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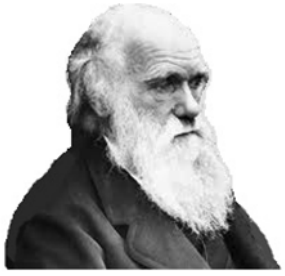

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G6PD Activity in Malaria Infected Children in Owerri

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ABSTRACT

G6PD activity in malaria infected children in Owerri was studied. Blood samples were collected from fifty one (51) children hospitalized in Federal Medical Center (FMC) and analyzed using standard Medical Laboratory methods. Results revealed that out of the 51 children examined; 26 were male and 25 were females. 39 representing 76.47% of the total malaria infected children numbers were G6PD deficient while 12 representing 23.53% had their G6PD normal. Two different plasmodium species and their percentage occurrences were observed as follows; *Plasmodium falciparum* (78.43%) and *Plasmodium malariae* (21.57%). Result further showed that male children were more ($P < 0.05$) deficient than females with percentage levels of 61.53% and 38.47% respectively. Children between the ages of 49-60 months was observed to be more ($P < 0.05$) G6PD deficient with percentage 25.64%, while those between 0-12 months were least G6PD deficient with percentage of 8.3% ($P < 0.05$). There is therefore urgent need for public enlightenment on public health implications, need for proper hygiene as well as strategies for preventing and controlling mosquito bites which as a result curses malaria which has a high risk of degenerating Glucose-6-phosphate dehydrogenase (G6PD) deficiency in children.

***Keyword*:** G6PD, Malaria, *Plasmodium falciparum, Plasmodium malarae,* Children

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathological disease in humans. This disease is described as a widespread, heritable X-chromosome linked abnormality [1]. It is estimated that it affects approximately 400 million people worldwide [2]. This disease is seen most frequently in approximately all of Africa, Asia and the countries near the Mediterranean Sea [3].

G6PD enzymes were demonstrated to play an active role in survival of erythrocytes. It is known that in the pentose phosphate pathway of erythrocytes, glucose-6-phosphate dehydrogenase, (G6PD) enzyme provides the production of NADPH and GSH. GSH, produced by pentose phosphate pathway can react with H_20_2 and reduce it to H_20. This prevents the generation of oxidative stress within red blood cells, oxidative stress can be induced in erythrocytes whose G6PD enzymes are deficient. In individuals whose G6PD enzymes is deficient, different kinds of haemolysis from mild to severe are seen bound to differences in variants of the disease [4].

In epidemiological studies, it was shown that the prevalence of G6PD deficiency significantl related to malaria. Malaria is known as a parasite disease that affects 300-500 million people all over the world. It is wide spread in tropical and subtropical regions of Asia, Africa and American continents [5].

2. MATERIALS AND METHODS

2. 1. Study Area

The work was done in Owerri Imo State, and south Eastern Nigeria. Owerri is located in Eastern heart-land with a population of 127,213 [6], people of mainly Igbo ethnic group and few other tribes which make up 62,990 males and 64,223 females. Owerri lies within latitudes 5°25’ and 5°29 N and 6°59 and 7°30 E. Owerri is a cosmopolitan town in Imo State, and is the capital of Imo State. Owerri is one of the major commercial cities of south Eastern Nigeria, characterized, by a wide distribution of hotels, fuel stations, institutions, banks, hospitals, markets and so on. The subjects therefore, represent a group of G6PD deficient children (0-5years) infected with malaria.

2. 2. Study Population

A total of fifty one (51) subjects were recruited for the study which includes; 26 males and 25 females who had cases of malaria infection. All the subjects were children within age of 0-5 years. The sample size was calculated using percentage prevalence.

2. 3. Advocacy and Mobilization and Pre-Survey Contacts

Before the work preceded all ethical clearance from the authorities were collected, as well as informed consent from the prospective study subjects. Dates were fixed for sample collection with my study subjects, which the venue was the hospital’s laboratory.

2. 4. Selection Criteria

Children, who presented with fever, had peripheral blood film examination for *Plasmodium falciparum*. Those who had malaria parasitaemia with no other obvious cause for the fever (such as respiratory infections) were also recruited.

2. 5. Exclusion Criteria

Patients who were later found not having any significant plasmodium species were excluded from the study. Those with underlying hepatic diseases were also excluded.

2. 6. Parameters for Study

The parameters screened for were:

a. Malaria parasite (MP) test using Normal Malaria Protocol diagnostic using leishmann and Giemsa staining technique.
b. Glucose-6-phosphate dehydrogenase activity using Randox G6PD quantitative *invitro* test.

2. 7. Study Design

The study was a cross-sectional research that involved G6PD activity in malaria-infected children. Samples were collected for the various test, and divided into modules as follows;

(A) Module 1: Testing for malaria parasite;

Capillary blood was tested for malaria parasite using Malaria Normal protocol, and only the samples that detected the presence of Plasmodium were used for G6PD analysis.

(B) Module 2: Venous washed red cells were estimated for G6PD activity.

2. 8. Laboratory Procedures

All reagents were commercially purchased and the manufacturer's Index Procedure was strictly adhered to.

Methodology for Malaria Parasite Using Normal Malaria Protocol to Check and Detect the Presence of Plasmodium Species in the Patient's Blood.
Principle

Malaria antigen from the lysed blood sample reacts with anti-malaria monoclonal antibody conjugated to colloidal gold complex migrates along the nitrocellulose membrane where it becomes bound by a line of specific monoclonal antibody producing a pink line in the test result area.

Procedures

Before the test, the finger of subjects was cleansed with a swab containing appropriate disinfectant. The finger was pricked with a lancet, and blood sample was collected with loops provided. Thick blood films were stained using Giemsa staining method while thin blood

films were stained using Leishmann staining method. Stained slides were examined under the light microscope using x100 objective lens (immersion oil) (Meeusen *et. al*., 2001). Thick blood films were used to determine the parasite densities while thin blood films were used to identify the parasite species and infective stages. Slides with malaria parasite <3 in a high power field were scored scanty; 3-10 as (+); 10-19 as (++); >20 as (+++) or more according to the degree of infection (parasitemia) [7].

Preparation of thick and thin blood films

Preparation of thick film was done using a clean grease free slides and a drop of blood; allowed to dry and stained using Giemsa stain. Preparation of thin film was done using a clean grease free slides and a drop of blood; lightly filmed on the slide and allowed to dry and stained using Leishman stain.

2. 9. Determination of G6PD Activity;

Using *Randox Invitro* Test Method [8].

Principle;

The enzyme activity is determined by measurement of the rate of absorbance change at 340nm due to the reduction of $NADP^+$.

$$\text{G-6-P} + NADP^+ \xrightarrow{\text{G6PDH}} \text{Gluconate-6-phosphate} + NADPH + H^+$$

Procedures

0.2 ml of EDTA anticoagulated blood from each subject was washed with 0.9% NaCl solution. Each was centrifuged after each wash for 10 minutes at 3000 rpm. The process was repeated 3 times for all the subjects. The washed-centrifuged red cells were suspended in 0.5 ml of solution 4 (Digitonin) and was left to stand for 15 min. at +4 °C and then centrifuged again. The supernatant gotten from the centrifuged mixture was used in the assay within 2 hours as follows

Table 1. Reagents and Volumes.

Reagents	Volume
G-6-PDH Buffer	3 mls (3000 µl)
G-6-PDH NADP	0.10 ml (100 µL)
Haemolysate	0.05 ml (50 µl)

It was mixed and incubated for 5min. at 37 °C, and a 0.05 ml of G-6-PDH substrate was added. It was further mixed and the absorbance was taken at 1, 2 and 3 minutes respectively using a spectrophotometer.

Calculation: The G6PD activity can be represented as follows;

ΔA X 30476 nm/min (mu/electhrolytes per ml/blood)

(Randox *invitro* Test Kit, 2015)

3. RESULT

3. 1. Overall G6PD deficiency in the study

In the study, a total of (51) fifty one malaria infected children (0-5years) blood samples were examined for G6PD deficiency. Out of the fifty one samples, 39 were deficient while 12 were normal, giving a percentage deficient to normal infants in the ratio of 76.47:23.53per cent. The result is shown in Figure 1

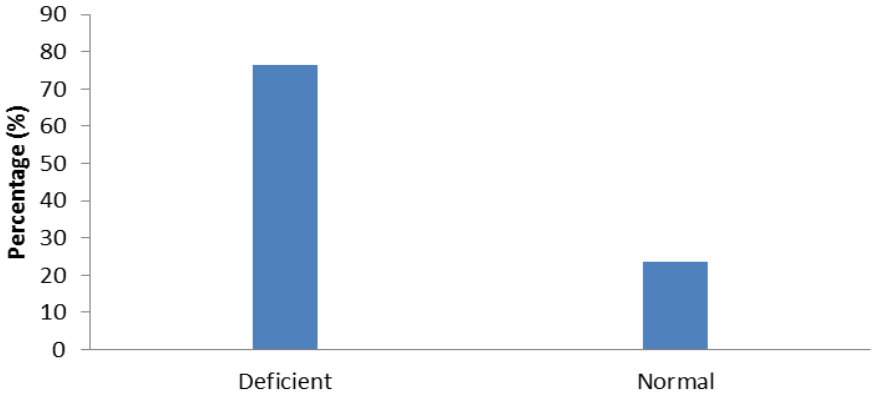

Fig. 1. Overall G6PD deficiency in the study

3. 2. Parasitological parameters

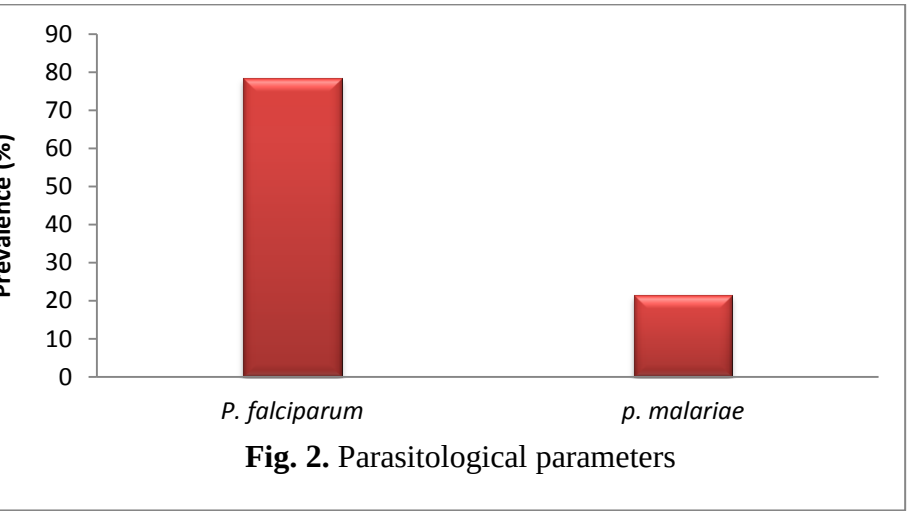

Fig. 2. Parasitological parameters

In the parasitology examination of malaria two species of plasmodium was isolated which include *Plasmodium falciparum* and *Plasmodium malariae*. *Plasmodium falciparum* was present in 40 (78.43%) and *P*. malariae in 11 (21.57%) children; species distribution is shown in Figure 2. Significant differences were found between P. falciparum and *P*. malariae.

3. 3. Overall sex-related prevalence of deficiency in the study area

Out of the fifty one children examined, twenty five were males while twenty six were females. Out of the twenty five males examined, twenty four (24) were G6PD deficient while out of the total of twenty six females examined; fifteen (15) were G6PD deficient. This gives a percentage prevalence of 61.53% and 38.47% of males and females, respectively. The result is shown in Figure 3

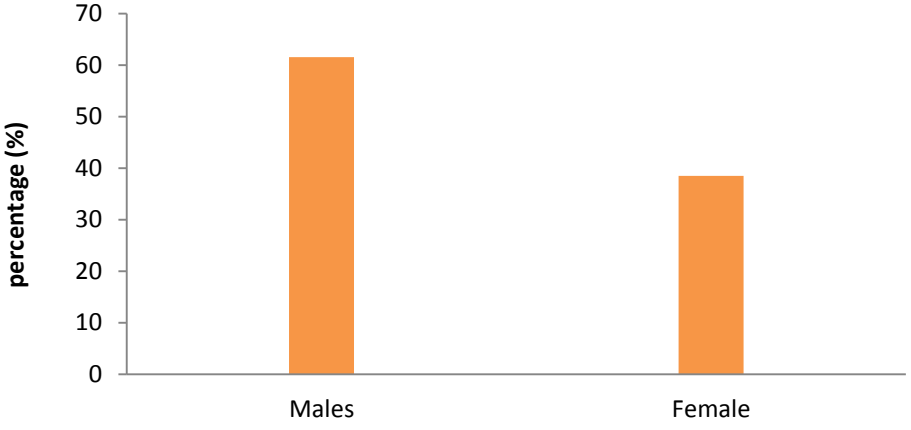

Fig. 3. Overall sex-related prevalence of deficiency in the study area

3. 4. Overall age-related prevalence of G6PD deficiency in the study area

In the study, malaria patient (children) between the ages of 0 to 60 months was examined for G6PD deficiency. Out of the 10 children in 0-12 months examined, 5 were G6PD deficient. Of the children between the ages of 13-24 months, a total of 10 were examined and 7 of them were G6PD deficient. Of the children between the ages of 25-36months, 10 were examined and 8 were G6PD deficient. Of the children between the ages of 37-48 months, 10 of them were examined and 9 were G6PD deficient and of the children between the ages of 49-60 months, 11 of them were examined and 10 were G6PD deficient. The children between the ages of 49-60 months had the highest percentage deficiency prevalence of 25.64% ($P < 0.05$), followed by children between the age 37-48months with 23.08% deficiency, followed by children between the ages of 25-36 months with 20.51% deficiency, followed by children between the age 13-24months with 17.95% deficiency and children between the ages of 0-12months that has the least prevalence of 8.3% ($P < 0.05$). The result is shown in Figure 4.

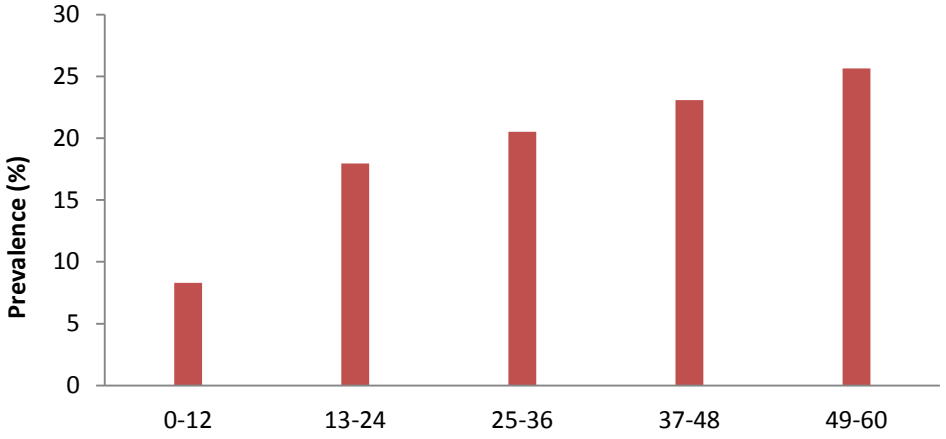

Fig. 4. Overall age-related prevalence of G6PD deficiency in the study area

3. 5. Age and sex-related prevalence of infection in the study area

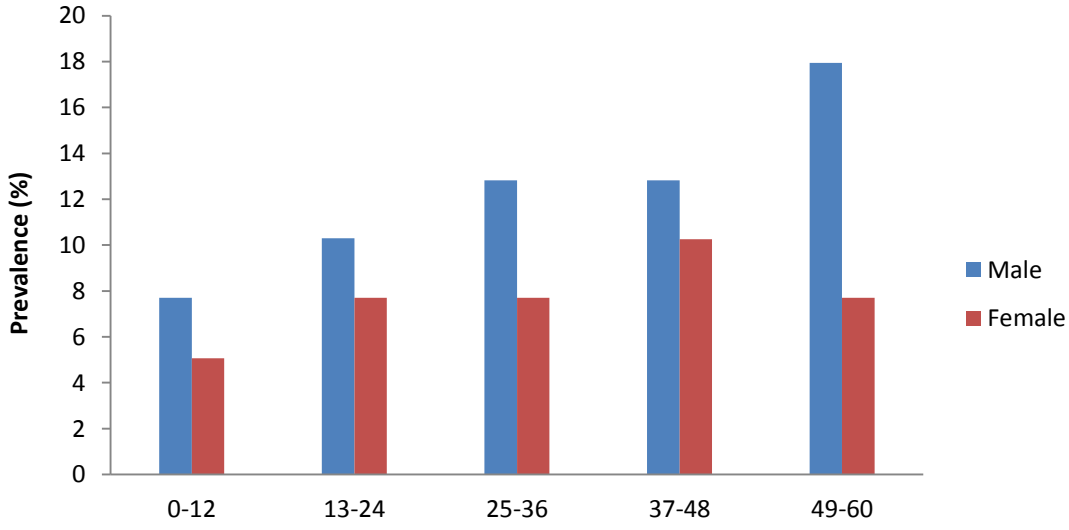

Fig. 5. Age and sex-related prevalence of infection in the study area

Of the children between the age of 0-12 months, 10 were examined out of which 5 of them were G6PD deficient. Out of this 3 were male and 2 were female giving a G6PD deficient of 7.70% and 5.07%, respectively. Children between the age of 13-24 months, 10 of them were examined and 7 G6PD deficient. Out of this 4 were male while 3 were female giving a G6PD deficiency of 10.30% and 7.70%, respectively. Of the children between the ages of 25-36 months, 8 of them were G6PD deficient out of 10 examined 5 of them were male while 3 were female giving a prevalence of 12.82% and 7.70%, respectively. For children between the age of 37-48months, out of the 10 examined, 9 were G6PD deficient, out

of this, 5 were males while 4 were females, which gives a G6PD deficiency percentage prevalence of 12.82% and 10.25%, respectively. For children between the age of 49-60months, 10 of them were G6PD deficient out of 11 examined; 7 were male while 3 were female which gives G6PD deficient percentage prevalence of 17.94% and 7.70%, respectively. The result is shown in Figure 5.

4. DISCUSSION

Nigeria being a country moving towards elimination of malaria has a high prevalence of G6PD deficiency ($P < 0.05$). The result of this study revealed that malaria fever still remains the major morbidity and mortality in children between the ages of 0-5years by which reduces the G6PD of the children which agrees with the study of Nimol *et al*. [9]; who also recorded G6PD deficiency in malaria children. Primaquine is an essential drug for malaria fever [10, 11]. Analysis for malaria parasite revealed the higher occurrence of *Plasmodium falciparum* (78.43%) and *Plasmodium malariae* (21.57%) is presence in the patients examined for G6PD deficiency. Observation from the overall sex related prevalence in Fig .3. suggests that male children have the higher occurrence of this G6PD deficiency more than females. Report from other developing regions like Enugu [12] also indicates that males are more affected than females. The results obtained from age related prevalence in Fig 3.4 suggests that children that's between 3-4 and 4-5years stand a greater risk of contracting malaria fever which can possibly lead to G6PD deficiency with percentage prevalence of 23.08% and 25.64% respectively ($P < 0.05$), this is in agreement with other researchers [13,14]. From the result in Fig. 5, there were more infections in older male and female infants than younger ones ($P < 0.05$). Moreover, male infants were also observed to be more G6PD deficient than the females ($P < 0.05$). This trend of results can also be attributed to the same reason for results in Fig. 3 and Fig 4. The study established that there is high prevalence of G6PD deficiency in children in Owerri. The high proportion of malaria-infected, children reinforces the importance of not only detecting G6PDd but also to measure the haemoglobin concentration before starting treatment.

5. CONCLUSION

G6PD activity in malaria infected children in Owerri studied. From the study, the following were observed that; there is high prevalence of G6PD deficiency in the study area. Genera of *Plasmodium* associated in the study area includes; *Plasmodium falciparum* and *plasmodium malariae*. Among the two Plasmodium, *Plasmodium falciparum* had the highest prevalence rate ($P < 0.05$). It was equally observed that the male children were more deficient in G6PD more than the females ($P < 0.05$). Children between 49-6months of age were infected more ($P < 0.05$).

Recommendation

There should be good sanitation practices in the area of the study. There should be adequate health education in our schools and homes. Children suspected to be expose to mosquito which is the consecutive agent of malaria fever should always use mosquito treated

net as recommended by the Federal Ministry of Health. Used water and water storage should be properly covered to avoid the inhabitation of mosquito Infants should be guided and should not be allowed to move about freely as this may expose them to mosquito either in the day or night. It is equally recommended that Nigerian Medical Association should embark on a massive public enlightenment programs through the mass media on the dangers of drug abuse.

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