Prevalence of Glucose -6- Phosphate Dehydrogenase (G-6-PD) Deficiency in Sokoto: Liver Function and Oxidative Stress Biomarkers in Deficient Individual

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Authors' contributions

This work was carried out in collaboration between all authors. Author TO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author IJ performed sample and data analyses. Authors DMB and MAN reviewed the manuscript and supervised the study. Author MKD managed the experimental process and literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in the pentose phosphate pathway (PPP) and plays an essential role in the oxidative stress response by producing Nicotinamide adenine dinucleotide phosphate (NADPH), the main intracellular reductant.

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Deficient individuals suffer from mild chronic haemolytic episode which could be exacerbated on exposure to oxidant drugs.

Aim: The aim of the study was to determine the prevalence of G-6-PD deficiency in Sokoto, assess liver function, lipid peroxidation and antioxidants status in G-6-PD deficient individuals.

Place and Duration of Study: The study was undertaken at the Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, between February and April 2015.

Methods: G-6-PD screening in 1000 individuals (603 males and 397 females) using Methaemoglobin Reduction Method was carried out, liver function and oxidative stress biomarkers were then evaluated in 60 deficient individuals (30 males and 30 females) and 60 individuals with normal G-6-PD status as controls using standard techniques.

Results: 376 (37.6%) subjects were found to be G-6-PD deficient, 128 (12.8%) of the males and 248 (24.8%) of the females screened were deficient. G-6-PD deficient individuals have significantly low ($p<0.05$) total protein (TP), aspartate transaminase (AST) and alkaline phosphatase activities when compared to control group but the decreases were within the reference range, while albumin (Alb), total bilirubin (TB) and conjugated bilirubin (CB), alanine transaminase (ALT) and alkaline phosphatase (ALP) values showed no significant difference ($p > 0.05$). Significantly high ($p<0.001$) malondialdehyde (MDA) and low total antioxidant potential (TAP) values were obtained in G-6-PD deficient individuals compared to controls.

Conclusion: The prevalence of G-6-PD deficiency in Sokoto is high, hence screening for G-6-PD deficiency before administration of oxidant drugs in G-6-PD deficient subjects may be necessary. G-6-PD deficient individuals may also be at the risk of developing oxidative stress induced diseases.

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme that catalyses the first reaction, the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, and concomitantly reduces NADP+ to NADPH, which is the rate-limiting and primary control step of the NADPH generating portion in the Pentose Phosphate Pathway (PPP). Thus, G6PD acts as a guardian of cellular redox potential during oxidative stress [1]. Nicotinamide adenine dinucleotide phosphate (NADPH) is a functionally important metabolite that is commonly used for reductive biosynthesis and maintenance of cellular redox potential. It is a required cofactor in reductive biosynthesis of fatty acids, isoprenoids, and aromatic amino acids [2,3,4]. NADPH is also used to keep glutathione in its reduced form. Reduced glutathione (GSH) acts as a scavenger for dangerous oxidative metabolites in the cell, and it converts harmful hydrogen peroxide to water with the help of glutathione peroxidase (GSHPx) [5]. Perturbed NADPH production increases sensitivity to reactive oxygen species (ROS) and provokes apoptosis and necrosis thus highlighting the role of G-6-PD in defending against oxidative damage [6,7,8]. Numerous pathways are known to maintain cellular NADPH levels. The major NADPH-producing enzymes in the cell are glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in the pentose phosphate pathway (PPP), malic enzyme (ME) in the pyruvate cycling pathway, and isocitrate dehydrogenase (IDH) in the tricarboxylic acid (TCA) cycle [9]. Activity of IDH1, ME1, and 6PGD remains unchanged during oxidative stress, while G6PD is the only NADPH-producing enzyme that is activated [1]. As erythrocytes lack the citric acid cycle, the Pentose phosphate shunt is the only source of NADPH.

G6PD deficiency is a hereditary X- linked disorder and the most prevalent enzyme defect in humans and affects an estimated 400 million people worldwide, especially in populations historically exposed to endemic malaria [10]. The most common clinical manifestations are neonatal jaundice and acute haemolytic anaemia, which is caused by the impairment of the erythrocyte's ability to remove harmful oxidative stress triggered by exogenous agents such as drugs, infection, or fava bean ingestion [8,10]. Haemolytic anaemia caused by infection and subsequent medication is a clinically important concern in patients with G6PD deficiency. This issue has been a primary focus for many decades in relation to efforts to understand the impact of *Plasmodium* infection (malaria) and antimalarial drugs [11,12].

Global prevalence of G-6-PD deficiency was reported to be about 4.9%, 3.4% in America, 4.7% in Asia, 3.9% in Europe, 6.0% in Middle East, 2.9% in Pacific and 7.5% in Africa [13]. In Turkey, the frequency of G-6-PD deficiency among students was 1.2% [14], Oman, 25% in males and 10% in females [15], Pakistan, 1.8% [16] and in Iraq 6.1% [17]. Luzzatto and Gordon-Smith [18] reported the prevalence of G-6-PD deficiency of 4 – 26% in Nigeria with the male population having about 20 – 26%. Oduola *et al.* [19] reported 26.7% as prevalence of G-6-PD deficiency in Ile-Ife, while Akanni et al. [20] reported a prevalence rate of 19.5% among prospective and suitable blood donors in Osogbo with G-6-PD deficiency.

Because G6PD acts as a guardian of cellular redox potential during oxidative stress, G-6-PD deficient individuals may be prone to oxidative stress induced disorders; since haemolysis of red blood cells may also be aggravated in G-6-PD deficient subjects especially when oxidant drug is ingested, conjugating ability of liver may be disturbed. Hence, the present study was designed to determine the prevalence of G-6-PD deficiency in Sokoto and to assess liver function profiles and lipid peroxidation in deficient subjects.

2. MATERIALS AND METHODS

2.1 Subjects

One thousand apparently healthy volunteers of the study population were recruited for the study to establish the prevalence of G-6-PD deficiency in Sokoto. This sample size was arrived at using the formula described by Oyejide [21] and Singha [22], n= $(z_1$-a$)^2$ (p) (1-p) /d^2. The target population were adolescents and adults. The adults were hospital employees, students of Tertiary Health and Educational Institutions, prospective blood donors, individuals in different occupational groups; and the adolescents were students of secondary schools within Sokoto metropolis.

2.2 Study Design

This study was a descriptive cross-sectional study. The study was carried out within 3 months, February – April, 2015. After establishing prevalence of G-6-PD deficiency, liver function test, total protein (TP), Albumin (Alb), total bilirubin (TB), conjugated bilirubin (CB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP); and total antioxidant potential(TAP) and Malondialdehyde (MDA) were determined in 60 selected G-6-PD deficient individuals and 60 individuals with normal G-6-PD status that served as control.

2.3 Blood Collection

Seven ml of blood was collected from each individual by clean venipuncture, 3 ml dispensed into EDTA (ethylene diamine tetra acetic acid) specimen bottles for G-6-PD screening and the remaining 4 ml dispensed into lithium heparin specimen bottles for biochemical analysis.

2.4 Methods

2.4.1 Assay in whole blood

G-6-PD screening was performed using Methaemoglobin Reduction Method [23]. The screening was carried out on the day of blood collection.

2.4.2 Assay in plasma

Total and conjugated bilirubin were estimated by Malloy and Evelyn, total protein and albumin by Biuret and Bromocresol Green (BCG) methods respectively; AST, ALT activities were determined using Reitman-Frankel method, alkaline phosphatase by nitrophenyl phosphate method of Bassey et al. [24]. Lipid peroxidation was measured by plasma malondialdehyde estimation colorimetric method of Shah and Walker's [25] and total antioxidant potential by copper reducing antioxidant assay method of Sashindran et al. [26].

2.5 Data Analysis

The data obtained from this study were analyzed using the statistical package for social science (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). The data were represented as the mean ± standard deviation (S.D). Student T-test at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when $p < 0.05$.

3. RESULTS

The prevalence of G-6-PD deficiency in Sokoto is 37.6%, with male and female having G-6-PD deficiency of 12.8% and 24.8% respectively

(Tables 1 and 2). The values obtained for liver function profile revealed no significant difference (p>0.05) except ALP (p<0.05), TP (p<0.01) and AST (p<0.01) (Table 3). Statistically significant increases in MDA (p<0.001) and decrease in TAP (p<0.001) concentrations were found among G-6-PD deficient subjects as compared to normal controls (Table 4).

Table 1. Prevalence of G-6-PD deficiency in Sokoto

Subjects	Number	%
Normal	624	62.4
Deficient	376	37.6
Total	1000	100

Table 2. Prevalence of G-6-PD deficiency in both gender

Gender	Screened subjects	Deficient subjects	%
Male	603	128	12.8
Female	397	248	24.8
Total	1000	376	37.6

% = Percentage, G-6-PD= Glucose-6-phosphate dehydrogenase

Table 3. Liver function parameters in G-6-PD deficient subjects

Parameters	Normal	Deficient group
T Bil (µmol/l)	15.29±0.27	14.59±0.32
C Bil (µmol/l)	3.18±0.80	3.02±0.10
ALT (U/l)	3.31±0.23	3.67±0.19
AST (U/l)	5.35 ±0.38	3.95±0.20**
ALP (U/l)	159.65±10.75	131.10±6.17*
ALB (g/l)	40.20±0.69	38.70±0.61
TP (g/l)	68.70±0.95	64.30±0.82**

*Values are presented as Mean±SEM. **Values bearing asterisks differ significantly (p<0.01) from the normal group, *Values bearing asterisks differ significantly (p<0.05 from the normal group, T Bil=Total bilirubin, C Bil=Conjugated bilirubin, ALT=Alanine transaminase, AST=Aspartate transaminase, U/L=Unit per litre*

4. DISCUSSION

In the present study, the prevalence of G-6-PD deficiency in Sokoto was established to be 37.6%, with male and female having G-6-PD deficiency of 12.8% and 24.8% respectively (Tables 1 and 2). The prevalence was high, the reason for this finding is not known but the prevalence of G-6-PD deficiency varies from one part to another all over the world. Our result also showed that G-6-PD deficient individuals have increased MDA and reduced TAP values than people with normal G-6-PD status. Since MDA is a byproduct of lipid peroxidation, this may signify increase lipid peroxidation in G-6-PD deficient individual. Antioxidants are substances which at low concentration significantly inhibit or delay the oxidative process, while often being oxidized themselves. Endogenous and exogenous antioxidants are used to neutralize free radicals and protect the body from free radicals by maintaining redox balance [27,28,29]. Plasma antioxidant in G-6-PD deficient individuals might have been consumed in neutralizing oxidative process, and this may be why we observed low values. Prolonged exposure to free radicals, even at a low concentration, may responsible for the damage of biologically important molecules and potentially lead to tissue injury [27,28,29]. Oxidative stress causes different diseases via four critical steps; membrane lipid peroxidation, protein oxidation, DNA damage and disturbance in reducing equivalents of the cell; which leads to cell destruction, altered signalling pathways. Oxidative stress has been implicated in various diseases like cancer, cardiovascular diseases, neurological disorders, diabetes, and ageing [27,28,29].

From this study, liver function profiles of G-6-PD deficient individuals were not significantly different from values obtained for control group except AST, ALP and total protein that were significantly reduced in G-6-PD deficient individuals than the control group, but all the values were within the reference range. Hence, our findings revealed that liver functions in G-6-PD deficient individuals are not impaired.

Table 4. Oxidative stress biomarkers in G-6-PD deficient subjects

Parameters	Controls (n=60) Mean±SEM	Deficient subject (n=60) Mean±SEM
Malondialdehyde (nmol/L)	44.80±3.70	85.60±8.30***
Total antioxidant potential (µM CRE)	286.71±20.26	174.27±17.86***

***Statistically significant (p< 0.001) as compared with normal controls
CRE=Copper reducing equivalents

5. CONCLUSION

In conclusion, the prevalence of G-6PD deficiency is high in Sokoto, it is suggested that patients that need therapy that can precipitate haemolytic crisis should be screened for G-6-PD deficiency before treatment. From this study, MDA is high and TAS is low in G-6-PD deficient individuals, the benefit of antioxidant diet which may prevent oxidative stress induced diseases in G-6-PD deficient individuals will be investigated.

6. LIMITATION OF THE STUDY

The study was self-funding, we would have increased our sample size for liver function profiles and oxidative stress markers in G-6-PD deficient individuals.

ETHICAL APPROVAL

This study was conducted in accordance with the Declaration of Helsinki. The study participants gave their informed consent and the research protocol was approved by the Ethics and Research Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto, and the Ministries of Health and Education, Sokoto, Sokoto State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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