Prevalence Of Glucose-6-Phosphate Dehydrogenase Deficiency in People Living in Katsina Metropolis

Osibemhe M., 1,2 and Hauwa’u A. A., 2*

1Department of Biochemistry, Faculty of Basic Medical Sciences, Edo State University Uzairue, Edo State, Nigeria (Sabbatical).
2Department of Biochemistry and Molecular Biology, Faculty of Life Science, Federal University Dutse-Ma, Katsina State, Nigeria.

Email: hauwauabuaminus@gmail.com; Tel: 08035957414

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most prevalent human enzyme defects observed in malaria endemic areas. The study included 212 participants, with 142 (67%) male and 70 (33%) female, aged between 14 to 59 with a mean age of 24.20±9.35 years. The study was granted ethical approval by the Katsina State Ministry of Health. Each subject gave his/her written informed permission after counseling. G6PD deficiency, malaria parasite, reduced glutathione (GSH), glutathione peroxidase (GPx), packed cell volume (PCV) and hemoglobin (Hb) were assayed using standard procedures. Among the 212 participants tested in the study, 40 (19%) were found to be G6PD deficient and 172 (81%) participants were normal. Among 212 participants tested, 75 (35.4%) were infected with malaria parasite out of which 13 (17.3%) were G6PD deficient and 62 (82.7%) were normal. Among the 40 G6PD deficient subjects, 33 (82.5%) were male while 7 (17.5%) were females. GSH concentration was lowered significantly in G6PD deficient subjects compared to the normal subjects. GPx, Hb and PCV showed no significant difference (p>0.05) in the deficient subjects compared to the normal subjects. The results of this study suggest that G6PD deficiency is quite common with a prevalence of 19% in the population of Katsina metropolis. The data also shows that males are more likely to be affected by G6PD deficiency than females.

Keywords: Hemoglobin, G6PD, glutathione peroxidase, GSH, malaria.

1.0 INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme found in the cytoplasm of all cells. The oxidation of glucose-6-phosphate to 6-phosphogluconate is catalysed by glucose-6-phosphate dehydrogenase (G6PD) in the pentose phosphate pathway, which is also known as the hexose monophosphate shunt (Kassahun et al., 2023; Albagshi et al., 2020). In order to achieve this goal, nicotinamide adenine dinucleotide phosphate, often known as NADPH, is synthesised from NADP. As a result of the generation of NADPH, red blood cells are shielded from the effects of oxidative stress (Ibrahim et al., 2022; Bello et al., 2016). Reducing glutathione from oxidised glutathione and keeping the intracellular reduced glutathione pool stable both need NADPH, an important co-factor in many biosynthetic reactions (Satyagraha et al., 2021; Isaac et al., 2013). In the absence of reduced glutathione, oxidised haemoglobin will crystallise into Heinz bodies. On the other hand, reduced glutathione acts as a scavenger for free radicals and assists in the conversion of oxidised haemoglobin into free haemoglobin (Eyo et al., 2023; Satyagraha et al., 2021; Isaac et al., 2013). In the meantime, the function of the G6PD enzyme is depicted in the picture below.
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most familiar enzyme defect in humans (Tassew et al., 2022; Bello et al., 2016; Isaac et al., 2013). Alving and his colleagues in 1956 were the first to discover G6PD deficiency during their research on whether hemolytic anemia would be present in Africans being treated with the antimalarial, Primaquine. Later, it was known that G6PD deficiency not only occurred in Africans but was also prevalent worldwide. Recently, it was found that more than 400 million people are G6PD deficient globally (Alangari et al., 2023; Ibrahim et al., 2022; Garcia et al., 2021; Jinyoung et al., 2018). It usually affects males and is inherited as an X-linked recessive condition. G6PD deficiency is characterized by neonatal jaundice and hemolytic anemia, both of which can lead to neurological problems and even mortality (Eyo et al., 2023; Kassahun et al., 2022; Tassew et al., 2022; Eugenia et al., 2019; Isaac et al., 2013).

It is known that G6PD deficiency confers resistance to severe malaria (Oduola et al., 2022; Jinyoung et al., 2018). Although there is no clear understanding of how G6PD deficiency protects against severe malaria, there are postulations that linked it to the effects of G6PD deficiency on the physiology of RBC (Babalola et al., 2022; Tassew et al., 2022; Jinyoung et al., 2018; Bello et al., 2016). Unless exposed to oxidative medicines (primaquine, dapsone, sulfamides, quinolones, certain antibiotics and some analgesics), certain diseases, or after eating fava beans, most persons with G6PD deficiency have no symptoms. G6PD deficiency is treated primarily by limiting exposure to oxidative agents that cause haemolytic anaemia (Tassew et al., 2022; Garcia et al., 2021; Jinyoung et al., 2018). Certain medications, including primaquine, dapsone, sulfamides, quinolones, certain antibiotics, and some analgesics, have been described as haemolytic triggers that cause haemolytic anaemia in G6PD-deficient individuals (Sathupak et al., 2021; Eugenia et al., 2019; Chu et al., 2018). Primaquine is a very effective anti-malaria drug that can reduce the transmission of malaria by eliminating gametocyte of *P. falciparum* and reducing the number of hypnozoites of *P. Vivax* and *P. Ovale* (Babalola et al., 2022; Sathupak et al., 2021; Eugenia et al., 2019; Chu et al., 2018).

Reactive oxygen species (ROS) cause oxidative stress when their synthesis and buildup in cells and tissues exceeds the body’s capacity to detoxify them (Swastika et al., 2020). To survive with the formation of ROS, red blood cells and all other cells have an antioxidant defence system that includes enzymatic and non-enzymatic antioxidants. The paramount antioxidant molecule of intracellular environment is reduced glutathione (GSH), which has many physiological roles. Glutathione peroxidase (GPx) is a cytosolic enzyme which catalyses the reduction of hydrogen peroxides to water and oxygen using GSH as a co-factor. In red blood cells, GSH-Px is the most effective antioxidant against oxidant stress and has paramount importance in phagocytosis of cells (Swastika et al., 2020). The incidence of G6PD deficiency in Nigeria varies widely between previous researches. G6PD deficiency was discovered to be 14.4% among 118 children requiring paediatric care who were seen at the Emergency Paediatric unit at Usman Danfodiyo University Teaching Hospital in Sokoto, Nigeria (Isaac et al., 2013). In Kano, Nigeria, Sagir & Umma, (2018) found that 20.4% of healthy blood donors lacked the G6PD enzyme. Bello et al. (2016) found that G6PD deficiency was present in 16% of Plasmodium falciparum-infected children in Katsina state, Nigeria. However, our study among residents of Katsina metropolis cuts across a wider age bracket and accounts for the deficiency of G6PD in healthy individuals and malaria infected ones.

### 2.0 MATERIALS AND METHOD

#### 2.1 Study Area

Katsina LGA, Katsina State, was the site of the research. Katsina is one of Nigeria's northwestern states. The population of Katsina LGA is 524,000 according to the current metro area population of Katsina in 2023. Its total area is approximately 142km² (55 sq mi), its coordinates are 12°59'20"N and 36°03'8"E. Katsina is located approximately 260km (160 mi) east of the city of Sokoto and 135km (84 mi) northwest of Kano, close to the border with Niger Republic. Katsina LGA has an annual average temperature of 30.89°C (87.6°F), which is 1.43°F hotter than the rest of Nigeria (Weather and Climate, 2023). The warmest months are March to June, with an average daily temperature above 36.7°C (98°F). The coldest months are December to January, with an average low temperature of15°C (59°F) and high of 28.9°C (84°F). The rainy season is...
from June to October, the month with the most rain in Katsina is August. Malarial transmission is high during the wet season and low during the dry season. The figure below shows the selected area of the study.

![Figure 2: Map of Katsina State Showing the Selected Area of the Study](source: Geographic Information System (GIS) Laboratory FUDMA (Adapted from Google Earth Imagery 2023))

2.2 Study Design
This study was designed to determine the prevalence of G6PD deficiency in people living in Katsina metropolis which comprises of 212 participants, 142 (67%) males and 70 (33%) females. The participants gave their consent in writing. This research was given ethical approval by the Health Research Ethical Committee (HREC) of the Ministry of Health in Katsina State, Nigeria.

The formula below was used to get the above sample size

\[
\text{Sample size} = \frac{Z_{1-\alpha/2}^2 P(1-P)}{d^2} \quad \text{(Charan & Biswas, 2013)}.
\]

\[Z_{1-\alpha/2}^2 = \text{standard normal variate. At 5% type 1 error (p < 0.05) it is 1.96 and at 1% type 1 error (p < 0.01) it is 2.58. In majority of studies p values are considered significant below 0.05 hence 1.96 is used in formula.}\]

\[P = \text{Expected proportion in population based on previous studies or pilot studies.}\]

\[d = \text{Absolute error or precision. (Charan & Biswas, 2013).}\]

Using the formula above

\[Z_{1-\alpha/2}^2 = 1.96\]

\[P = 0.16 \quad \text{(Bello et al., 2016)}\]

\[d = 0.05\]

\[
\text{Sample size} = \frac{(1.96)^2 \times 0.16 \times (1 - 0.16)}{0.05^2} = \frac{3.8416 \times 0.16 \times 0.84}{0.0025} = 206.52
\]

2.3 Sample Collection and Method
Three milliliters of whole blood was drawn (Venipuncture sample) from each study subject into Ethylene Diamine Tetra-acetic Acid (EDTA) tubes. The blood samples collected were transported to Ramal medical laboratory, Katsina for the analysis.

G6PD deficiency was detected using the Randox G6PD quantitative in vitro test

**Principle:** The principle is based on reduction of NADP⁺ by G6PD present in red blood cells. The NADPH generated fluoresces under ultraviolet light at a wave length of 340 nm.

G-6-P+ NADP⁺ → G6PD → Gluconate-6-P+ NADPH + H⁺

The activity of G6PD enzyme was determined by the rate of change in absorbance. Red blood cell G6PD value of ≥2.9 U/gHb was regarded as normal, while those with red blood cell G6PD value of <2.9 U/gHb were regarded as deficient and those with red blood cell G6PD value of <1.6 U/gHb were regarded as severely deficient (Isaac et al., 2013).

GSH was measured using standard procedure
**Principle:** The principle is based on the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce 2-nitro-5-mercapto-benzoic acid (TNB), which possesses its absorption maximum at 412nm (Nse et al., 2022).

GSH concentration was calculated using the formula:

\[
\text{GSH mmol/l} = \frac{A_{\text{sample}} \times \text{Conc of standard}}{A_{\text{standard}}}
\]

Concentration of standard GSH = 0.20mM

GPx was measured using standard procedure

**Principle:** The principle is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colour disposition that can be detected at 430 nm (Daramola et al., 2018).

The activity of glutathione peroxidase (GPx) was calculated using the formula:

\[
\text{GPx activity (mg protein/ml enzyme)} = \frac{\text{OD/min} \times \text{Vt} \times \text{Df}}{E \times \text{Vs} \times \text{Y}}
\]

OD/min = Absorbance of test  
Vt = Total volume of reaction mixture  
Df = Dilution factor  
Vs = Volume of sample  
E = Molar extinction coefficient of purpurogallin (12M⁻¹Cm⁻¹)  
Y = Mg of protein.

The hematocrit technique was used to determine packed cell volume (PCV).

**Principle:** Packed cell volume (PCV) is a measure of the ratio of the volume occupied by the red cells to the volume of whole blood in a sample of capillary, venous or arterial blood. The ratio is measured after appropriate centrifugation and is expressed as a decimal fraction (Gunn et al., 2002).

The sample was mixed well and the capillary tubes were filled to two-third. The dry end was sealed with plasticine clay. The capillary tubes were placed in opposite slots of microhematocrit centrifuge with the clay-filled ends against gasket. The capillary tubes are centrifuged for five (5) minutes. The capillary tubes were placed unto the hematocrit reader and the results were read and expressed in percentage.

Haemoglobin was determined using Cyanmethemoglobin method

**Principle:** When blood is diluted in buffered solution of potassium ferrocyanide (KFeCN) and potassium cyanide (KCN), the potassium ferrocyanide converts haemoglobin (Hb) to methemoglobin (metHb). Potassium cyanide further converts metHb to cyanmethemoglobin. The absorbance of the solution is measured at a wavelength of 540nm (Bernard et al., 2011). Haemoglobin is derived from the formula below:

\[
\text{Haemoglobin (g/dl)} = \frac{A_{\text{test}} \times \text{Conc of standard} \times \text{Dilution factor}}{A_{\text{standard}}} 
\]

A microscopic technique was used to identify the malaria parasite. Giemsa is a Romanowsky stain that is frequently used in parasitology to stain parasites that feed on blood, including malaria. Giemsa has both basic and acidic dyes, which are attracted to the basic and acidic elements of blood cells, respectively. The cytoplasm, granules, and other essential parts of cells are stained by the acidic dyes Eosin and Azure Variable. The acidic components, particularly the cell nucleus, are stained by the fundamental dye methylene blue. Romanowsky stains' staining responses are pH-dependent, which is why the stains are diluted in buffered water with a certain pH (Cheesbrough, 2010).

**Procedure:**

- Thin blood smear was prepared and allowed to air dry
- The film was placed on a staining rack and 1–2 drops of moisture-free methyl alcohol (methanol) was added and allowed to dry on the film.
- The blood film was covered with undiluted stain but the slide was not allowed to flood. The number of drops required to cover the film was counted.
- Twice the volume of pH 7.2 buffered water (i.e. twice the number of drops as stain) was added. Without it overflowing, it was allowed to stain for ten minutes. After ten minutes the stain was washed off with tap water.
- The back of the slide was wiped cleaned and it was placed on a draining rack for the smear to dry. After the smear was dried the results were checked microscopically.
2.4 Statistical Analysis
Graph Pad and Microsoft Excel 2010 were used to statistically analyze the data. Data were presented as means and standard error of means. The differences between the deficient and normal participants were compared using a student t-test. Differences were considered significant at P<0.05.

3.0 RESULTS
The purpose of this study was to ascertain the prevalence of G6PD deficiency among people living in Katsina metropolis. The study recruited 212 subjects made up of 142 (67%) males and 70 (33%) females, aged 14-59 years with a mean age of 24.20 ± 9.35 years.

3.1 Prevalence of G6PD Deficiency in People Living in The Study Area
Figure 4 depicts the prevalence of G6PD deficiency among the participants. Among the 212 subjects tested in this study, 40 (19%) were found to be G6PD deficient while the remaining 172 (81%) subjects were normal.

3.2 Prevalence of G6PD deficiency among malaria infected subject
The study found out that among 212 participants tested, 75 (35.4%) were infected with malaria parasite. Among the 75-subject infected with malaria parasite, 13 (17.3%) were G6PD deficient and 62 (82.7%) were normal (Figure 5). Therefore, prevalence of 17.3% was observed. Among the 62 normal subjects infected with malaria 39 (52%) have a parasitic count of + (1-10 parasite per 100 HPF), 14 (18.7%) have ++ (11-100 parasite per 100 HPF and 9 (12%) have +++ (1-10 parasite per each HPF). 13 (17.3%)
G6PD deficient subject infected with malaria have a parasitic count of + (1-10 parasite per 100 HPF).

Figure 5: Prevalence of G6PD deficiency among malaria infected subjects

3.3 Prevalence of G6PD deficiency among participants based on sex
According to this study's findings, among the 40 deficient subjects, 33 (82.5%) of the males had G6PD deficiency, compared to 7 (17.5%) of the females (Figure 6).

Figure 6: Prevalence of G6PD deficiency based on sex

3.4 Prevalence of G6PD deficiency based on selected areas
The results showed that the percentages of deficient subject among the selected areas of Katsina metropolis were statistically similar; the percentage of deficient patients were: 20.8%, 18.9%, 18.9% and 17% for north, south, east and west respectively (Figure 7).

Figure 7: Prevalence of G6PD deficiency based on the selected areas

3.5 G6PD activity, Anti-Oxidant Enzymes and Hematological Indices among the normal and deficient subject.
The results of G6PD activity, reduced glutathione (GSH) and glutathione peroxidase (GPx) are presented in Table 1. Haemoglobin (Hb) and packed cell volume (PCV) concentrations are presented in Table 2. The activity of G6PD in the deficient subject was significantly (p<0.05) reduced compared to the normal subjects. GSH concentration was significantly (p<0.05) decreased in deficient subjects compared to the normal subjects. GPx activity, Hb and PCV levels showed no significant difference (P>0.05) in deficient compared to the normal subjects.

Table 1: G6PD activity, GSH concentration and GPx activity in normal and deficient subject.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal n=172</th>
<th>Deficient n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (U/gHb)</td>
<td>9.636±0.450a</td>
<td>1.547±0.163b</td>
</tr>
<tr>
<td>GSH (mmol/l)</td>
<td>1.344±0.147a</td>
<td>0.659±0.047b</td>
</tr>
<tr>
<td>GPx (U/mg)</td>
<td>4.781±0.218b</td>
<td>5.097±0.480b</td>
</tr>
</tbody>
</table>
Values are presented in mean ± SEM. Significant differences exist between values in the same row with various superscripts. Values in the same row with the same superscript are not substantially different. G6PD = Glucose-6-phosphate dehydrogenase, GSH = Reduced glutathione and GPx = Glutathione peroxidase

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal n=172</th>
<th>Deficient n=40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>13.59±0.204a</td>
<td>13.59±0.103a</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>40.73±0.306a</td>
<td>41.10±0.614a</td>
</tr>
</tbody>
</table>

Values are presented in mean ± SEM. Significant differences exist between values in the same row with various superscripts. Values in the same row with the same superscript are not substantially different. Hb = Haemoglobin and PCV = Packed cell volume.

4.0 DISCUSSION
In this study, a prevalence rate of 19% G6PD deficiency was observed among residents of the study region. This is consistent with some preceded studies done in Nigeria and other parts of the world. Williams et al., (2013), Isaac et al., (2013), and Bello et al., (2016) published earlier research in Nigeria that suggest a prevalence of 15.3%, 14.4%, and 16%, in the southwestern Nigeria, Sokoto state and Katsina state respectively. Thus, a study by Sagir & Umma (2018) among healthy blood donors in Kano showed a prevalence of 20.4%. G6PD deficiency occurs mostly in people of African heritage and range in from 3.6% to 28.0% (Sagir & Umma, 2018). Report from Assefa et al., 2018 indicated that the incidence in Ethiopia was 8.9%. According to a research, the incidence in malaria-endemic municipalities in Brazil is 5.6% (Jaobi et al., 2022). Moreover, report from Dechyoitin et al. (2021) among females from previously malaria endemic regions in northeastern Thailand found prevalence of 18% by fluorescent spot test and 27.0% by PCR-based genotyping. The present study recruited 212 participants, among the 212 participants; 75(35.4%) were found to be infected with malaria with parasitic count ranging from + (1-10 parasite per 100 HPF), ++ (11-100 parasite per 100 HPF and +++ (1-10 parasite per each HPF). Out of the 75 participants infected with malaria, it was found that 13 (17.3%) were G6PD deficient with parasitic count of + (1-10 parasite per HPF), this shows that the G6PD deficient subject have low parasitic count. Thus, this is in accordance with the hypothesis that suggest that G6PD deficiency confer resistance against severe malaria (Tassew et al., 2022; Eugenia et al., 2019; Jinyoung et al., 2018; Bello et al., 2016; Isaac et al., 2013). G6PD deficiency is a natural selection, a number of epidemiological studies report that it is common in areas where malaria is widespread (jinyoung et al., 2018). In this study, male subjects 33 (82.5%) had a higher prevalence of G6PD deficiency than female subjects 7 (17.5%). Because it is an X-linked recessive genetic condition characterized by unusually low levels of G6PD, G6PD deficiency primarily affects males. Because the mutation would need to occur in both copies of the gene to induce the condition in females, the deficiency is uncommon in females, but in males just one defective copy of the gene is required for the disease to emerge (Ibrahim et al., 2022; Isaac et al., 2013). The findings of the study is in accordance with literature that indicate that the sex of the patient is crucial, and men are at higher risk than women (Tassew et al., 2022; Jinyoung et al., 2018; Bello et al., 2016; Isaac et al., 2013). The lack of statistical correlation between G6PD deficiencies among the selected areas in this study demonstrates that G6PD deficiency is independent on the area of the participants within Katsina metropolis. This is also in accordance with a study conducted by Bello and his colleagues that suggested that between the three senatorial zones of Katsina state there is no statistical correlation of G6PD deficiency (Bello et al., 2016). As expected, the activity of G6PD in the deficient subject was significantly (p<0.05) lowered when compared with the normal subjects. This is in consistent with other studies conducted in Nigeria and other part of the world (Tassew et al., 2022; Eugenia et al., 2019; Jinyoung et al., 2018; Bello et al., 2016; Isaac et al., 2013). The reduced concentration of GSH in G6PD-deficient RBC as compared to normal RBC observed in this study was not surprising. It may be due to the fact that in RBC, oxidized glutathione (GSSG) is reduced to reduced
glutathione (GSH) by glutathione reductase, an NADPH dependent enzyme. In RBC NADPH is dependent on G6PD enzyme because NADPH is produced through the conversion of glucose-6-phosphate to 6-phosphogluconate which is catalysed by G6PD. With the reduced activity or deficiency of G6PD, NADPH will either be absent or low therefore reduction of GSSG to GSH will be low (Alangari et al., 2023; Ibrahim et al., 2022; Tassew et al., 2022; Oduola et al., 2018). The non- significant change observed in the levels of Hb and PCV of G6PD deficient subjects as compared to normal subjects in this study show that both the deficient subject and the normal subject have no any sign of anaemia. This may be suggestive of asymptomatic G6PD deficiency (Alangari et al., 2023; Tassew et al., 2022; Oduola et al., 2018; Bello et al., 2016; Isaac et al., 2013).

5.0 CONCLUSION
The results of this study suggest that G6PD deficiency is quite common with a prevalence of 19% in the population of Katsina metropolis. The data also shows that males are more likely to be affected by G6PD deficiency than females. Decrease levels of GSH was observed in G6PD deficient subjects while no any significant variation was observed in GPx activity, Hb levels and PCV levels.

6.0 ACKNOWLEDGEMENTS
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