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Relation of Nutritional Status, Sickle Cell Trait, Glucose-6-Phosphate Dehydrogenase Deficiency, Iron Deficiency and Asymptomatic Malaria Infection in the Niger Delta, Nigeria

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This study aimed at investigating these complex interactions with a view to ascertaining the risk or benefit of acquiring these factors in a malaria endemic part of Nigeria. In a cross sectional study, 240 asymptomatic, non-hospitalized children aged 1-8 years of both sexes were assessed for nutritional status (using anthropometric indexes), malaria parasite, hemoglobin, white blood cell count, hemoglobin electrophoretic pattern, glucose-6-phosphate dehydrogenase deficiency and serum ferritin concentrations. Fifteen percent of the children were malnourished (BMI Z score <-2), 12.5% were iron deficient (serum ferritin <12 ng mL^{-1}), 12.5% were HbAS, 87.5% HbAA while 5% of the children were G6PD deficient. The mean parasite density of the study population was 1.14×10^3 parasites μL^{-1} and the risk of malaria infection was higher in iron replete children than those who were iron deficient (RRR = 0.33, $\chi^2 = 2.825$, $p<0.05$). The risk was age-related, higher in the under fives than the 5-8 years group (RRR =1.7, $\chi^2 = 2.910$; $p<0.02$). There was an association between low serum iron concentration and sickle cell trait ($\chi^2 = 35.890$; $p = 0.056$), no statistical significant risk was observed with nutritional status, hemoglobinopathy and G6PD deficiency. This study provides an observational support that iron deficient children are to some extent protected against malaria infection and malnutrition places children below five years of age at risk of malaria related morbidity.

Key words: Nutritional status, iron deficiency, G6PD deficiency, malaria, HbAS, HbAA

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INTRODUCTION

A large percentage of child deaths related to malaria are attributable to under nutrition and deficiencies of vitamin A, zinc, iron and folate (Steketee, 2003) Nutritional status strongly influences the disease burden of malaria, however this relationship remains unclear and controversial (Nyakeriga *et al*., 2004). Many individuals at risk of malaria have micronutrient deficiencies that may hamper protective immunity. Nutrition appears to influence susceptibility to malaria and affects the course of the infection (Shankar, 2000). Published data from developing countries indicate that children generally fail to achieve their genetically determined potential growth because of poor diet and infection (Ulijaszek, 1994; Waterlow, 1994). On the other hand, several studies have suggested that malnourished children are protected to some degree against malaria (Walker *et al*., 1996; Nyakeriga, 2005).

In malaria endemic areas, host factors especially the genetic red cell disorders, including sickle cell trait and enzyme deficiencies (glucose-6-phosphate dehydrogenase deficiency) have been shown to confer natural protection against malaria infection hence the term natural immunity. All these factors affect parasite survival and provide resistance in malaria endemic areas, the so called malaria hypothesis (Berkley *et al*., 1999; Newton *et al*., 2000).

Glucose-6-phosphate dehydrogenase is an enzyme in the hexose monophosphate shunt responsible for the generation of reduced glutathione. This reduced glutathione protects sulphydryl group of hemoglobin and the red cell membrane from oxidation by the oxygen radicals. Defects in the shunt leads to inadequate protection against oxidation, resulting in oxidation of sulfhydryl groups and precipitation of haemoglobin as Heinz bodies and in lysis of the red cell membrane (Frank, 2005).

In order to elucidate the complex web of interactions between nutritional status, sickle cell trait and G6PD deficiency, a study was undertaken in a malaria endemic part of the Niger Delta region of Nigeria. The study aimed at investigating the relationship between iron status, sickle cell trait, G6PD deficiency and malaria with a view to ascertaining the risk or benefit of acquiring these conditions in a malaria endemic area.

MATERIALS AND METHODS

Study area and population: This study was conducted between March 2005 and April 2006 in Rumueme, Port Harcourt. The geographical location is latitude 4°31^1-5°31^1 and longitude 6°30^1-7°21^1. The typical deltaic wetlands and mangrove forests that climatize the area provides enough breeding grounds for mosquito and malaria transmission throughout the year, although the majority of clinically evident infections occur after long and short periods of rainfall, that in general occur during months of October- November and March to July every year, respectively. The study population consisted of 240 children (1-8 years) of both sexes recruited from households and schools.

A cross sectional study design was used in this study. Eligibility criteria for this study were as follows; (1) axillary temperature of ≤37.5°C, (2) age 1-8 years, (3) absence of symptoms suggestive of malaria or any other systemic illness (4) parental consent.

This study received ethical approval form the Rivers State University of Science and Technology and informed consent was received from each parent before samples were collected for analysis. All the children were weighed using the YAMATO digital scale (China). Heights were measured in children ≥1 year with a Leicester height measure (MS instruments). The temperature of each child at the time of blood collection was taken using DGL digital thermometer (MODE ECT- 1, Germany).

Collection of blood samples: Four milliliter of whole blood was drawn with syringe (5 mL) through venepuncture using the antecubital vein and dispensed into an ethylenediamine tetra acetic acid (EDTA) and was used for malaria parasite estimation, hemoglobin electrophoresis and glucose-6-phosphate dehydrogenase deficiency while the remaining two milliliter in the syringe was used for serum ferritin concentration determination.

Methods: Malaria was estimated by microscopy with well stained Giemsa smears (thick and thin) using 100x oil immersion. Thick and thin blood smears were stained by fresh working Giemsa stain according to standard procedure. Parasite densities were recorded as a ratio of parasites to white blood cells (500 WBCs) from thick smears. Densities (parasites μL^{-1}) = parasites/500 WBCs × WBC count of individual subjects.

White blood cell count was done by diluting a well mixed whole blood with Turk's solution in the ratio of 1 in 20 (950 µL of Turk's solution to 50 µL of whole blood. The haemocytometer was then filled with an aliquot of this mixture and allowed to settle for one minute prior to performing the count, white blood cells were counted with ×10 objective with reduced light in the four corner squares of the counting chamber. The member of white blood cells/cubic milliliter was calculated as follows: cells counted × dilution factor × chamber depth/area of chamber counted.

Haemoglobin electrophoretic pattern of the participants were determined using haemoglobin electrophoresis by cellulose acetate membrane at pH 8.9 as described by Brown (1993). A small quantity of haemolysate of venous blood from each of the subjects was placed on the cellulose acetate membrane and carefully introduced into the electrophoretic tank containing Tris-EDTA-Borate buffer at pH 8.9. The electrophoresis was then allowed to run for 15-20 min at an electromotive force of 160 V. The results were read immediately. Haemolysates from blood sample of known haemoglobin (i.e., AA, AS, AC) were included as controls.

A qualitative determination of G6PD in red cells of participants was carried out using the G6PD deficiency screen reagent set (Pointe scientific, USA. Lot # 513704). A red cell haemolysate was prepared by adding 50 μL of whole blood to 2.5 mL deionised water, mixed gently and allowed to stand for 5 min. Into 13×100 mm test tubes labeled positive, negative controls and samples were dropped 500 μL of reconstituted G6PD screen reagent. 1.0 mL of the haemolysate was then added into each tube and gently shaken to mix. One milliliter of mineral oil was then gently layered on top of the reaction mixture. The tubes were observed at 15 min interval for up to 1 h for colour change from deep purple to red/orange, Negative samples did not show any colour change.

Nutritional status of the children were determined using anthropometrical indexes. Z-scores were determined using the CDC Epi Info V 6.04 to classify children as undernourished (BMI Z <- 2), well nourished (BMI Z-0.5 to 2), overweight (BM Z>2), Haemoglobin level of participants were estimated using the cyanmehaemoglobin method with reagents bought from Pointe Scientific Inc, USA (Catalog #H 7504-500).

Statistics: Data were arranged in a 2×2 contigency table and analyzed using the statistical package for social science (SPSS) (Version 11.0, Chicago, IL.USA). Descriptive statistics of continuous variables were expressed as Mean±Standard deviation. The students t-test was used for the comparison of means. Relative Risk Ratio (RRR) and Chi-square values were calculated using standard formulae. Non parametric test (Kruskal-Wallis) was employed for the analysis of skewed data. Significant level was set at $p \leq 0.05$.

RESULTS

Only the temperature and haemoglobin values showed significant difference between the parasitized and non-parasitized children ($p<0.02$ and $p<0.01$, respectively) (Table 1).

Table 2 shows the nutritional status of the children based on BMI Z scores. 15% were undernourished, 13.75% at risk of under nutrition, 35% well nourished and 36.25% overweight. There was no significant relationship existing between haemoglobinopathy, iron status and malaria as shown in Table 3. The risk of malaria infection was higher in iron replete children than those who were iron deficient and this was statistically significant (RRR = 0.33; χ^2 = 2.825; $p<0.05$). The risk was also higher in the under fives than the 5-8 years children (RRR = 1.7; χ^2 = 2.910; $p<0.02$). Malnutrition, haemoglobinopathy and G6PD deficiency were not found to exert any significant risk for acquiring malaria infection (Table 4).

Table 1: Characteristics of study participants

Characteristics of study participants	Parasitized n = 66 mean±SD	Non-parasitised n = 144 mean±SD	p-value
Temp (°C)	37.3±4.3	36.8±0.8	<0.02
Height (cm)	111.5±17.2	111.6±18.0	>0.05^{ns}
Weight (kg)	15.1±1.9	15.2±1.7	>0.05^{ns}
BMI (kg m^{-2})	15.2±2.2	15.5±3.1	0.77^{ns}
Haemoglobin (8 day L^{-1})	10.8±1.9	11.3±1.7	<0.01*
Parasite density	1.14×10^3 Parasites μL^{-1}	-	
WBC	5.4±2.3	5.3±2.4	20.27^{ns}

BMI: Body Mass Index, WBC: White Blood Cell, *Statistically significant, ns: Not Significant

Table 2: BMI Z scores of 240 children aged 1-8 years in relation to age groups and sex

	BMI Z-scores			
Demographics Age	<-2 n (%)	<-1 to 1 n (%)	-0.5 to 2 n (%)	>2 n (%)
<5 years	18(7.5)	9(3.75)	39(16.24)	30(12.5)
5-8 years	18(7.5	24(10.0)	45(18.75)	57823.75)
Total	36(15.0)	33*(13.75)	84*(35.0)	87(36.25)
Sex				
Boys	21(8.75)	18(7.5)	35(15.0)	45(18.75)
Girls	15(6.25)	15(6.25)	48(20.0)	45(18.75)
Total	36(15.0)	33(13.75)	84(35.0)	87(36.25)

Z-scores <-2: Undernourished, Z-scores <- 1 to 1 at risk of under nutrition, Z scores-0.5 to 2 = Well nourished, Z-scores > 2 = Over weight

Table 3: Relationship between iron status, sickle cell trait and malaria Hb electrophoretic pattern

Study variables	AA (n %)	AS (n %)	Total (n %)
P. falciparum	60(25.0)	6(2.5)	66(27.5)
Iron deficient (SF<12 ng mL^{-1})	30(12.5)	3(1.3)	33(13.8)
Iron replete (SF 12-<20 ng mL^{-1})	18(7.5)	3(1.3)	21(8.8)
Adequate iron (SF 20-100 ng mL^{-1})	144(60.0)	21(8.8)	165(68.8)
Risk of iron excess (SF 101-300 ng mL^{-1})	15(6.3)	3(1.3)	18(7.5)
Excess iron (SF > 300 ng mL^{-1})	3(1.3)	0(0)	3(1.3)

Chi-square (χ^2) test = 0.378; p = 0.984^{ns}, Pearson correlation (R) = 0.028; p = 0.803^{ns}

Table 4: Risk analysis of malaria parasite infection in relation to iron deficiency, nutritional status, sickle cell trait and G6PD Deficiency

Study variables	Parasitized n (%)	Non-parasitised n (%)	Total n (%)
Iron deficiency (SF<12 ng mL^{-1})	6(2.5)	24(10.0)	30(12.5)
Iron replete (SF 12-20 ng mL^{-1})	12(5.0)	9(3.75)	21(8.75)
RRR = 0.33; χ^2 =2.825; p<0.05; 95% CI 0.6-2.0.			
Undernourished (BMI Z <- 2)	12(5.0)	24 (10.00	36 (15.0)
Well nourished BMI Z + 1 to 2)	27 (11. 25)	57 (23. 75)	84 (35.0)
RRR = 1.03; χ^2 = 0.032; p>0. 05^{ns}; 95% Cl 0. 34-2.37			
AS	6 (2.5)	24 (10.0)	30 (12.5)
AA	60 (25.0)	150 962.5)	210 (87.5)
RRR = 0.66; χ^2 = 0. 354; p>0.05 . 95% Cl 0.4-1.4			
G6PD deficiency	3(1.3)	9(3.8)	12(5.0)
G6PD Normal	62(26.3)	165(68.8)	228(95.0)
RRR = 0.8; χ^2 = 0.013; P = 0.9^{ns}; 95%Cl 0.4-1.6			
<5 years	39(16.25)	60(251.0)	99(41.25)
5-8 years	33(13.75)	108(45.0)	414(58.75)
RRR = 1.7; χ^2 = 2. 910; p<0.02; 95% C I 0.9-2.8			

RRR: Relative Risk Ratio

DISCUSSION

The prevalence of sickle cell trait in present study population was 12.5%. Given the high prevalence of asymptomatic malaria and iron deficiency in our study population (Jeremiah *et al*., 2007, 2008), we wanted to test the hypothesis that sickle cell trait protect individuals from becoming iron deficient and malaria infection. Subsequently, the association between biochemical indices of iron status and these sickle cell traits were investigated. Further, we found that iron deficiency was associated with age, being more prevalent in younger children. Overall, our results showed that children with sickle cell trait were not protected from being iron deficient (χ^2 = 0.378, p = 0.984). Instead, we observed an association between low serum iron concentration and sickle cell trait (χ^2 = 35.890, p = 0.056). These observations are discussed in the light of previous reports. Iron deficiency and malaria are singly or in combination the most common causes of anemia. However, the aetiology of malaria is multifactorial, involving host factors such as haemoglobinopathies (sickle cell trait). The interaction between haeoglobinopoathy, iron status and malaria is complex and rather controversial. While a large body of evidence from the literature shows that milder sickle cell trait is associated with protection against malaria, the sever forms of haemoglobinopathy have been shown on the other hand to be associated with anaemia and on the other hand protection against iron deficiency through increased iron absorption (Hershko *et al*., 1982).

There is no consensus concerning the specific mechanism of protection against malaria infection by any of the haemoglobinopathies, but variant haemoglobin has been associated with various pathophysiological condition. For example, HbS is associated with auto-oxidation of haemoglobin and β-thalassemia with ineffective erythropoiesis (Schrier, 2002). Nevertheless, several mechanisms have been postulated including reduced invasion of variant red cells by the parasite (HbE, HbC and HbH and/or consequent reduced growth, reduced cytoadherence of infected red blood cells as well as reduced rosetting of uninfected red cells and increased clearance of the infected red cell via phagocytosis. (Roberts and Williams, 2003).

Another possible mechanism through which haemoglobinopathy protection could protect against malaria is through iron deficiency. While the role of iron in protection against malaria remains controversial, there is some evidence that iron deficiency might protect against malaria infection. In line with this, it has been reported that iron supplementation of pregnant women with sickle cell trait (HbAS) increased their susceptibility to malaria (Menendez *et al*., 1995). This implies that iron deficiency might contribute to protection against malaria seen in HbAS condition. In this study there was an association between low serum iron and sickle cell trait. We speculate that these observations if corroborated by future longitudinal studies, iron deficiency might be yet another mechanism through which sickle cell trait might protect against malaria. There was a reduced risk of malaria infection in the iron deficient children as compared to the iron replete children (RRR = 0.33, χ^2 = 2. 825, p>0.05) suggesting that iron deficiency was associated with protection against malaria to an extent.

The relationship between malnutrition and malaria was also examined in this study. The prevalence of under nutrition (defined as BMI Z scores <-2) was observed to be 15.0%. When two groups (undernourished) and well nourished group were subjected to risk analysis, it was observed that there was no significant difference (RRR = 1.03, χ^2 = 0.32 p>0.05) The effect of nutritional status on malaria became manifest only when age was introduced into the analysis (RRR = 1.7, χ^2 = 2.910, p<0.02). Malnutrition and malnutrition associated adverse effects cuts across all ages. However, in malaria endemic areas, the greatest impact is seen in the younger children, less than the age of five years (Snow *et al*., 1999) Recent estimates combining prevalence data and the population attributable risk factors revealed that most malaria deaths were attributed to under nutrition in children less than five years of age (Caulfield *et al*., 2004). This study corroborates other reports in Nigeria that malnutrition and malaria are two twin factors that are responsible for most

infant mortality rates. (Ezedinachi and Ejezie, 1990; Ejezie and Ezedinachi, 1992; Ekanem *et al.*, 1994; Olanrewaju and Johnson, 2001).

With respect to glucose-6-phosphate dehydrogenase deficiency (G6PD), this study observed 5% deficient children and 95% normal G6PD. The distribution of this genetic trait among the parasitized and non-parasitized group was not significant. No association either was established with malaria. The G6PD deficient cells lack the ability to resist sustained oxidative stress adequately and hence the free radical producing parasite is a challenge to such cells. This situation is thought to make them more susceptible to phagocytosis. Also, oxidative stress induced by the parasite plus the normal red cell oxidative stress particularly unquenched by the enzyme deficiency, results in a environment in which normal parasite growth is limited (Nagel, 2004).

A study conducted in Mali by Guindo *et al.* (2007) among a population of children with severe malaria indicated a population of children with severe malaria indicated a protective effect of the G6PD A- allele in heterozygous females and in males hemizygous for the wild type allele. This finding was also similar to the report of Ntoumi *et al.* (2003) who concluded that G6PD A- heterozygous females are protected against all forms of *Plasmodium falciparum malaria.*

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