

A Possible Protective Role of Sickle Haemoglobin against Severe Malaria in Bamenda, Cameroon

Awah, F. M. and Uzoegwu, P.N.

Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

Corresponding author: Awah, F. M., Department of Biochemistry, University of Nigeria, Nsukka

Abstract

A possible protective role of sickle gene against severe malaria was investigated in 186 sickle heterozygotes (HbAS), using 420 dominant homozygotes (HbAA) as control. These subjects were glucose-6-phosphate dehydrogenase non-deficient and respectively selected from 456 HbAS and 1,516 HbAA individuals, whose haemoglobin genotypes were determined by electrophoresis on cellulose acetate membrane at alkaline pH. Subjects were monitored for their annual frequency and severity of malaria attack as well as haematological profiles. The overall incidence of the sickle gene in the study population was 25.6 %. Dominant homozygotes (HbAA) suffered malaria attack relatively more often and had significantly higher ($P<0.05$) parasitaemia, more severe clinical malaria symptoms and lower haematological profiles than sickle heterozygotes. Percentage HbS in sickle heterozygotes varied from 28 % – 46 %. There was a statistically significant positive correlation between percentage HbS and haemoglobin concentration, packed cell volume, mean cell volume, mean cell haemoglobin concentration and platelet count indicating a direct relationship. A negative correlation was obtained between percentage HbS and white blood cell counts, percentage parasitaemia, parasite density, annual frequency of malaria attack and severity of clinical malarial symptoms showing an inverse relationship. These results seem to indicate that, among the sickle heterozygotes, there still exist variations in malaria protection defined by the percentage HbS. The higher the percentage HbS in sickle heterozygotes the less severe the malaria attacks. It could therefore be suggested that sickle heterozygous state does not prevent plasmodial infection per se but ameliorates the course of falciparum malaria by protecting against severe malarial anaemia and high-density parasitaemia.

Keyword: Malaria protection, Percentage sickle haemoglobin, Haematological profile, Bamenda, Cameroon

Introduction

Malaria, a tropical disease caused by the mosquito-borne haematoprotzoan parasite of the genus *Plasmodium* and transmitted by the female anopheles mosquito, kills more people than most communicable diseases. At least 90% of the deaths from malaria occur in Sub-Saharan Africa (Tsuji *et al.*, 2001; Greenwood and Mutabingwa, 2002). Between 300 – 500 million clinical cases and an estimated mortality of over one million deaths are reported each year with children <5 years old being the most affected and adults >18 years old the least affected groups (Felger *et al.*, 2003). The major pathophysiological features of malaria cut across the classical clinical syndromes of cerebral malaria and severe malarial anaemia (Marsh *et al.*, 1995). Anaemia in *P. falciparum* malaria is clearly multifactorial. It includes bone marrow suppression in addition to haemolysis and there is a strong argument that erythrocyte destruction and ineffective erythropoiesis play equal parts in the etiology in malarial anaemia. Severe anaemia occurs 1.42 - 5.66 million times annually and kills 190,000 - 974,000 children less than five (5) years old annually (Sean and Joel, 2001).

Sickle gene is one of most commonly inherited haemoglobinopathy in the world. Sickle haemoglobin (HbS) is caused by a "typographical error" in the genetic code, in which thymine replaces adenine in the DNA encoding β -globin gene. Consequently, valine replaces glutamate at the sixth amino acid position in the β -globin product (Koch *et al.*, 2000). The high frequency of HbS

gene in some malaria endemic areas parallels the historical incidence of malaria infection and it's believed to provide selective advantage to heterozygotes against fatal malaria (Allison, 2002). Some studies have shown that percentage HbS in sickle heterozygotes vary inversely with the parasite densities in plasmodial-infected individuals (Stirnadel *et al.*, 1999; Uzoegwu and Onwurah, 2003). The actual mechanism by which HbS gene conveys resistance to malaria is still to be unraveled. Some scientists attributed the sickle gene resistance to phagocytic-oxidative burst generated during plasmodial infection (Delmas-Beauvieux *et al.*, 1995; Ebert and Stern, 2001), while others attribute the resistance to genetic factors (El-Hazmi and Warsy, 1997). However, Cooke and Hill (2001) proposed that the resistance could be due to impaired entry of parasites into the RBCs and their impaired growth while Carlson *et al.* (1994) attributed it to impaired rosette formation. The resistance is also attributed to the leakage of potassium needed by the parasite for survival, thereby causing parasitic death (Bindon, 2004). Recent studies in West Africa show that HbAS provides significant protection against high malarial morbidity and mortality, severe malarial anaemia, cerebral malaria, and high-density parasitaemia while having less effect on infection *per se* (Hill *et al.*, 1991; Aidoo *et al.*, 2002). However, none of these proposals is conclusive. Although protection against mortality has been indirectly estimated using gene frequencies, the degree of protection has not been determined using cohort studies. In this study, therefore, the current incidences of HbS

gene, its effect on malarial severity and the role of different percentage *HbS* in protecting sickle heterozygotes against severe malaria was investigated in different age brackets in Bamenda, Cameroon with an intent to throw more light into the role this factor could play in the protection against malaria attack.

Materials and Methods

Subjects and blood sample collection: A total of 2,043 subjects aged one (1) to seventy (70) years, who sought malaria treatment in the Bamenda Provincial Hospital, as well as other volunteers were screened for Hb genotype, examined and tested for malarial infection. Blood samples (5 ml) were collected by venipuncture into sample tubes containing EDTA (1.0 ± 0.15 mg/ml of blood) as an anticoagulant and then rocked gently to mix. Thumb pricking with sterile injection needle (21G) was also used to obtain blood samples for routine monthly malaria diagnosis in the cohort groups. Glucose-6-phosphate dehydrogenase non-deficient cohort groups comprising of 186 sickle heterozygotes (*HbAS*) and 420 dominant homozygotes (*HbAA*) were monitored for malaria infection for a period of one year (December 2004 – November 2005). Selection of the subjects was based on their accessibility, willingness to submit themselves for monthly monitoring for malaria infection, malaria diagnosis and full blood count analysis whenever one was sick.

Preparation of Tris-EDTA-borate buffer: Stock solution of Tris-EDTA-Borate buffer for haemoglobin electrophoresis was prepared by dissolving 14.4g Tris-(hydroxymethyl)-aminomethane (1.2×10^{-2} M Tris, Sigma), 1.5 g ethylene diamine tetracetate (4.0×10^{-3} M EDTA, Sigma) and 0.9 g boric acid (1.0×10^{-2} M borate, BDH) in about 800 ml of distilled water, the pH adjusted to 8.6 and the solution then made up to 1 litre. Working Tris-EDTA-Borate buffer was prepared by making a one-in-ten dilution of the stock solution in distilled water to obtain a final concentration of 1.2×10^{-3} M Tris, 4.0×10^{-4} M EDTA and 1.4×10^{-3} M borate (pH 8.6).

Cellulose acetate strips: Appropriate strips of cellulose acetate paper (Shandon, UK) were cut out from the large sized ones and then soaked in the working Tris-EDTA-Borate buffer solution until used. The used cellulose acetate strips were re-soaked in the same buffer for washing and re-used three or four times.

Preparation of haemoglobin lysate: Uncoagulated blood was centrifuged at $3,000 \times g$ for ten minutes to separate the red blood cells from plasma. The upper layer was aspirated out while the sediment was washed three times by re-suspending in equal volumes of normal saline (0.85% w/v of NaCl) (BDH Ltd) and then re-centrifuged at the same speed. The packed cells were re-suspended in equal volume of normal saline. A portion of the suspended cells (20 μ l) was then mixed with 80 μ l of distilled water in a 1:4 dilution to lyse the cells. The solution was gently

shaken for two minutes and then centrifuged at $3,000 \times g$ for 20 minutes. The resulting supernatant, Hb lysate, was used for the genotype test while the precipitate was discarded. The Hb lysate was stored in the refrigerator ($2 - 4^\circ\text{C}$) until used within four days. In some instances, 20 μ l of uncoagulated whole blood was mixed with 60 μ l of distilled water and the resulting Hb lysate spotted directly for Hb genotype determination.

Clinical symptoms in the cohort group during malaria infection: The cohort groups were observed for clinical symptoms during malarial infection by the medical doctors in the hospital in the area of study. The intensity and severity of the symptoms were rated from 1 to 5 (+ to +++++) in the increasing order of severity.

Malaria diagnosis: Malaria was diagnosed based on clinical symptoms of malaria such as intermittent fever, severe headache, diarrhea, respiratory distress, and severe vomiting and then confirmed by microscopic examination of Giemsa stained (BDH Ltd) thick and thin blood films (oil immersion, X100).

Glucose oxidase reagent for fasting blood sugar: The glucose reagent (Teco Diagnostic) was reconstituted by mixing each bottle of reagent with 50 ml of distilled water. The reconstituted reagent contained 15 μ l/ml glucose oxidase, 1.2 μ l/ml peroxidase, 4.0 μ l/ml mutarotase, 0.38 mM 4 - minoantipyrine, 10 mM p- hydroxybenzene sulfonate and non-reactive ingredients. The reagent was stored at $2 - 8^\circ\text{C}$ in amber containers.

Determining haemoglobin (Hb) genotype: Haemoglobin genotypes were determined by cellulose acetate membrane electrophoresis (CAME) of Evans (1971) as modified by Uzoegwu (2001).

Full blood count: Haematological indices [Hb concentration, red blood cell (RBC) counts, white blood cell count (WBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), packed cell volume (PCV), platelet (PLT) count and red cell distribution width (RDW)] were determined using an automated haematology analyzer (CELL-DYN 1200; Abbott, USA).

Determination of target cell percentage: The target cell percentage was determined according to the method of Cheesbrough, (2000) and calculated thus: Target Cell Percentage = Average number of target red cell counted / Total number of red cells counted in fields $\times 100$

Determination of proportion of sickle Hb in heterozygotes: The percentage sickle haemoglobin (% *HbS*) was determined in sickle heterozygotes (*HbAS*) according to the method of Al-Shakour and Al-Suhali (2002) as follows: Percentage *HbS* = $0.874 \text{ Hb} + 0.936\text{MCH} + 0.916$ Target Cell Percentage

Determination of percentage parasitaemia and parasite density: Percentage parasitaemia was determined by counting the number of infected RBCs in 1,000 RBCs in a Giemsa or Leishman stained thin blood film using a tally counter according to the method of Greenwood and Armstrong, (1991). The percentage parasitaemia was computed thus: Percentage Parasitaemia = Total number of infected RBCs / Total number of RBCs (infected and uninfected) x 100. Parasite density was estimated by counting the number of intra-erythrocytic parasites in 10,000 RBCs (approximately 36-45 oil immersion microscopic fields) (Greenwood and Armstrong, 1991). The parasite density per μl was then calculated as follows: Parasite density = RBC count / μl x Number of Parasites per 10,000 RBCs / 10,000

Determination of serum fasting blood sugar: Glucose reconstituted reagent (1.5 ml) was pipetted into clean cuvettes labeled blank, standard and tests and incubated for 5 minutes at 37 °C. Then 10 μl of standard and subjects serum were added to the respective cuvettes and incubated for another 10 minutes. The amount of coloured complex formed was proportional to glucose concentration and was photometrically (Lexington, USA) measured at the wavelength of 520 nm against the reagent blank. The glucose concentration in the subject's serum was then calculated using the formula: Fasting blood sugar = Absorbance of standard / Absorbance of test x Conc. of standard (mg/dl)

Statistical Analysis: Normally distributed data were compared using the paired t-test and correlation using Pearson correlation coefficient. The statistical package SPSS for window was used for analysis. A conservative P<0.05 was chosen for the level of significance.

Results

Annual frequency of malaria attack in sickle heterozygotes and dominant homozygotes: As per the data extracted from the questionnaires and hospital files of hospital attendees, 66.89 %, 18.92 % and 14.19 % of sickle heterozygotes (HbAS) were treated for malaria attack less than 2 times, 2 – 5 times and more than 5 times per annum respectively as compared with 16.83 %, 32.00 % and 51.17 % dominant homozygotes (HbAA) for the same period of time (Table 1).

Table 1: Number of annual malaria attacks per HbAA and HbAS genotypes

Hb genotype	Annual frequency of malaria attack						Total	
	< 2 times		2 – 5 times		> 5 times		N ^a	%
HbAA	52	12.38	109	25.95	259	61.67	420	100
HbAS	139	74.73	34	18.27	13	7	186	100

Malaria surveillance (December 2004 to November 2005): Malaria infection rate was relatively high with peak periods in April and October while June recorded the lowest incidence of malaria infection (Figure 1). Malaria attacked

every age group indiscriminately, though it was more severe in children less than five years old than in adults. The plasmodial species infections in different age brackets are presented in Table 2.

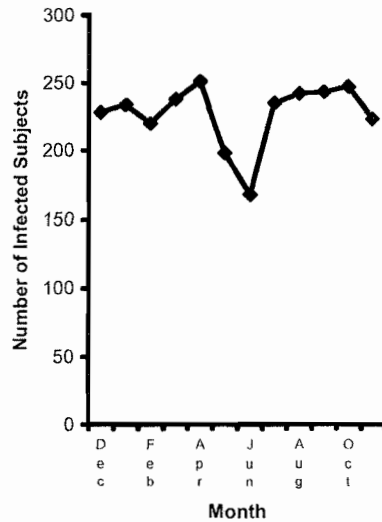


Figure 1: Monthly malaria surveillance within the period of study

Clinical symptoms during malarial infection in the cohort groups under investigation: Results of clinical examination showed that, a greater proportion of HbAA subjects suffered more frequent and more severe malaria attack when compared with sickle heterozygotes (HbAS). Sickle heterozygotes were also observed to have significantly fewer episodes of severe malarial anaemia and higher parasite density (Hb<7g/dl and >10,000 parasites/ μl) and also manifested severe clinical malarial symptoms less often than the normal HbAA subjects (Table 3).

Severity of malaria attack in different Hb genotype groups: More severe malarial anaemia episodes were significantly associated with HbAA than HbAS subjects, who in turn were associated with reduced risks of high-density parasitaemia (>10,000 parasites/ μl) than HbAA subjects (Table 4).

Haematological profile and parasitaemia in P. falciparum infected HbAA and HbAS subjects:

Table 5 compares the haematological profile of 420 HbAA and 186 HbAS subjects. It was observed that, Hb concentration, PCV, RBC and MCHC were significantly higher (P<0.05) in HbAS subjects than in HbAA individuals. PLT counts were also significantly higher (P<0.01) in HbAS than HbAA subjects. However, MCV, MCH and RDW were higher in HbAS than in HbAA subjects but not statistically significantly (P>0.05). The WBC counts, parasite densities, percentage parasitaemia and annual frequencies of malaria attack were found to be significantly higher

Table 2: *Plasmodium* infection in different age brackets

Age	Sex	N ^o infected	Species infection		
			<i>P. falciparum</i>	<i>P. malariae</i>	Mixed infection (<i>P. f</i> and <i>P. m</i>)
<5 years	M	239 (25%)	236 (97.9%)	3 (1.3%)	3 (0.8%)
	F	169 (17.6%)	168 (99.4%)	1 (0.6%)	0 (0%)
5 – 18 years	M	160 (16.6%)	155 (96.8%)	3 (1.9%)	2 (1.3%)
	F	211 (22%)	207 (98.1%)	3 (1.4%)	1 (0.5%)
> 18 years	M	97 (10.1%)	93 (95.8%)	2 (2.1%)	2 (2.1%)
	F	84 (8.7%)	80 (95%)	3 (3.5%)	1 (1.5%)
Total		960	937 (97.6%)	15 (1.6%)	8 (0.8%)

Table 3: Clinical symptoms during *P. falciparum* infection in the cohort groups

Clinical characteristic	Crude incidence/1000 persons per month	
	HbAA (n = 420)	HbAS (n = 186)
Severe malarial anaemia episodes (Hb<7g/dl + >10,000 Parasitaemia)	9.5	-
All cause severe anaemia episodes (Hb<7g/dl + Any level parasitaemia)	21.43	10.75
High density parasitaemia episodes (>10,000 Parasitaemia)	11.9	5.37
Cerebral malaria episodes	13.0	2.8
Hypoglycaemic episodes	27.3	13.2
Coma episodes	7.6	1.4
Seizure episodes	2.9	-
Respiratory distress episodes	12.1	2.7
Severe headache episodes	60.2	35.9
Diarrhoea episodes	38.6	13.0
Severe vomiting episodes	46.2	20.8

Table 4: Severity of malaria attack in various Hb genotype groups

Hb (g/dl)	Severity	N ^o of cases observed per month	
		HbAA (n = 420)	HbAS (n = 186)
< 6	++++	9	2
6 – 8	+++	18	6
8 – 10	++	21	11
10 – 12	+	30	17
Parasite density (N ^o /μl)	Severity	N ^o of cases observed per month	
		HbAA (n = 420)	HbAS (n = 186)
> 10000	+++++	5	1
7000 – 10000	++++	9	3
3000 – 7000	+++	18	7
1000 – 3000	++	13	5
<1000	+	39	22

Table 5: Haematological parameters and parasitaemia in *P. falciparum* infected HbAA and HbAS subjects

Haematological indices / Parasitaemia	HbAA (n = 420)	HbAS (n = 186)	
Hb (g/dl)	11.1 ± 1.3	12.5 ± 2.3	*
PCV (%)	25.8 ± 5.4	29.1 ± 5.6	*
RBC (M/μl)	3.4 ± 0.7	3.9 ± 0.9	*
MCHC (g/dl)	43 ± 3.04	43.7 ± 1.4	*
PLT count (K/μl)	282.8 ± 104.7	303.1 ± 124.8	**
MCV (fl)	74.9 ± 0.5	75.3 ± 8.3	φ
MCH (pg)	32.4 ± 3.9	32.6 ± 2.1	φ
RDW (%)	13.5 ± 2.8	13.4 ± 2.0	φ
WBC count (K/μl)	7.4 ± 3.5	6.9 ± 4.05	*
Parasite density (N ^o /μl)	4,019 ± 467.1	1,037.6 ± 650.3	*
Av. annual frequency of malaria attack	≥ 4	≤ 2	*
% Parasitaemia	0.5 ± 0.2	0.2 ± 0.06	*

Values are presented as: Mean ± standard deviation; * = Significant (P<0.05) ** = Significant (P<0.01) φ = Not significant (P>0.05)

(P<0.05) in HbAA than in HbAS subjects. *P. falciparum* was found to infect both sickled and non-sickled cells. However, most of the parasites were found in non-sickled cells since very few cells were sickled. *P. falciparum* was seen to strived more in dominant homozygotes (HbAA) than in sickle heterozygotes (HbAS) as indicated by the high percentage parasitaemia and parasite densities.

Percentage sickle haemoglobin in heterozygotes: The percentage sickle haemoglobin (%HbS) was observed to vary from 28% – 46% in heterozygotes (Table 6).

Haematological profile of malaria infected sickle heterozygotes per percentage HbS: Table 7 showed significant differences (P<0.05) in the haematological parameters assayed in plasmodial

Table 6: Distribution of Sickle Heterozygotes according to Percentage HbS

Percentage HbS	Number	Percentage (%)
Groups A ($\leq 30\%$ HbS)	24	12.91
Group B (31 – 40% HbS)	53	28.49
Group C ($> 40\%$ HbS)	109	58.60
Total	186	100

Table 7: Haematological profile and parasitaemia of plasmodium infected sickle heterozygotes with different concentrations of sickle haemoglobin

Haematological /parasitaemia profile	Group A HbS% $<30\%$ (n = 24)	Group B HbS% 30 – 40% (n = 53)	Group C HbS% $> 40\%$ (n = 109)	
Hb (g/dl)	7.6 \pm 0.4	9.9 \pm 1.9	13.5 \pm 1.7	*
PCV (%)	18.6 \pm 1.6	24 \pm 5.5	31.1 \pm 4.8	*
RBC (M/ μ l)	3.6 \pm 1.0	3.4 \pm 0.8	4.0 \pm 0.6	ϕ
MCHC (g/dl)	41 \pm 1.3	41.4 \pm 3	44.2 \pm 4.4	*
Platelets (K/ μ l)	306 \pm 169.3	347 \pm 183.6	383 \pm 92.4	**
MCV (fl)	58 \pm 3.5	70 \pm 6.0	76.6 \pm 5.1	*
MCH (pg)	24 \pm 1.2	29.3 \pm 2.7	33.6 \pm 2.7	*
RDW (%)	16 \pm 0.9	15.6 \pm 2.9	12.2 \pm 1.2	ϕ
WBC (K/ μ l)	7.6 \pm 6.9	7.3 \pm 3.4	7.3 \pm 3.5	ϕ
Target cells (%)	0.1 \pm 0.05	0.3 \pm 0.18	0.5 \pm 0.2	*
HbS %	28.3 \pm 0.5	35.6 \pm 1.8	42.6 \pm 2.2	ϕ
Parasite density (N ^a / μ l Blood)	2,900 \pm 571.6	1,125 \pm 525.8	680.1 \pm 63.1	*
Av. annual frequency of malaria attack	> 4	2 – 3	≤ 1	*
% Parasitaemia	0.35 \pm 0.1	0.2 \pm 0.06	0.1 \pm 0.02	*

* = Significant ($P < 0.05$) ** = Significant ($P < 0.01$) ϕ = Not significant ($P > 0.05$)

infected sickle heterozygotes with different sickle haemoglobin concentrations. As sickle haemoglobin concentration increased, Hb concentration, PCV, MCV, MCH, MCHC and target cells % increased showing a direct relationship ($r = 0.57$, $p = 0.01$). The result further shows that as the concentration of sickle haemoglobin in sickle heterozygotes increases, RDW, WBC counts, the annual frequency of malaria attack, parasite density and percentage parasitaemia decreased showing an inverse relationship ($r = 0.07$, $p = 0.54$).

Discussion

The possible protective effect of sickle haemoglobin against plasmodial infection was investigated in the malaria endemic population of Bamenda in North West Cameroon, with 22.3% and 3.3% incidences of sickle cell trait and sickle cell anaemia respectively. These incidences are lower than those (29.9% and 3.91%) reported for Mamfe, South West Cameroon (Uzoegwu, personal communication, 2004). *P. falciparum* was the most prevalent (93.9%) malaria causing species in the population. The higher plasmodial infection observed in children under 5 years old (42.6%) than in individuals between 5 and 18 years and those above 18 years of age (38.6% and 18.8% respectively) could be attributed to the higher vulnerability of children to malaria infection. Adults could have mounted much stronger immune response to the plasmodial parasite than children. A higher susceptibility of males to malaria than females as observed in this study could be due to hormonal or genetic differences between the two sexes. The highest

malarial infection, which occurred in April, could be attributed to the prevailing rainy season, relatively high humidity and warm temperature in this month, which favours the breeding of anopheles mosquitoes. The low infection observed in June could be due to the characteristic unfavourable cold weather, usually associated with the month, which could militate against mosquito breeding. Sickle

gene seems to have less effect on malaria infection *per se*, since parasites were observed in both sickled and non-sickled RBCs. However, our observation of significantly lower ($P < 0.05$) parasite density, lower percentage parasitaemia, relatively lower frequency of manifested malaria attack and less severe clinical malarial symptoms in HbAS than HbAA subjects could suggest decreased susceptibility of HbAS subjects to malaria. This seems to suggest that malaria parasites survived more in the HbAA than in the HbAS subjects. Parasite density could be reduced in sickle heterozygotes by stronger immune response by the

reticuloendothelial system, which could have been provoked by the presence of the malaria parasite, during which the sickle haemoglobin condenses and polymerises in the red blood cells triggering sickling in HbAS RBCs. In this condition, both the parasites and the RBCs are removed by the reticuloendothelial system. The observation that some HbAA subjects had low frequency of malaria or did not suffer malaria at all could reflect the differences in immune defence, while some sickle heterozygotes (HbAS) suffered malaria frequently, could probably implicate the concentration of HbS or other genetic factors in the protection of individuals against malaria attack. This selective protection against severe malaria disease could be supported by the fact that sickle cell trait is associated with protection against severe malarial anaemia and high-density parasitaemia (Aidoo, 2002). The protection implied above could be due to impaired entry of plasmodial parasite into the red cells or the reduced proliferation of the parasite in the HbAS red cells.

Cohort HbAA subjects were observed to be more often admitted to hospital for the treatment of malaria than HbAS subjects at 5:1 ratio. Severe malaria disease is associated with a number of symptoms including intermittent fever, severe headache, diarrhoea, coma, respiratory distress, anaemia and hypoglycaemia (Berkley *et al.*, 1999). In this study, more episodes of severe malarial anaemia (Hb < 7 g/dl) and high parasitaemia ($> 10,000$ parasites/ μ l), cerebral malaria, hypoglycaemic episodes, coma, seizure, respiratory distress, severe headache, diarrhea and vomiting were observed in well nourished HbAA subjects compared to G6PD non-deficient sickle

heterozygotes. Since *falciparum* malarial mortality is related to Hb concentration and parasite densities (Field, 1949), *HbAS* subjects had lower malarial mortality rate compared to *HbAA* individuals. However, parasitaemia might be an unreliable index to the disease severity since severe disease may occur in the face of undetectable parasitaemia where the parasites are sequestered and their growth cycle is tightly synchronised. The severe anaemia episodes were more predominant in children below 5 years old while the cerebral malaria episodes were predominant in older children, in consonance with the observation of Snow *et al.* (1997). Coma, a prominent feature of severe malaria was seen only in an *HbAA* subject. Hypoglycaemic episodes, most common in children with severe malaria (Molyneux *et al.*, 1989) were more common in *HbAA* subjects compared with sickle heterozygotes though not significant. This may be due to increased consumption of glucose in the peripheral tissues by the parasites (Dekker *et al.*, 1997).

The significantly higher ($P < 0.05$) Hb concentration, PCV, RBC, MCHC, PLT counts but insignificantly higher ($P > 0.05$) MCV, MCH, and RDW in sickle heterozygotes compared with dominant homozygotes, of the same age bracket during malaria attack, substantiates the reports that relative increase in haematological indices of *HbAS* pregnant women may be a protective mechanism against malaria infection during pregnancy (Awodu *et al.*, 2002). The relatively lower levels of haematological indices in *HbAA* subjects could indicate excessive intravascular haemolysis of parasitised and unparasitised RBCs during *falciparum* infection, which could cause the observed severe anaemia. The lower platelet count observed in *HbAA* subjects may indicate a more severe plasmodial infection or parasite burden since thrombocytopenia may be useful as a sensitive but not specific marker of active infection, contributing to disease pathology in human malaria by platelets forming clumps with plasmodial infected erythrocytes (Lou *et al.*, 2001, Pain *et al.*, 2001). The significantly higher WBC count ($P < 0.05$) in *HbAA* compared to *HbAS* subjects during the progress of malaria could be indicative of a more acute malaria in *HbAA* subjects since more WBCs are mobilized. This suggestion is supported by the report that leucocytosis, monocytosis, increased neutrophil counts and increased number of circulating lymphocytes are associated with severe or acute malaria (Molyneux *et al.*, 1989).

The fact that a greater population (58.6%) of the sickle heterozygotes in Bamenda were observed to have sickle haemoglobin concentration above 40% could explain the observation of generally low parasite density, percentage parasitaemia and relatively lower frequency of malaria attack in most sickle heterozygotes. The observation that sickle heterozygotes with higher *HbS* content had higher haematological indices than those with lower proportion of *HbS* concentration is in consonance with the report of Al-Shakour and Al-Suhali (2002). The lower Hb concentration and PLT count as well as higher parasitaemia in heterozygotes with less than 30%

HbS concentration, is indicative of more severe malaria as observed compared to sickle heterozygotes with percentage *HbS* between 31–40% and those with percentage *HbS* greater than 40% who had higher Hb concentration, PLT count and lower parasitaemia, indicative of less severe malaria. This seems to confirm the reports of Uzoegwu (2001) and Uzoegwu and Onwurah (2003) in Uga and Nsukka Communities respectively that sickle heterozygotes with higher sickle haemoglobin content had lower frequencies of malaria attack and lower parasite densities. Sickle haemoglobin could therefore play a protective role during malaria attack. The presence of target cells in peripheral blood is characteristic of most haemoglobinopathies although not specific to them since target cells had been observed in liver disease and post-splenectomy conditions (Cheesbrough, 2000). In haemoglobinopathies, the presence of target cells is related to the presence of abnormal haemoglobin, in such instance, their occurrence may be the initial indication that abnormal haemoglobin is present. As such, as it is expected, the number of target cells increased as the amount of abnormal *HbS* increased. The positive correlation between percentage *HbS* and haemoglobin concentration and the negative correlation with parasitaemia is indicative of the fact that higher concentration of *HbS* in sickle heterozygotes is advantageous in protecting against severe malaria. The higher the percentage *HbS* the greater the protection afforded.

To understand clearly the protective role of sickle gene against malaria, it is suggested that, the mechanism of intravascular haemolysis of both parasitised and unparasitised red blood cells in plasmodial infection be elucidated, which is presently an on-going study.

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