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G6PD activity in whole blood distinguishes the pattern of vascular complications in type 2 diabetes

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ABSTRACT

Background: Hyperglycemia in the second type of diabetes significantly contributes to oxidative load, which in turn promotes the evolution of vascular complications. Since Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in antioxidant defense, our study aimed to evaluate the correlation between G6PD activity and the presence and pattern of vascular complications in individuals with type 2 diabetes. **Materials and Methods**: The total blood G6PD activity and fasting blood glucose (FBG) levels were compared between two groups of patients and a group of 44 healthy individuals. The first group of patients included 80 type 2 diabetic patients without vascular complications, while the second group comprised 44 type 2 diabetic patients with macrovascular complications and 36 type 2 diabetic patients with microvascular complications. HbA1c and creatinine values were too compared between the two patient groups. FBG and creatinine levels were measured using colorimetric methods, HbA1c was assessed by a fluorescence immunoassay, and total blood G6PD was determined using a kinetic method assay. Statistical tests (One-way ANOVA test, Tukey's test, and Pearson's correlation) were conducted using IBM SPSS Statistics 26, and $P< 0.05$ was considered significant. **Results**: The study revealed a rise in G6PD activity among patients with macrovascular complications, while its activity decreased in patients with microvascular complications. Additionally, G6PD activity exhibited a negative correlation with creatinine levels in patients with microvascular complications. **Conclusion**: This study suggests that measuring G6PD activity in whole blood could serve as a differential and prognostic marker for vascular complications. This study also demonstrates, for the first time, that high creatinine levels may indicate low antioxidant activity in type 2 diabetic patients with microvascular complications.

Keywords: Type 2 diabetes, G6PD, Oxidative stress, Vascular complications, Creatinine.

1. Introduction

Uncontrolled high glucose levels in diabetes mellitus result from disruptions in the secretion or function of insulin. Diabetes has several categories, with two main types: type 1 and type 2. Peripheral insulin counteraction and the progression of various vascular problems in its patients characterize type 2 diabetes[1, 2].

Vascular complications in type 2 diabetes can lead to deterioration in the organs supplied by these vessels. These vascular complications are classified into microvascular complications and macrovascular complications[1]. The most critical microvascular complications are those that lead to nephropathy, neuropathy, or retinopathy, while coronary artery disease (CAD), peripheral arterial disease (PAD), and cerebrovascular disease are considered the most significant macrovascular complications[3].

Numerous studies have indicated the role of oxidative stress in the pathological mechanism leading to the development of vascular complications in diabetic patients[4]. The heightened oxidative stress in diabetic patients is attributed to the predominance of oxidant

production over antioxidants[5, 6]. Excess production of oxidants can damage endothelial cells, induce inflammation, and increase adhesion factors and oxidized lipoproteins, all of which contribute to atherosclerosis that is the main pathogenic factor of vessels[7].

G6PD is an enzyme located in the cytoplasm that plays a role in the pentose phosphate pathway. Its enzymatic activity is essential for the cell's antioxidant system as it produces NADPH. NADPH is essential for the production of reduced glutathione, which is considered the primary source of antioxidant capacity in most cells, such as red blood cells (RBCs)[8-10].

Since G6PD is a crucial enzyme in antioxidant defense and plays an essential role in modulating the vascular redox state, our aim in this investigation was to study its activity and correlate it with the occurrence and type of vascular complications in type 2 diabetic patients.

2. Materials and Methods:

2.1. Study Population:

The Faculty of Pharmacy at Aleppo University approved this study (ethical approval certificate No. 12/V, 18.02.2022) in accordance with the Declaration of Helsinki and its subsequent revisions since 1975. The samples were collected from the National Center of Diabetes Mellitus in Aleppo, Syria. The exclusion criteria included G6PD deficiency or a previous disorder in any endocrine gland, such as the thyroid.

After obtaining informed consent, peripheral blood samples were collected from 204 individuals, comprising 160 patients with type 2 diabetes and 44 healthy individuals.

Type2 diabetic patients were divided into two groups: The first group consisted of 80 patients without vascular complications, while the second group comprised 44 patients with macrovascular complications and 36 patients with microvascular complications.

Among the patients, 26 had cardiovascular (CVD) complications, 12 had high blood pressure, 1 had a stroke, and 5 had diabetic foot issues, all of which are considered macrovascular complications. Among the patients with microvascular complications, 10 had diabetic retinopathy, 7 had diabetic nephropathy, and 19 had diabetic neuropathy.

2.2. Blood samples:

Venous blood (5 mL) was taken following an overnight fasting period of 10–12 hours. Each sample was distributed into two tubes: one containing EDTA 2K for the estimation of G6PD activity and HbA1c, and the other containing heparin for the estimation of fasting blood glucose and creatinine.

2.3. Methods:

G6PD activity was measured using the G-SIX KIT method from Coral Clinical Systems, a division of Tulip Diagnostics (P) Ltd. in India (Catalog Number GSX1/0817/VER-02). Briefly, G6PD catalyzes the oxidation of glucose-6-phosphate, converting NADP to NADPH. The conversion rate of NADP to NADPH is measured by the increment in absorbance, which corresponds to the G6PD activity in the sample.

FBG and creatinine levels were measured using the BioSystems kit with the Glucose Oxidase/Peroxidase method (Catalog Number M11503i-18) and the BioSystems kit with the JAFFÉ method (Catalog Number M11502i-21), respectively.

The HbA1c Rapid Quantitative Test (using fluorescence immunoassay, FinecareTM, Catalog Number W207) was employed to measure HbA1c levels.

2.4. Statistical Analysis:

Data were expressed as mean ± standard deviation (SD) and were analyzed using a one-way ANOVA test, Tukey's (post hoc tests), and Pearson's correlation analysis in IBM SPSS Statistics 26. P-values below 0.05 were considered significant.

3. Results:

Table 1 summarizes the key characteristics of the study participants. The table shows that there were almost minor differences in the

average age and percentage of smokers across all groups. The participants in all groups consisted of both males and females, as indicated by the sex distribution. Additionally, the duration of diabetes since diagnosis was significantly longer in the group of patients with macrovascular complications compared to the other patient groups (P<0.05).

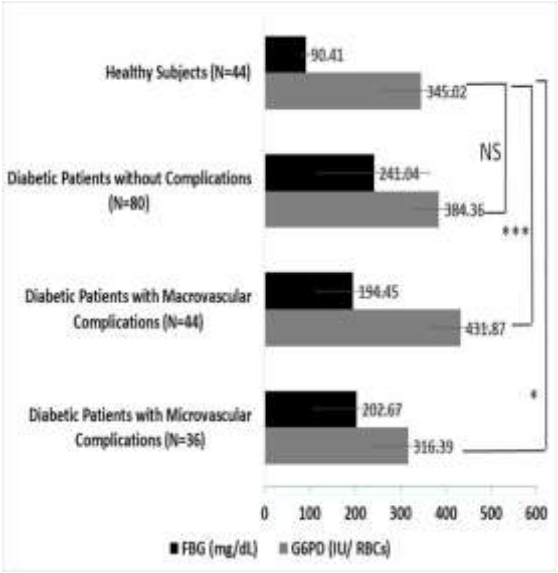

Figure 1: *Comparison of FBG values and G6PD activity was conducted between the Patient groups and the Healthy subjects group. The Tukey's test was used to determine statistical significance with NS indicating non-significance, * indicating P<0.05, and *** indicating P<0.001. The chart displays the mean ± s.d.*

Parameter	All Samples (N=180)	All Diabetic Patients (N=160)	Diabetic Patients with Microvascular Complications (N=36)	Diabetic Patients with Macrovascular Complications (N=44)	Diabetic Patients without Complications (N=80)	Healthy Subjects (N=44)
Age (years; mean ± SD)	56.3 ± 10.1	56.2 ± 9.4	56.3 ± 8.1	62.7 ± 7.1	57.4 ± 10.1	49.5 ± 9.6
Sex, Male (% of Male)	92 (45.1%)	81 (50.6%)	24 (66.7%)	20 (45.5%)	37 (46.3%)	11 (47.7%)
Sex, Female (% of Female)	112 (54.9%)	79 (49.4%)	12 (33.3%)	24 (54.5%)	43 (53.8%)	23 (52.3%)
Smoking (%)	59 (27.5%)	46 (28.7%)	6 (16.7%)	13 (29.5%)	27 (33.8%)	10 (22.7%)
Obesity (%)	22 (5.9%)	22 (7.5%)	6 (16.7%)	6 (13.6%)	0 (0%)	0 (0%)
Duration of diagnosis (years; mean ± SD)	-	9.7 ± 8.2	8.4 ± 7.8	13.0 ± 9.3	6.6 ± 6.3	-
Non-treated (%)	-	26 (16.2%)	2 (5.6%)	2 (4.5%)	22 (27.5%)	-
Treated with oral hypoglycemic drugs (%)	-	118 (73.8%)	29 (80.6%)	31 (70.5%)	58 (67.5%)	-
Treated with Insulin (%)	-	16 (10%)	5 (13.8%)	7 (16.0%)	4 (5.0%)	-

Table 1.*Basic characteristics of the participants.*

As shown in Table 2 and Figure 1, statistical analysis using the one-way ANOVA test revealed significant differences in the G6PD activity and FBG levels among the study population. The statistical analysis also revealed a significant difference in creatinine values among patients, but HbA1c levels did not exhibit an expected significance (Table 2).

Laboratory Parameters	Diabetic Patients with Microvascular Complications (N=36)	Diabetic Patients with Macrovascular Complications (N=44)	Diabetic Patients without Complications (N=80)	Healthy Subjects (N=44)	P values*
G6PD (IU/10^9 RBCs) ± SD	316.39 ± 76.86	[illegible] ± 58.98	[illegible] ± 57.77	[illegible] ± 82.17	<0.05
G6PD (IU/g Hb) ± SD	10.64 ± 2.73	[illegible] ± 2.85	[illegible] ± 2.30	[illegible] ± 3.11	<0.05
FBG (mg/dL) ± SD	202.67 ± 90.86	[illegible] ± 78.44	[illegible] ± 121.23	[illegible] ± 7.62	<0.05
HbA1c (%) ± SD	7.89 ± 2.12	[illegible] ± 1.92	[illegible] ± 2.56	-	0.304
Creatinine (mg/dl) ± SD	1.08 ± 0.49	0.81 ± 0.17	0.775 ± 0.19	-	<0.05

*One way ANOVA test

Table 2. Mean values of participants' laboratory parameters.

As illustrated in Table 3, Tukey's tests for FBG levels revealed that the significant findings indicated by the One-way ANOVA test were due to elevated FBG levels in all patients compared to healthy controls, and decreased FBG levels in patients with macrovascular complications compared to those without vascular complications.

Similarly, Tukey's tests revealed that the high G6PD activity in the group of patients with macrovascular complications was statistically significant compared to the other groups (P<0.05). Additionally, the low G6PD activity in the group of patients with microvascular complications was also found to be significant compared to the other groups (P<0.05). While the G6PD activity did not differ significantly between the group of patients without vascular complications and the group of healthy individuals (Table 3).

Laboratory Parameters	Group (1)	Group (2)	*P-values
G6PD (IU/10^9 RBCs)	Microvascular Complications	Macrovascular Complications	0.000***
	Microvascular Complications	No Complications	0.000***
	Microvascular Complications	Healthy Subjects	0.012*
	Macrovascular Complications	No Complications	0.001**
	Macrovascular Complications	Healthy Subjects	0.000***
	No Complications	Healthy Subjects	0.236
G6PD (IU/g Hb)	Microvascular Complications	Macrovascular Complications	0.000***
	Microvascular Complications	No Complications	0.000***
	Microvascular Complications	Healthy Subjects	0.002**
	Macrovascular Complications	No Complications	0.000***
	Macrovascular Complications	Healthy Subjects	0.000***
	No Complications	Healthy Subjects	0.475
FBG	Microvascular Complications	Macrovascular Complications	0.979
	Microvascular Complications	No Complications	0.169
	Microvascular Complications	Healthy Subjects	0.000***
	Macrovascular Complications	No Complications	0.040*
	Macrovascular Complications	Healthy Subjects	0.000***
	No Complications	Healthy Subjects	0.000***
Creatinine	Microvascular Complications	Macrovascular Complications	0.000***
	Microvascular Complications	No Complications	0.000***
	Macrovascular Complications	No Complications	0.749

Table 3. Tukey's tests (Post Hoc tests) for multiple comparisons.
(* P<0.05, ** P<0.01, *** P<0.001)

The elevated level of creatinine in the group of patients with microvascular complications was statistically significant compared to those in the other two groups of patients. In contrast, its level did not differ between patients with macrovascular complications and patients without vascular complications (Table 3).

Table 4 shows that G6PD activity did not correlate with FBG or HbA1c levels (P>0.05), but it was negatively correlated with creatinine levels (P<0.05). Furthermore, there was no significant correlation between G6PD activity and the date of diagnosis (P=0.259).

	Pearson coefficient	P-values
G6PD (IU/10^{11} RBCs) & FBG	٠,٠٠٦	٠,٩٢٥
G6PD (IU/10^{11} RBCs) & HbA1c	0.016	0.839
G6PD (IU/10^{11} RBCs) & Creatinine	**-0.225**	**0.004**
G6PD (IU/10^{11} RBCs) & Date of diagnosis	0.046	0.565
G6PD (IU/g Hb) & FBG	0.004	0.960
G6PD (IU/g Hb) & HbA1c	0.050	0.532
G6PD (IU/g Hb) & Creatinine	**-0.182**	**0.021**
G6PD (IU/g Hb) & Date of diagnosis	0.090	0.259

Table 4. Pearson correlation between the parameters in patient groups.

4. Discussion:

Macrovascular and microvascular complications are the primary contributors to the morbidity and mortality of patients with type 2 diabetes[3]. The molecular mechanisms underlying the development of these complications are numerous[3,4]. Long-term exposure to high glucose concentrations leads to dysfunction of vascular endothelial cells, resulting in the development of complications in blood vessels of various sizes[11]. Numerous studies have indicated that the destruction of endothelial cells due to oxidative stress induced by hyperglycemia is the primary factor in the development of vascular complications in diabetic patients[12, 13].

Endothelium combats oxidants by increasing the production of antioxidants through enzymes such as G6PD. The X-linked gene encodes G6PD, which primarily functions to produce NADPH, a crucial cofactor in reductive biosynthesis[8-10]. G6PD activity can be affected by inherited or acquired factors. As an example of acquired factors, the application of a high concentration of glucose to cultured endothelial cells caused a decrease in G6PD activity through cAMP-dependent protein kinase A (PKA), leading to an increase in reactive oxygen species (ROS) within the endothelial cells. Therefore, the exogenous overexpression of G6PD restored the redox state and protected endothelial cells from oxidant-induced damage[14, 15]. In contrast, some studies have indicated a protective effect of G6PD deficiency on cardiovascular diseases[16].

The data reported in this paper indicates that, despite good glucose control, the duration of diabetes was longer in the group of patients with macrovascular complications compared to other groups of patients. Thus, the results suggest that the duration of exposure to high blood sugar is a determining factor in the development of macrovascular lesions and may reflect the time needed for atherogenesis, which primarily affects the large and medium vessels[17, 18].

The results obtained here suggest that G6PD activity in whole blood is affected by various factors that differentiate diabetic macrovascular complications from diabetic microvascular complications. However, it does not change in diabetic patients without vascular complications compared to healthy controls. The elevated G6PD activity in patients with macrovascular complications may be attributed to the enzyme being released into the bloodstream from damaged endothelial cells. This hypothesis is supported by the low G6PD activity in patients without vascular complications and in healthy controls, compared to that in patients with macrovascular complications. On the other hand, the low G6PD activity in patients with microvascular

complications may result from various mechanisms, such as the interaction between endothelial cells and pericytes in the microvasculature. Pericytes produce numerous cytokines in response to the oxidative stress induced in the diabetic endothelium of small vessels[19]. These cytokines stimulate signaling pathways in stressed endothelial cells and in RBCs that may modulate the activity of certain kinases (e.g. PKA and Fyn), resulting in a decrease in G6PD activity[15, 20]. A recent study showed that RBCs contribute to the development of endothelial dysfunction in patients with type 2 diabetes by increasing the activity of Arginase through a mechanism dependent on reactive oxygen species (ROS)[21]. Therefore, the close interaction between RBCs and endothelial cells may be an important factor in the mechanism causing the microvascular disorder. This interaction may also lead to a decrease of G6PD in both RBCs and endothelial cells[21]. Furthermore, a recent Exome-Wide Association Study (EWAS) identified the connections between different genes and each type of vascular complication. Therefore, the mechanism by which diabetes causes vascular complications or modifies G6PD activity varies at the genetic level, depending on the size of the vessel[22].

Lastly, we observed no difference in the value of HbA1c between patient groups, indicating that HbA1c is an independent variable and reflects the mean glycemia over a 2–3 months period[23, 24]. In addition, no correlation was found between G6PD activity and HbA1c, which supports the findings of a previous study indicating that HbA1c did not correlate with markers of oxidative stress[25].

Although patients with nephropathy constituted only 20% of those with microvascular complications, the creatinine levels in this group were significantly elevated compared to the levels in the other two groups of patients. Interestingly, G6PD activity was inversely correlated with creatinine levels. This intriguing result can be explained by the fact that creatine, which is broken down into creatinine, exhibits antioxidant activity. Due to the decreased G6PD activity in patients with microvascular complications, there is more degradation of creatine, leading to a higher level of creatinine[26].

5. Conclusions

This study demonstrated the disparity in G6PD activity between the two types of vascular complications in type 2 diabetic patients, indicating distinct mechanisms causing vascular damage depending on the size of the vessel, as well as different approaches to combating oxidative stress. Our results suggest that measuring the G6PD activity in whole blood can be used to monitor patients with type 2 diabetes and predict the development of vascular complications. Larger studies must be conducted to demonstrate the inverse relationship between creatinine and G6PD activity in patients with diabetic microvascular complications in order to uncover the molecular mechanism that could link creatinine and oxidative stress in diabetic patients.

6. Disclosure of potential conflicts of interest

The authors report no conflicts of interest.

7. Acknowledgments

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8. Contributions

FK collected the samples, performed the experiments, analyzed the data, and wrote the manuscript. **MA** analyzed the data and wrote the manuscript. **MSJ** designed the study, wrote the manuscript and analyzed the data.

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