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Research Article

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Prevalence of Glucose 6 phosphate dehydrogenase deficiency and its association with malaria infection among patients attending Federal medical Centre Owerri

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Abstract

The study was done to investigate the prevalence of G6PD deficiency among gender and the association of G6PD deficiency with malaria infection. 100 subjects were used in this study, 50 male patients and 50 female patients; 5mls of whole blood sample was obtained front patients by standard phlebotomy and was dispensed into HOT A container. Methaemoglobin method for G6PD deficiency screening was done using 2mls of blood, malaria screening was done using RDT malaria test strip following the manufacturers protocol. Statistical analysis was performed for social science (SPSS) windows version 20.0 of the 100 individuals tested a total of 16% were G6PD deficient while 84% had normal G6PD status, 12% were males and 4% were females with a prevalence of 24% among the males and 8% among the female population, of the 16 G6PD deficient individuals, 25% were malaria positive and 75% were malaria negative, this suggest the influence of G6PD deficiency on malaria infection. 24% of the normal individuals were malaria positive in contrast with 76% being malaria negative. In conclusion G6PD deficiency is more prevalent among the male subjects, and G6PD deficiency tends to have a protective effect against malaria.

Keywords: prevalence, glucose 6 phosphate dehydrogenase deficiency, malaria infection

Introduction

G6PD is a cytoplasmic enzyme that is found in all cells of the body. The active form of the enzyme is either a dimer or a tetramer consisting of identical subunits. The primary structure of a single subunit polypeptide chain consists of 515 amino acids (Luzzatto and Mehta, 2009). G6PD B is the enzyme found in most people worldwide and it is identical to that of the chimpanzee and gorilla (Luzzatto and Battistuzzi, 2005). In populations outside of tropical and semi tropical regions it is virtually the only G6PD isoenzyme, except for rare private mutations, as no variants have reached polymorphic frequencies (> .1%) in populations in these areas (Luzzatto and

Battistuzzi, 2005). Of all human loci, the G6PD locus is the most polymorphic. Over 300 variants have been fully described (Luzzatto, Battistuzzi, 1985; Beutler, 1990; Luzzatto and Mehta, 2009). In their comprehensive review, Luzzatto and Mehta indicate that 77 of these variants have reached polymorphic frequencies (> .1%). Eleven of these polymorphic alleles have normal activity and 66 of the variants have decreased activity and are called deficient variants (Luzzatto and Mehta, 2009). In a more recent overview Beutler (2002)states that over 400 variants have now been described, but does not provide an indication of how many of these variants have reached polymorphic frequencies. Enzyme variation has traditionally been classified by means of electrophoretic mobility determined by starch gel electrophoresis and as a function of enzyme variant activity compared to the activity of G6PD B. The standard for activity is the rate at which NADP+ is reduced by glucose-6-phosphate with G6PD B as the catalyst. On this basis, enzyme variants are classified as fast, normal, and slow in terms of electrophoretic mobility, and as Classes I—Y based on enzyme activity relative to G6PD B (Luzzatto and Mehta, 2009). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an incomplete hereditary X-linked hemolytic disease. This enzymopathy is widespread in the tropics and subtropics, synchronizing with endemic or formerly endemic malarial; which suggests that G6PD deficiency may have arisen, spread, or maintained in frequency through natural selection by malaria. Although it is hard to detect G6PD deficient patients as most affected people are asymptomatic until they are exposed to triggers, more than 400 million people are thought to be G6PD deficient2. G6PD catalyzes the reaction in the pentose phosphate pathway that generates reduced form of NADPH, which is in turn responsible for glutathione (GSH) homeostasis. GSH is an antioxidant, and together, these processes make cells more able to resist and control oxidative stress. Inability of the erythrocytes to maintain GSH homeostasis results in oxidative stress and affects the integrity of the RBCs, giving rise to haemolysis. Optimum RBC redox status is required by malaria parasites for their survival, replication, and development6. This factor is diminished in G6PD deficient RBCs, supporting the protection hypothesis. G6PD deficiency is transmitted X-chromosomally. Females can thus be homozygously deficient or heterozygously deficient, whereas males are hemizygously deficient. Heterozygously-deficient women have a mixed population of erythrocytes, owing to random inactivation of one of the two X chromosomes, known as lyonization. One of the erythrocyte populations is G6PD deficient; the other has normal G6PD function. The DNA sequence of 140 known mutations has been established (Beutler and Vulliamy 2002). Most mutations are point mutations and small deletions that cause structural defects in the enzyme. Large deletions or expression defects are not known in the gene or its promoter, except one. One mutation causes a frame shift. The lack of severe mutations indicates that total G6PD deficiency is lethal. In most cases, mutations cause instability of the enzyme or altered activity, usually by decreased affinity of G6PD for its substrates, NADP1 or glucose-6-phosphate (G6P) (Luzzatto, 2006).

Materials and Methods

Study area

The study area federal medical center Owerri Imo State, Southern Eastern Nigeria.

Sample size

The sample size was gotten using cross sectional (qualitative variable).

Study subjects

A total of 100 apparently healthy individuals comprised of 50 males and 50 females attending FMC were recruited for this study.

Study design

This was a cross sectional study designed to determine the prevalence of G6PD and its

association with malaria infection among the patients attending FMC Owerri. The patients were conveniently enrolled into the study. Quantitative test data was obtained through screening for G6PD and malaria status of the subject.

Study population

The chemical analysis was done in Federal Medical Centre Owerri. A total of hundred (100) subjects were recruited for the study in Federal Medical Centre Owerri municipal of Imo State. The test group comprised of known patients, while the control group comprised of apparently healthy non- subjects.

Selection criteria

All the recruited subjects were known patients that have not being under treatment for a long time and have given their consent of participation.

Inclusion criteria

In this study, I included all sufferers that weren't under treatment and those that have being on treatment but not for a long time.

Exclusion criteria

I excluded sufferers that has being on treatment for a longer period of time.

Advocacy and Pre-Survey Contacts

Ethical clearance was applied with letter of introduction from the head of Department Medical Laboratory Science Imo State University, Owerri, Imo State. And an ethical approval was obtained from the Medical ethics and research committee of Federal Medical Centre, Owerri.

Sampling techniques

Apparently healthy individuals were enrolled for the study.

Specimen collection and processing

five millimeters of whole blood was collected from each study participants and two millimeters of the blood emptied into S-Monovette,ethylene-diaminetetra-acetic acid(k2EDTA) anti coagulanted vacutainer tubes. The anticoagulated blood was stored in a refrigerator till required for use in the screening of subjects for G6PD deficiency (qualitative test). All samples collected were analyzed within 48 hours.

Procedure

Two mls(2mls) of anticoagulated (EDTA) blood was added to the tube containing 0.1ml of the freshly prepared sodium nitrate, Glucose reagent and 0.1ml methylene blue and mix gently by inverting the tube marked(test). The control tube was prepared by adding 2mls of blood without the sodium nitrate Glucose reagent and methylene blue marked (normal reference tube). 2mls of blood was also added to another tube containing 0.1ml of sodium nitrate-Glucose reagent without methylene blue and mix gently marked (deficient reference tube) [Cheesbrough,2010]. All the tubes were incubated at 37°C for 90 minutes and after incubation for 3hours. At 37 degrees, it was separated into 3 tubes and l0mls of distilled water was added to all the tubes and the colors were compared.

Protocol Table

TUBES	TEST	NORMAL	DEFICIENT
		REFERENCE	REFERENCE
Sodium nitrate	0.1ml		0.1ml
Glucose reagent	0.1ml		
Methylene bluereagent	0.1ml	-	-
Sample	2mls	2mls	2mls
Distilled water	10mls	10mls	10mls

Statistical analyses

The data collected were entered into the data editor of Statistical Package for Social Science (SPSS Version 22) software. Analysis was based on simple percentages or proportions. A chi-square test at a 95% confidence level was allowed to test for association of G6PD deficiency between male and female subjects. A p value of <0.05was considered significant in all statistical analysis.

Method

The first step of the test procedure involves mixing the patient's blood with a lysing agent in a test strip or well. This ruptures the red blood cells, releasing more parasite protein. Dye-labeled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labeled antibody, or antigen, is bound at the control line. Blood and buffer, which have been placed on strip or in the well, are mixed with labeled antibody and are drawn up the strip across the lines of bound antibody. If antigen is present, some labeled antibody-antigen complex will be trapped and accumulate on the test line. Excess-labeled antibody is trapped and accumulates on the control line. A visible control line indicates that labeled antibody has traversed the full length of the strip, past the test line, and that at least some free antibody remains conjugated to the dye and that some of the capturing properties of the antibodies remain intact. The intensity of the test band will vary with the amount of antigen present, at least at low parasite densities (antigen concentration), as this will determine the amount of dye particles which will accumulate on the line. The control band intensity may decrease at higher parasite densities, as much of the labeled antibody will have been captured by the test band before reaching the control.

Results

Prevalence and Gender Distribution of G6PD deficiency

Of the 100 individuals tested, a total of 16(16%) were G6PD deficient, while 84(84%) had normal G6PD status. Of the 16 G6PD deficient individuals, 12 were males (12%) and 4(4%) were females. The prevalence was 24% among the male population and 8% among the female population.

Prevalence of G6PD and association with malaria infection

Of the 16 G6PD deficient individuals, 25% were malaria positive and 75% were malaria negative. This suggests an influence of G6PD enzyme deficiency on malaria parasite infections. 24% of the normal individuals were malaria positive in contrast with 76% being malaria negative.

Prevalence of malaria and association with G6PD deficiency

Of the 100 individuals tested a total of 3% were malaria positive G6PD deficient males; 1% was malaria positive G6PD deficient females. 9% of the test subjects were G6PD deficient males that tested negative to malaria. 3% of the populations were G6PD deficient malaria negative females.

Table 4.1 Prevalence and Gender Distribution of G6PD deficiency

Gender	G6PD deficient (T)		Normal (C)	
	N	Freq (%)	N	Freq (%)
Male	12	24	38	76
Female	4	8	46	92
Total	16		84	

Prevalence and Gender Distribution of G6PD deficiency

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Table 4.2 Prevalence of G6PD and association with malaria infection

	G6PD deficient (T)	Normal (C)	Males	Females	Male T	Male C	Female T	Female C
MP +ve	4	20	9	4	3	30	1	34
MP -ve	12	64	41	46	9	8	3	12
TOTAL	16	84	50	50	12	38	4	46
Freq	16%	84%	50%	50%	12%	38%	4%	46%

Prevalence of G6PD and association with malaria infection

Of the 16 G6PD deficient individuals, 25% were malaria positive and 75% were malaria negative. This suggests an influence of G6PD enzyme deficiency on malaria parasite infections. 24% of the normal individuals were malaria positive in contrast with 76% being malaria negative.

VARIABLES		
	MP +ve	MP –ve
G6PD deficient (T)	4	12
Frequency	25%	75%
Normal (C)	20	64
Frequency	24%	76%

Prevalence of malaria and association with G6PD deficiency

Of the 100 individuals tested a total of 3% were malaria positive G6PD deficient males; 1% was malaria positive G6PD deficient females. 9% of the test subjects were G6PD deficient males that tested negative to malaria. *3%* of the populations were G6PD deficient malaria negative females.

Discussion

A significant association was observed between gender and G6PD status in patients with malaria with the incidence of G6PD deficiency being higher in males than in females. This is similar to the findings of other studies sand is likely due to G6PD deficiency being an X-linked hereditary disease; with the male tending to be at greater risk than female as the males has only one X-chromosome. Hemizygous males and homozygous normal females are most and least commonly affected.

Of the 100 individuals tested, a total of 16(16%) were G6PD deficient, while 84(84%) had normal G6PD status. Of the 16 G6PD deficient individuals, 12 were males (12%) and 4(4%) were females. The prevalence was 24% among the male population and 8% among the female population.

Studies showed that in plasmodium falciparium-infected red blood cells (RBCs), the flavoenzyme glutathione reductase (GR) regenerates reduced glutathione, which is essential for antioxidant defense. GR utilizes NADPH produced in the pentose phosphate shunt by glucose -6-phosphate dehydrogenase (G6PD). Hereditary G6PD deficiency is frequent in malaria endemic areas and provides protection against severe malaria. Of the 16 G6PD deficient individuals, 25% were malaria positive and 75% were malaria negative. This suggests an influence of G6PDenzyme deficiency on malaria parasite infections. 24% of the normal individuals were malaria positive in contrast with 76% being malaria negative.

Conclusion

This study showed a prevalence of G6PD deficiency in a malaria-endermic area. This study also supported the assertion that patients with G6PD deficient red cells had no protection against malaria infection. These findings will help to recognize and diagnose malaria patients with a G6PD deficiency, as well as to identify the risks and protective factors against malaria in endermic regions.

G6PD deficiency causes problems primarily when the deficiency is complicated by the treatment of malaria.

Treatment can cause (severe) hemolysis in G6PDdeficientpatients. Therefore, patients should be screened for G6PD deficiency before treatment with these potential hemolytic agents. The test used for this purpose should be inexpensive and easy to perform, but also should diagnose heterozygous women reliably. The fluorescent spottest, the spectrophotometric assay, and the cytochemical assay are all relatively inexpensive.

The fluorescent spot test is the least expensive, and the cytochemical assay is the most expensive. The fluorescent spot test is easiest to perform but is very unreliable for the detection of heterozygous women. The cytochemical assay detects heterozygous women reliably, but is cumbersome.

For the best of both worlds, the authors recommend the use of two different tests for the detection of G6PD deficiency in men and women: the inexpensive fluorescent spot test is appropriate for the detection ofG6PD deficiency in men, whereas a simplified cytochemical assay should be used for the detection ofG6PD deficiency in women to discriminate between healthy, heterozygously-deficient, and homozygously deficient women.

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