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Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency among *Plasmodium falciparum* Infected Children in Kaduna State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author BMB carried out the investigated the work, performed statistical analysis, and wrote the first draft of the manuscript. Authors AA and AAH managed the analyses of the study and literature searches. Author KBD designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) is an inherited genetic disorder characterized by reduced or absent activity of the G6PD enzyme. It is prevalent globally, especially in regions where malaria is endemic. The G6PD enzyme plays a critical role in maintaining cellular redox balance and protecting red blood cells from oxidative stress. This study was conducted in

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Kaduna North Local Government Area, aim to identify G6PD deficiency among children aged 1-5 years. The study utilized several methods, including rapid diagnostic tests for detecting *Plasmodium falciparum*, automated blood analyzers for measuring red blood cell parameters, quantitative G6PD kits to assess enzyme activity, and spectrophotometer assays. Among 207 children who tested positive for Plasmodium falciparum, 33 (15.94%) were identified with G6PD deficiency in the 1-5 years age group. The highest prevalence was observed in age group 5 (33.33%), while age groups 3 and 4 had the lowest prevalence (12.12%). Males had a higher prevalence (66.67%) compared to females (33.33%), with Barau Dikko Teaching Hospital reporting the highest prevalence rate (16.98%) among the hospitals studiedThe study found that 70.21% of cases exhibited the lowest G6PD enzyme activity (0-4.4 range) U/g Hb, while 19.15% showed normal activity (4.5-13.5 range) U/g Hb, and 10.64% had activity above 13.5U/g Hb. Understanding the genetic underpinnings and implementing appropriate management strategies are essential for effectively managing individuals affected by G6PD deficiency.

Keywords: Glucose-6-Phosphate Dehydrogenase (G6PD); malaria; plasmodium falciparum; children; Kaduna State; Nigeria.

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is a cytoplasmic enzyme found in all the cells, and it is responsible for the oxidation of glucose-6-phosphate to 6-phosphogluconolactone. This reaction also converts the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) into its reduced form, NADPH. NADPH is crucial for the reduction of oxidized glutathione (GSSG) back to its reduced form (GSH) via the enzyme glutathione reductase. This process is vital for protecting blood cells from oxidative damage by neutralizing peroxides as well as other reactive oxygen species [1].

Red blood cells (RBCs) are susceptible to damage from both external and internal oxidants. Consequently, any defects in glutathione metabolism or the hexose monophosphate pathway, such as those resulted from a deficiency or impaired function of the enzyme glucose-6-phosphate dehydrogenase (G6PD), compromise the RBCs' ability to defend against oxidative imbalance. This can result in hemolytic anemia of varying degrees of severity and neonatal hyperbilirubinemia, a significant contributor to neonatal mortality and morbidity [2]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked inherited disease, is the most significant among these enzyme defects. In the 1950s, it was initially identified in black people, who exhibited varying susceptibility to hemolytic anemia following the primaquine administration for malaria treatment [3,4].

G6PD deficiency is currently the commonest inherited erythrocyte disorder in subtropical regions and Mediterranean countries [5]. Migration has played a role in spreading G6PD deficiency globally, resulting in varying prevalence among diverse ethnicity. The condition predominantly affects males with a single X chromosome carrying the deficient gene and females with two copies of the gene having decreased activity of enzyme. However, females diagnosed of Turner's syndrome and about 10% of those who are carriers could also experience hemolysis when exposed to substance that can triggers oxidative stress which include chemicals, medications, infections, or 'fava' beans [6].

The clinical outcomes of G6PD deficiency vary widely, from no symptoms to persistent hemolysis throughout life, depending on the remaining enzyme activity. Major manifestations include drug-induced hemolysis, non-spherocytic hemolytic anemia, favism, and severe, prolonged neonatal jaundice that can lead to neurological complications or mortality [7].

About 80% of preterm neonates and over 60% of full-term neonates exhibit clinical jaundice, characterized by elevated serum total bilirubin levels, in the first week of life [8]. Jaundice in G6PD-deficient neonates is influenced by various factors, including delayed maturation of liver enzymes needed for bilirubin processing and elimination. Additional contributors include neonatal immaturity, feeding methods, infections presence, and exposure to environmental substances like naphthalene-containing mothballs, herbal remedies, or antiseptic powders used on the umbilical cord [9].

Hyperbilirubinemia resulting from G6PD deficiency can manifest from the first day of life and is often serious with long duration. While

closely monitored cases rarely result in mortality, managing hyperbilirubinemia remains challenging in resource-limited settings, posing risks of severe neurological complications linked to toxicity of bilirubin [8,10]. In the United States, G6PD deficiency affects 1.6% of females and 2.5 % of males, with most cases being moderately severe. Particularly affected are the African American males, showing a prevalence of around 10% in the population generally and 22.5 % among jaundiced newborns. The deficiency prevalence reaches 70% in Kurdish Jews, 5% in China, within the range of 3% and 6.9% in Southern Russia and Pakistan, and drops to 0.1% in Japan [11,12]. In West Africa, the prevalence of G6PD deficiency is in variation based on the specific region [13].

The prevalence of G6PD deficiency in Nigeria varies between 4% and 26%, with approximately 20% to 26% of the male population being affected [14]. This is supported by 20% prevalence rates in Jos North Central and 14.4% in Sokoto, North West Nigeria have been reported [15]. The prevalence of G6PD deficiency among jaundiced newborns in Nigeria, influenced by the method of enzyme assay utilized, usually within the range of 20.5% and 35.3%. This rate is notably higher compared to Americans of African descent [16].

In red blood cells lacking G6PD, P. falciparum development is impeded, which lowers the risk of severe illness and slows the pace of parasite reproduction. In children between the ages of 6 and 12, G6PD deficiency was linked to a decreased prevalence of asymptomatic *P. falciparum* carriage [12].

2. MATERIALS AND METHODS

2.1 Study Design

In 207 children ages 1 to 5 years, this study sought to determine the prevalence of G6PD deficiency among those who were admitted or presented with *P. falciparum* malaria at selected hospitals between September and November of 2022. After counselling, parents or guardians gave their written, informed consent. The Ministry of Health in Kaduna State, Nigeria's Health Research Ethical Committee (HREC) gave ethical permission.

2.2 Study Area

The study was conducted in Kaduna North Local Government Area of Kaduna State (Fig. 1). It is located between latitude 10:35 North and longitudes 7:25 East. It is boarded by Igabi Local Government to the South, West and East of Kaduna State. It is characterized by two weather regimes. During the rainy season, the weather is warm, oppressive, and often overcast, while the dry season is characterised by hot weather with partly cloudy skies. From the beginning to the end of the year, temperatures usually in the range of 55°F to 95°F, rarely dropping below 50°F or exceeding 102°F. The state's natural vegetation consists largely of guinea savanna-characterised woodlands [17].

2.3 Sample Collection Method

Following the Plasmodium falciparum Rapid Test Device screening for malaria parasites, a laboratory technician extracted 2.0 ml of venous blood from every kid in the study population who tested positive for malaria at the designated hospitals. In order to perform a G6PD deficiency screening using the BioSystem Quantitative in vitro test (S.A Costa Brava 30, 08030 Barcelona (REF code: 571), the samples were collected in EDTA (Ethylene Diamine Tetra-acetic Acid) tubes and promptly transferred in an ice-cooler box to the Precision Biomedicals Laboratory [18].

2.4 Screening of Samples

2.4.1 Screening of malaria falciparum

The diagnosis of malaria was performed using the FIRST RESPONSE Malaria pf (HRP2) Ag rapid diagnostic test (RDT) kit. The assessment was performed following the manufacturer's instructions. To perform the test, a blood sample of 5 µL was collected using the provided specimen transfer device and added to the "1" well of the cassette in the test kit. Then, two drops (60 µL) of the buffer solution were added to the "2" well. The test cassette was allowed to develop for 20 minutes [19].

2.4.2 Determination of red blood cell and hemoglobin

The RBC count and hemoglobin content of the samples were determined using automated techniques. Sysmex percentage absolute counting and Sysmex CyFlow Counting were used [20].

2.4.3 G6PD enzyme detection using test kits

The G6PD assay was conducted using the BioSystem (S.A Costa Brava 30, 08030

Barcelona (REF code: 571) based on the reaction principle described by Beutler. In this assay, NADPH is produced from NADP+, and the spectrophotometric measurement of NADPH at 340 nm was used to determine G6PD activity. A G6PD kit was obtained from Fortress Diagnostics (with product code BXC0571). The kit functions on the reaction principle described by Beutler, in which NADPH is produced from NADP+. The NADPH produced is measured spectrophotometrically at 340 nm. The assay was conducted following the manufacturer's protocol. Initially, 0.2 mL of whole blood was washed three times with 2 mL aliquots of 0.9% solution of NaCl and spun at 3,000 rpm for 10 minutes each time. The washed RBCs were then suspended in 0.5 mL solution of digitonin and left to stand at 4°C for 15 minutes before being centrifuged again. The resulting supernatant was incubated with substrate and cofactor at 37°C for 10 minutes in an electric oven. Subsequently, the mixture was analysed using an ultraviolet/visible spectrophotometer for the enzyme assay, which was performed within 2 hours of preparation. G6PD activity was quantified and expressed in international units per gram of haemoglobin (IU/g Hb) [21].

2.5 Polymerase Chain Reaction

Polymerase Chain Reaction was used to amplify G6PD using PCR four genes. Samples were enrolled in PCR (Accupower Hotstart PCR premix, Bioneer; Deutsch, 1978). The templates were added about 2uL, with specific primer1 (2uL) and distilled 16uL water to the premix to make a 20uL reaction for the 1st round G6P OUT reversed (Bioneer) and template 2uL was added the PCR conditions: (Thermal cycler PTC 100, MJ Research Pre-Denaturation): 5mins at 95^{0}C, denaturation: 40 secs at 94^{0}C, annealing 40 sec at 54^{0}C, extension: 40 sec at 72^{0}C. 35 cycles and final extension: 5min at 72^{0}C. The result was run on 2% agarose gel.

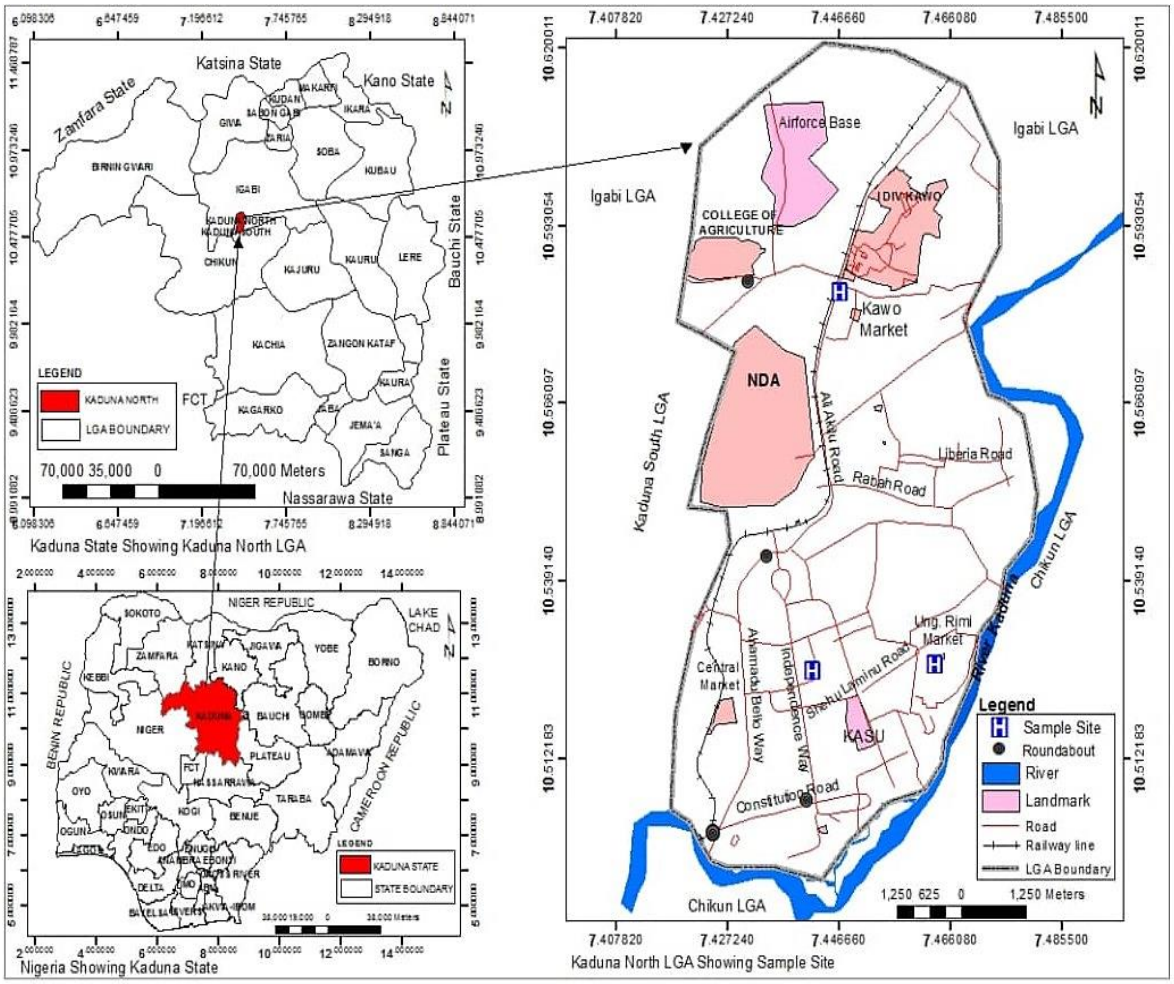

Fig. 1. Map of Kaduna North Local Government Area Showing Sampling Sites

Calculation

$$\text{G6PD (IU/g Hb)} = \frac{\text{G6PD activity derived from analyser (Mu / ML)}}{100 \times \text{Hb (g/Dl)}}$$

Values ≤4.4 U/g Hb were considered deficient.
Values >4.5 were considered sufficient (normal)

To preserve the blood for haematological parameter measurement, G6PD enzyme assay, and malaria diagnosis, the collected blood was introduced into a labelled bottle containing the dipotassium salt of ethylenediaminetetraacetic acid (EDTA). EDTA is commonly used as an anticoagulant to prevent blood clotting and preserve the blood sample for laboratory analysis. The labelled bottle with the EDTA-treated blood sample was then prepared for further testing and analysis [18].

2.6 Statistical Analysis

The data obtained from the study of spectrophotometric measurement of G6PD enzyme activity was analysed using descriptive statistics from Microsoft Excel 2017. The result evaluation of the data was based on the reference provided by the company 4.5-13.5 U/g Hb. Anything value below the range is considered deficient, and the result was presented in histogram, percentage and frequency distribution.

3. RESULTS

3.1 Overall Prevalence of *Plasmodium falciparum* Children Sampled in Selected Hospital in Kaduna North L.G.A.

Fig. 2 shows the overall prevalence of *Plasmodium falciparum* among the children sampled in the selected hospitals. Results shows that of the 207 children examined, 33 (15.94%) children were positive for *Plasmodium falciparum.* Out of these number, 22 (66.67%) were male while 11 (33.33%) were female.

3.2 Prevalence of *Plasmodium falciparum* among Children Sampled Based on Age Groups

Table 1 shows the prevalence of *Plasmodium falciparum* among children sampled based on age. Results shows that the highest prevalence was observed among children age 5 years, this is followed by children age 2 years. The least prevalence was observed among children of age groups 3 and 4 years. Results is presented in Table 1.

Table 1. Prevalence of *Plasmodium falciparum* among Children Sampled Based on Age Groups

Age Group	Number Examined	Number of positive (%)
1	40	6 (18.18)
2	32	8 (24.24)
3	40	4 (12.12)
4	37	4 (12.12)
5	58	11 (33.33)
Total	207	33 (15.94%)

3.3 Prevalence of *Plasmodium falciparum* among Children Sampled Based on Gender

Prevalence of *Plasmodium falciparum* based on gender is presented in Table 2. Results shows that male had the highest prevalence of 66.67% compared to female with prevalence of 33.33%.

3.4 Prevalence *Plasmodium falciparum* among Children Sampled in Selected Hospitals

The prevalence of *Plasmodium falciparum* among children sampled in selected hospitals shows that Barau Dikko had the highest prevalence of 16.98% while other hospitals sampled had the least prevalence of 15.39%. Results is presented in Table 3.

3.5 Prevalence of G6PD Deficiency Based on the G6PD Enzyme Activity

The distribution of G6PD deficiency based on the activity of the G6PD enzyme is depicted in this assay; in Table 4, NADPH is produced from NADP+, and the spectrophotometric measurement of NADPH at 340 nm is used to determine G6PD activity. The data was evaluated based on reference ranges provided by the company4.5-13.5 U/g Hb. The children's samples that fall less than the reference range 0-4.4 u/gHb were deficient with 33 samples, and it covered 70.21% of the overall 47 samples tested to be positive for malaria falciparum. 9 sample fell within 4.5-13.5U/g Hb representing 19.15% of the positive samples and greater than 13.5% is also referred to be normal with five children's samples. About 10.64% samples making up the overall 47 samples.

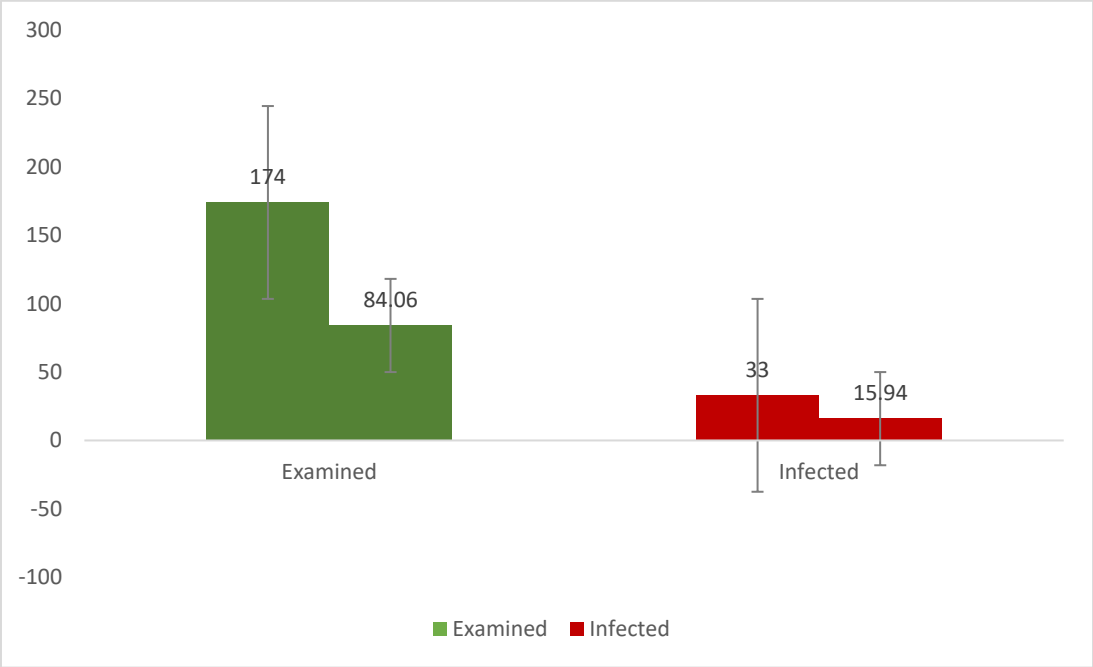

Fig. 2. Overall Prevalence of *Plasmodium falciparum* among Children Sampled in Selected Hospital in Kaduna North L.G.A.

Table 2. Prevalence of *Plasmodium falciparum* among children sampled based on gender

Gender	Number Examined	Number Positive (%)
Male	115	22(66.67)
Female	92	11(33.33)
Total	207	

Table 3. Prevalence *Plasmodium falciparum* among children sampled in selected hospitals

Hospital Sampled	Number Examined (%)	Number of Positive (%)
Barau dikko	53 (83.02)	(9)16.98
Badarawa	52 (84.02)	(8)15.38
Ungwan Rimi	52 (84.02)	(8)15.38
Kawo	52 (84.02)	(8)15.38
Total	207	

Table 4. Prevalence of G6PD deficiency based on the G6PD Enzyme Activity from 0-13.5(U/g Hb)

G6PD Enzyme Activity (U/g Hb)	Enzyme	Percentage (%)
0-4.4	33	70.21
4.5-13.5	9	19.15
>13.5	5	10.64
Total	47	

3.6 Results of Molecular Detection of Glucose-6-Phosphate Dehydrogenase Deficiency Genes

The visualization of bands at 300bp (Plate I) indicates the presence of wild type 202 (W1), while 422bp also infers wild type 376 as (W2).and also band viewed at 600bp indicates 202 MUTANT type (M1) with 800bp shows 376 MUTANT type as (M2) 4 samples with lower G6PDD enzyme activity was amplified.

3.7 DNA Sequence Variation of G6PD A-202 M1 and NG-009015.2

In this study nonsynonymous polymorphism of variant of G6PD A-202: NG-009015.2 of M1 1years old male. Mutation was observed in the query sequence with reference to the subject. There was a deletion of c16416T, c16417A, c16429A, c16446T, c16447G, c16516C, c16515C, c16520C, c16549A, c16555G and substitutions at position c16430G>T, c16431A>T, c16437A>T, c16441C>T,c16452A>C, c16469A>T, c16475G>T, c16489T>C, insertion of G was also observed between position c16421 and c16422 and that of A at position c16447 and 16448.this leads to the change in amino acids that will be translated in position.c16417I>K, c16420Y>Q, c16423P>A, c16426T>P, c16429I>P, c16432W>K, c16530W>V, c16533L>A, c16536F>V, c16539 R>P, c16542D>G c16545G>W, c16548L>P, c16557E>P . The only synonymous polymorphism is c16554P, c16551L, c16408K, c16410K, c16413K.

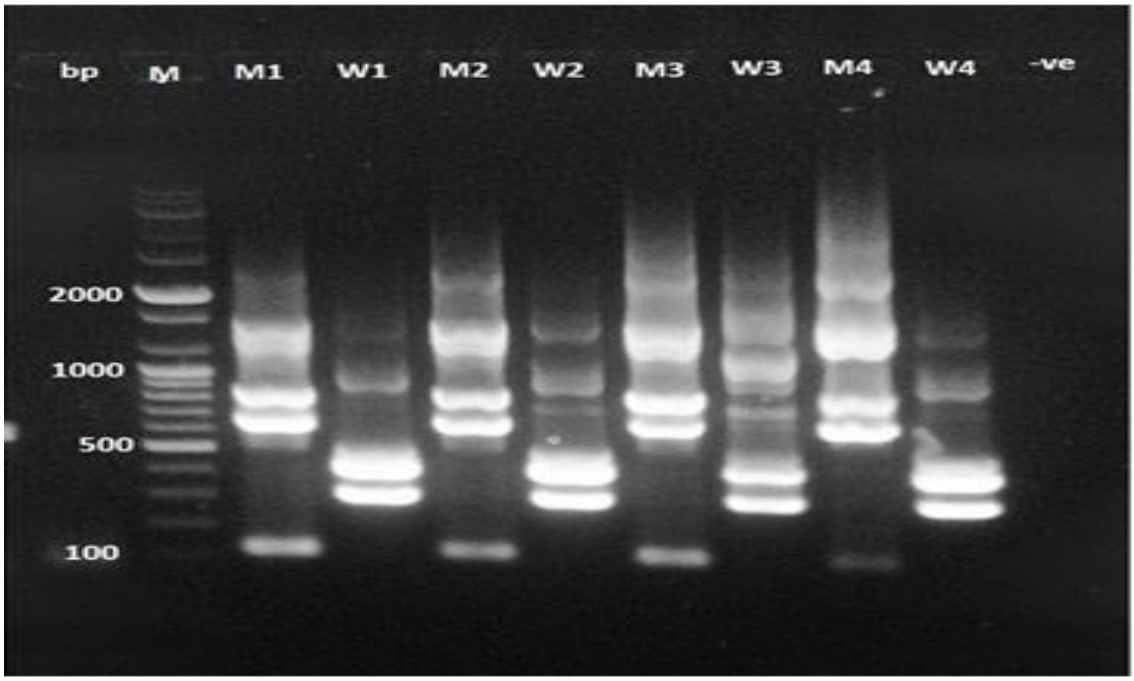

Plate I. Amplified PCR Products of G6PD 202 and 376 genes

Where;
M1 =MUT TYPE202/600bp
M2=MUT TYPE 376/800bp
W1=WILD TYPE202/300bp
W2=WILD TYPE 376/422bp
M = molecular ladder
Bp = base pair
-v = negative control

3.8 M1 MUT TYPE A and B 202

ACTTTCGGCAGAGGCCATCCCGGAACAGCCACCTGAGGGAGGGACAGTGTAACCAGTGCGGCA
GAGTAGGACCAGGCCTGGCCCTGACGGGAGGTCACAGGGGAAAGTGGGGATACATTTACCTTTG
GTGGGGCTTGCTTCTTCTTAATTGAATTTCCCTGTTTTCTCCTGAAACGGGGAATGTATAAATGCTG
TGACCAAGCTACATACATCATCCTCCACCCTGAATCTCCTGGTCTTACTCTGATTACTACTGGGCC
AACATGGTTTGGTTGCCTGATATACCTAAAAACCCCATCATTCAGCTGAGGATGACCTCCATCCCT
GACCCACAAAACCTATTCTAATCTCCTGCCTGCTCCCTCTAAACCTGAAAAACCTGTTCAAAAAAC
ATTACCCCACCATCTGCCATGTGGCCCCTCCACTGCCCCTGACACCTCCCGCTTACATTAGGAAG
AAATGGCTGAAAAACTGGAATAATGTTCCCTTTCCTAAGGGGAAAAACCGGATATGCTTCTGCCCA
AAACACCTTCCTCATGTTTATGACCGTTCCCCCTCAAGA.

CAGAAGGCCATCCCGGAACAGCCACCTGAGGGCAGGGACAGCTGTAACCAGTGCGGGAGAGTA
GGACCAGGCCTGTCCCTGGCGGGAGGTCACAGGGGCAGGGGGGACACACTTACCAGATGGTG
GGGTATTTCTTCTTCTTGGCCAAATTTCCCTGTTTTATCCTGAAAAGGGGGATGTAAAATGCTGCTG
ACACACCTACATACATCATCCTCCACCCTTGATCTCCTGGTCCTACTCCTGATCACTACTGGGCCA
CAACATGGTTTGGTTGCCTGATATACCTAAAAACCCCATCATTCAGCTGAGGAAGACCTCCGTCCC
TGACCCACAAACCTATTCTAATCTCCTGCCTTCCCCCTCTAAACCTGAAAATTCTGTTCAAAAAACC
ATCTACCCCCCCAATGCCATGTGCCCTTCACTGCCCCTGACCTCTCCCGCTTACATTAGGAAAAAA
CATGGCTCCCCAATATGGATTATATTCCCTTTCCTAAGGAGAAAAAGGGCCATATCTTCTGCCCACA
AAACGCCTTCTATGTTTATGACGTTCCCCCTCAAA.

3.9 M4 MUT TYPE 376

GGTAGGAGGCTGCATCATCGTACTGGCCAGCCACATAGGAGTTGCGGGAAAAAGTCCTCCTGTT
GACTTCTCCTCTGGGGTGGCCTGGGAGACACGGACAGACAGACACACGACGATGTCAGCCCCT
CTTTTGAGTCCGTGGTGTTCTGCCCCAGGGGAGTGGAGGGTCTTCCCTGGAGGCCGGGAGGGG
CCCTAAATCAGTGTCCCCCCCACACTGGGTTCGCCCCATCTTACAGTCTGCACATCCAGAGGGAG
GGAGGCCAAAGCAGCAGCACAACACTGCCCCAGGTGGGACCCCTGTGCCTTAGACAGAGGCCA
ATTTCAGATATTTTACCTGGGAAAATACACTGGAAAATCTCTCTCCAAAATATGACACCCAACTATGA
TTGGGGGAGAAAACCCGAACACTGGAGGGGACCTGTGGGTCCTGGTCACGGGGTGGTAATGGG
GGTCTCAAGGAATACCACACAGGGGGGGGGGCTGGAAAGAGAACTCCCCGATGGGATGGGGGA
AGCCCCCCAACTGCCTTGGGGGCTTGTGAGATGGAAAACCGGGTGGGGGGAGGCCTCCCCCC
CCCTGAAAGGTTCCTCTCTTGGGAATCCCCACTGGAGGTGAGGCATACCCCAATGAAGTTGGTTT
TCGGTAAAGGTTCCGACACAAGCCCTGTAGAGGGCGGGCATGATCTACCCATGGGGGGGGAAGA
GGA

Fig. 3. Sequence of G6PD M1: MUT 202 Male aged1 and M4: MUT 376 Female aged 3

DNA Sequence Variation of G6PD B-202 M1 and NG-009015.2

The other G6PD A-202 variant of M2 female exhibit a synonymous polymorphism despite the deletion of certain nucleotides at position c16201G, c16232C, c16234A, c16247G, c16249G, c16252G, c16254C, c16271C, c16281G, c16309A, c16346G, c16359G, c16362G, c1365G, c16373T, c16446T, c16449T, c16494C, c16515C. And substitution at position c16155A>G, c161556A>T, c1171A>T, c16172G>C, c16173A>T, c16174G>C, c16175G>C, c16176A>C, c16181G>C, c16191C>A, c16192G>T, c16195A>T, c16198C>T, c16204C>A, c16216A>G, c16222C>A, c16227C>A, c16228T>A c16240G>C, c16241A>T, c16242G>A, c16256G>T, c16257A>T, c16272T>G, c16275C>T, c16301G>T, c16302A>C, c16311G>T, c16316T>C, c16317A>T, c16318G>C, c16320G>A, c16354G>T, c16355G>C, c16367T>A, c16370G>T, C16371T>A, c16373G>T, c16379C>A, c16383G>T, c16384G>C, c16385A>C, c16386G>T, c16388C>T, c16389G>T, c16390G>T ,c16397A>T, c16398C>T, c16400G>A, c16417A>T, c16489C>T, c16491G>A .and one insertion of G between position c16275 c16276.

Despite the level of substation deletion and insertion the only mutation that lead to change in amino translation was observed in position c16399N>D and c16417K>I.all the other amino acid translated in the subject sequence was synonymous to that of the query sequence which are;

c16496G,c16402L,c16405A,c16408K,c16411K,c16414K,c16420Y,c16423P,c16426T,c16429I,c16432W,C16530W,c16533W,c16536F,c16539F,c16545G,c16542D,c16548L,c16551L.

DNA Sequence Variation between G6PD A-376 M4 and NG-009015.2

A nonsynonymous polymorphism of variant of G6PD A-376: NG-009015.2of M4 4years old female mutation was observed in the query sequence with reference to subject. There was deletion of some certain nucleotides like c16670G, c16680A, c16692C, c16693G, c16695A,

c16698G, c16702G, c16705G, c16728G, c16734A, c16736G, c16738G, c16740G, c16743G, c16752G, c16753G, c16754G, c16755G, c16756C, c16757G, c16758G, c16759G, c16760G, c16761C, c16762G, c16766G, c16772G, c16774G, c16797C, c16798G, c16837C, c16739A,

c16841G, c16842A, c16844G, c16876C, c16891C, c16892G, c16909G, c16918G, c16926G, c16934G, c16945G, c16965C, c16987G, c17021C, c17025G, c17037A, c17049C, c17050C, c17051G, c17052T, c17053G, c17054C, c17055C, c17056C, c17057C, c17065G, c17068G, c17070C, c17076T, c17084A, c17087T, c17137T, c17140C, c17156A, c17160A,c17206G, c17216A, c17223G, c17227C, c17256A, and substitution at position c16661G>T, c16662C>T, c16665G>A, c16672A>C, c16675T>G, c16683T>C, c16710T>C, c16711G>C, c16714G>A, c16729G>C, c16746G>A, c16768G>C, c16829A>T, c16854C>G, c17211T>A, c16750A>T. This scenario leads to the change in amino acid that will be translated in position c17207S>L, c17210K>T, c17213G>K, c17216G>E, c17219L>D, c17222F, c17231A>R, c17234T>N, c17237P>S, c17240M>Y, c17243W>V, c17246L>A, c17249A>G, c17252S>Q, c17255T>Y, c17258M>D, c17261M>D, c17264Q>A, c17267P>A, c17270S>P, c17273T>Y.
The only synonymous polymorphism is c17189A, c17192T, c17195P, c17198E, c17201E, c17204K, c17225F,

4. DISCUSSION

In the study, of the 207 children tested though the number of positive sample is small compared to other studies, but nonetheless, the overall prevalence of 33 (15.94%) was recorded. In a related study, [15] also showed a lower prevalence (14.4%) of *Plasmodium falciparum* among children in Sokoto, North-West Nigeria. In contrast, the work [16] other works also revealed high prevalence (20%) of *Plasmodium falciparum* among children in Jos, North Central Nigeria [22].

The study also revealed high prevalence of *Plasmodium falciparum* among 5 years' age group compared to others, this could be due to the fact that the immune system of children can be compromised due to various factors which can make them more susceptible to infections and disease. Some of these factors are nutritional deficiency, congenital immune deficiency, environmental factors and medications. The study shows that male had high prevalence of *Plasmodium falciparum* (66.67%, 22 out of 33) out of 207 compared to females (33.33%, 11 out of 33). this may be as a result of the fact that more males were sampled at the course of sample collection than females. This finding is in agreement with the work [12] where males (59.57%) had high prevalence than female (40.43%) in Katsina State, Nigeria. Contrary, the work of Shenkutie [23] showed that *Plasmodium falciparum* is prevalent among females (4.8%) compared to the male (2.8%). Higher prevalence (16.98%) of *Plasmodium falciparum* was observed in Barau Dikko teaching hospital compared to the other hospitals. This could be due to several factors like demographic of the population and geographic location.

This study also revealed that children that were positive for *Plasmodium falciparum* had lower G6PD enzyme activity and falls within the range of 0-4.5 U/g HB, with 33(70.21%) others falling within the range of 4.5-13.5 U/g HB 9(19.15%) and > 13.5 U/g 5(10.64%). this shows why most of the positive samples has lower G6PD enzyme activity because of the highest prevalence obtained within the range of 0-4.5 U/g HB with 33(70.21%) in this study.

This study identified the prevalence of G6PD deficiency among children with *Plasmodium falciparum* in Kaduna North. Malaria-endemic countries pose a significant challenge to the prevalence of malaria in glucose-6-phosphate dehydrogenase deficiency individuals. The mechanism of *Plasmodium falciparum* malaria resistance in people with G6PD deficiency is thought to be linked to impaired antioxidant defense, resulting in membrane damage in the early stages of parasitised red blood cells. This triggers a rapid removal of the parasites by phagocytosis, preventing their further development [24]. The geographical malaria distribution corresponds closely to the deficient distribution of G6PD variants [25].

Worldwide, a high frequency of infection occurs in malaria-endemic areas. As a result, it has been claimed that G6PD deficiency may offer a natural defense against malaria infection [26]. However, some studies have reported varying rates of G6PD deficiency in some parts of Nigeria. For instance, 14.4% of cases have been reported in Sokoto, North-West Nigeria. Reports also have also made known a 20% prevalence in Jos, North Central, of Nigeria [15,16].

The findings in this study correlate with the previous prevalence of G6PD deficiency studies, which show a wide geographical distribution, ranging from very rare among indigenous populations of Northern Europe to over 20% in portions of Southern Europe and Asia, and up to

40% in some areas of Southeast Asia, Africa, and the Middle East [27]. In recent years, a case-control study involving over 2,000 African children conducted by [28] demonstrated a notable decrease in the risk of contracting malaria among individuals with the African form of G6PD deficiency, ranging from 46% to 58%. The research proposed that the evolutionary benefit of malaria resistance was counter balanced by the drawbacks associated with insufficient levels of G6PD. This helped to keep the number of malaria cases stable in areas where it is common [28].

In this study, G6PD deficiency of Male M1 MUT TYPE 202 A, which has about 562 base pair's showed many forms of non-synonymous polymorphism this led to numerous changes in the amino acid translated and M1 MUT TYPE 202 B which also has about 559 base pair has more synonymous polymorphisms and fewer amino acid changes, M4 MUT TYPE 376 has non-synonymous polymorphisms and fewer amino acid changes it contains about 706 base pair. This observation corresponds with earlier reports emphasizing the importance of the patient's sex, with males being at greater risk of severe G6PD deficiency than females [29].

5. CONCLUSION

The study revealed a significant occurrence (15.94%) of G6PD deficiency among children with *Plasmodium falciparum* malaria who reside in Kaduna North, L.G.A. The findings consistently point to a high prevalence of G6PD deficiency in this population.

Among the children studied, *plasmodium falciparum* was notably more prevalent in males 22(66.67%) compared to females 11(33.33%). Additionally, a significant majority of G6PD deficiency cases (92.92%) occurred during early childhood, specifically between the ages of 1 and 5 years, with age 5 having a higher prevalence of about (33.33%). which falls within the range of 0-4.5 U/g HB with 33(70.21%). This could be influenced by the mutation of the G6PD Gene, leading to reduced enzyme activity.

In this study, several mutations on G6PD M1 MUT TYPE A, B 202 and G6PD M4 MUT TYPE 376 were observed in different positions due to deletion insertion and substitution, which led to changes in amino acid and lack of the G6PD enzyme due to the change of the dimer interface of the enzyme.

Though the number of sample size is small it is recommended to increase the number of samples. The study has established the occurrence of G6PD Deficiency among *Plasmodium falciparum-infected* children in Kaduna North L.G.A with a higher prevalence in males (66.67%) than females (33 .33%) and higher among age group 5 years compared to others.

The study has revealed several mutations deleterious of mostly G Nucleotides and in some polymorphic incidences A, C, and T.

A notable change in the amino acids translation shows mostly why there were changes in the homodimer of the active G6PD enzyme due to the mutation, and it could lead to disrupting the mutation and it could lead to disrupting the protein folding by altering the binding of structural NADP molecule.

6. RECOMMENDATIONS

1. It is crucial to Understand G6P Dehydrogenase it function and deficiency by studying the enzyme function including the role of G6PD in the pentose phosphate pathway and its importance in protecting cells from oxidative stress. With how G6PD Deficiency Explore the genetic basis and clinical manifestations of G6PD deficiency, including its impact on red blood cells and its association with hemolytic anemia.
2. The present study shows that enzymatic activity alone may not accurately predict clinical outcomes and genotypic information must be considered.
3. Additional research is required to give a better understanding of the severity of the G6PD Deficiency by Utilizing advanced technologies by the use of bioinformatics tools to analyze genetic data and predict the effects of mutations on G6PD gene and it functions. Also, to employ cell and animal models to study the physiological effects of G6PD deficiency and test new treatments that will help in managing the symptoms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office of this journal.

ETHICAL APPROVAL

Ethical approval was sought from Kaduna State Ministry of Health and Barau Dikko Teaching Hospital, Kaduna State University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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