Assessment of G6PD Activity among Diabetic Patients and Its Relationship with Hyperglycemia, Wad Madani, Gezira State, Sudan

Afraa Kamil Ahmed1, Yosria Mohammed Elsiddig1, Khalid Abdelsamea Mohamedahmed1, Sami Yousif Gamar1, Yousif Abdelhameed Mohammed2, Zeinab H. Alfaham1, Osman Khalaf Alla Saeed3, Asad Adam Abbas4*

1Faculty of Medical Laboratory Sciences, University of Gezira, Wad Madani, Sudan
2National Cancer Institute, University of Gezira, Wad Madani, Sudan
3Faculty of Medicine, University of Gezira, Wad Madani, Sudan
4Faculty of Medicine & BNNICD, University of Gezira, Wad Madani, Sudan

Email: *asadadam@live.com


Received: October 1, 2022
Accepted: November 18, 2022
Published: November 21, 2022

Copyright © 2022 by author(s) and Open Access Library Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Abstract
Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme in the pentose phosphate pathway (PPP) which reduces NADP to NADPH while oxidizing glucose-6-phosphate. NADPH subsequently acts as a reducing agent, allowing oxidized glutathione to be converted to reduced glutathione, which protects against oxidant damage. Objectives: This study aimed to assess G6PD activity in Sudanese diabetic patients and its relationship with hyperglycemia. Materials and Methods: This study was a case control study done during the period from 2015 to 2018. Included 245 diabetic patients, compared to 245 age matching adults non-diabetic as control, male and female. Erythrocyte G6PD activity was determined using a quantitative spectrophotometric assay of enzyme by commercial kit. Blood glucose was conducted using a spectrophotometric procedure by Glucose Oxidase/Peroxidase test. Results: The study proved that, the incidences of low activity of G6PD enzyme among diabetic patients were 21.6%, and there was no evidence of deficiency or low activity in controls P. value (0.000). According to G6PD activity normal value, 53 (21.6%) were low activity and 192 (78.4%) were normal activity, and there was no complete deficiency at all in studied population. The relation between G6PD activity and high blood glucose level revealed a highly significant difference with P. value (0.006). Conclusions: The study concluded that G6PD low activity is one of the risk factors for diabetes mellitus, as well as hyperglycemia.
1. Introduction

Diabetes mellitus is a metabolic disorder marked by hyperglycemia caused by insulin metabolism problems. If diabetes hyperglycemia is not effectively treated, it can lead to long-term damage, dysfunction, and failure of several organs, including the eyes, kidneys, nerves, heart, and blood vessels [1].

In diabetic patients, an increase in oxidative stress has been noted, which could be attributed to an increase in oxidant-producing processes or a decrease in antioxidant defense mechanisms [2].

The World Health Organization (WHO) classified three kinds of diabetes mellitus in 1999. The Type 1 DM; which was previously referred to as “insulin-dependent diabetes mellitus” (IDDM) or “juvenile diabetes” [3] is caused by the body’s inability to manufacture insulin and currently necessitates the use of insulin injections or an insulin pump. The majority of cases of type 1 diabetes are immune-mediated, with beta cell loss resulting from a T-cell-mediated autoimmune attack [4]. The Type 2 DM; which was previously referred to as non-insulin dependent diabetes mellitus (NIDDM) or “adult-onset diabetes” is claimed to be caused by insulin resistance, a disease in which cells fail to adequately utilize insulin, which can be paired with an absolute insulin deficit or diminished insulin output [5]. Patients with type 2 diabetes are frequently, but not always, beyond the age of 40 when they first present [6]. The third form, gestational diabetes, occurs when a pregnant woman without a history of diabetes has a high blood glucose level may precede development of type 2 DM [7]. Despite the fact that type 1 diabetes has a peak incidence around the time of puberty, about 25% of cases appear after 35 years of age [8].

Glucose-6-phosphate dehydrogenase (EC1.1.1.49; D-Glucose-6-phosphate: NADP + oxidoreductase) is the rate-limiting enzyme of the pentose phosphate pathway. Because it produces the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), the major cellular reductant and fuel for glutathione recycling within the cells, it is necessary for antioxidant defense [9].

G6PD regulates cell metabolism and has been linked to a variety of illnesses, including diabetes, aldosterone-induced endothelial dysfunction, and cancer. G6PD’s main function is to be a key generator of NADPH, a hydrogen carrier required by many important cellular systems such as glutathione recycling, nitric oxide generation, and the cytochrome p450 system, and others [10]. There is growing evidence that G6PD activity is more important for NADPH synthesis.
than for ribose formation in the defense against oxidative stress [11]. It was discovered that diabetes mellitus and high glucose levels reduce G6PD activity [12].

The link between G6PD deficiency and diabetes is still being researched. Experimental evidence supports the concept that hyperglycemia causes a reduction in G6PD activity. When comparing the prevalence of G6PD deficit in people with diabetes to the background rate in the general population, thorough screening of G6PD activity revealed an increased prevalence of G6PD deficiency in people with diabetes [13].

The cause of reduced G6PD activity in diabetic patients who do not have a gene mutation is unknown. A G6PD deficiency could result not only from G6PD gene mutations but also from alterations in factors that control G6PD activity, namely, 1) Hormones or growth factors; 2) Oxidative stress; 3) Post-translational regulation. G6PD activity has been demonstrated to be induced by hormones and growth factors such as insulin, estrogen, and epidermal growth factor (EGF), G6PD activity may also be boosted by vitamin D3 [14].

2. Methods
2.1. Objective

The purpose of this study aimed to assess G6PD activity in Sudanese diabetic patients and in age-matched non-diabetic controls and to ascertain whether diabetes mellitus and hyperglycemia lead to G6PD deficiency or low activity.

2.2. Methodology

This is a prospective case-control study. The case-control ratio was 1:1, includes 245 diabetic patients, compared to 245 age matching adults non-diabetic as control.

Conducted in Aldaraja diabetic Centre which is responsible for follow up by regular care and treatment for diabetes. Established in 1997. Directed by health insurance-Ministry of Welfare Social Development.

2.3. Inclusion Criteria

All Sudanese patients with diabetes mellitus.

Age ranged 30 - 90 years old.

2.4. Exclusion Criteria

Non-diabetic patients, ages less than 30 years, any level of non-Sudanese ancestry.

The approval for this study was obtained from the ministry of health. Ethical clearance was obtained from the Research Ethical Committee (REC) of the faculty of medicine, university of Gezira. Verbal consent was taken from each study subject.

Erythrocytes’ G6PD activity was determined for all studied cases admitted to the diabetic Centre and for the controls, using a Quantitative spectrophotometric assay of enzyme by commercial kit. The results were interpreted according to the normal reference value for normal control as follow: Normal activity: 80 -
80 \text{mu}/10^9 \text{cells}, \text{Enzyme deficiency: 0 - 11 \text{mu}/10^9 \text{cells}, Enzyme low activity: 11 - 80 \text{mu}/10^9 \text{cells}}.

The values of G6PD activity were obtained for cases and controls. The differences between cases and controls for quantitative variables were analyzed by using the Statistical Package for Social Sciences (SPSS) software version 20.0, descriptive analysis was done using (mean \pm standard deviation, frequency, percentage of each value, Odd ratio, 95 \% CI, Pearson Correlation), to determine the significance of the association between G6PD activity and diabetes and hyperglycemia, $P$ values $< 0.05$ were considered to be statistically significant.

3. Results

3.1. Demographic Characteristics of Studied Participants

Among the 490 study participants, 245 (50\%) were diabetic patients while 245 (50\%) were non-diabetic age and gender matching controls. Among the diabetic patients, 105 (42.9\%) were male and 140 (57.1\%) were female. All the participants were between 30 and 90 years. The demographic characteristics of the patients are summarized in Table 1.

3.2. Laboratory Investigations of Studied Participants

The studied group consisted of 245 diabetic patients and 245 non diabetic as control. According to G6PD activity normal values the statistical analysis of results determined the G6PD activity among diabetic patients, results showed that a highly significant difference in G6PD activity between studied populations and control group with p. value (0.000) shown in Table 2. Where the study showed that there was no impact on G6PD activity in control group.

The percentage presentation of G6PD activity status among studied patients was found low activity in 53(21.6\%) out of 245 diabetic patients, and 192(78.4\%) were normal activity of G6PD, and there was undetectable levels of G6PD activity total deficiency reported as all. shown in Table 3.

Blood glucose measurement was conducted using a spectrophotometric procedure by Glucose Oxidase/Peroxidase test, Table 4 showed the Frequency

Table 1. Demographic characteristics of participants.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Participants</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>105</td>
<td>42.9</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>140</td>
<td>57.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>245</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Participants</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 - 50 Year</td>
<td></td>
<td>56</td>
<td>22.9</td>
</tr>
<tr>
<td>51 - 70 Year</td>
<td></td>
<td>153</td>
<td>62.4</td>
</tr>
<tr>
<td>71 - 90 Year</td>
<td></td>
<td>36</td>
<td>14.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>245</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 2. G6PD activity between studied group and control group.

<table>
<thead>
<tr>
<th>G6PD activity mu/10^6 cells</th>
<th>Subjects</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>245</td>
<td>116.55</td>
<td>35.700</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>245</td>
<td>127.81</td>
<td>16.160</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Percentage presentation of G6PD activity status among studied population.

<table>
<thead>
<tr>
<th>G6PD activity</th>
<th>Frequency</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>53</td>
<td>21.6</td>
</tr>
<tr>
<td>Normal</td>
<td>192</td>
<td>78.4</td>
</tr>
<tr>
<td>Total</td>
<td>245</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. Distribution of studied population according to blood glucose level status.

<table>
<thead>
<tr>
<th>blood glucose level mg/dL</th>
<th>Frequency</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>60</td>
<td>24.5</td>
</tr>
<tr>
<td>High</td>
<td>185</td>
<td>75.5</td>
</tr>
<tr>
<td>Total</td>
<td>245</td>
<td>100.0</td>
</tr>
</tbody>
</table>

distribution of studied population according to blood glucose level; 24.5% were normal level & 75.5% were high level of blood glucose. The relation between G6PD activity & high blood glucose level was found to be; odd ratio (5.4), P. value (0.006), with (95% CI) 1.63 to 17.96 reported in Table 5, from a total of 185 of hyperglycemic (high level of blood glucose) patients there were 50 patients with low G6PD activity. From 60 with normal level of blood glucose diabetic patients there were only 3 with low G6PD activity. And the Pearson Correlation showed negative highly significant correlation between hyperglycemia and G6PD activity, Pearson correlation −0.418, Sig. (2-tailed) 0.000.

None of the studied cases with G6PD low activity had shown clinical manifestations of favism or drug-induced hemolysis.

4. Discussion

Glucose-6-phosphate dehydrogenase (G6PD) is an antioxidant enzyme that cat-
alyzes the rate limiting step of the pentose phosphate pathway (PPP) which is critical for the synthesis of NADPH and pentose sugar. G6PD activity is required for the function and survival of many cells [15]. Furthermore, G6PD activity has been found to be changed in a variety of diseases, including cardiovascular disease, cancer, skin problems, and diabetes [16]. Indeed, hyperglycemia has been found to cause a significant reduction in G6PD activity in the liver, kidneys, and red blood cells in animal models [10]. While it has been demonstrated that a decrease in G6PD activity is linked to ketosis-prone diabetes (KPD) [17]. As a result, the enzyme plays a role in the development of oxidative damage in diabetic mellitus, albeit the amount of its involvement in T2D-related oxidative stress is unknown. Despite this, little research has been done on the modification of G6PD activity in relation to the degree of hyperglycemia. More importantly, information on the human-based studies on the actual relationship between the altered G6PD activity and hyperglycemia is scarce in the literature and even the few studies were limited to only in Taiwan [18], Pakistan [19], Egypt [20], and India [15] with no data from other parts of the world especially the black populations at the sub-Saharan Africa.

Epidemiological evidence from around the world suggests that glucose-6-phosphate dehydrogenase (G6PD) deficiency may be a risk factor for diabetes mellitus, and there is widespread agreement that G6PD activity is influenced by diabetes and hyperglycemia.

A hospital in Saudi Arabia researched glucose-6-phosphate dehydrogenase deficiency and diabetes mellitus in a report published by Niazi G A [21] This hospital-based investigation found a statistically significant greater prevalence of glucose 6 phosphate dehydrogenase (G6PD) activity changes in Saudi diabetic patients (12.4%) compared to the healthy population control (2.0%). (P. value less than 0.008). Because diabetic patients have a higher rate of G6PD deficiency. The nature of this link, according to the study, is difficult to explain. It is recommended that all diabetic patients be tested for this enzymopathy in order to avoid using medicines or toxic substances that can cause hemolytic consequences.

Another agreement research was undertaken in Syria, this time at the Al Assad. University Hospital in Lattakia’s Department of Laboratory Medicine. It included 100 patients at Al Assad University Hospital with the goal of observing the link between glucose-6-phosphate dehydrogenase deficiency and diabetes mellitus. It was found that diabetes individuals, particularly those who are uncontrolled, are more likely to acquire G6PD deficiency or low activity, and that uncontrolled diabetic patients had varying levels of G6PD activity.

In this study, we found that the prevalence of low activity of G6PD enzyme among Sudanese diabetic patients was 21.6% compared to age matching non-diabetics control group, p.value (0.000). This agrees also with a study done by Nadira A. et al. [22], they studied (G6PD) status in Type 2 diabetes mellitus. Their study concluded that G6PD deficiency may be one of the risk factors for Type 2 diabetes mellitus irrespective of blood glucose control status.
In terms of the link between hyperglycemia and G6PD activity, hyperglycemia is a disorder characterized by high glucose levels in the blood, and it is more common in adults over the age of 40. Hyperglycemia is known to produce the creation of free radicals, which may be the primary cause of oxidative stress in the absence of the G6PD enzyme, resulting in diabetic damage due to an improper antioxidation process. The findings of the study revealed from a total of 185 hyperglycemic patients there were 50 patients with low G6PD activity, with odd ratio (5.4) compared with normal level of blood glucose with P. value (0.006), with (95% CI) 1.63 to 17.96. Of 60 with normal blood glucose diabetic patients there were only 3 with low G6PD activity. These findings agree with the study of the relationship between the degree of hyperglycemia and G6PD activity, which was investigated in diabetic patients attending Aminu Kano Teaching Hospital, Kano, Nigeria [23] conducted by Mohammed Auwal et al., concluded that reduced G6PD activity is associated with poor glycemic control.

5. Conclusions
The study concluded that:
• G6PD low activity is one of the risk factors for diabetes mellitus, irrespective of blood glucose control status, and G6PD low activity could be etiologically associated with diabetes.
• Diabetic hyperglycemia leads to decrease G6PD activity and may lead to serious complications. This issue itself aggravates diabetic injury due to inappropriate antioxidation process.

6. Recommendation
It is suggested that:
• All patients with diabetes should be routinely screened for this enzymopathy to provide appropriate medical care.
• Supplementation of an adequate dose of vitamin C & E as an antioxidant to diabetic patients with low G6PD activity may be beneficial to avoid diabetic’s injury.

Conflicts of Interest
The authors declare no conflicts of interest.

References


Clinical Endocrinology & Metabolism, 90, 4446-4451.
https://doi.org/10.1210/jc.2004-2545


