J. of Bio. Res & Biotech. 1, (1), 97 - 114 (Jan/June, 2003)

Correlation of Lipid Peroxidation Index With Sickle Haemoglobin Concentrations In Malaria-Positive and-Negative Statuses of AA, AS and SS Individuals from the University of Nigeria, Nsukka Community

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Abstract
The incidences of different haemoglobinopathy genes were determined in 6406 volunteers from the University of Nigeria, Nsukka, community. Results revealed 3.81% and 29.04% incidences of sickle cell anaemia (SCA) and Sickle gene trait respectively in the community. The concentrations of plasma malondialdehyde (MDA), lipid peroxidation index, of a total of 260 selected normal homozygotes (HbAA), sickle heterozygotes (HbAS) and subjects with homozygous Sickle Cell Anaemia (HbSS), were further assayed when they were infected and then when they were not infected with *Plasmodium falciparum* (p.f) parasite. The correlaction of MDA concentrations and Sickle haemoglobin (HbS) doses of the genotypic groups revealed that the differences in MDA concentrations computed when the individuals were infected and when they were not infected were directly proportional to their corresponding doses of HbS in AA, AS and SS genotypic groups. This result was confirmed by the direct proportion exhibited by the mean plasma MDA concentration differences (MDACDs) of groups of heterozygotes during plasmodial infection and when they were not infected with their corresponding mean percentage HbS concentrations. The results further demonstrated that the percentage HbS concentration of various heterozygote groups were inversely proportional to their corresponding parasite densities and their annual frequencies of malaria attack. These results not only demonstrated the high incidences of SCA and trait in the university community but also could explain further why most sickle heterozygotes suffer less malarial attack than the normal heterozygotes as well as explain the variations usually observed in the frequency of malarial attack among sickle gene carriers.

Key words: Sickle cell disease, Malaria, Sickle Hb concentration, Lipid peroxidation

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Introduction

Sickle cell anaemia, which presents haemolytic anaemia and vasocclussive episode, is one of the most prevalent disorders in Africa. (Charache, Lubin & Reid 1989, Nigeria, being the most populous country in Africa, is known to have the greatest number of sickle cell disease patients in the world (Santer, 1976). Sickle cell anaemia (SCA), the most lethal type of sickle cell disease (SCD), is caused by mutant forms of Beta-globins (HbSS) in which neutral amino acid, valine (Val), replaces an acidic amino acid, glutamic acid (Glu), in the sixth amino acid sequence of the Beta-globin chain (β 6Glu ---> val) of haemoglobin (Ingram, 1958). Two mutant haemoglobins (HbSS), inherited one from each parent, could polymerise under reduced oxygen environment or during infections to form higher order aggregate which could grow, distort and rigidify the egg-shape of normal red blood cells into sickle shape. The consequence of this is that the sickle rigid red blood cells could stick to the endothelium of the blood vessels and block the microvasculature to affect the normal flow of blood and consequently prevent further supply of blood to the tissue or organ affected. This event could lead to organ or tissue damage, pains and perhaps death (Kim-Shapiro, et. al., 1999). Malaria, on the other hand, is widely spread in Nigeria, as it is in other countries of Africa. It is caused by intracellular protozoon parasite, *Plasmodium species* transmitted by female anophele mosquitoes when they suck blood from their victims.

Sickle gene carriers, with the haemoglobin (Hb) genotype of AS, are known to be protected against malaria attack (Allison, 1956; Fleming et., al., 1979; Luzzatto, 1979; Young et. al., 1990). Even within the heterozygotes, there are variations in their protection against plasmodial infection (Uzoegwu, 2001). The mechanism by which this selective protection is achieved is unclear. Some scientists attribute the protection to the ability of HbS to retard or impede the development of intraerythrocytic parasities (Pasvol, 1980). Other scientists favour the phagocytic action of activated macrophages against abnormally formed sickle erythrocytes during sickle cell crisis or during parasite infections (Friedman, 1978; Luzzatto, 1979), while yet others implicated phagocytosis-induced oxidative burst, generated during plasmodial infection or sickle cell crises (Clark and Hunt, 1983; Ochenhouse and Shear, 1984; Shocker et. al., 1986; Eze, 1991; Delmas-Beauvieux et. al., 1995). Some other scientists involved genetic factors (Luzzatto and Pinching, 1990).

Excess amounts of reactive oxygen species (ROS) are known to be produced in SCA (Stern, 1993; Gladwin et. al., 1999) and in malarial state (Hunt and Stocker, 1990; Eze, 1991; Mishra *et. al.*, 1994) as well as many other disease conditions (Smith, 1989; Durosinmu *et. al.*, 1991; Gladwin *et al*, 1999) and even in normal individuals (Halliwell and Guttridge, 1992). Some other scientists had also demonstrated enhanced levels of malondialdehyde, the main by-product of lipoperoxidation (Livrea *et. al.*, 1998), in the sera of plasmodium-infected SCA patients and normal subjects when compared to those not infected (Uzoegwu, 2001).

The role of reactive oxygen species in diseases are complex (Hancock et. al., 1997), ranging from immune surveillance of the host (Kwon et. al. 1991; Karupiah et. al., 1993) to damages to host tissues (Eze, 1991; Radi *et. al.*, 1991). The destruction of intra-erythrocytic

malaria parasites by ROS may result in the protection of the host whilst the destruction of the host erythrocytes could lead to disease pathology.

However, none of these proposed mechanisms of protection of sickle heterozygotes against malaria attack was conclusive in explaining the reasons why sickle gene carriers suffer less malaria attack usually observed within the heterozygote group. This study is therefore an attempt to throw more light into the reasons for which heterozygotes are protected against malaria attack compared to the HbAA individuals as well as to further attempt to proffer reasons for the variation always observed in the frequency of malaria attack among the sickle gene carriers.

Materials and Methods

Study Plan

Haemoglobin electrophoresis and blood antigen tests were performed with 6406 randomly collected blood samples from volunteer - residents of the University of Nigeria, Nsukka Campus, who attend the University of Nigeria Medical Centre Clinic. Two thousand four hundred and sixty (2460) volunteers made up of 1383 males (AA=897, AS=425, SS=28 SF = 23), age range, 6-35 yars, and 1079 females (AA=727, As =312, SS=17, SF=23), Age range 13-31 years, were screened for the presence of plasmodial parasite infections in their blood. The extent of lipid peroxidation, indexed by serum malondialdehyde (MDA) level, was evaluated in 260 male and female individuals (AA = 51, As = 166 SS = 43) age range, 18-32 years, selected from 2460 volunteer who showed monoinfection with plasmodium falciparum. Malondialdehyde was determined in these individuals during plasmodial infection and then when uninfected. In addition, sickle harmoglobin (HbS) concentration for each heterozygote and parasite loads were determined for 160 out of the 166 sickle heterozygotes, while their frequencies of malarial attack were monitored for twelve months. The 160 sickle heterozygotes selected, were made up of 112 males, age range 18-32 years and 48 females, age range, 19-30 years.

The selection of the 160 volunteer heterozygote was based on accessibility of and proximity to the individuals as well as their willingness to allow the monitoring of plasmodial infection for one year. The selected heterozygotes were grouped into three (A=19-30%, B=31-40% and C=41-50%) based on the HbS percentage concentration assayed for each individual sickle gene carrier (table 4).

Collection of Blood Samples

Blood samples (5ml) were drawn from individuals by venipuncture into glass tubes containing heparin as anticoagulant. Haemolysates for genotying were prepared from 1ml of whole blood by mixing the blood with equal volume of distilled water to lyse the cells and release the haemoglobin types to be separated. Plasma was prepared from the remaining quantity (4ml) of blood by centrifugation at 3000g for 10 minutes with Hittich centrifuge. The golden

yellow top layer which showed no sign of haemolysis was collected, used immediately or stored in deep freezer in aliquots of 120ul until used.

Diagnosis of Malarial Parasite Infection

Plasmodial parasite infection was diagnosed by clinical examination for malaria symptoms in individuals and by parasitological method of examination of Giemsa stained thick and thin blood films for plasmodial parasites. Geometric mean of parasite density per microlitre of blood was determined for 99 males and 46 females with plasmodium falciparum monoinfections based on individual count (WHO, 1991).

Haemoglobin Electrophoresis

Different haemoglobin types of haemolysates prepared from 6406 blood samples were separated by haemoglobin electrophoretic method of Evans (1971), using cellulose acetate strips as support and working Tris-EDTA-Borate buffer (pH 8.9) as running buffer. Different haemoglobin standards were used as controls. The separated haemoglobin spots were visualised directly as pink spots or otherwise stained and then the cellulose acetate paper destained with distilled water until the background is white. In some cases, the haemoglobin spots were also visualised directly as pink spots.

Percentage Levels of HbA and HbS in Carriers

Percentage levels of HbA and HbS in sickle gene carriers were estimated by elution method of Baker and Silverton (1985) as outlined below with slight modifications.

Haemoglobin electrophoresis was performed with the haemolysates of the selected individuals who had shown HbAS genotype. Ten microliters of each haemolysate was spotted, in duplicate, on cellulose acetate strip and the electrophoresis performed. After the electrophoretic separation of the haemoglobin types, the HbA and HbS bands were cut off from the strip and individually eluted with 1ml of phosphate buffered saline (BDH, England) for 30 minutes. The cellulose acetate cuts were then removed from the solution. Two hundred microliters (200nl) Drabkins solution (0.5g Potassium Ferricyanide, 0.1254g Potassium Cyanide and 1.4g Potassium dihydrogen Phosphate, (Sigma) dissolved in one litre of distilled water), were added to each eluate to develop a characteristic yellowish green colour after 30 minutes incubation at room temperature. The optical density of the coloured solution was detected at 540nm using a mixture of equal volumes of Drabkins solution and PBS as blank. Equivalent concentrations of haemoglobin types were extrapolated from the standard curve constructed with bovine haemoglobin standard (sigma). The percentage levels of HbA and HbS were then computed from the results.

Lipid Peroxidation Assay

Lipid peroxidation assay was performed using the thiobarbituric acid (TBA) spectrophotometric method of Albro and his co-workers (1986) as modified by Das and his colleagues (1990) and as summarised below.

Standard curve was first constructed using serial dilutions of 1, 1', 3, 3' - tetraethoxypropane (TEP) (sigma). TEP reacts with hydrochloric acid to release malondialdehyde (MDA), the main aldehydic product of and index for lipid peroxidation (Delma-Beauvieux et. al., 1995; Jiankang et. al., 1997). MDA reacts with thiobarbituric acid, in acid condition, to yield a red chromogen (Yeo et. al., 1994) which is extracted with butan-1-ol. The optical density of the chromogen (2ml) was read at 532nm against butan-1-ol background. The concentrations of TEP dilutions were then plotted against the respective optical densities (1/100 dilution of 24.6mg/ml TEP stock solution contained 2.46mg MDA/ml or 15nmol MDA) (Albro et. al., 1986).

The MDA content of each plasma sample was evaluated with 5µl plasma by mixing with 10% ($^{w}/_{v}$) trichloroacetic acid (TCA), incubated for 10 minutes and then added in sequence, 1.25ml 0.05M H_2SO_4 and 1.5ml 0.67% TBA. The assay mixture was thoroughly mixed, incubated in boiling water for 1 hour and then cooled under running tap water. The thiobarbituric acid reactive substances (TBARS), mainly MDA, was finally extracted by mixing with 2ml butan-1-ol, and centrifuging the assay mixture at 3000g for 10 minutes. The optical density of the top butan-1-ol layer (extract) was immediately read at 532 against butan-1-ol background. The MDA concentration of each sample was extrapolated from the standard curve.

Table 1: Distribution of genotype groups, gene frequencies of A, S, C and F and population probability in the University of Nigeria population (Gene frequencies were calculated according to the method of Burns (1970). Probabilities of occurrence of Hb. Genotypes are calculated using the equation $(P + q)^2 = 1$, (Udeozor and Kaine 1986) , p, q, r, x = frequencies of HbA, HbS, HbC and HbF).

Genotype	Number	Incidence (%)	Genes	Gene Frequency	Population Probability	
AA	4287	66.92	A	0.8149	P^2	0.6641
AS	1860	29.04			2(Pq)	0.2780
AC	6	0.09	S	0.1706	2(Pr)	1.14×10^{-2}
SS	79	1.23			q^2	2.91×10^{-2}
SF	165	2.58	C	0.0007	2(Px)	4.40×10^{-2}
SC	.3	0.05			2(qr)	1.20×10^{-4}
CC	0	0.00	F	0.0129	r^2	4.90×10^{-7}
3 band	6	0.09	3 Band	0.001		
Total	4406	100		1		

Results

From Table 1, it could be observed that the incidences of sickle cell anaemia (SS) and sickle gene trait (AS) were 3.81% and 29.04% respectively. However, the sickle cell subjects with persisting foetal haemoglobin showed an incidence of 2.58% while those without foetal haemoglobin exhibited the incidence of 1.23%. HbC gene carriers were identified in only 0.09% of those tested. While sickle haemoglobin C disease (SC disease) revealed an incidence of 0.05%, haemoglobin CC disease was not identified in the community at all, confirming the rarity of this disease in the eastern states of Nigeria (Lehmann and Nwokolo, 1959).

Table 2. Prevalence of Malaria and Percentage specie infection in male and female volunteers from the University of Nigeria, Nsukka Community

Sex	Geno type	No Tested	No of. Mal. Positive	Percentage of Malaria positive (%)	Specie Infection					
					P. f	Per centage Infection (%)	P.m	Per-centage Infection (%)	Pf + Pm	P e r - centage Infection (%)
Male										
	AA	897	535	59.64	501	55.85	11	1.23	23	2.56
	AS	425	121	28.47	115	27.06	2	0.47	4	0.94
	SS	28	23	82.14	23	82.14	0	0	0	0
	SF	33	27	81.82	26	78.79	0	0	1	3.03
Total		1383	706	51.05	665	48.69	13	0.94	28	0.02
Female										
	AA	725	337	46.48	324	44.69	1	0.14	12	1.66
	AS	312	87	27.88	83	26.60	1	0.32	3	0.96
	SS	17	11	64.71	11	64.71	0	0	0	0.0
	SF	23	12	52.17	23	64.71	0	0	0	0.0
Total		1077	447	41.43	441	100	2	0.19	15	1.39
G r a n d Total		2460	1153	46.87	1106	40.87	15	0.61	43	1.75

Malaria Parasite Infection

The results presented in Table 2 show that 46.87% of the 2460 volunteers tested in the community revealed the presence of plasmodial parasites in their blood. The male percentage infection rate (51.05%) was observed to be greater than that of the female (41.43%) giving an impression that males were more susceptible to plasmordial parasite infection than females. Table 2 also shows that 44.96% and 0.61% of the population revealed monoinfections with P*lasmodium falciparum (P.f)* and *Plasmodium malariae* (P.m) respectively while mixed infection with P*f* and *Pm* recorded a prevalence of 1.75%.

Plasmodium falciparum therefore is the major plasmodial specie that caused malarial infection among the people of the community. It was also observed that both male and female sickle heterozygotes (genotype HbAS) appeared to be less susceptible to plasmodial infection than normal homozygotes (Genotype HbAA). Furthermore, most of the subjects with SCA were infected with *P.f* parasite indicating the fragile susceptibility of sickle cell anaemia patients to malaria parasites infections. However, the SCA subjects with persisting foetal haemoglobin seemed to be slightly less susceptible to plasmodial infection than those without any foetal haemoglobin. In addition male subjects with SCA, displayed higher susceptibility to malaria than female SCA patients.

The data presented on table 4 showed that female heterozygotes have reduced parasite load as well as reduced annual frequency of malaria attack than their male counterparts.

Concentration of Sickle Haemoglobin in Carriers

The evaluation of the percentage concentration of HbS in 112 male and 48 female sickle gene carriers (HbAS) revealed a range between 19.50% and 46.13% HbS in the heterozygotes of both sexes. This result is at variance with a report that HbS concentration in an individual never exceeded 45% of the total Hb of the carrier. (Fleming, et. al., 1979). However the result is in consonance with that reported by Okafor in 1995, in which 46% HbS was the maximum reported for Obowo community, Imo State, Nigeria. The mean HbS percentage concentration for each group, A to C, was computed to be A= 22.62, B=36.15 and C = 42.95% (Table 4). normal haemoglobin percentage concentration was revealed to be between 53.77% and 79.75% HbA.

The result of the mean parasite load and the frequencies of malaria attack monitored for one year, determined for each of the heterozygote groups, revealed that group A with the lowest HbS percentage concentration manifested the highest parasite load and highest frequency of malaria attack, whilst group C heterozygotes with the highest mean HbS level exhibited lowest parasite load and least frequency of malaria attack.

Table 3: Mean MDA concentration difference (MDACD) between *P.f.*-infected subjects and when they were not infected (Number in bracket indicates number tested, values indicate mean MDA ± SD of values of the number tested)

Status of *Plasmodium falciparum* (P.f) infectionAA	AA	AS	SS
	[redacted]	nM. MDA	
P.f.-negative	3.78 ± 0.08(76)	4.58 ± 0.11(100)	6.17 ± 0.06 (50)
P.f.-positive	4.05 ± .12(71)	6.22 ± 0.06(92)	9.15 ± 0.8(32)
MDACD	0.27	1.64	2.98
Sickle Dose	0	1	2

Lipid Peroxidation

The result of the lipid peroxidation assay for male and female volunteers revealed that MDA concentrations of all the genotype groups were elevated at plasmodial infections when compared with the values at no infection. Furthermore, the mean difference between the MDA levels at plasmodial infection and the values at no infection was highest in sickle cell anaemia subjects (HbSS) than the values observed for other genotype groups. The mean MDA concentration difference (MDACD) for sickle cell anaemia subjects was computed to be highly significant ($p < 0.01$) when compared with the mean value computed for normal subjects (HbAA) and also significantly higher ($p < 0.05$) than the mean value computed for sickle heterozygotes. MDA concentration difference between *Plasmodium falciparium* – positive and -*P. f* negative situations exhibited direct proportionality with HbS doses of AA, AS and SS individuals (Table 3, fig 1). In addition, female heterozygotes exhibited lower MDACD than male counterparts (fig. 2). Furthermore, the mean sickle haemoglobin concentrations of the three heterozygote groups increased with the increase in MDACD, while their corresponding parasite loads and the annual frequencies of malarial attack decreased appropriately (Table 4).

Table 4: Group of Heterozygotes (A-C) (based on HbS percentage conc. ranges), their corresponding lipid peroxidation index, parasite load and annual frequency of malaria attack. (Number in bracket indicates the number of heterozygotes tested).

Group	Mean Percentage HbS conc. (%)		MDA (nM MDA) *P.f.*-Negative	MDA (nM MDA) *P.f.*-positive	MDACD (nM MDA)	Parasite (No./ul Blood)	Annual Frequency of Malaria attack
A	22.62	M	3.23 ± 0.18(18)	3.49 ± .02(52)	0.26	5.1×10^3	>6
		F	2.67 ± 0.8(9)	2.75 ± .06(31)	0.18	4.1×10^3	>4
B	36.15	M	4.71 ± .12 (29)	5.43 ± .04(42)	0.62	1.3×10^3	2-4
		F	3.86 ± 0.1(11)	4.40 ± 0.10(30)	0.54	0.6×10^3	2
C	42.95	M	6.34 ± 0.13(42)	7.37 ± 0.21(42)	1.03	2.5×10^2	≤1
		F	5.67 ± .07(23)	6.52 ± 0.14(23)	0.85	1.2×10^2	<1

Discussion

The incidences of sickle cell anaemia (3.81%) and sickle gene carriers (29.04%) as revealed in this study were not in accord with but higher than those reported by some scientists (Nwokolo, 1960; Kaine, 1982, 1983) among the Igbos of Eastern Nigeria. But these incidences compared well with those reported for the Sudan Savannah region of Gariki Community Kano District, Nigeria (Fleming et. al., 1979). The Igbos of Eastern Nigeria inhabits the University of Nigeria, Nsukka Campus since the bulk of the students and other residents are drawn from the eastern states of Nigeria since the observed incidences are high in this community, one would have expected a reduction of the incidence of sickle cell anaemia community considering the fact that the ignorance rate among the people could have been less in this enlightened community. It is therefore surprising to observe such unprecedented high incidence of sickle cell anaemia in this community considering their level of education and enlightenment that could have minimised haemoglobin incompatible marriages. The high incidence of sickle cell anaemia observed in this community could therefore confirm the observation of gross ignorance of SCA and its presentations by both elites and non-elites in Nigeria (Uzoegwu, 1995). However, the greater number of sicklers with persisting concentration of foetal haemoglobin (HbF), (2.58% as compared to 1.23% without HbF), explained the reason for many sickle cell individuals surviving up to the age range involved this survey, HbF could possibly ameliorate crisis in sickle cell subjects and hence make them live normal life (El-Shafei et. al., 1992). The high incidence of sickle gene carriers such as observed in this study could have given rise to higher incidence of sickle cell anaemia since the high risk of haemoglobin incompatible marriages are likely to exist among the members of this community observed to be sufficiently unknowledge of this disease. The low incidences of HbC gene carriers and SC diseases coupled with the absence of HbCC

disease observed in this study could confirm the rarity of HbC gene among Igbos of Eastern Nigeria (Nwokolo, 1960; Udeozor and Kaine, 1986).

Malaria is highly endemic in the study community, with the percentage prevalence of 46.87%, (1106/2460), 44.96% of the infection being caused by *Plasmodium falciparum*. This result is consistent with the observation that *Plasmodium falcriparum* is the major infecting plasmodial specie that causes malaria in West Africa (WHO, 1984). It was further observed that although the concentrations of sickle haemoglobin (HbS) were similar in both male and female heterozygotes, females seemed to have been less susceptible to malaria parasite infection as their parasite load and annual frequencies of malarial attack were less than those observed for their male counterparts. This observation is confirmed by an observation in this study that male subjects with SCA displayed higher susceptibility to malarial attack than their female counterpart these results could be explained by the fact that females were better immuned to malaria attack and a variety of other parasitic diseases than males (Brabin and Brabin, 1991). This seeming female advantage was attributed to hormonal or genetic difference or both (Landgraf et. al., 1994).

The result of the mean serum malondialdehyde concentrations evaluated for AA, AS and SS volunteers during *Plasmodium falciparum* infection compared to the values during no infection suggested increased lipid peroxidation during the progress of malaria. (Table 3: fig 2). This increase is not surprising as malaria parasites had been reported to induce oxidant stress in their hosts (Hunt and Stocker, 1990). Increased reactive oxygen species had been reported in plasmodial parasite infections (Clark, 1987; Eze, 1991; Mishra, et. al., 1994). Reactive oxygen species when in excess, could mediate lipid peroxidation as observed in this study. In addition, the MDA concentration differences MADCDs of different genotype groups increased with the rise in their sickle haemoglobin doses, being highest in sickle cell anaemia subjects who have double haemoglobin dose while the increase is least in HbAA individuals with no HbS dose at all.

Similarly, these results showed that the MDACD varies directly with sickle haemoglobin dose of a genotype group implicating HbS in lipid peroxidation process in malaria parasite-infected individuals. This result was corroborated with another result in this study in which MDACD were as well directly proportional to the mean HbS contents of different groups of heterozygotes.

The reasons for these observations could not be far-fetched given that sickle haemoglobin could negatively affect the plasmodial survival in the hosts, perhaps by way of activating macrophages which could kill the parasites by phagocytosis or the activated macrophages releasing hydrogen peroxide (H_2O_2) in a phagocytosis-associated respiratory burst (Ockenhoused and Shear, 1984). In addition the key event in the destruction of phagocytosed bacteria by polymorphonuclear leucocytes was the production of superoxide radical ($.0^-_2$) (Babior and Crawley, 1983). Superoxide radical can exert deleterious effects directly or by engendering more potent oxidants (Fridovrich, 1988) such as H_2O_2 and some other soluble factors such as alpha tumour necrosis factor (TNF-a) (Kwiatkowski, 1995),

Lymphokines (Ockenhouse and Shear, 1984), nitric oxide (NO) (Bank et. al., 1998) and its stable metabolic product, plasma nitrite (Rees et., al., 1995). These soluble factors had been known to be produced in sickle cell condition. Alpha Tumour necrosis factor had been demonstrated in blood of subjects infected with *Plasmodium falciparum* vivax (Kwiatkowski et. al., 1990; Karamaweera, et. al., 1992) and could also be induced by endotoxin-like antigens released by blood-stage parasites (Bentler and Grann, 1993). Lymphokines, observed to be elaborated in spleens of malaria parasite-infected mice (Ockenhouse and Shear, 1984) were found to stimulate normal macrophages for enhanced phagocytosis, to protect the host against malaria parasite (Schlichtherle et. al., 1996). Hydrogen peroxide (H_2O_2), produced during malaria or in sickle cell anaemia crisis, could subsequently generate more highly reactive hydroxyl radical (OH^o) (Play Fair et. al., 1985) or other radicals as final effect or molecules to mediate the killing of plasmodial parasites (Clark and Hunt, 1983), thereby reducing the parasite number which decreases parasite load in the host. Reactive oxygen intermediates had been known to destroy plasmodial parasites (WHO, 1984). The parasite killings could reduce the parasite number which decreases parasite load in the host. Reactive oxygen intermediates had been known to destroy plasmodial parasites (WHO, 1984). The possibility of the reductioin of the number of parasites by reactive oxygen intermediates could account for the reduced malarial parasite load observed in this study during enhanced lipid peroxidation process. In additional, *Plasmodium falciparum*-activated blood phagocytes were known to produce reactive oxygen intermediates (Kharazmi et. al., 1987) which may cause parasite death or damage to the host tissues when in excess, thereby contributing to the pathology of malaria. Excess ROS produced during malaria or sickle cell crises, could subsequently engage in lipid peroxidation of host tissues, thereby leading to the production of such products as malondialdehyde (Buffinton et. al., 1988). It is not therefore surprising to observe, in this study, elevated levels of MDA in AA, AS and SS subjects infected with *Plasmodium falciparum* parasite.

The highest mean value of MDACD observed in sickle cell anaemia subjects, when compared with the values in AA and AS individuals, could at least, in part, be attributed to the presence of the double sickle haemoglobin dose in sickle cell subjects. During plasmodial infection, oxygen content of the infected cells is greatly reduced. This apparent hypoxia could lead to the exposure of hydrophobic regions surrounding the sixth valine amino acid in the βeta-globin sub-unit (Gladwin et. al., 1999), leading to the precipitation of sickle haemoglobins and the subsequent sickling of the SS red blood cells. These events are minimized in AS subjects and non-existent in normal AA individuals. Sickle erythrocytes are more fragile than normal ones (Wyler, 1983). They are therefore, more liable to faster haemolysis than normal erythrocytes. Rapid haemolysis could result to precipitation of abnormal deposition of more ferritin-like iron which had been implicated in the enhancement of oxidative stress in sickle cell erythrocytes (Hebbel, 1990). Consequently, heightened lipid peroxidation could occur under enhanced oxidative stress. These facts may be supportive to the observation that sickle erythrocyte membrane manifested increased fanton activity than

normal red blood cell membrane (Hebbel et. al., 1982). Furthermore, excess reactive oxygen species had been known to be generated in sickle cell disease state (Stern, 1993; Gladwin et. al., 1999). As much as twice the number of superoxide radical was generated during the release of oxygen from oxyhaemoglobin than was released during the same process in normal oxyhaemoglobin A (Hebbel et. al., 1988). This release was associated with the oxidation of ferric ions and the release of more deleterious reactive oxygen species, hydroxyl radical (Wever et. al., 1973) in Fanton and Haber-Weis reaction. If therefore, more excess ROS are generated by SS erythrocytes than by normal AA erythrocytes, it is logical to think that higher oxidative stress could be created and hence more lipid peroxidation process could occur in sickle cell anaemia subjects than in normal individuals, thereby leading to the generation of higher MDA level in SS than in AA subjects as observed in this study.

The results presented in Table 4 and figure 2 indicate that group A heterozygotes with lowest sickle haemoglobin percentage concentration manifested not only lowest mean MDACD but also highest parasite density and highest annual frequency of malaria attack when compared with groups B and C heterozygotes bearing lower HbS percentage concentrations. Similarly, group C heterozygotes with highest sickle haemoglobin content exhibited highest mean MDACD but lowest parasite load and lowest annual frequency of malarial attack when compared with the values for groups A and B heterozygotes. It could, therefore, be deduced from these results that sickle haemoglobin content might have a direct variation with lipoperoxidation process but could inversely vary with parasite load and frequency of malarial attack. The presence of less plasmodial parasites in a host is more likely to cause less malaria attack than the situation in a higher population of plasmodial parasite. This investigation might have not only proffered further explanation why sickle heterozygotes are less susceptible to malarial attack than dominant homozygotes (HbAA) but also explained the variation, in malarial attack, usually experienced within sickle heterozygote group.

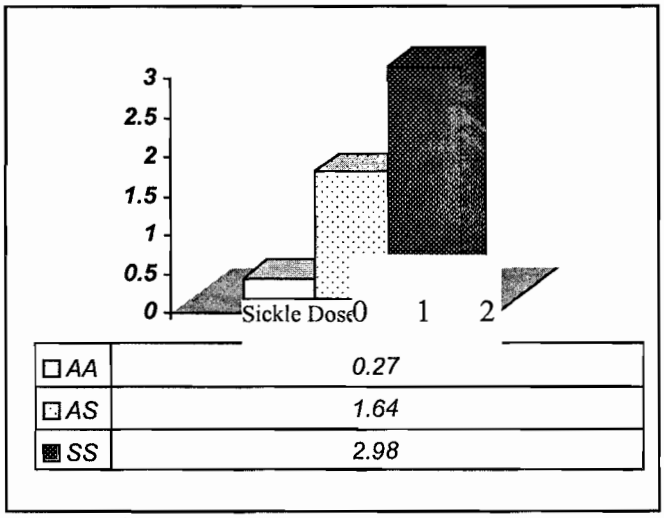

Fig. 1: Histogram showing differences in the levels of MDA when the subjects were infected with *Plasmodium falciparum* and when they were not infected (MDACD) plotted against sickle haemoglobin doses of AA, AS and SS subjects.

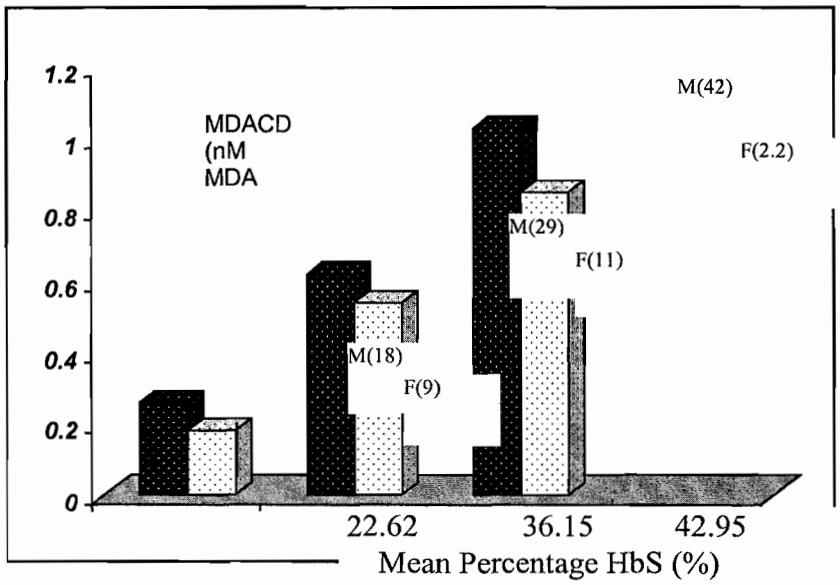

Fig. 2: Different groups of heterozygotes (A-C) against corresponding MDA Conc. Diff. Between P.f. - infected subjects and when they were not infected in males and in females (*Number in bracket represents the number of individuals tested).*

Acknowledgements

This investigation was fully supported with the generous grant from ICGEB, Italy, Grant Number CRP/Nig/96/018. The wonderful assistance given by Prof. A Falasche and Dr. Alex Ochem is highly appreciated. Mr. Alphosus Nneji of the Dept. of Microbiology, University of Nigeria Nsukka is sincerely acknowledged for the parasitological diagnosis of malaria parasites.

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