searching for treatments; 40 patients on insulin plus oral hypoglycemic drugs; and the other patients were on different oral hypoglycemic modalities of treatment 28 patients on sulfonylureas with metformin (SU+M); 24 patients on dipeptidyl peptidase-4 inhibitor with metformin (DP+M); 20 patients on Sodium-glucose cotrans-porter-2 (SGLT2) inhibitors with metformin (SG+M) and 15 patients on pioglitazone with metformin (PIO+M).

2.3. Biochemical measurements

Six milliliters of venous blood were used to determine the levels of total serum cholesterol, triglycerides (TG), low-density lipoprotein (LDL), glycated hemoglobin (HbA1c), and random blood sugar (RBS). The serum from the biochemical tube containing clot activator separation gel was allowed to clot and then centrifuged at 3,000 rpm for 10 minutes. One ml of serum was stored in a deep freezer at -35℃ for measurements of oxidized nitric oxide (NO_x), endoglin (ENG), intercellular adhesion molecule (ICAM-1), and glutathione (GSH).

Commercially available biochemical kits were used by the Roche Cobas c 111® instrument to determine random blood sugar (RBS), total cholesterol, triglycerides (TG), and low-density lipoprotein (LDL). Glycated hemoglobin (HbA1c) was measured using ion-exchange high-performance liquid chromatography (BioRad D-10)®.

In vivo or in an aqueous solution, nitric is easily oxidized to yield NO2. The NO_2^- react with chromogenic substance to produce a crimson azo compound. The concentration of azo compound is linearly related to NO concentration. Indirect measurement of NO concentration by measuring optical density values of oxidized nitric oxide (NOx) concentration was done using a colorimetric assay method kit from Elabscience® and the instrument ChemWell-T® Spectrophotometer. The Elabscience® Assay Kit was used to measure the amounts of endoglin (ENG), intercellular adhesion molecule (ICAM-1), and glutathione (GSH). Measured using an enzyme-linked immunosorbent assay (ELISA) and Thermo ScientificTM MultiskanTM FC Microplate Photometer instrument.

2.4. Statistical data

The present study was statistically evaluated using the Chi-square method for analysis and demographic evaluation. One-way analysis of variance with Bartlett's post hoc analysis for multiple comparisons was utilized using GraphPad Prism for Windows (version 8.0).

3. RESULTS

Overall, 182 participants enrolled in the present study. Thirty-five patients were newly diagnosed with T2DM without treatment for DM, and 127 patients with T2DM were already on different antidiabetic medications for three months. The demographic characteristics of the study groups are shown in Table 1. Significant differences were detected among the treated groups regarding age

Table 1. Demographic information of Participants.

Groups	Age(years) (Mean±SD)	Gender		BMI (Mean ± SD)	Marital Status		Smoking	
		Male	Female				Yes	No
Group 1 (NC)	36.33 ± 10.49	13	7	28.16 ± 4.866	15	5	4	16
Group 2 (DMC)	44.95 ± 12.67	18	17	31.51 ± 4.547	34	1	5	30
Group 3 (INS+M)	59.28 ± 8.732	10	30	31.06 ± 4.453	39	1	3	37
Group 4 (SU+M)	55.79 ± 13.37	9	19	29.71 ± 4.233	27	1	1	27
Group 5 (DP+M)	56.00 ± 10.08	8	16	31.82 ± 4.790	23	1	2	22
Group 6 (SG+M)	54.85 ± 14.20	9	11	32.12 ± 5.526	19	1	3	17
Group 7 (PIO+M)	54.47 ± 14.80	5	10	32.02 ± 4.818	13	2	1	14
***P* Value**	<0.0001	0.0024		0.1031	0.4633		0.8265	

BMI: body mass index; NC: normal control; DMC: diabetes mellitus control; INS+M: insulin with metformin; SU+M: sulfonylureas with metformin; DP+M: dipeptidyl peptidase-4 inhibitor with metformin; SG+M: Sodium-glucose cotransporter-2 (SGLT2) inhibitors with metformin; PIO+M: pioglitazone with metformin, SD: standard deviation, the data are expressed as mean±SD values. Statistically significant *P*-values are in red-color typing.

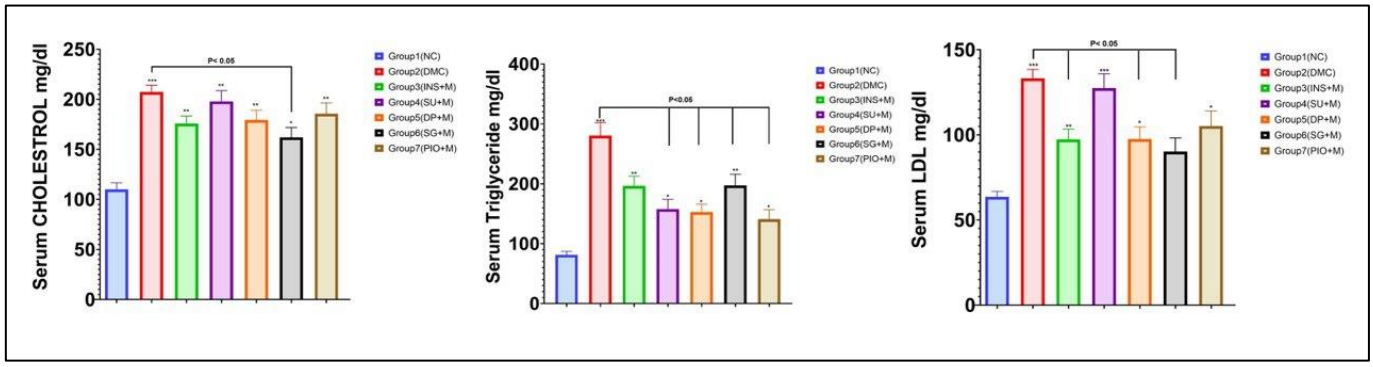

Figure 1. The serum concentration of total cholesterol, triglycerides, and low-density lipoprotein: LDL in different treatment modalities of diabetic patients among groups. The data were analyzed using a one-way ANOVA with Bartlett's post hoc test. Data were denoted as $^{*}P<0.05$, representing a significant difference compared to a normal control group; $^{**}P<0.001$; $^{***}P<0.0001$.

Table 2. Clinical characteristics of Participants.

Groups	HBA1C (Mean±SD)	RBS (Mean±SD)	Blood pressure (Mean±SD)	
			SBP	DBP
Group 1 (NC)	4.380 ± 0.105	98.93 ± 2.598	113.3 ± 1.93	77.27 ± 2.277
Group 2 (DMC)	10.180 ± 0.352**	334.60 ± 20.18**	154.4 ± 4.24**	86.69 ± 2.359
Group 3 (INS+M)	9.628 ± 0.345**	244.10 ± 14.18*	143.5 ± 3.99**	81.48 ± 3.944
Group 4 (SU+M)	9.918 ± 0.352**	231.70 ± 17.12 *	143.0 ± 4.04**	84.82 ± 1.909
Group 5 (DP+M)	7.963 ± 0.252*	213.70 ± 13.36 *	134.2 ± 4.24*	80.04 ± 2.770
Group 6 (SG+M)	8.595 ± 0.487**	205.50 ± 16.14 *	148.5 ± 6.55**	77.00 ± 2.000*
Group 7 (PIO+M)	7.479 ± 0.233*	201.60 ± 11.14 *	123.1 ± 7.60	70.93 ± 2.100*
***P* Value**	<0.0001	<0.0001	<0.0001	<0.05

HbA1c: glycated hemoglobin; RBS: random blood sugar; SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation, and the data are expressed as mean±SD values. Data were denoted as $^{*}P<0.05$, representing a significant difference compared to the normal control group; $^{**}P<0.001$.

Table 3. Contingency table analysis using Chi-square and Fisher's exact test.

Groups	Blood pressure		LDL		TG		HBA1C	
	Normotensive %	Hypertensive %	Norm %	High %	Norm %	High %	Norm %	High %
Group 1 (NC)	95.0	5.0	95.0	5.0	100.0	0.0	95.0	5.0
Group 2 (DMC)	37.1	62.9	57.0	43.0	14.3	85.7	8.6	91.4
Group 3 (INS+M)	77.1	22.9	85.7	14.3	57.1	42.9	14.3	85.7
Group 4 (SU+M)	57.1	42.9	53.6	46.4	46.4	53.6	10.7	89.3
Group 5 (DP+M)	66.7	33.3	75.0	25.0	25.0	75.0	33.3	66.7
Group 6 (SG+M)	90.0	10.0	75.0	25.0	25.0	75.0	35.0	65.0
Group 7 (PIO+M)	66.7	33.3	73.3	26.7	53.4	46.6	53.3	66.7
***P* Value**	<0.0001	<0.0001	0.0080	0.0080	<0.0001	<0.0001	<0.0001	<0.0001

LDL: low-density lipoprotein; TG: triglycerides; HbA1c: glycated hemoglobin. The data are expressed as %: percentage values. High percentages are in red-color typing. $P<0.05$ represents a significant difference.

and gender. At the same time, no significant differences were detected between groups in terms of BMI, marital status, or smoking habits.

Regarding the lipid profile, as can be seen in Figure 1. Our data demonstrated a highly significant increase in serum total cholesterol, TG, and LDL concentration in DMC compared to the normal control. In contrast, patients in Group 6 who had been treated with sodium-glucose cotransporter-2 (SGLT2) showed the lowest cholesterol and LDL concentrations comparable to DMC. Patients in Groups 4, 5, and 7 showed a significant reduction in serum TG levels compared to the diabetic control group. LDL levels were significantly reduced in Groups 3,5 and 7 compared to DMC.

Clinical characteristics are seen in Table 2; glycosylated hemoglobin (HbA1c), random blood sugar (RBS), and blood pressure were different among groups. As expected, patients in Group 2 had higher HbA1c, RBS, and blood pressure compared to the remaining groups. Groups 5 and 7 showed HbA1c, RBS, and blood pressure drops, but still more than normal ranges. Actually, in spite of a highly significant increase in systolic blood pressure among diabetic patients in Groups 3, 4, 5, 6, and 7 compared to normal control, it is still within or near normal values.

A contingency table analysis (Table 3) explains the percentage of hypertensive patients and those with a bad lipid profile in the present work. Patients in Groups 2 and 4 showed the highest abnormal percentage compared to the remaining groups. The data suggest that patients in Groups 2, 3, and 4 appeared to be associated with an abnormally high percentage of HbA1c compared to other groups.

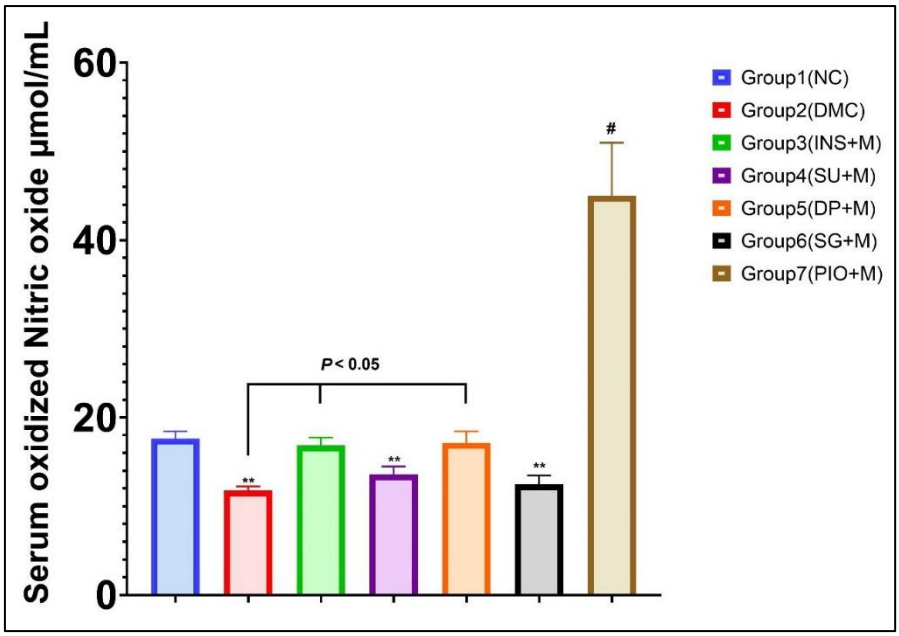

Figure 2. The serum concentration of oxidized nitric oxide in different treatment modalities of diabetic patients among groups. The data were analyzed using a one-way ANOVA with Bartlett's post hoc test. Data were denoted as # a significant difference among groups; $^{**}P<0.001$.

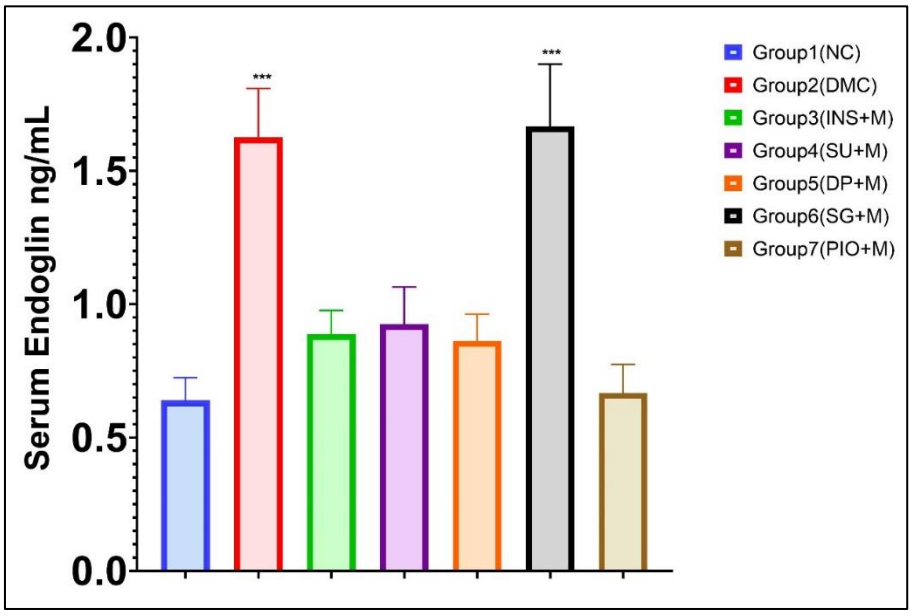

Figure 3. The serum concentration of endoglin in different treatment modalities of diabetic patients among groups. The data were analyzed using a one-way ANOVA with Bartlett's post hoc test. Data was denoted as $^{***}P<0.0001$, a significant difference among groups.

Nitric oxide is considered a key regulator of endothelium function. In this study, patients in Group 7 who were given pioglitazone had significantly higher levels of NOx in their blood compared to the other groups, as shown in Figure 2. Unlike other groups, the concentration of NO_x in diabetic patients in Group 2 was significantly decreased ($P<0.001$) compared to the normal control group. The same finding was detected in Groups 4 and 6. This reflects endothelial dysfunction.

In Figure 3, newly diagnosed diabetic patients (Group 2) and those treated with SGLT2 and metformin (Group 6) showed significantly higher serum endoglin levels compared to other treated diabetic and normal control groups.

Concerning serum ICAM-1, newly diagnosed diabetic patients and all diabetic patients on treatments in all groups showed significant elevation of ICAM-1 levels compared to the normal control group, with the highest level observed in Group 2 $P<0.001$, as presented in Figure 4.

In order to get complete information about the cause and effect of antidiabetic drugs on endothelial function, serum glutathione concentrations were measured in all diabetic and control groups. There was a significant reduction in serum GSH levels in Group 2 and Group 6 compared with the remaining groups. Groups 3 and 5

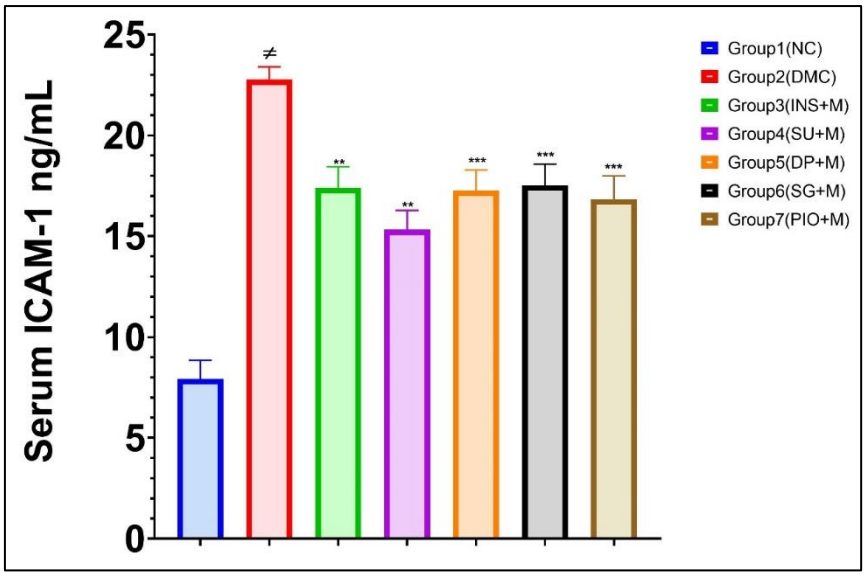

Figure 4. The serum concentration of ICAM-1: Intercellular Adhesion Molecule-1 in different treatment modalities of diabetic patients among groups. The data were analyzed using a one-way ANOVA with Bartlett's post hoc test. Data were denoted as ≠a significant difference among groups; $^{**}P<0.001$; $^{***}P<0.0001$.

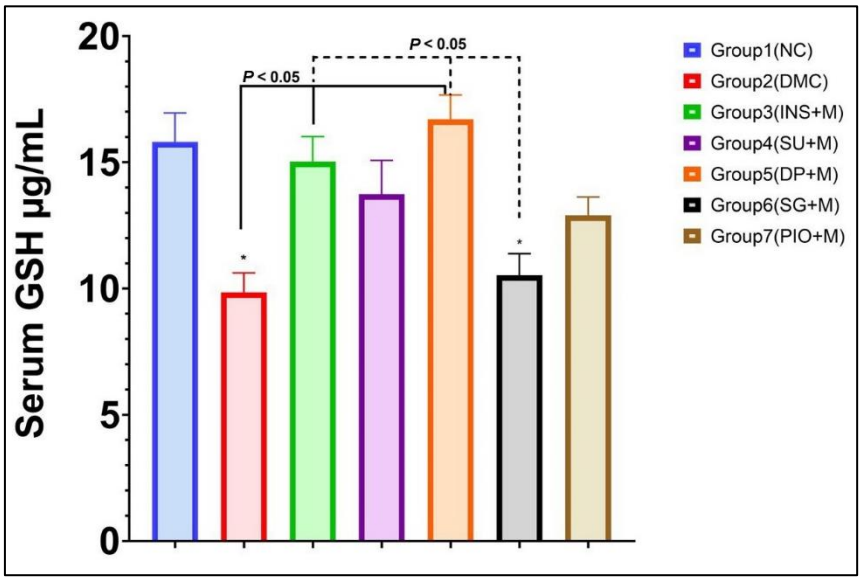

Figure 5. Serum glutathione concentrations of subjects in the study groups. The data were analyzed using a one-way ANOVA with Bartlett's post hoc test. $P<0.05$ is considered significantly different (n=). Data was denoted as $^{*}P<0.05$, representing a significant difference compared to a normal control group.

appear to be comparable with the normal control group, with the highest values regarding GSH concentration, as illustrated in Figure 5.

4. DISCUSSION

Diabetes mellitus is correlated with endothelial dysfunction and seems to be a consistent observation among DM patients. Endothelial dysfunction biomarkers have played a significant role in predicting the development of diabetic vascular complications and in identifying therapeutic targets. Therefore, it is important to select diabetic treatment modalities that enhance or strengthen endothelial function through different mechanisms. The present study showed no significant differences in BMI, marital status, or smoking between all groups, while diabetic patients need to consider the impact of obesity on developing microvascular complications. The present finding is supported by the Rivellese et al. (2000)[26] study, which found no significant variation in the BMI being very similar at the end of each treatment period. In contrast, Doghish et al. (2019)[12] found that the BMI of both patient groups was significantly different from the control group, but there was no significant difference between patient groups.

We also found a highly significant total cholesterol, TG, and LDL level was observed in Group 2 compared to NC and diabetic groups with different modalities. The

finding is consistent with past studies by Kachhawa et al. (2016)[27], which reported an increase in cholesterol levels, TG, and LDL in the type 2 DM patient group compared to a control group. In fact, due to the impaired function of lipoprotein lipase, which is localized in endothelial cells, diabetic hyperlipidemia results from an imbalance of elevated serum levels of TG, LDL, and decreased HDL levels, and this leads to an early manifestation of atherosclerosis and progression, thereby contributing to the development of endothelial dysfunction and vascular complications. In contrast, patients treated with SGLT2 in Group 6 showed the lowest cholesterol concentration, LDL, comparable to normal control. The present finding also supports Calapkulu et al. (2019)[28] studies, which concluded that dapagliflozin positively affects the lipid profile by reducing total cholesterol, LDL, and triglyceride levels. In contrast with our results, a recent meta-analysis by Sánchez-García et al. (2020)[29] indicated that SGLT2 inhibitors substantially increase total cholesterol and LDL levels while decreasing triglyceride concentrations. These findings imply a limited direct benefit of these antidiabetic medications for treating hyperlipidemia in patients with T2DM.

The study also revealed that Group DMC had higher HbA1c, RBS, and blood pressure than the other groups, which was predictable. Alkandari (2022)[30] found that untreated patients with diabetes and hypertension were more likely to experience an increase in HbA1c and blood pressure, which is in good agreement with the results of the present study. Among diabetic patients in Groups 3, 4, and 6, our results showed a highly significant increase in systolic blood pressure and HbA1c compared to normal control. Regarding Group 3, the present finding also supports the Yki-Järvinen et al. (2001)[31] study, which concluded that insulin in combination with metformin had no significant effect on improving glycemic control by measuring HbA1c and reducing blood pressure. According to the finding in Group 4, the study result agrees with Cook et al. (2005)[32], which found that after six months of the initiation of sulphonylurea, the median HbA1c deteriorated at a similar rate to that observed with metformin monotherapy. The result in Group 6 contradicts numerous studies' findings that SGLT2 inhibitors significantly reduce blood pressure, with greater systolic reductions than diastolic reductions (Rosenwasser 2013)[33].

Also, we found that Groups 2 and 4 had the highest abnormal percentage of hypertension, HbA1c, with a bad lipid profile compared to other groups. Similar to our findings, Ozder et al. (2014)[34] reported that HbA1c was significantly correlated with TG and LDL levels, and the Zheng et al. (2019)[35] study showed that in T2DM patients, elevated TG levels are firmly associated with impaired glycemic control. Mansour et al. (2020)[36] study found that hypertension was observed in 42.0% of patients, whereas hyperlipidemia was observed in 70% with high HbA1c percent.

Interestingly, our results showed that treated patients with pioglitazone showed a highly significant increase in serum NO_x concentration compared to the other groups. Pioglitazone, as a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist, the activation of PPAR-γ enhances insulin sensitivity, reduces inflammation, and decreases oxidative stress. These effects indirectly suppress atherogenesis and improve endothelial function, which is associated with the upregulation of NO production[37]. This is supported by the Nishio et al. 2008[38] study, which revealed that treatment with pioglitazone significantly decreased endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS) "is an enzyme that is upregulated in inflammatory conditions and converts arginine to citrulline and NO" by enhancing adipocytokine levels and may have enhanced endothelial function. The present finding also supports the Yu et al. (2013)[39] study, which concluded that pioglitazone administration led to an increase in NO production, which was an independent measure of the improvement in endothelial dysfunction.

In Groups 2, 4, and 6, our results showed that the concentration of NO_x was significantly decreased. Contrary to that result, the studies by Ueba et al. (2005)[40] concluded that glimepiride induces NO production in human coronary artery endothelial cells, and the use of glimepiride in T2DM may have the potential to prevent coronary artery disease in addition to lowering glucose levels. Durante et al. (2021)[41] demonstrated that SGLT2 inhibitors increase the bioavailability of NO, which is intimately related to vascular health.

Regarding ENG, our results show that Groups 2 and 6 had significantly higher serum endoglin levels compared to other treated diabetic and normal control groups. The present finding also supports the study, which concluded that soluble endoglin is significantly elevated in the early stages of T2DM[12]. However, regarding the treated group with SGLT2 and metformin, our results did not agree with Fun et al. (2017)[42], which found that with the medium dose of dapagliflozin, there was a trend toward reduction of endoglin. Endoglin may play an essential role in certain phases of diabetic vascular disease. The concentration of endoglin may decline over time, which may be due to decreased production or increased formation of complexes with other unidentified substances in the circulation[43].

The measurement of ICAM-1 was conducted to examine the presence of adhesion molecules as a means to assess endothelial dysfunction comprehensively. All groups showed significantly elevated serum ICAM-1 levels compared to the normal control group, with the highest level observed in Group 2. He et al. (2022)[44] found that the elevation of pro-inflammatory cytokines included ICAM-1 in newly diagnosed patients with T2DM who did not receive treatment, which is in good agreement with the results of the present study. Chronic

hyperglycemia is strongly associated with the upregulation of inflammatory mediators in several molecular pathways, according to research demonstrating that glucose directly upregulates ICAM-1[45].

There was a clear trend of decreasing serum GSH levels in untreated diabetes control and SGLT2 treatment groups compared with the remaining groups. The present finding also supports the Hakki et al. (2013)[46] study, which concluded that GSH levels were diminished in subjects with newly diagnosed diabetes compared to other groups. However, regarding Group 6, our result reflects the findings of Iannantuoni et al. (2019)[47], which indicated that T2DM receiving SGLT2 treatment experiences a simultaneous decrease in mitochondrial superoxide production and an increase in glutathione levels. According to our results in the present study, some antidiabetic medications restore vascular redox homeostasis by decreasing oxidative stress and increasing GSH levels, thereby contributing to the enhancement of endothelial function.

5. CONCLUSION

Newly diagnosed patients with diabetes have a high risk of developing endothelial dysfunction that leads to atherosclerosis and cardiovascular disease development. Moreover, that is obvious in the increasing blood pressure, HbA1c, RBG, and serum concentrations of cholesterol, TG, and LDL. As expected, the measured biomarkers for the endothelial function in these patients were abnormal by decreasing NO_x and GSH levels and increasing ENG and ICAM-1, which appear to indicate the development of endothelium dysfunction. Regarding the treated groups, although the results differed between the treatment groups, there was an apparent effect on some indicators of endothelial dysfunction, with a clear preference for the pioglitazone treatment group appearing to be comparable with the normal control group by decreasing blood pressure and the lowest from other treated groups in HbA1c. Also, the group that was given pioglitazone had the highest level of NOx compared to the normal control group. This is good for keeping the endothelium from failing, but the group that was given SGLT2 had the least impact on these biomarkers.

6. ACKNOWLEDGMENT

We appreciate the patients who took part in our study.

Conflict of interest
None to declare.

Funding
None to declare.

Ethics approval
This study was approved by the research ethics committee of the Basrah directorate (approval number 663) and the University of Basrah College of Medicine (approval number 030406). After receiving appropriate information about this study, informed consent was obtained from all study participants before their enrollment in the study.

Author contribution
All authors contributed to the idea and design, data collection, data analysis and interpretation, article writing or critical revision for significant intellectual content, and the final approval of the version to be published.

Article info:
Received August 5, 2023
Received in revised form October 27, 2023
Accepted December 10, 2023

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