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PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN MALARIA INFECTED BLOOD SAMPLE OF CHILDREN AGED 1-5 YEARS IN YENAGOA, BAYELSA-STATE

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ABSTRACT
Glucose-6-Phosphate dehydrogenase (G6PD) deficiency is an X - linked inherited disorder which occurs when G6PD enzyme is absent or defective. And awareness of G6PD deficiency is pivotal in choice of certain pediatrics' medications that can induce hemolytic anemia. This study preliminarily investigated G6PD deficiency in malaria infected blood samples of children aged between 1to5 years; using molecular technique. Rapid diagnostic tests were carried out to investigate the presence of malaria infection. DNA extraction, PCR amplification and sequencing were then carried out to identify the presence of G6PD deficiency in the sample. From the result, a male child tested positive for G6PD deficiency (i.e 4% of total sample); while the others (females) were not deficient in G6PD. This suggests a low prevalence of G6PD deficiency amongst children aged 1 - 5 attending clinic in Yenagoa.

KEYWORDS: Glucose-6-Phosphate dehydrogenase, G6PD deficiency, prevalence, Age, sex.

INTRODUCTION

Glucose-6-phosphate dehydrogenase is an enzyme in the pentose phosphate pathway which is a metabolic pathway that supplies reducing energy to cells such as erythrocytes. G6PD catalyses the first step in the pathway and generates nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH generated is used by the enzyme gluthathione reductase to reduce the amount of reactive oxygen species. G6PD maintains the level of NADPH produced and in turn NADPH maintains the level of gluthathione in cells which helps protect the erythrocytes (Red blood cell) against oxidative damage. A defect or absence of Glucose-6-phosphate dehydrogenate is known as Glucose-6-phosphate dehydrogenate deficiency. G6PD deficiency is an inherited X-link disorder genetically transmitted by a sex-linked gene of intermediate dominace causing hemolytic anemia and neonatal jaundice (Beutler, 1983; Viroj, 2005).

The occurrence and discovering of mechanism of G6PD deficiency, linked to treatment of malaria in history spanned over 30 (Cordes, 1926; Carson et al, 1956; Otto Warburg and Christian, 1932; Gennaro Sansome and Giuseppe Segni, 1957).

Malaria is an endemic disease in Sub-Sahara Africa. It is a tropical disease caused by plasmodium parasite and it is transmitted by female Anopheles mosquito

It is widespread in tropical and subtropical regions of Asia, America and American continents (William et al, 2013).

There were 249 million cases of malaria in 2022 compared to 244 million cases in 2021. The estimated death stood at 608000 in 2022 compared to 610000 in 2021 with 80% being 5 years old or less (World Malaria Report, 2022).

Four African countries accounted for just over half of all malaria deaths worldwide; Nigeria (26.8%), the Democratic Republic of Congo (12.3%), Uganda (5.1%) and Mozambique (4.2%).

Nearly every minute a child under five died of malaria and many of these deaths are preventable and treatable (UNICEF, 2021). In 2019, WHO revised its approach to calculating the number of deaths attributed to malaria. With the revised WHO calculation, malaria accounts globally for 7.8 percent of the fraction of deaths in children aged less than 5 years (rather than 4.8 percent as reported previously).

Three (3) species of parasite associated with more severe malaria cases are Plasmodium falciparum, Plasmodium vivax, and Plasmodium knowelsi (Bello et al, 2016). Whereas Plasmodium ovale and Plasmodium malariae causes a milder form of malaria.

Different human populations that are geographically close might have diverse prevalent rates for G6PD deficient genes; even as G6PD deficiency reportedly affects 10% of the world (i.e. 200 - 400 million people globally (Cappellini and Fiorelli, 2008; Williams et al, 2013). An analysis in 2012 estimated an overall genetic frequency for G6PD deficiency to be about 8% across malaria endemic areas (Nkhoma et al, 2009; World Health Organization, 2020).

In Nigeria, prevalence of Glucose-6-phosphate dehydrogenase deficiency ranges from 4% to 26% with male having a frequency of 20-26% (Ademowo et al, 2002; Egesi et al, 2008). A study carried out in Katsina among children aged 0-5 reported an overall prevalence of 16.2% (Bello et al, 2016). Another study carried out in Oshogbo, Nigeria among 200 blood donors and 86 jaundiced neonates indicated G6PD deficiencies of 19.5% and 47.7% respectively (Akanni et al, 2010).

However in this study, the prevalence of Glucose-6-phospate deficiency in infected malaria blood sample was investigated for children aged 1-5 in Yenagoa, Bayelsa State of south southern Nigeria; with specific objectives of assessing the age and sex profile of the condition.

METHODS

This study was carried out in Nucleometrix Research Laboratory, Yenagoa, Bayelsa state.

Study Design: A cross sectional study design was adopted in this study.

Sampling Technique: Random sampling technique was used to select 25 samples, thus 25 blood samples were collected from Tobis Clinic, Yenagoa, Bayelsa.

Sample Size

Taro Yamane formula with 95% confidence level was used to determine the sample size of this research (Yamane, 1973).

Calculation of sample size using the Taro Yamane method

$$n = \frac{N}{1+N(e)2}$$

Where n = Sample size required
N = Population size
e = Allowable error which is between 0.01 – 0.05
Assuming N = 100 and e = 0.1, therefore

$$n = \frac{100}{1+100\,(0.1)\,2}$$

Therefore, n = 50

Sample Processing

Rapid Diagnostic Test

Rapid diagnostic tests (RDTs) for malaria are immunochromatographic assays which detect malaria antigens in the blood along a membrane containing specific antimalarial antibodies (Muth et al, 2007). RDTs were developed out of a need to establish the presence of malaria parasitaemia at the point of care even where there are no laboratories or skilled microscopist (Ezumma et al, 2011). Anticoagulated blood was evenly mixed by gently swirling and then sample dropper (5µl) dipped into the blood sample to draw 5µl of blood. This was loaded into the sample port, with addition of 4 drops of buffer solution into the buffer port in the test device. Then reading and interpreting of results was done after 20 minutes.

Molecular Identification

1. DNA extraction (Chemical Method)

The samples were lysed by adding four volumes of genomic lysis buffer to the samples (4:1). They were vortexed for 6 seconds, then they were let stand at room temperature for 5 minutes. The mixture of individual samples was transferred to a correspondingly labelled Zymo-spin II CR column in a collection tube, it was centrifuged at 10,000 rpm for 1 minutes and the flow through was discarded. The Zymo-spin II CR column was transferred to a new collection tube. DNA pre-wash buffer 200µl was added to the Zymo-spin II CR column and was centrifuged for one minute, g-DNA wash buffer 500µl was added to the Zymo-spin II CR column and centrifuged for one minute. The spin column was then transferred to a clean microcentrifuge tube, and 90µl DNA elution buffer was added, it was incubated at room temperature for two minutes and it was then centrifuged at 10000 rpm for 30 seconds with a view to eluting the DNA.

2. DNA quantification

The extracted genomic DNA was quantified using Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile nuclease free water and blanked using DNA elution buffer. Two microlitres of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

3. G6PD First round amplification

G6PD 1 was carried out using G6PD OF: 5'-CCCAGGCCACCCCAGAGGAGA -3' and G6PDOR : 5'- CGGCCCCGGACACGCTCATAG-3' on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 25 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 94ºC for 5 minutes; denaturation, 94ºC for 45seconds; annealing, 64°C for 30 seconds; extension, 72ºC for 45 seconds ; for 35 cycles and final extension, 72ºC for 1 minute. The PCR product was used to prepare the nested PCR.

4. Amplification G6PDIF genes

G6PDIF genes were amplified using the G6PDIF: 5-CCACCACTGCCCCTGTGA-3' and G6PDIR: 5'-GGCCCTGACACCACCCACCTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 94ºC for 5 minutes; denaturation, 94ºC for 45 seconds; annealing, 64ºC for 30 seconds; extension, 72ºC for 45seconds for 35 cycles and final extension, 72ºC for 1 second. The product was resolved on a 1% agarose gel at 200V for15 minutes and visualized on blue light imaging system for a 300bp product size.

RESULTS

Table 3.1 A total of 25 sample (infected malaria blood) was obtained from Tobis Clinic, Yenagoa, Bayelsa state within the age of 1- 5. 5(20%) samples were collected from age 1; 2(8%) male and 3(12%) female. 7(28%) samples from age 2; 4(16%) male and 3(12%) female. 3(12%) samples from age 3; 1(4%) male and 2(8%) female . 5 samples from age 4; 3(12%) male and 2(8%) female. Finally from age 5, 5(20%) sample was collected. 3(12%) male and 2(8%) female.

Table 3.1: Distribution of Malaria Infected Blood Sample.

Age {Years}	Male	Female	Total
1	2(8%)	3(12%)	5(20%)
2	4(16%)	3(12%)	7(28%0
3	1(4%)	2(8%)	3(12%0
4	3(12%)	2(8%)	5(20%)
5	3(12%)	2(8%)	5(20%)
Total	**13(52%)**	**12(48%)**	**25(100%)**

Table 3.2 Only 1(4%) male of the 25 sample collected was deficient in Glucose-6- phosphate dehydrogenase.

Table 3.2 Prevalence of Glucose-6- phosphate dehydrogenase deficiency in infected malaria blood sample.

Age {Yrs}	Male		Female		Total	
	NE	**NI**	**NE**	**NI**	**NE**	**NI**
1	2	0	3	0	5	0
2	4	1	3	0	7	1
3	1	0	2	0	3	0
4	3	0	2	0	5	0
5	3	0	2	0	5	0
Total	**13(52%)**	**1(4%)**	**12(48%)**	**0(0%)**	**25(100%)**	**1(4%)**

Where:
NE = Number Examined
NI = Number Infected

DISCUSSION

Table 3.1 represents the distribution of samples collected within the age of 1 – 5. From age 1, 5 samples accounting for 20% of the total sample were collected {2(8%) male and 3(12%) female}. In age 2, 7 samples were obtained accounting for 28% of the total sample {4(16%) male and 3(12%) female}. A total of 3 samples were collected from age 3 accounting for 12% of the total sample {1(4%) male and 2(8%) female}. In age 4, a total number of 5 samples were collected accounting for 20% of total sample {3(12%) male and 2(8%) female}. Finally from age 5, 5 samples were obtained accounting for 20% of total sample {3(12%) male and 2(8%) female}.

Table 3.2 represents the prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency in infected malaria blood sample. Among the 25 samples collected; there was a prevalence of 4% G6PD deficiency while 96% of the samples were normal which agrees with previous reports. In previous report in Nigeria, the prevalence of G6PD deficiency ranged from 4% - 20% (Ademowo et al, 2002). The occurrence of G6PD deficiency was seen in a male sample of age 2. No female sample was deficient in G6PD; they all had the enzyme intact. This further supports the history of G6PD as an X – linked disorder carried by genes on the X chromosome; and the fact that male hemizygotes and female homozygotes are most frequently affected (Amiwero C.E and Olatunji, 2012). From my findings there was no effect of age on G6PD deficiency and also no statistical relationship between G6PD deficiency and malaria.

A study carried out in Katsina, Nigeria among children aged o – 5 reported an overall prevalence of 16.2% (Bello et al, 2016). Another study carried out in Sokoto, Nigeria among 118 children visiting the pediatrics emergency unit of Usman Danfodio University Teaching Hospital for pediatric care indicated G6PD deficiency for 14.4% (Isaac *et al*, 2016).

It can then be said that there is a low prevalence of G6PD deficiency in children aged 1 – 5 in Yenagoa, Bayelsa State.

CONCLUSION

This study has shown a low prevalence of G6PD deficiency in children aged 1 – 5 in Yenagoa, Bayelsa State which may be as result of ethnicity and region. It could also be due to limited samples for the study. However Bayelsa state is a mosquito endemic area and as such there is a possibility of haemolysis crisis for patient deficient in G6PD enzyme that may take quinine containing antimalarial drug which can trigger the haemolysis. Therefore there is a need for the inclusion of G6PD screening as a routine test in the laboratory.

Limitations of the Study

As a preliminary study, there was limited access to diverse population and sample size; and no grant was accessed for funding.

Recommendation

This study has laid a foundation that G6PD deficiency is present among children in Bayelsa state which can be the basis for G6PD as a routine test in health facilities in Bayelsa state, therefore recommendations include;

* Further research on the prevalence of G6PD deficiency in Bayelsa state using larger population and sample size
* Government's provision of funds for molecular diagnostic tools in health care sectors to enable accurate detection of G6PD deficiency and wider range/scope of research.

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APPENDIX I
MATERIALS AND REAGENTS

- Specimen, Blood
- Wire loop
- Cotton wool
- Applicator stick
- Agarose gel
- Micropipette
- Eppendorf tubes
- Heating block
- Vertexing machine
- Centrifuge
- Incubator
- Autoclave
- Thermal cycler/Polymerase reaction (PCR) machine
- Transilluminator
- Nanodrop machine
- Icepack
- Electrophorectic machine
- Distilled water
- Conical flask
- Casting tray
- Microwave oven
- Primers
- Deoxynucleoside triophosphates (DNTPs)
- Magnessium chloride
- Buffer
- DNA template
- Taq Polymerase

APPENDIX II
AGAROSE GEL PREPARATION

In making 100ml of 1% agarose, the following was done
1g of agarose powder was dissolved in 100ml of Tris-boris EDTH (TBE) in a conicasl flask. The conical flask was placed in a microwave and allowed to heat for 8 minutes at a high temperature. The mixture was allowed to cool at 50^0c and 1µl of ethidium bromide was added and swirled for proper mixture. A casting tray was set and allowed to rest for 30 minutes.

PCR Cocktail
PCR Cocktail Preparation
The cocktail was prepared by adding the following
Master Mix = 15✕ 12 samples =180µl

Forward primer = 0.6✕ 12 samples = 7.2µl

Reverse primer = 0.6 ✕12 samples = 7.2µl

Water = 1.8✕ 12 samples = 141.6µl

Final volume = 30µl