

Molecular Detection of Glucose-6-Phosphate Dehydrogenase Deficiency in Katsina State, Northern Nigeria

ABSTRACT

Aims: To determine the prevalence of glucose-6-phosphate dehydrogenase deficiency and its variant (G6PD A⁻) among children diagnosed with *Plasmodium falciparum* malaria in Katsina state, Nigeria.

Study design: Cross-Sectional Studies.

Place and duration of study: General Hospitals Katsina, Dutsin-ma, Daura, Baure, Malumfashi and Funtua of Katsina state, Nigeria from June, 2020 to December, 2020.

Methodology: A total of 200 blood samples were collected from the study subjects after getting the ethical approval and informed consent. Their socio-demographic information and clinical presentations were also noted with the aid of questionnaire. G6PD deficiency was detected using G6PD qualitative test. Molecular characterization of African A⁻ Variants was carried out using PCR and Sanger sequencing. Phylogenetic studies were carried out to analyze the relationship between the types of mutations found in Nigeria and other countries.

Results: The G6PD qualitative test shows that 35(17.5%) samples were G6PD deficient which indicates significant association ($P < 0.05$) between G6PD and malaria. The PCR and sequence analysis of the 35 G6PD deficient samples shows the presence of G202A mutations in only 7(20.0%) samples. However, the BLAST analysis of the nucleotide sequences has shown 98.73% - 100% homology with other sequences of G6PD from the NCBI database. The bioinformatics analysis revealed G6PD mutations which indicate a Guanine to Adenine mutations at amino acid number 68 substitution of valine to methionine.

Conclusion: This study has shown a high prevalence of G6PD deficiency among children diagnosed with *Plasmodium falciparum* malaria in Katsina State, North-western Nigeria. Polymerase Chain Reaction, NCBI blast, Phylogenetic and Bioinformatics analysis of the deficient samples shows that G202A mutation in relation to the deficient children was not statistically significant ($p > 0.05$), hence does not appear to have a role in G6PD deficiency among children in the selected area of Katsina state, Nigeria though our findings were limited by the small sample size.

Keywords: BLAST, G6PD, G202A mutation, Children, *Plasmodium falciparum*, Polymerase Chain Reaction, and NCBI

1. INTRODUCTION

“Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme found in the cytoplasm of all cells catalyzing the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH” [1]. “NADPH enables cells to neutralize oxidative stress that can be activated by several oxidant agents, and to preserve the reduced form of glutathione” [2]. “Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defence against oxidative damage is dependent on G6PD” [3, 4].

“Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency has been shown to protect against malaria infection which affect 241 million people worldwide with an estimate of 627 000 malaria deaths in 2020 of which 95% in the African region and 80% were children under 5 years of age” [5]. “Malaria is the 3rd leading cause of death for children under five years worldwide, after pneumonisa and diarrheal disease” [6].

"The geographical distribution of malaria is remarkably similar to the world distribution of deficient G6PD variants" [7]. "It is postulated that the high frequency of G6PD deficiency has arisen because G6PD deficient variants confer some protection or resistance against malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*" [8]. Deficient individuals and heterozygous female carriers of deficient alleles have been shown to have a significant selective advantage against severe malaria.

"Malaria is a febrile sickness rooted by sporozoa of the genus *Plasmodium*, four species of which infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The malaria parasites undergo a transformational cycle in the female anopheles mosquito, which is the vector. They are transferred to the human host following a bite by the mosquito, quickly enter the liver where they undergo a transformational phase of different duration among the four species (pre-erythrocytic phase), and then enter the red blood cell (intra-erythrocytic phase) where they continue their replication cycle. The asexual erythrocytic parasite is the stage in the life cycle that causes disease. This is characterized by fever, chills and sweats (which vary in regularity among the various species), anaemia, expansion of the liver and spleen" [9].

"The mechanism of *Plasmodium falciparum* malaria resistance in deficient individuals is likely linked to an impaired antioxidant defence in deficient ring-stage parasitized red cell involving membrane damage, which activates a profuse removal of parasite by phagocytosis before it developed to trophozoite-stage parasitized red cell and to schizonts" [10].

Regardless of the clinical and epidemiological importance of the interplay between G6PD deficiency and malaria, the magnitude of its incidence and significance has not been accurately measured. Examining and identification of G6PD deficient individuals will help in curtailing such incidence, through proper selection of treatment, patient counseling, and avoidance from disease-precipitating drugs such as anti-malarial and other agents.

2. MATERIAL AND METHODS

2.1 Subjects

The research work was a cross-sectional research design, in which subjects aged ≤ 5 years that are not related admitted or presented with *Plasmodium falciparum* malaria to the selected hospitals from June, 2020 to December, 2020 were screened for G6PD deficiency. Only one subject was participated from each family. This study was conducted in a total of 200 subjects. Of these, 119 (59.0%) were males and 81 (40.50%) females. Moreover, 120 (60.00%) subjects come from the rural areas of the study population while 80 (40.00%) were from the urban areas. Majority of the children in study population were within the range of 0-12months 62 (31.00%) and decreases as the months increases i.e. 13-24, 25-36, 37-48 and 49-60months with the following numbers and percentages 49 (24.50%), 34 (17.00%), 30 (15.00%), and 25 (12.50%) respectively.

Venous blood samples (2.0mls) were withdrawn from each subjects of the study population by the laboratory technician and ethical guidelines was followed. The samples were collected in EDTA tubes and immediately transported in cold storage box to the Laboratory of General Hospital Dutsin-ma for G6PD screening. The positive deficient samples were kept in a solar refrigerator before sampling is completed after which they were taken to Centre for Biotechnology Research, Bayero University Kano for molecular analysis.

2.2 Qualitative Assays for G6PD

The activity of the G6PD enzyme was measured qualitatively using the G6PD diagnostic kit (Biorapid Diagnostics Nig. Ltd.) according to the manufacturer's instructions. G6PD, which is present in red blood cell hemolysate, works on glucose-6-phosphate and lowers NADP⁺ and, when combined with Premium Motor Spirit (PMS), reduces the blue-colored 2, 6-dichlorophenol indophenol to a colorless form, preserving the hemolysate's original cherry red color. The rate of decolorization is proportional to the activity of the enzyme.

2.3 Molecular Studies

DNA was extracted from G6PD-deficient blood using Nucleic Acid Extraction Kit II (Geneaid International) as stated in the manufacturer's guidelines. Purified DNA was solubilized in a TE buffer (10 mM Tris– HCL, 1 mM EDTA, pH 8.0) and stored at -20 °C. The PCR reaction was carried out for detection of African mutation using one pair of oligonucleotides as follows: Forward primer 5' TACAGTCGTGCCCTGCCCT 3' and reverse primer 5' CCGAAGCTGGCCATGCTGG 3'. The PCR reaction to amplify G202A was initiated by denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 61 °C for 30 seconds, and extension at 72 °C for 45 s and final extension at 72 °C for 5 min. Amplification was done using one unit of *Taq* DNA polymerase per μ L in a final volume of 23 μ L.

The DNA sequencing of the amplified PCR results of G202A gene were carried out by the Dideoxy/Chain Termination method at Inqaba Biotec West Africa LTD. This method was developed by Frederic Sanger and Alan Coulson in 1977. The automated cycle sequencing founded on Sanger's principle, CEQ™ 200XL DNA Analysis System by BECKMAN COULTER Company, was used to sequence the PCR results.

2.4 Statistical Analysis

The data generated from this research were analysed by descriptive statistics (mean, standard deviation, percentage), Pearson correlation by 2 tailed-tests of significance, and comparing means by paired samples t-test using IBM Statistical Package for Social Sciences (SPSS) 20.0 software. The statistical level of significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Prevalence of G6PD Deficiency

This research work is regarded to be as one of the few outlines which have been done in the study area, to access the occurrence of African variant of G6PD mutations. The pervasiveness of G6PD deficiency between the total subjects was 17.5% (35 out of 200). Males were 11.5% (23 out of 119) compared to 6.0% (12 out of 81) for females. Additionally, the variance in pervasiveness of G6PD deficiency among male and female were analytically not notable ($P > 0.05$). The number of G6PD deficient subjects based on age encountered in this study showed no remarkable dissimilarity ($P > 0.05$) between the age group. However, children within the age-group of 0-12 months have the highest deficiency, 14 (22.6%) while those between the age of 49-60 months shows the least deficiency 03 (12.0%). Moreover, the prevalence of G6PD deficiency did not differ between the sampling area, ($P > 0.05$). The prevalence was 34.30%, 34.30% and 31.40% for Katsina Central, Katsina North and Katsina South respectively.

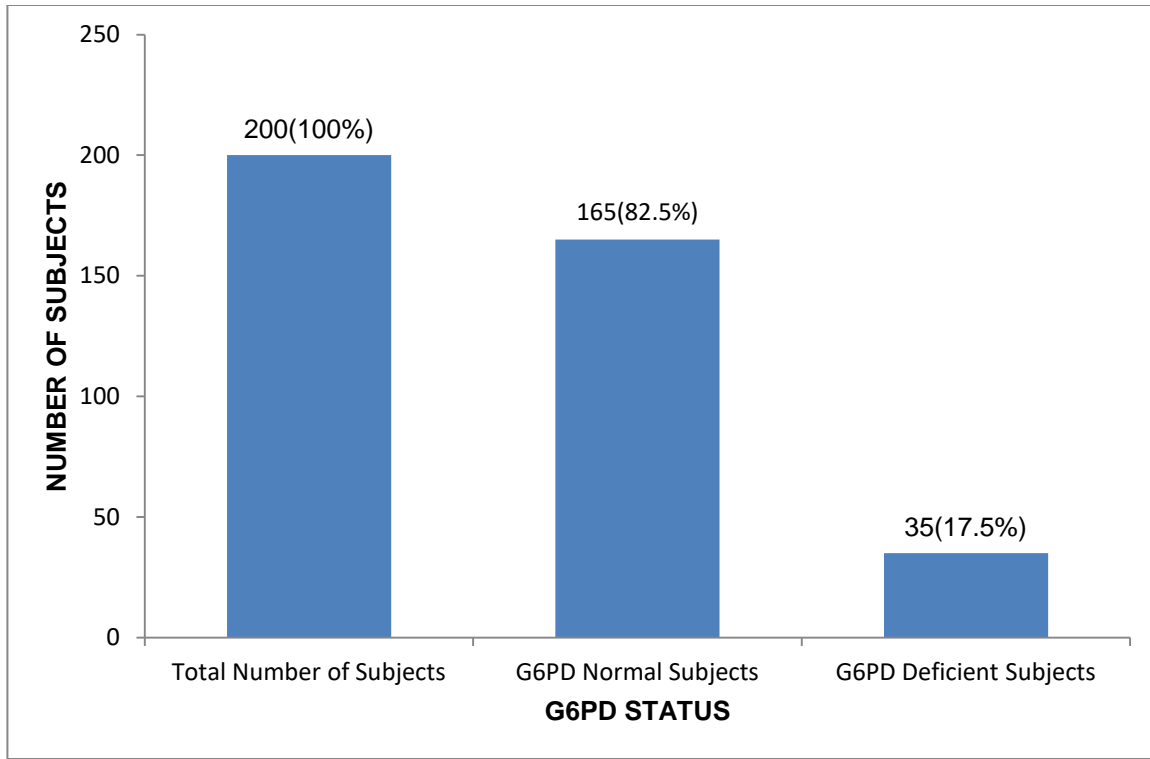


Figure 1. Prevalence of G6PD deficiency among children diagnosed with Plasmodium falciparum malaria

3.2 Prevalence of G6PD Mutations

The dispersal of G202A mutations in Katsina state was acquired from a total of 35 G6PD deficient samples. Polymerase chain reaction of the 35 deficient subjects indicates the existence of G202A mutation in only five samples, upon sequencing (forward and reverse) of the five deficient samples two samples failed quality control but the remaining three samples shows high percentage identities, small gaps and low e-values, hence the sequences are homologous. Nucleotide blast of sample 7 forward sequence shows 98.73% identity and the reverse sequence shows 100% identity while the nucleotides blast of sample 10 and 12 both forward and reverse sequences shows 100% identity.

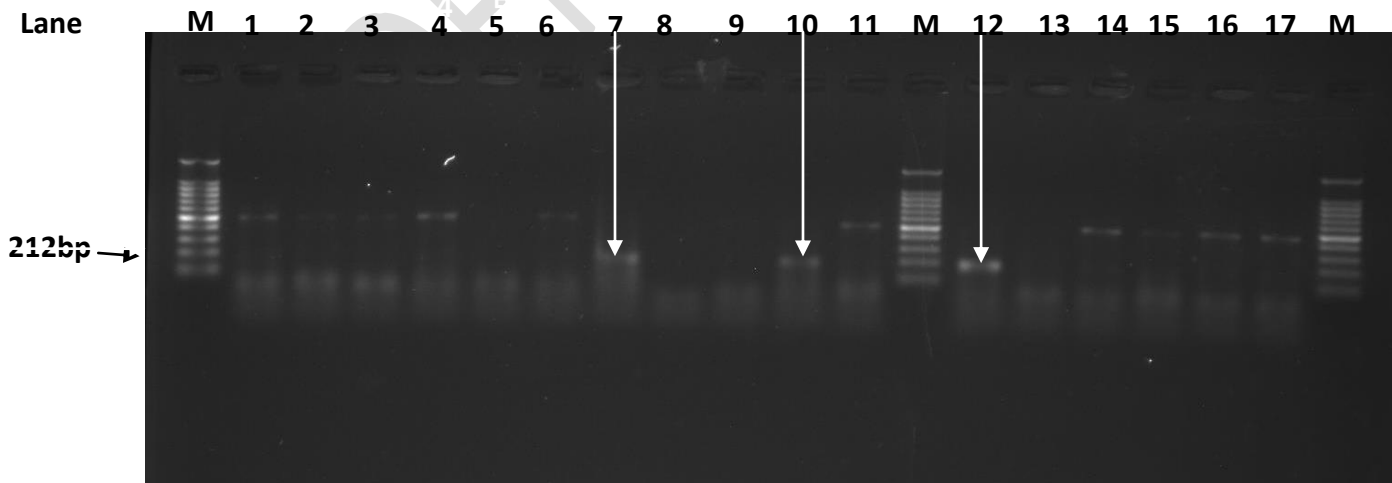


Fig. 2. Gel picture of the PCR products showing the 3 positive samples for African variant mutation (G202A) in lane 7, 10, 12; Lane M: Molecular marker and Lane 1, 2, 3, 4, 5, 6, 8, 9, 11, 13, 14, 15, 16, 17 shows negative samples for G202A mutation. The horizontal arrow shows the position of the base pairs in the molecular marker.

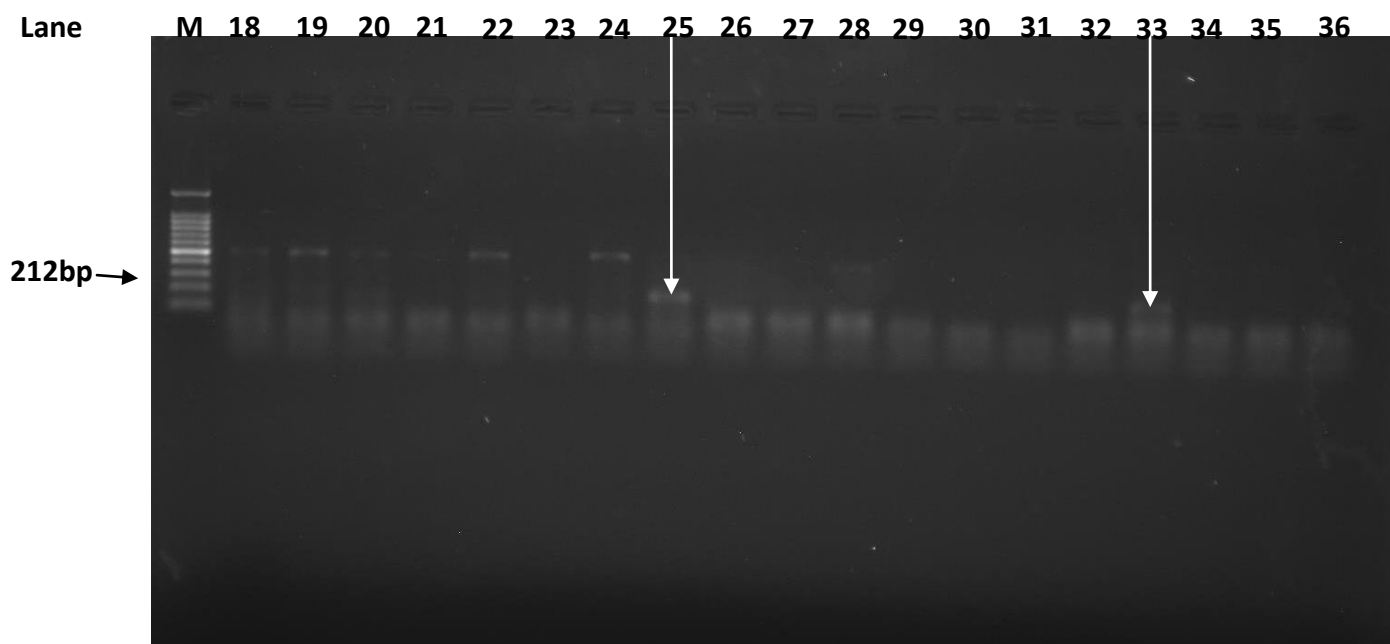


Fig. 3. Gel picture of the PCR products showing the 2 positive samples for African variant mutation (G202A) in lane 25, 33; Lane M: Molecular marker and Lane 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35 shows negative samples for G202A mutation and Lane 36: free nuclease water (Negative control). The horizontal arrow shows the position of the base pairs in the molecular marker.

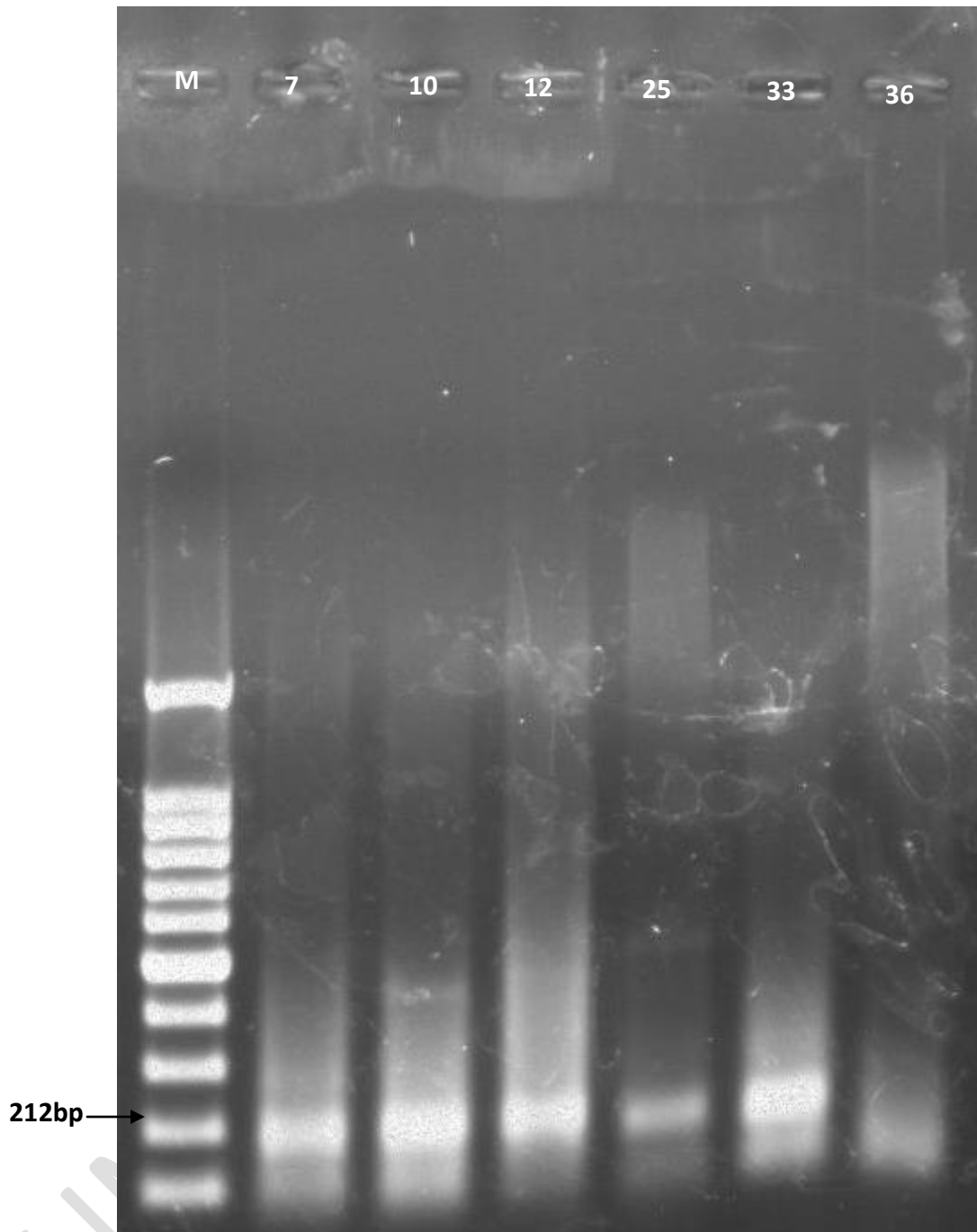


Fig. 4. Gel picture of the PCR products showing the 5 positive samples for African variant mutation (G202A) in lane 7, 10, 12, 25, 33; Lane M: Molecular marker and Lane 36: free nuclease water (Negative control). The horizontal arrow shows the position of the base pairs in the molecular marker.

In this research we noted a prevalence of 17.5% (35/200) among the 200 subjects diagnosed with *Plasmodium falciparum* which is statistically significant. In observational research, it was shown that the prevalence of G6PD deficiency is lined to malaria. Malaria is familiar as a parasitic disease that attacks 241 million people worldwide [11, 12]. The prevalence rate observed from this research is in uniformity with past reports in Nigeria and other parts of the world. "In one of the past reports in Nigeria, the prevalence rate of G6PD deficiency lies from 4% to 26%. Moreover, a work by [13] in Sokoto, Nigeria among 118 children visiting the Emergency Paediatric unit of Usman Danfodiyo University Teaching Hospital for paediatric similar care stipulated G6PD deficiency of 14.4%". "Also a work by [14] in

Oshogbo, Nigeria among 200 blood donors and 86 jaundiced neonates shows G6PD deficiencies of 19.5% and 47.7%, respectively”.

“This work also stipulates that a significant number of prevalence with G6PD deficiency was among the children within 1 year old (31.25%) followed by children within 2 years old (25.00%), those within 3 years old (18.75%) and those within 4 and 5 years old that shows the same prevalence (12.50%). This is statistically significant and is in uniformity with the study of [13] where the majority of subjects with G6PD deficiency in their work were in the following order, 2- to 3- and 4- to 5-year age-groups”. However, this is in contrast with the past report of [15] which is based on sex and G6PD deficiency interaction that sex does not have any alterant effect on G6PD deficiency. Also, another report [15] within children in Malaysia stipulated that sex was not a remarkable oracle linked with actual G6PD enzyme levels.

In this research, a greater prevalence rate of G6PD deficiency was also noticed in male children (62.5%) in contrast to female children (37.5%) and is statistically significant. G6PD deficiency is an X-linked recessive hereditary disease marked by abnormally low levels of G6PD. The deficiency is X-linked since the X chromosome carries the gene for G6PD enzyme; therefore this deficiency mostly affects males. G6PD deficiency is inherited from females who have one copy of the causative gene on one of their X chromosomes. Males who inherit the causative gene from the mother have G6PD deficiency, while females who receive the gene are carriers (carrier females generally do not show any characteristic symptoms). The deficiency is rare in females because the mutation would have to be present in both copies of the gene to trigger the disorder, while in males only one abnormal copy of the gene is required for epitome of the disease. This is in uniformity with past reports that shows that the sex of the patient is crucial and that males are at high risk based on complications than females [17, 18]. A work by [19] “among males resident of Jos, Nigeria indicated the prevalence rate of 20% G6PD deficiency. G6PD deficiency is essential as it is known that red blood cells that are deficient in G6PD are immune to *Plasmodium falciparum* storming since the parasite require the enzyme for its normal abidance in the host cell. This deficiency offers a careful safeguard against *Plasmodium falciparum* malaria” [20, 21].

“This research work shows that there was no interdependence statistically between G6PD deficiencies with either of the Senatorial zones i.e the sampling areas (Central, North and South senatorial zones). Therefore this shows that G6PD deficiency does not hang on the locality of the subjects within the state. Despite of the senatorial zone of origin a child may have the G6PD deficiency or not. The geographical distribution of G6PD deficiency proposes that some polymorphisms give out immunity to *Plasmodium falciparum* malaria” [22]. “This phenomenon has been studied mainly for the African variant (G6PD A-), showing that it also confers immunity against lethal falciparum malaria” [23]. “The higher prevalence of G6PD deficiency in malaria endemic countries is a sign that malaria infection has applied a giant selective pressure in many human populations” [20, 7]. “In *Plasmodium falciparum* infection it has been illustrated that shorter half-life and rapid clearance of red blood cells of G6PD deficient subjects make them less susceptible to malaria ambush from these parasites” [24].

“In UAE the frequency of G6PD deficiency in national populations is higher significantly (7.4%) unlike the non-nationals (3.8%) ($p < 0.001$) [5]. This reflects high occurrence of the deficiency in the region”. “Past research on G6PD deficiency between UAE national males living in Al-Ain have documented rates of 9.1 to 11% [25, 26] which is a little bit larger than the rate of 7.4% observed in the present research”. The occurrence of G6PD deficiency in the UAE is lower than that in Kuwait (19%), Bahrain (21%), and Oman (27%) [27, 28]. However, the variation of G6PD deficiency within various geographical locations in Oman lies between 8.7 and 29% [29] while the frequency of G6PD in the UAE population is much larger than that in the populations living in some other Mediterranean countries like Italy (1 to 2%) [30], Spain (1%) [31], Turkey (1.2%) [32]. “The occurrences of G6PD deficiency which have been documented all over the Eastern Mediterranean region lies between 3.6% in Jordan to 39.8% in Eastern Saudi Arabia” [33].

“One of the broadest review in India, shows that the large differences of G6PD deficiency has been observed ranging from 0% - 30.7% between the various caste, ethnic, and linguistic groups of India. The distribution in different areas reported rate of G6PD deficiency extending between 0% - 30.70% in Eastern India to 0% - 27.9% in Western India. The frequency of G6PD deficiency was reported extending from 0% - 23.21% in Northern India to 0% - 18% in Southern India. However, it was reported from 1.86% - 15.71% in North-eastern India and 0% - 19.23% and in Central India, the rate in the island regions of India was observed to be less” [34].

Molecularly, Polymerase chain reaction of the 35 deficient samples indicates the presence of G202A mutation in only five samples. Sequencing reaction of the five deficient samples, though two out of the five failed quality control, the remaining three samples shows high percentage identities, small gaps and low e-values, hence the sequences are homologous. There is no significant difference statistically in the presence of this mutation among the three samples. The nucleotide sequences obtained from the sequenced genes (both forward and reverse) are 99%, 100%, 100%,100%,100% and100% respectively.

4. CONCLUSIONS

G6PD deficiency is one of the most known X-link inherited hemolytic effects observed, affecting around 400 million people globally. The paramount important of G6PD is to safeguard the red blood cells from oxidative injury. The most important way for deterrent and minimization in the frequency rate of clinical symptoms of G6PD deficiency is to prevent oxidative agents such as infection, fava beans and oxidative drugs that instigate hemolysis, also examining of neonates for prompt detection of the deficiency.

These situations extent life-frightening storylines for all G6PD deficient individuals with different genetic variants. Hence, individuals that are required to use antimalarial drugs should be diagnosed appropriately for their likelihood to have the deficiency. For proper control and treatment, either an authentic test for screening G6PD deficiency or an anti-malarial drug that can be safely given to G6PD deficiency patients is needed.

This research has shown a high frequency 35(17.5%) of G6PD deficiency between children diagnosed with *Plasmodium falciparum* malaria residing in Katsina state in Northwestern Nigeria which shows significant linkage ($p<0.05$) between G6PD deficiency and Malaria. Also children within the age-group of 0-12 months have the highest deficiency, 14 (22.6%) while those between the age of 49-60 months shows the least deficiency 03 (12.0%).

Polymerase Chain Reaction, NCBI blast, Phylogenetic and Bioinformatics analysis of the deficient samples shows that G202A mutation does not materialize to have any impact on G6PD deficiency between children in the study area of Katsina state, Nigeria even though our observations were restricted by the small-scale sample magnitude.

Ethical Approval

The ethical approval for this study was obtained from Katsina State Ministry of Health Ethical Research Committee (MOH/ADM/SUB/1152/1/276) that grants ethical clearance for research that involves human subjects.

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Javier FB., Magda C., Sánchez LQ., Lilian C. Glucose-6-Phosphate Dehydrogenase (G6PD): Response of the human erythrocyte and other cell to the decrease in their activity. *Colombia Médica*. 2007; 38:1.
2. Filosa S., Fico A., Paglialunga F., Balestrieri M., Crooke A., Verde P. et al. Failure to increase glucose consumption through the pentose-phosphate pathway results in the death of glucose-6-phosphate dehydrogenase gene-deleted mouse embryonic stem cells subjected to oxidative stress. *Biochem J*, 2003;**370**(3):935–943.
3. Santana, MS, Arcanjo, ARL, Rocha, MAF, Sardinha, JFJ, Alecrim, WD. Association of methemoglobinemia and glucose-6-phosphate dehydrogenase deficiency in malaria patients treated with primaquine, *Revista da Sociedade Brasileira de Medicina Tropical*. 2007; 40:533–536.
4. Santana MS, de Lacerda MVG, Barbosa MdGV, Alecrim WD, Alecrim MdGC. Glucose-6-Phosphate Dehydrogenase Deficiency in an Endemic Area for Malaria in Manaus: A Cross-Sectional Survey in the Brazilian Amazon. *PLoS ONE*. 2009;4(4):e5259. doi:10.1371/journal.pone.0005259.
5. Saleh AA., Eiman A.Z., Shakir H., Amira MA., Huda SB., Abdul-Hamid AZ. et al. Molecular Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency in Abu Dhabi District, United Arab Emirates. *Trop J Pharm Res*. 2014;13(5):731-737.
6. World Health Organisation. 2021;World Malaria Report, 2021.
7. Louicharoen C., Patin E., Paul R., Nuchprayoon I. Witoonpanich B, Peerapittayamongkol C. et al. Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians, *Sci*. 2009; 326:1546-1549.
8. Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency; the genotype-phenotype association. *Blood Rev*. 2007; 21:267-283.
9. Cappellini MD. and Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet*. 2008;371:64-74.
10. Barisic M., Korac J., Pavlinac I., Krzelj V., Marusic E., Vulliamy T. et al. Characterization of G6PD deficiency in southern Croatia: description of a new variant, G6PD Split. *J Hum Gen*. 2005; **50**:547-549.
11. Haworth J., Wernsdorfer W., McGregor I. The global distribution of malaria and the present control effort. *Malaria: principles and practice of malariology*. 1988; 2(4):1379-1420.
12. Sutherland CJ., Tanomsing N., Nolder D. Two nonrecombining sympatric forms of the human malaria parasite *Plasmodium ovale* occur globally. *J Infect Dis*. 2010; 201(10):1544-1550.
13. Isaac IZ., Mainasara AS., Erhabor O., Omojuyigbe ST., Dallatu MK., Bilbis LS. Glucose-6-phosphate dehydrogenase deficiency among children attending the Emergency Paediatric Unit of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. *Int'l J Gen Med*. 2013;6:557–562.
14. Akanni EO., Oseni BSA., Agbona VO. Glucose-6-phosphate dehydrogenase deficiency in blood donors and jaundiced neonates in Oshogbo, Nigeria. *J Med Lab Diag*. 2010;1(1):1– 4.
15. Pinna A., Solinas G., Masia C., Zinellu A., Carru C., Carta A. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in nonarteritic anterior ischemic optic neuropathy in a Sardinian population, Italy, *Inves. Ophthalm. and Vis. Sci*. 2008;49(4):1328–1232.
16. Wang FL., Boo NY., Ainoon O., Wong MK. Comparison of detection of glucose-6-phosphate dehydrogenase deficiency using fluorescent spot test, enzyme assay and molecular method for prediction of severe neonatal hyperbilirubinaemia, *Sing. Med. J*. 2009;50(1):62–67.
17. Munyanganizi R., Cotton F., Vertongen F. Red blood cell disorders in Rwandese neonates: screening for sickle cell disease and glucose-6-phosphate dehydrogenase deficiency. *J. Med. Screen*. 2006;13(3):129–131.
18. Nader C., Mehran K., Amir HK., Mohammad HF., Behrang S., Mohammad R. The efficacy of a neonatal screening programme in decreasing the hospitalization rate of patients with G6PD deficiency in southern Iran. *J Med. Screen*. 2010;17(2):66-67.
19. Egesie D., Joseph DE., Isiguzoro I., Egesie UG. Glucose-6-phosphate dehydrogenase (G6PD) activity and deficiency in a population of Nigerian male residents in Jos. *Nig. J Physiol. Sci*. 2013;23(1–2):9–11.
20. Ruwende C., Khoo SC., Snow RW. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. *Nat*. 1995; 376:246-249.
21. Tishkoff SA., Varkonyi R., Cahinhinan N. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Sci*. 2001; 293:455-62
22. Howes RE. et al. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Med*. 2001;9
23. Guindo A., Fairhurst RM., Doumbo OK., Wellem TE., Diallo DA. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. *PLoS Med*. 2007;4:e66. (DOI: 10.1371/journal.pmed.0040066).

24. Cappadoro M., Giribaldi GO., Brien E. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by Plasmodium falciparum may explain malaria protection in G6PD deficiency. *Blood*. 1998;92:2527–34.
25. Bayoumi RA., Nur-E-Kamal M., Tadayyon M., Mohamed K., Mahboob B., Qureshi MM. Molecular characterization of erythrocyte glucose-6- phosphate dehydrogenase deficiency in Al-Ain district, United Arab Emirates. *Hum Hered*. 1996; 46:136–141.
26. Abdulrazzaq YM., Micallef R., Qureshi MM., Dawodu A., Ahmed I., Khidr A. et al. Diversity in expression of glucose-6- phosphate dehydrogenase deficiency in females. *Clin. Genet*. 1999;55(1):13-19.
27. Daar S., Vulliamy T., Kaeda J., Mason PJ., Luzzatto L. Molecular characterization of G6PD deficiency in Oman. *Hum Hered*. 1996;46(3): 172-176.
28. Shaker Y., Onsi A., Aziz R. The frequency of glucose-6- phosphate dehydrogenase deficiency in newborns and adults in Kuwait, *American Journal Human Genetics*, 1966;18:609–613.
29. White J., Ghristie B., Nam D., Daar S., Higgs D. Frequency and clinical significance of erythrocyte genetic abnormalities in Omanis. *J Med. Genet*. 1993;30:396–400.
30. Smith M. The incidence of G6PD deficiency in a population of Greek, Italian and Yugoslav origin in Australia, *Med. J*. 1979;25: 485-486.
31. Cladera SA., Oliva BE., Torrent QM., Bartolozzi CE. Prevalence of glucose -6- phosphate dehydrogenase deficiency in a student population on the island of menorca. *Sangre (Barc)*.1997; 42: 363-367.
32. Keskin N., Ozdes I., Keskin A., Acikbas I., Bagci H. Incidence and molecular analysis of G6PD deficiency in the province of Denizli, Turkey. *Med Sci Monit*. 2002;8: 453-456.
33. Beutler E. The molecular biology of G6PD variants and other red cell enzyme defects. *Ann. Rev. Med. J*. 1992;43: 47-59.
34. Shah II., Jarullah J. Jarullah B. Prevalence of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in India: A Systematic Review, *Advan. in Biosci. Biotech*. 2018;9: 481-496.
35. Black FL., Salzano FM. Layrisse Z. Restriction and persistence of polymorphisms of HLA and other genetic traits in the Parakana Indians of Brazil, *Ameri. J. of Physc. Anthropol*. 1980;52:119–132.