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The Significance of Glucose-6-Phosphate Dehydrogenase Deficiency in *Plasmodium falciparum* Malaria: A Case Study of Patients Visiting the Central Regional Hospital, Cape Coast, Ghana

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Abstract: Glucose-6-phosphate dehydrogenase (G-6-PD) is a cytoplasmic enzyme involved in the metabolism of glucose. It has been reported that G-6-PD deficiency confers protection against *falciparum* malaria. Contrary to this, other researchers have reported that the presence of G-6-PD-deficiencieny does not necessarily prevent individuals from developing malaria. *Plasmodium falciparum* malaria, sickle cell anaemia/ trait and G-6-PD status were assessed in a total of 142 patients (70 had malaria and 72 were non-malaria participants) who visited the Central Regional Hospital in Cape Coast. The prevalence of *Plasmodium falciparum* infection was 98.6% in G-6-PD non deficient individuals, 1.4% in moderately deficient patients and 0% in G-6-PD severely deficient individuals. The prevalence value of malaria infection for the deficient patients (severe and moderate) was significantly ($p = 0.005$) lower than that for non deficient individuals. Based on the data obtained, it can be concluded that G-6-PD deficiency may play a role in the prevention of malaria and its development into a severe form.

Key words: Malaria · G-6-PD deficiency · *Plasmodium falciparum*

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G-6-PD) enzyme is one of the many cytoplasmic enzymes involved in cellular metabolism. It catalyzes the oxidative dehydrogenation of glucose-6-phosphate to 6-phosphogluconate during the Pentose Phosphate Pathway (PPP) [1]. The oxidized form of the coenzyme nicotinamide adenine dinucleotide phosphate ($NADP^+$) is converted to reduced nicotinamide adenine dinucleotide phosphate (NADPH) during the PPP. Implicitly, the PPP plays an essential role in the maintenance of cellular redox status. The PPP occurs mainly in the liver, adipose tissue, lactating mammary glands, red blood cells (RBCs), adrenal cortex and other endocrine glands concerned with steroids hormone synthesis [2]. Through a series of reactions, the PPP converts glucose-6-phosphate (G-6-P) to ribulose-5-phospate, a precursor of many important molecules like nucleic acids, adenosine triphosphate (ATP), coenzyme A (CoA), nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD) [3]. The two main reaction steps involved in the production of NADPH are the conversion of glucose -6- phosphate to 6-phosphogluconolactone and the conversion of 6-phosphogluconate to ribulose-5-phosphate in the oxidative stage of the PPP.

The reaction steps in PPP which are involved in NADPH production are significant to RBCs in that it is the only metabolic pathway that supplies NADPH in RBCs which is important in mopping free radicals [3]. The NADPH produced in RBCs is used to convert oxidized glutathionine (GSSG) to reduced glutathionine (GSH) which is required to reduce hydrogen peroxide (H_2O_2), a free radical generated in small amounts during normal cell

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metabolism and in large amounts as a result of metabolism of certain drugs and foods like fava beans. In detoxifying H_2O_2, GSH itself is oxidized to GSSG [4]. Thus GSH also prevents reactive oxygen species (ROS) and peroxides from building up to toxic levels within the RBCs [5].

G-6-PD deficient individuals are limited in their ability to generate NADPH and consequently GSH. In G-6-PD deficiency, the sulfhydryl groups in the globin portion of hemoglobin are oxidized leading to its denaturing as a result of the reactive oxygen species (ROS) in the cells [2]. Individuals with G-6-PD deficiency disease accumulate H_2O_2 and other ROS released as by-products of metabolism of oxidants in their RBCs which in turn denatures the hemoglobin by oxidation and subsequently precipitates it leading to Heinz body formation. The ultimate effect is lysis of the red blood cell membrane. Although hemolysis is predominantly extravascular, membrane injury may be sufficient to cause cell disruption within the circulation giving rise to hemoglobinemia and hemoglobinuria [3].

The G-6-PD gene is expressed in most tissues [1] and a defect in the gene reduces the amount of G-6-PD enzyme or alters its structure which makes it unable to play its protective role effectively. This enzyme deficient condition is inherited in an X-linked recessive pattern making males more susceptible to the disorder than females [4].

The prevalence of G-6-PD deficiency disease is high in malaria endemic areas [6, 7]. In Ghana, as far back as 1989, the incidence of G-6-PD deficiency was known to be 15-25% [8]. Current data on the prevalence of G-6-PD deficiency disease in Ghana is not yet available. Generally, G-6-PD deficiency has been associated with protection to malaria infection by *falciparum* parasites [9]. This has led to the theory by Genesia *et al.* [10] that carriers of G-6-PD deficiency disease may incur moderate protection against malaria infection. However, Bienzle *et al.* [11] did not find any evidence that G-6-PD deficient subjects had any greater protection against *falciparum* malaria.

In Ghana studies by Mockenhaupt *et al.* [12], has shown that possession of moderate G-6-PD deficiency provides a means of protection against *Plasmodium falciparum* malaria. Meanwhile, Guindo *et al.* [13] found that G-6-PD hemizygous (severe deficient) males were found to be protected against malaria and little or no such protection was present in G-6-PD heterozygous (moderate deficient) females. It is not known whether in Ghana hemizygous G-6-PD deficient patients may have protection against malaria. In view of this, the study was conducted to determine the significance of G-6-PD deficiency in *falciparum* malaria incidence in patients visiting the Central Regional Hospital, Cape Coast, Ghana.

MATERIALS AND METHODS

Study Area and Selection of Subjects: The study was carried out at the Central Regional Hospital, Cape Coast, Ghana. Patients who had malaria and those without malaria were recruited into the study. Each participant, after granting consent, was screened for sickle cell status, parasitemia and G-6-PD status. A total of 142 subjects were enrolled into the study. This comprised 70 malaria and 72 non-malaria subjects.

Blood Sampling: Venous blood (4ml) was collected from each participant into tubes containing EDTA after which malaria, G-6-PD deficiency and sickle cell anaemia and trait status of each participant were determined.

G-6-PD, Sickle Cell and Fasting Blood Glucose Assays: Fasting venous blood (10 mL) sample was collected and separated into two tubes containing fluoride and acid-citrate-dextrose (ACD) after which, Fasting blood glucose (FBG) was determined using the principle of enzymatic oxidation in the presence of glucose oxidase using an automated chemistry analyzer (ATAC 8000 Random Access Chemistry system). The qualitative methemoglobin (MetHb) reduction test [14] was employed in determining G-6-PDD deficiency status (severe deficient, moderate deficient and non deficient). The principle behind the method is that determination of MetHb is dependent upon the change of optical density at 640 nm wavelength when sodium cyanide is added to a solution containing MetHb, which converts the MetHb to cyanmethemoglobin. The sodium metabisulphite reduction test was used to determine the sickle cell status of participants [15]. This is a test for sickle cell anemia or trait in which red blood cells are deoxygenated by the addition of a reducing agent - sodium metabisulphite. This reducing agent when added to blood lowers the oxygen tension of blood thus erythrocytes of persons with sickle cell anemia or trait will assume a sickle shape.

The Giemsa staining method was used to determine the presence of parasitemia [15].

Inclusion and Exclusion Criteria: Subjects included in the study were malaria and non-malaria subjects.

Patients suffering from sickle cell anaemia/trait were excluded from this study; because research has shown that apart from G-6-PD enzyme deficiency, sickle cell trait (heterozygous) provides resistance to *Plasmodium falciparum* malaria infection [16].

Statistical Analysis: Cross tabulations and Chi-square test were used to analyse the data using SPSS Version 16 (SPSS Inc., Chicago, IL, USA) and presented on a percentage frequency distribution table. The statistical difference was tested at 5% significance level.

RESULTS

Sex Characteristics of the Study Population: Out of the investigated 142 subjects, 65 were females and 77 were males. There was no significant difference between male and female subjects (p=0.314). The 72 participants not suffering from malaria, were used as controls, with 50 of them being males whilst 22 were females. The males were significantly higher than the females (p<0.001). The remaining 70 malaria-positive participants on the contrary, recorded a significantly higher (p<0.001) female participation of 43 compared to 27 for males (Table 1). The sickle cell test showed that none of the subjects recruited for the study had sickle cell anaemia or trait.

Characteristics of G-6-PD Status in Non-malaria Subjects: Among the 50-non malaria male patients, 9 (18%) severely expressed G-6-PD deficient enzyme, while 41 (82)% had normal G-6-PD enzyme. In the female group, only 3 (13.6%) manifested G-6-PD moderate deficient enzyme, while 19 (86.4%) of them had normal G-6-PD enzyme activity. None of the females who participated in the study was severely deficient of the G-6-PD gene. The prevalence of severe deficient enzyme was found to be 18% in the male subjects, 12.5% in the study sample, moderate deficient G-6-PD enzyme was 13.6% among females and 4.2% in the study sample (Table 2).

Characteristics of G-6-PD Status in Malaria Subjects: Amongst the females, 2.3% showed G-6-PD moderate deficient phenotype. Table 3 shows that 69 malaria patients representing 98.6% were found to have normal G-6-PD enzyme activity. It was also found that the females had the highest number of malaria cases and none was G-6-PD severe deficient.

Table 1: Sex characteristics of the study subjects

Sex	Malaria	Non-malaria	Total
Males	27	50	77
Females	43	22	65
Total	70	72	142

Results are presented as counts

Table 2: G-6-PD deficiency distribution in the non-malaria participants

Sex	Severe deficient	Moderate deficient	Non deficient	Total
Male	9 (18.0)	0 (0.0)	41 (82.0)	50 (100)
Female	0 (0.0)	3 (13.6)	19 (86.4)	22 (100)
Total	9 (12.5)	3 (4.2)	60 (83.3)	72 (100)

Results are presented as counts and percentage count

Table 3: G-6-PD deficiency distribution in malaria participants

Sex	Severe deficient	Moderate deficient	Non deficient	Total
Male	0 (0)	0 (0)	27 (100)	27 (100)
Female	0 (0)	1 (2.3)	42 (97.7)	43 (100)
Total	0 (0)	1 (1.4)	69 (98.6)	70 (100)

Data presented as counts and percentage count

Table 4: Prevalence of malaria in G-6-PD severe deficient, moderate deficient and non-deficient subjects

G-6-PD status	Number of malaria subjects	Prevalence Rate%
G-6-PD Severely Deficient	0	0
G-6-PD Non-Deficient	69	98.57[a]
G-6-PD Moderately Deficient	1	1.43

Significant difference among the prevalence rate in G-6-PD non deficient,G-6-PD severely deficient and G-6-PD moderately deficient individuals is represented as (a) (P value < 0.05)

Prevalence of Malaria in G-6-PD Deficient and Non-deficient Patients: There was a high significant difference (p=0.005,) in the prevalence of malaria among severe, moderate and non-deficient G-6-PD individuals (Table 4). Amongst the 70 malaria patients studied, the prevalence of malaria was 98.6% in G-6-PD non deficient patients. A much lower percentage (1.4 %) was recorded for G-6-PD moderately deficient patients while no case of malaria was reported amongst severely deficient patients.

DISCUSSION

G-6-PD deficiency disease is one of the most common enzymopathies in the world. According to Allison and Clyde [6], the prevalence of G-6-PD deficiency disease is high in malaria endemic areas. In Ghana, there is scanty available information on the interaction of *falciparum* malaria infection and G-6-PD deficiency state.

In this study, the 18% prevalence of G-6-PD deficiency among male non-malaria patients is in conformity with the prevalence rate recorded in Ghana by World Health Organization [8]. No case of a severe deficient individual was recorded for females in this study. Out of 43 female malaria participants, only one was found to be G-6-PD moderate deficient. This result is in agreement with investigations by Mockenhaupt *et al.* [12] in Ghana which provided evidence that G-6-PD moderately deficient women are protected against malaria caused by *Plasmodium falciparum.* However, it is contrary to that of Guindo *et al.* [13] who reported that G-6-PD hemizygous (severe deficient) males were found to be protected against malaria but little or no such protection was present in G-6-PD heterozygous (moderate deficient) females.

The study also revealed that out of 27 male malaria subjects, none was severely deficient of G-6-PD. This indicates that G-6-PD deficiency could play a protective role in malaria prevention especially in the males and this is in conformity with a report by Guindo *et al.* [13] that G-6-PD hemizygous (severe deficient) males are protected against malaria. The incidence of *Plasmodium falciparum* infection in G-6-PD non-deficient individuals in this study was much higher than in moderate (heterozygous) and severe (homozygous / hemizygous) G-6-PD deficiency. This observation supports that of Ruwende *et al.* [9] who reported that G-6-PD deficiency in African children can reduce the risk of malaria infection by 46-58% in both, the heterozygous females and hemizygous males.

One of the possible explanations given to this is that, cells infected with *Plasmodium falciparum* parasite are cleared more rapidly by the spleen [9]. Another explanation is that the growth of *falciparum* parasites is inhibited in G-6-PD deficient red blood cells since G-6-PD deficient red blood cells are unable to generate the ribose derivatives needed by the parasite for nucleic acid synthesis. The production of the key derivative 5 – Phosphoribosyl – 1 – Pyrophosphate (PRPP) is inhibited by low levels of intracellular reduced glutathionine (GSH). This is what may be responsible for the overwhelming clearance rate of parasitized red blood cells, which ultimately results in death [3]. Moreover, the absence of the G-6-PD enzyme causes an increase in advanced glycated end products (AGEs) due to a build up of glucose concentration unable to be cleared by the Pentose Phosphate Pathway. This build up facilitates oxidative stress of cells which includes *Plasmodium falciparum*-infected red blood cells [9]. These phenomena are what might give G-6-PD deficient patients an evolutionary advantage.

Limitations of the Study: The lack of genotypic assessment is a major limitation. Moreover the use of a more sensitive molecular biology tool than just qualitative test may confirm more G-6-PD deficiency positives than observed in the study. Another possible study limitation in this study was that it was not conducted in the way to prove protection thus G-6-PDD cannot be said to protect but rather a factor for the protection from malaria. There are many factors that affect malaria protection, so it is impossible to make definitive conclusions about G-6-PD deficiency as a protective factor in development of malaria. Thus further studies on larger study groups with several variables are needed to address those issues, particularly the use of molecular markers associated with G -6-PD deficiency.

CONCLUSIONS

It is concluded from this study that G-6-PD deficiency may play a role in the prevention of malaria and subsequently prevent the development of the disease condition into its severe form.

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