Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: July 31, 2024

Citation: Jelani I, Abdullahi HL., 2024. Molecular Characterization of Glucose-6-Phosphate Dehydrogenase deficient variants in Kano North-West Nigeria. Medical Research Archives, [online] 12(7). https://doi.org/10.18103/mra.v12i7.5312

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DOI: https://doi.org/10.18103/mra.v12i7.5312

ISSN: 2375-1924

RESEARCH ARTICLE

Molecular Characterization of Glucose-6-Phosphate Dehydrogenase deficient variants in Kano North-West Nigeria

Jelani, I*[1], Abdullahi HL[2], Alhassan AJ[3], Dalhatu MM[4], Dangambo MA[3], Abacha FZ[5], Haruna S[6], Mahmud RI[7], Yandutse MI[8], Hamza HD[8], Abdu R[9], Musa A. H[5]

[1]Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346, Sokoto, Nigeria
[2]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Bayero University, Kano, P.M.B. 3011, Kano, Nigeria
[3]Department of Biochemistry, Faculty of Basic Medical Sciences Bayero University, Kano, P.M.B. 3011, Kano, Nigeria
[4]Biochemistry Department, Aliko Dangote University of Science and Technology, Wudil, Kano P.M.B. 3244, Nigeria
[5]Department of Medical Laboratory Science, College of Medical Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria
[6]Department of Medical Laboratory Science, Faculty of Allied Health Science, College of Medicine, Ahmadu Bello University, Zaria, P.M.B. 1096, Samaru Zaria, Nigeria
[7]Kebbi State College of Health Sciences and Technology Jega, P.M.B. 9003, Kebbi, Nigeria
[8]Department of Chemical Pathology, Federal Teaching Hospital, Katsina, P.M.B. 2121, Katsina, Nigeria
[9]Ministry of Defence, Ship House, P.M.B. 196, Abuja, Nigeria

*jelani.ismaila@udusok.edu.ng

ABSTRACT

Studies from sub-Saharan Africa where malaria is endemic have observed high incidences of Glucose-6-phosphate dehydrogenase A$^-$ (G-6-PD A$^-$) deficiency that sporadically leads to manifest haemolytic anaemia. The severity of this disease depends on the genetic variant involved, thus, pinpointing the type of G-6-PD variants will help in the management of G-6-PD deficiency. This is not exactly known among Hausa and Fulani ethnics in Northern Nigeria, hence the need for the study. Two ml of venous blood samples were collected from twenty (20) G-6-PD deficient individuals and dispensed into EDTA bottle to study the coding region of G-6-PD gene by using PCR-Sequencing techniques. Sequencing of the G-6-PD-deficient samples revealed two major polymorphic variants; A376G substitution (exon-5: Asn126Asp) and A376G/G202A substitution (exon-4: Val68Met) commonly observed among Hausa and Fulani ethnics. This is the first study of G-6-PD mutations in Kano, North-west Nigeria. The G-6-PD deficiency in Hausa and Fulani ethnics were associated with African variants (202A/376G G6PD A$^-$ allele) in exon 4 and no mutation was found within exon 6-7.

Introduction

The Glucose-6-phosphate dehydrogenase (G-6-PD) gene is located near the telomeric region of the long arm of the X chromosome (band Xq28), close to the genes for coagulation Factor 8, color blindness and X-linked dyskeratosis congenita[1-2] .The G-6-PD gene consists of 13 exons and 12 introns with nearly 20 kb in length. The first exon of the G-6-PD gene contains no coding sequence and intron 2 is extraordinarily long, extending for 9,857 bp [3-4].

Since G-6-PD deficiency is an X-linked condition; males are either G-6-PD normal or G-6-PD deficient hemizygotes, whereas females are G-6-PD normal homozygotes, deficient homozygotes, or heterozygotes. X chromosome inactivation is frequently nonrandom; varying proportions of red blood cells may have either G-6-PD normal or deficient. As a result, female heterozygotes will have a continuum of G-6-PD activity results [5].

Mutations in the G-6-PD gene ultimately lead to G-6-PD deficiency causing structural defects in the enzyme, instability of the enzyme or altered activity, usually by decreased affinity of G-6-PD for its substrates, $NADP^+$ or glucose-6-phosphate (G6P) [6-7].

Genotypic and molecular identification and characterization of G-6-PD variants is patchy globally with varying frequency across different populations[8]. They are mostly point mutations and small deletions which is heterogeneous as indicated by different studies that 186 G-6-PD mutations has been reported worldwide among which, 159 (85.4%) are single nucleotide substitutions (missense variants), 15 (8.0%) are multiple mutations (two or more substitutions), 10 (5.3%) are deletions, and two (1.0%) are mutations affecting introns[9,10,11]. In Mexico, global occurrence of G-6-PD mutations had been mentioned to be 217[12].

In West Africa, Scriver[13] has documented the range around 10–20% or more and the Study by Clark *et al.*[14] documented G-6-PD A^- (202A, 376G) been the most common deficient variant. This finding has been replicated among Yoruba ethics of western Nigeria with the frequency of 21.3% by Tishkoff *et al.*[15], 1% of G-6-PD Santamaria (376G/542T) and 10% of G-6-PD Betica-Selma (376G/ 968C) was reported among the Sereer population of Senegal by De-Araujo *et al.*[16], while the (376G/ 680T) variant is rare as reported by Beutler *et al.*[17]. The 202A allele frequency is substantially lower in Senegal (Sereer) 1.0% frequency[16], 3.9% in Sierre Leone (Mende), 5.7% in Ghana (Fante), 10.5% in Ghana (mixed), 10.5% in Sierre Leone (Temne), 18.9% in Ghana (Ga) by Tishkoff *et al.*[15]. The Gambia (mixed) reported 5.9% by Ruwende *et al.*[18], in Mali, 15.3% of (Dogon and Malinke) and 15.7% of (Bamako) was reported by Guido *et al.*[19] and Duflo *et al.*[20] respectively, 22.5% in Congo (Brazzaville) by Bouanga *et al.*[21], 13.0% in Uganda by Davis *et al.*[22] and 9.0–15.5% in Gabon by Mombo *et al.*[23] and Migot-Nabias *et al.*[24]. These discrepancies raise the possibility that alternative G-6-PD deficiency alleles may be relatively common in parts of West Africa, and may sometimes outnumber the 202A/ 376G G-6-PD A^- allele. In this study, our focus is to check the pattern of mutation in G-6-PD gene among Hausa and Fulani ethnic's residents in Kano metropolis.

Materials and methods

Study location

This study was performed at the Veterinary Epidemiology Laboratory, Usmanu Danfodiyo University Sokoto.

Study Participants and Specimen Collection

This study included 20 participants (12 males and 8 females) with G-6-PD deficiency in the age of 18-35 years were randomly selected for the study. The study participants was adults Hausa and Fulani who were artisans residing in Kano metropolis.

Each tube was labeled with a unique ID number assigned to each individual. Two ml of peripheral blood sample was collected from G-6-PD deficient subjects by venipuncture. The blood specimen was dispensed into ethylene diamine tetra acetic acid (EDTA) bottle for molecular analysis.

G-6-PD Mutation Analysis

The DNA was extracted from Peripheral blood specimen with G-6-PD deficiency using HiYieldPlus™ Genomic DNA Mini Kit for blood (Real Biotech Corporation-RBC, Taiwan) according to the manufacturer's instructions. The purity and concentration of DNA was achieved using Thermo Scientific Nanodrop One Microvolume UV-Vis Spectrophotometer (USA). The DNA concentrations were determined by measuring the absorbance at 260 nm wavelength (A260) and 280 nm wavelengths (A280). Purity was determined by calculating the ratio of absorbance at 260 nm and the absorbance at 280 nm (A260/A280). Two primer sets which covers part of the most polymorphic sites of the complete G-6-PD sequences (3, 4, 5 and 6, 7 exons) were adopted from Dallol *et al.* (2012) as provided in Table 1.

Two regions (exonic regions 3-5 and 6, 7) of the G-6-PD gene were amplified from the G-6-PD deficient samples, using the polymerase chain reaction. DNA amplification was performed using 35 cycles of denaturation at 95°C for 30 sec, initial annealing starting at 60°C for 30 sec, and extension at 72°C for 30 sec. A final extension step at 72°C for 5 min was performed to allow the newly synthesized fragments to complete replication. PCR products were analyzed by electrophoresis on 1% agarose gel, stained with ethidium bromide and the gels were then viewed with a UV transilluminator at 302 nm, and a photographic record was obtained (gel documentation with an integrated camera from omniDOC- Cleaver Scientific). PCR products were purified (Wizard SV gel) and further subjected to cycle sequencing using a BigDye Terminator v1.1/ v3.1 cycle sequencing kit (Applied Biosystems) in an ABI PRISM 310 Automated Sequencer (Applied Biosystems).

Sequencing data collected were viewed with Fintch TV App in ABI PRISM format. The mutation identification was done using the FASTA format of sequencing data to identify substituted base(s) (G and/or A at position 202 and alleles A and/or G at position 376 of G-6-PD) by aligning query sequence with wild type sequence with the aid of Snap Gene Software. ExPASy translate tool was used to convert nucleotides sequence into corresponding amino acids. Amino acids sequence from deficient participant was aligned in CLUSTALW tool with reference sequence (NM_001042351.2) to find the substituted amino acid.

Table 1: Primer sequences, gene fragments amplified and PCR product sizes for G-6-PD

Exon(s)	Forward primersequence (5'→3')	Reverse primersequence (5'→3')	Amplicon size (bp)
3, 4, 5	TGTCCCCAGCCACTTCTAAC	CTCATAGAGTGGTGGGAGCA	1125 bp
6, 7	AGGGGTTCAAGGGGGTAAC	TGCAGGGTGACTGGCTCT	563 bp

Results

G-6-PD variants were assessed in 20 (12 males and 8 female) phenotypically G-6-PD deficient individuals for mutations in the coding region of G-6-PD gene. PCR product from exon 3-5 is shown in figure 1. Molecular analysis revealed two major polymorphic variants; G-6-PD variant A+ (376G), and African variant A- (202A/376G) among Hausa and Fulani ethnics was commonly observed (Figure 2 and Table 2).

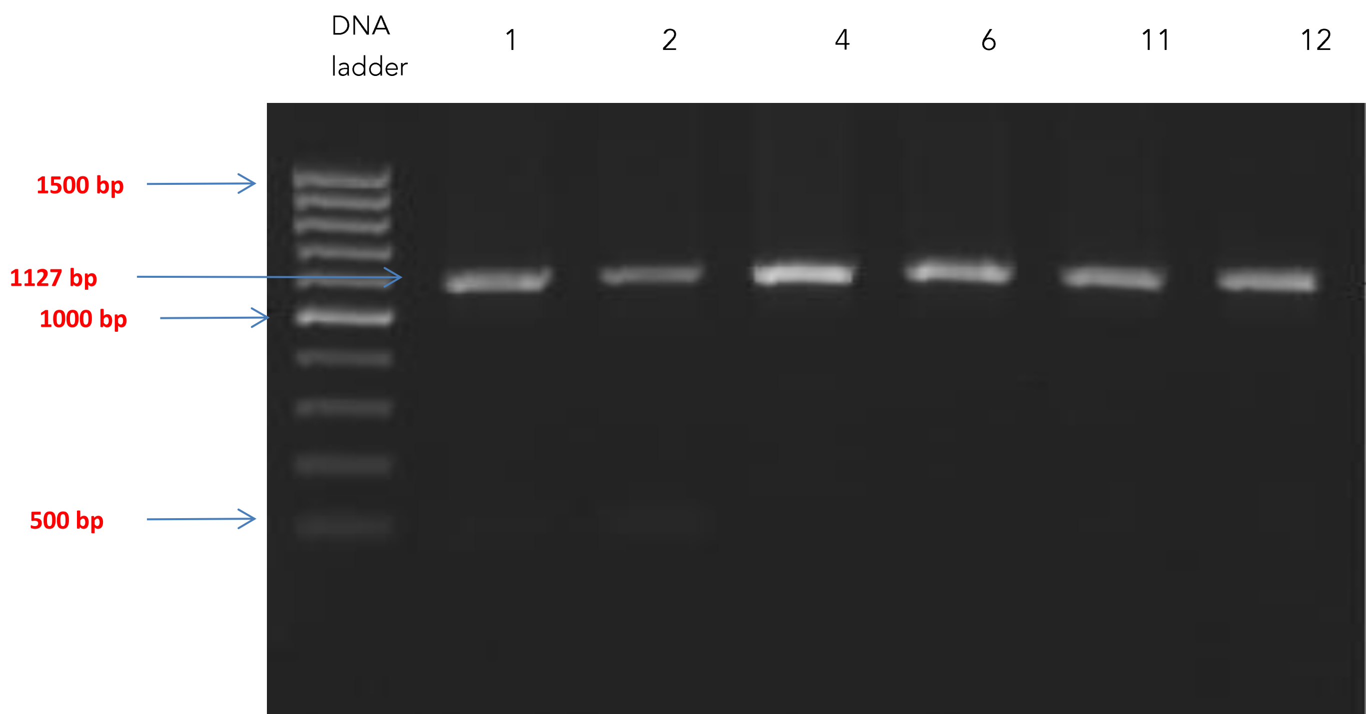

Figure 1: Gel electrophoresis showing PCR products of exons 3-5.

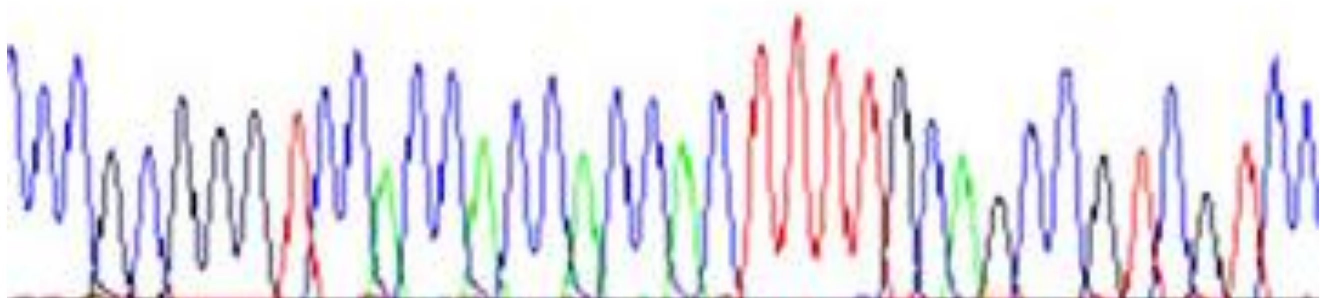

Figure 2: Electropherogram showing sequence analysis of PCR products of exon 4

Table 2 Glucose-6-phosphate dehydrogenase variants amongst deficient Hausa and Fulani ethnics in Kano

G-6-PD variants	Exon(s)	Base position	Base change	Amino acid position	Amino acid substitution
A+	5	376	A ➔ G	126	Asn ➔ Asp
A-	4	202	G ➔ A	68	Val ➔ Met
	5	376	A ➔ G	126	Asn ➔ Asp

A= adenine, G=Guanine, A ➔ G= Adenine changes to Guanine, G ➔ A= Guanine changes to Adenine, Asn=Asparagine, Asp= Aspartic acid, Val=Valine, Met= Methionine

Discussion

This work has confirmed the considerable genetic variants of G-6-PD deficiency in the Hausa and Fulani population. It serves as a baseline data for other molecular research in this area. The study revealed the G-6-PD variants of African type; (A$^-$) and A+ among Hausa and Fulani people living with the deficiency. This variants are polymorphic class of variants mostly found in three molecular variants; G-6-PD B, G-6-PD A$^+$ and G-6-PD A$^-$. G-6-PD B is known as the wild type while G-6-PD A$^+$ is a non-deficient type and G-6-PD A$^-$ is the deficient type. G-6-PD A$^+$ variant is related to a form of asymptomatic G-6-PD deficiency which involves the substitution of adenine by guanine (A/G) in the nucleotide (nt) 376 (exon 5) with a change at position 126 of asparagine to aspartic acid (Asn/Asp) amino acid residue[25]. The change at nt 376 alone gives rise to the non-deficient polymorphic variant G-6-PD A$^+$ but however demonstrated that the enzyme activity is not affected unless the nt 376 mutation is present [26].

The G-6-PD A$^-$ occurs from transitions of G to A occur in the nucleotide (nt) 202 (exon 4), and as a result the amino acid at position 68 should change from Val to Met and a G6PD A+ variant involves the substitution of adenine by guanine (A>G) transition in the nucleotide (nt) 376 (exon 5) with a change at position 126 of asparagine to aspartic acid (Asn>Asp) amino acid residue. is related to a form of asymptomatic G-6-PD deficiency[25]. The G-6-PD A$^-$ variant are expressed with different pattern in the gene as either single (e.g. G-6-PD Asahi, G202A) or double (**A**$^-$ G202A + A376G, **A**$^-$ A376G + G680T, **A**$^-$ A376G + T968C, Nefza and G6PD A$^-$) mutations.

These two variants are classified into Class III by World Health Organization. Class III variants cause moderate to mild enzyme deficiency (10 to 60% of normal activity) as repoted by LaRue *et al.*[27]. The G-6-PD A$^+$ (A376G) mutation observed in the study is a common variant, resulting in close to normal (~85%) enzyme activity of a non-deficient person, without significant clinical manifestations of G-6-PD-related haemolysis or appearing to confer resistance to malaria. This A$^+$ variant of G-6-PD has been reported in Ethiopia by Assefa *et al.*[28].

The African A$^-$ variant (G202A) is the more clinically important affecting enzyme functions in a similar fashion to those reported for Class I mutations[12]. They may be associated with different severity of clinical symptoms in the presence of antimalarial drugs such as primaquine, ranging from moderate to severe anemia that is most commonly associated with severe hyperbilirubinaemia. This agrees

with the report in a study by Ramírez-Nava *et al.*[12] and Faruok *et al.*[29]. Some of the participants who were G-6-PD deficient during the study complained that their post-treatment malaria condition were always worsen and more severe than pre-treatment states.

This variant is equally the most common G-6-PD variants worldwide. This was first described in Nigeria in 1967 and has continued up to 2002 by Gilles *et al.*[30] in 1967, Luzzatto and Allan[31] in 1968, Bienzle *et al.*[32] in 1972, Martin *et al.*[33] in 1979, Ademowo *et al.*[34] in 1995, May *et al.*[35] in 2000 and Ademowo and Falusi[36] in 2002. Other studies by May *et al. et al.*[35] and Ademowo and Falusi *et al.*[36] reported 24.2% in Hemizygous, 3.8% in Heterozygous female and 4.5% Homozygous female for its polymorphic frequencies. The findings in the current studies were similar to those observed previously in the same part of Nigeria by Gilles *et al. et al.*[30], Luzzatto and Allan[31], Bienzle *et al.*[32] Martini *et al.*[33], Ademowo *et al.*[34]. Similar results as the case with the current study were commonly reported worldwide, and in other studies in Burkina Faso by Meissner *et al.*[37] and Ouattara *et al.*[38], in Congo by Gampio-Gueye *et al.*[39], in Equatorial Guinea by Lin *et al.*[40], in Ethopia by Lo *et al.*[41], in Gambia, by Clark *et al.*[14], in Ghana, by Burchard *et al.*[42], in Mali, by Maiga *et al.*[43] among Fulani, in Mauritania, by Djigo *et al.*[44], in Namibia by Daniel *et al.*[45]. in Sengal by De-Araujo *et al.*[16], in Tanzania, by Enevold *et al.*[46] and Manjurano *et al.*[47] respectively, in America-Brazil- Mexico by Vizzi *et al.*[48], Pereira *et al.*[49], and Zamorano-Jimenez *et al.*[50], in India by Badens *et al.*[51], in Italy by Di-montemuros *et al.*[52], in Tunisia, by Benmansour *et al.*[53].

Although this study had limitation of small sample size and not able to sequence the whole genome or coding region in G-6-PD gene, but however it has confirmed two G-6-PD variants reported previously among Africans in Hausa and Fulani ethnics and entirely consistent with previous observations in Nigeria[15] (Yoruba ethnic) and Mali [43] (Fulani ethnic).

Conclusion

This is the first study of G-6-PD mutations in Kano, North-west Nigeria. Some of the female G-6-PD deficient participants complained of dizziness especially during their menstrual cycle and experienced a more severe post-malaria treatment condition. The G-6-PD deficiency in Hausa and Fulani ethnics were associated with African variants (202A/376G G6PD A$^-$ allele) in exon 4. There was no mutation found within exon 6-7.

Conflict of Interest:

None

Acknowledgement:

The authors thank all participants involved in the study for their cooperation. We also appreciate the kind gesture of Dr. Jibril A. H. from the Department of Veterinary Public Health and Preventive Medicine, Usmanu Danfodiyo University with regard to primer synthesis.

Funding:

This work was supported by Engr. Muhammad Bashir Jelani.

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