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Glucose-6-Phosphate Dehydrogenase Activity in Jaundiced and Non-Jaundiced Childern with Malaria Parasitaemia in Delta State, Nigeria

[1]N.E. Bini, [1]D.E. Madgdaline and [2]G.O. Avwioro
[1]Department of Anatomy, Delta State University, Abraka, Delta State, Nigeria
[2]Department of Medical Laboratory Service, Central Hospital Warri, Nigeria

Abstract: The impact of malaria and its influence on erythrocytes with regard to Glucose-6-Phosphate Dehydrogenase (G-6-PD), Haemoglobin (Hb) and consequent production of bilirubin were investigated in 150 children consisting of 50 apparently healthy children who were used as controls, 50 cases of malaria with jaundice and 50 cases of malaria without jaundice. *Plasmodium falciparum* was the commonest infection encountered. About 33 (66%) males and 17 (34%) females had malaria with jaundice, 34 (68%) males and 16 (32%) females had malaria without jaundice while 28 (56%) males and 22 (44%) females were used as control. Their blood samples were analyzed for G-6-PD activity, levels of haemoglobin, total bilirubin and percentage parasitaemia. Average G-6PD activity of 4.7±0.9 U g^{-1} Hb in malaria with jaundice was significantly lower than values obtained for malaria patients without jaundice 7.6±1.7 U g^{-1} Hb and both were significantly lower than the controls with the enzyme activity of 12.6±1.0 U g^{-1} Hb ($p<0.05$). A statistically significant correlation was found between G-6-PD activity and haemoglobin in malaria infested subjects but severe malaria infection was responsible for most haemolysis of the red cells. G-6-PD activity was found to be decreasing with severity of malaria infection with subsequent increase in total bilirubin values. Reduced values of G-6-PD activity increased red cell fragility with consequent lysis of the red blood cells.

Key words: Malaria Parasite (MP), Glucose-6-Phosphate Dehydrogenase (G-6-PD), Haemoglobin (Hb), bilirubin, enzyme activity

INTRODUCTION

Glucose-6-Phosphate Dehydrogenase (G-6-PD) catalyzes the rate limiting step of the pentose phosphate pathway providing reduced power to the cell in the form of reduced Nicotinamide Adenine Dinucleotide Phosphate (NAPDH) (Naylor *et al*., 1996). G-6-PD is the most common enzymopathy of humans, affecting >400 million people (Ezedinachi and Ejezie, 1990). Erythrocyte G-6-PD is a common enzymatic abnormality resulting in various clinical manifestations. Acute haemolytic anaemia association with oxidative stress is the most characteristic clinical manifestation of G-6-PD deficiency (Yuregir *et al*., 1994). G-6-PD plays an important role by protecting sulphydryl groups of the red cell membrane against oxidative stress by generating NADPH to maintain glutathione in the reduced form. Increased oxidative stress due to drugs or infection (malaria) leads to haemolysis of the red cells of patient with G-6-PD deficiency (Solberg, 1999; Szenberg *et al*., 1957). Malaria infection most especially at the erythrocytic stages imposes oxidative stress on the red cells, resulting in cell damage and subsequent production of oxygen free radicals. G-6-PD deficiency leads to abnormal rupture (breakage) of the red cells called anaemia (an abnormal low red cell count) (Martin *et al*., 1979). Decreased activity of G-6-PD below a critical level leads to loss of NADPH and reduced Glutathione (GSH) with concomitant precipitation of the denatured haemoglobin and Heinz body formation. Products of the haemoglobin breakdown may bind to the red cells membrane and cause lysis. G-6-PD deficiency in malaria and haemolytic anaemia can develop jaundice which is the yellowish discoloration of the white of the eye, skin and mucous membrane caused by deposition of bilirubin in the tissues (WHO, 2000).

MATERIALS AND METHODS

A total of one hundred and fifty children were studied comprising fifty who were jaundiced, fifty that were non-jaundiced subjects with malaria parasitaemia and fifty non-malaria apparently healthy controls. They comprise of males and females children of age range 1-10 years. The samples were collected from designated health centers and hospitals in Delta State, Nigeria. Subjects were not on any antimalairal drug at the time of collection of blood.

Corresponding Author: N.E. Bini, Department of Anatomy, Delta State University, P.O. Box 2072 Abraka, Delta State, Nigeria

G-6-PD: Erythrocyte G-6-PD activity was based on Kinetic Spectrophometric Method. Commercially prepared reagent (Randox Laboratory, USA).

Bilirubin: Bilirubin estimation was based on Jendrassik and Grof Method. Commercially prepared reagent (Randox Laboratory, USA).

Malaria parasite detection: The WHO (2000) procedure was adopted for detection and identification of malaria parasite from thick films. The thick blood film was stained for 10 min with 10% Giemsa stain buffered at 7.2.

Interpretation of result:

- 1-10 parasites per 100 HPF is +
- 11-100 parasites per 100 HPF is ++
- 1-10 parasites per HPF is +++
- >10 parasites per HPF is ++++

Haemoglobin estimation: Haemoglobin (Hb) estimation was carried out using spectrophotometry based on the method of Dacie and Lewis. The blood was suitably diluted (1 in 200) with cyanideferricyanide reagent and the absorbance was measured at 540 nm.

RESULTS AND DISCUSSION

Table 1 shows mean values of increasing Haemoglobin (Hb), decreasing mean values of total bilirubin and increasing mean values of G-6-PD activity of patients with malaria and jaundice, malaria without jaundice and control. There was significant difference ($p<0.05$) between the value obtained in malaria with jaundice and control of haemoglobin (g dL^{-1}), G-6-PD activity (U g^{-1} Hb) and total bilirubin (mg dL^{-1}). These were as a result of reduced degree of absence of malaria parasitaemia. High levels of total bilirubin were found in severe malaria parasiteamia resulting in haemolysis giving rise to total bilirubin (4.1±1.1), resulting from the effects of malaria parasite on the red blood cells. Table 2 also shows that children with an acute febrile illness tested show that high degree of malaria parasitaemia resulting in increased total bilirubin (4.1±1.1 mg dL^{-1}) due to haemolysis and decreased G-6-PD activity (5.2 U g^{-1} Hb) based on the effect of malaria parasite on the red blood cells. G-6-PD activity decreased with severity of infection but with increase in total bilirubin. There was no significant variation in the G-6-PD activity among the age groups in subjects with malaria parasitaemia ($p>0.05$) but there was significant variation of G-6-PD activity in subject with malaria parasitaemia when compared with the control ($p<0.05$).

The significantly low level of G-6-PD activity was observed in malaria with jaundice subjects than in control and mild/moderate infection and same observation in malaria without jaundice subjects and control ($p<0.05$) (Table 2). The severity of malaria infection was graded based on the percentage of malaria parasitaemia (%)/malaria parasite count. G-6-PD activity was not statistically different ($p>0.05$) in females (4.9±0.1 U g^{-1} Hb), males (4.6±0.9 U g^{-1} Hb) for patients with malaria with jaundice and females (7.8±1.8 U g^{-1} Hb), males (7.5±1.6 U g^{-1} Hb) for patients with malaria without jaundice (Table 3).

Table 1: Mean value of Hb, total bilirubin and G-6-PD in patients with malaria and jaundice and those with malaria without jaundice

Parameters	No. examined	Malaria with jaundice	Malaria without jaundice	Controls	p-value
Hb (g dL^{-1})	50	4.3±0.5	5.9±1.2	13.4±1.1	<0.05
Total bilirubin (mg dL^{-1})	50	3.4±0.6	1.3±0.3	0.9±0.3	<0.05
G-6-PD (U g^{-1} Hb)	50	4.7±0.9	7.6±1.7	12.6±1.0	<0.05

Table 2: G-6-PD activity and bilirubin level in malaria patients with or without jaundice according to age

	Malaria with jaundice				Malaria without jaundice				Controls			
Age in years	No. examined (50) (%)	G-6-PD (U g^{-1} Hb)	Total bilirubin	MP	No. examined (50) (%)	G-6-PD (U g^{-1} Hb)	Total bilirubin	MP	No. examined (50) (%)	G-6-PD (U g^{-1} Hb)	Total bilirubin	MP
1-2	17 (34)	4.7±1.0	4.1±1.1	+++	23 (46)	7.8±1.9	2.1±0.5	++	12 (24)	13.7±1.2	0.8±0.3	Nil
3-4	12 (24)	4.6±1.5	3.4±0.6	++++	10 (20)	7.6±1.4	1.3±0.5	++	8 (16)	13.0±1.1	0.9±0.2	Nil
5-6	13 (26)	4.6±1.1	3.2±0.5	++++	4 (8)	7.4±1.5	1.1±0.3	++	14 (28)	12.2±1.1	0.7±0.1	NIl
7-8	3 (6)	4.6±0.2	3.1±0.4	++++	6 (12)	8.4±2.2	1.2±0.4	+	4 (8)	11.8±0.4	0.8±0.1	Nil
9-10	5 (10)	5.2±0.9	3.0±0.6	+++	7 (14)	7.3±1.6	1.0±0.2	++	12 (24)	12.4±1.0	0.8±0.3	Nil

Table 3: G6PD activity in male and female patients with malaria parasitaemia

	Malaria with jaundice			Malaria without jaundice			No. of malaria (control)	
Sex	No. examined (50)	G-6-PD (U gw^{-1} Hb)	MP	No. examined (50)	G-6-PD (U g^{-1} Hb)	MP	No. examined (50)	G-6-PD (U g^{-1} Hb)
Male	33 (66%)	4.6±0.9	>1%	34 (68%)	7.5±1.6	<1%	28 (56%)	12.3±0.9
Female	17 (34%)	4.9±1.0	>1%	16 (32%)	7.8±1.8	<1%	22 (44%)	13.1±1.1

MP: Malaria Parasites

The result of this study indicates that patients with malaria accompanied with jaundice have lower G6PD activity as compared with malaria without jaundice and control subjects. The study refers principally to malaria infection caused by *Plasmodium falciparum* which was found to be most prevalent species of malaria parasite and which has been reported as the commonest cause of malaria in Nigeria (Ezedinachi and Ejezie, 1990). It is known that haematological parameters are in a dynamic process in the 1st year of postnatal life, related to the relatively young red blood cell population and alteration in active erythropoesis centres (Travis *et al*., 1980).

For this reason newborns and infants <1 year old were not include in this study. Manifestations of the malaria infection are protean but not all factors that contribute to the alteration in the normal function of many tissues are fully known (Anidi, 1990). The disruption of red cell metabolic activity at erythrocyte stage of malaria parasites could influence the traditional function of the red cell (oxygenation and deoxygenation) and may affect the activities of the reducing substance in the red blood cell leading to red cell lysis. This study supports reports by Sodeinde *et al*. (1975) and Ling and Wilson (1988) that G-6-PD activity were lower in severe malaria caused by *P. falciparum* than in control subjects. Similar conclusions were reached by Greene (1993) and Luzzatto *et al*. (1969). Since, world distribution of G-6-PD activity manifests geographic pattern, this study revealed a variation in G-6-PD activity compared with the research of Jaradat *et al*. (1996) and Missiou-Tsagaraki (1991). A different manifestation was seen in G-6-PD subjects due to the level of biochemical characteristic of the enzymes (Yoshida and Lin, 1973). The relatively low G-6-PD activity may coincide with the selective advantage it is known to provide against malaria infection (Kehinde and Akinyanju, 1991). In this study subjects with low haemoglobin level with high degree of malaria parasitaemia were found to have low G-6-PD activity which finding agrees with Bienle *et al*. (1972) that the rate of invasion of the red blood cell by malaria parasite were higher, especially those of malaria with jaundice patients which in turn affect the G-6-PD activity. The level of erythrocyte G-6-PD activity was significantly lower ($p<0.05$) in malaria patients than control (Table 1) most, especially in malaria with jaundice (Table 2). In each case studied among 50 children of both sexes having severe malaria (malaria with jaundice), there were no significant different ($p>0.05$) in G-6-PD activity of the age groups (Table 2). From severe malaria to mild/moderate infection there were significant difference ($p<0.05$) in the degree of parasitaemia.

CONCLUSION

Previous investigations have shown that if any of the groups (sex) of subjects has increase resistance against malaria is only relative rather absolute (Greene, 1993). In this study, G-6-PD activity of male subjects were slightly lower than in female subjects ($p>0.05$) (Table 3) which is in accord with Battistuzzi *et al*. (1977). The low G-6-PD activity in six of the patients in this study is in agreement with the findings of Luzzatto (1993) that *Plasmodium falciparum* might not be the major selective factor but defect in the red cell enzymes or other genetic abnormalities could be responsible.

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