Full Length Research Paper

A possible protective role of glucose-6-phosphate dehydrogenase deficiency and sickle haemoglobin genes against severe malaria in Madonna University, Elele Community

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The high frequency of the enzymopathy, glucose-6-phosphate dehydrogenase (G6PD) deficiency and haemoglobinopathy, sickle cell haemoglobin (HbS) in malaria endemic regions is believed to be due to a heterozygous advantage against severe malaria. However, data to prospectively confirm the selective protection in different regions are lacking. In this study, the frequency of these genetic defects, their extent of interaction and contribution to severe malaria protection was investigated in Madonna University community. The methaemoglobin method was used to determine the G6PD status while haemoglobin genotype was determined using cellulose acetate paper electrophoresis with Tris-EDTA-borate buffer (pH 8.9). Information on the subjects' history, frequency and severity of the malaria attack was gleaned from structured questionnaires and hospital files. A total of 13.25% of the subjects were observed to be deficient in G6PD. For haemoglobin genotype, 67.0% were dominant homozygotes (HbAA); 32.0% sickle heterozygotes (HbAS) and 1.0% recessive homozygotes (HbSS). G6PD deficient and HbAS subjects experienced less frequent malaria attack and none of them suffered severe malaria (MP+++ and MP++) within the past one year. A significantly lower number of the G6PD deficient and HbAS subjects suffered mild malaria (MP+) compared to the normal subjects. Also, less severe clinical malarial symptoms were observed in G6PD deficient and HbAS subjects compared to the normal. These results seem to support the hypothesis that inheriting the G6PD deficiency gene and sickle gene as heterozygous reduces the profligacy of malaria parasite and hence, protects against severe acute malaria while having less effect on infection per se.

Keywords: G6PD deficiency, sickle heterozygous, malaria protection.

INTRODUCTION

Malaria a tropical disease is caused by the protozoa of the genus Plasmodium species and transmitted by the female anopheles mosquito (Tsuji et al., 2001). Severe malaria is a multi-system disorder, which may arise from multiple poorly understood processes including acute haemolysis of infected and uninfected RBC and dyserythropoesis, as well as through the interaction of malaria infection with other parasite infections and with nutritional deficiencies (Newton et al., 1997). Immune processes and genetic traits have contributed in reducing the profligacy of the malaria parasite and a wide range of genetic polymorphisms have been developed to modify individual response to this lethal disease (Flint et al., 1998). The high frequency of genetic defects such as the genes for glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell haemoglobin (HbS) in malaria endemic regions is believed to be due to a heterozygous (HbAS) advantage against severe malaria (Allison, 2002).

Glucose-6-phosphate dehydrogenase [G6PD, EC 1:1:1:49] in human is an X–linked enzyme which plays an important role in the generation of reduced nicotinamide

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adenine dinucleotide phosphate (NADPH), which is the only source of reducing power in red blood cells (RBCs), where it is required to maintain the redox equilibrium and in particular to detoxify hydrogen peroxide (H_2O_2) and other compounds via reduced glutathione (GSH) (Mason et al., 2007). Reduced glutathione also maintains haemoglobin and other red cell proteins in a reduced active form, and possibly enhance the ability of the cells to withstand oxidative damage, particularly during infection and it exposure to oxidant drugs (Lui et al., 1994; Cheesbrough, 2000). G6PD is distributed in all cells; however, in G6PD deficiency the cells other than RBCs do not have significant deficiency. G6PD deficiency is one of the most common X-linked recessive hereditary enzymopathies affecting the erythrocyte metabolism (Luzzatto, 2006), featuring non-immune haemolytic anaemia in response to a number of causes. Patients with G6PD deficiency develops hemolytic anemia during acute malaria infection and when treated with certain therapeutic agent such as antimalarials, antipyretics and antibiotics which have oxidant properties. Increased oxidative stress in G6PD deficiency cells is well documented (Lui et al., 1994; Udomsak et al., 2003).

Haemoglobinopathies (disorders of haemoglobin) are the most common monogenic diseases and benign mutations. There are hundreds of haemoglobin variants identified, of which only three HbS, HbC and HbE have reached polymorphic frequencies (Weatherall and Clegg, 2001). Sickle haemoglobin (HbS) is due to "topographical" error in the genetic code in which thymine replaces adenine (GAG → GTG) in the DNA encoding the beta globin gene; consequently valine replaces glutamate at the 6th position in the beta globin product (Koch et al., 2000). The gene for HbS is distributed widely throughout sub-Saharan Africa and countries with African immigrants, where carrier frequencies range from 5 – 40% or more. Allison (2002) hypothesized that the sickle cell gene arose spontaneously in malarious zones where *Plasmodium falciparum* is endemic. This suggests that the gene might have evolved naturally to protect against malaria (Modiano et al., 2001).

Researchers have put forward several protective mechanisms via which G6PD deficiency and HbAS status may confer selective advantage against malaria, however, none is conclusive. The overall objective of this study was to determine the prevalence of the enzymopathy and haemoglobinopathy in a Madonna University Elele community population and the degree of protection offered to HbS heterozygotes and G6PD deficient subjects against severe malaria.

MATERIALS AND METHODS

Subjects and Blood Sample Collection

The research work was carried out in Elele, a malaria endemic area of Rivers State, Nigeria. The Madonna University Teaching Hospital (MUTH), Elele, was used as the base for the study. Four hundred (400) subjects aged five (5) to over fifty (50) years attending the MUTH and other student volunteers were screen for G6PD deficiency and haemoglobin genotype. Clinical records and patient files of some patients were obtained from the hospital while some were contacted at personal level to fill a structured questionnaire to identify resistant individuals, frequency and severity of malaria infection and to establish the general level of awareness about the genetic defects. Blood samples were collected by venepuncture in heparinized and ethylene diamine tetracetic acid (EDTA) tubes for screening.

Determination of G6PD Deficiency status (Methaemoglobin method)

The G6PD deficiency status was determined according to the method of Cheesbrough (2000) with slight modifications. Briefly, three glass tubes were labeled test, normal and deficient. Then 50 µL of freshly prepared sodium nitrite-glucose reagent was put in the test and deficient tubes. Thereafter, 50 µL of methylene blue was also put in the tubes and 1 mL of blood sample was now added in all three tubes. The tubes were cocked, mixed gently and incubated at 35–37 °C for 3 hours. Haemoglobin is oxidised to methaemoglobin by sodium nitrite while the redox dye-methylene blue activates the pentose phosphate pathway, resulting in the enzymatic conversion of methaemoglobin back to haemoglobin in those red cells with normal G6PD activity. In G6PD deficient cells there is no enzymatic re-conversion to haemoglobin. After incubation, 5mL of distilled water was pipetted into three (3) new tubes also labeled test, normal and deficient and then 50 µL of the content of each tube was mixed with the distilled water. Samples of normal G6PD activity had colour of test solution similar to the red colour of the normal tubes. In reduced G6PD activity (G6PD deficiency homozygotes) the colour of the test solution was similar to the brown colour of the deficient tubes while subjects were heterozygote when the test colour was midway between normal G6PD and G6PD

Table 1. G6PD Deficiency in Male and Female Population in Elele Community

Sex	Total N$^{\circ}$ Investigated	Prevalence (%)	
		Heterozygous G6PD Deficiency	Homozygous G6PD Deficiency
Male	244	5.75% (23)	2.50% (10)
Females	156	3.25% (13)	1.75% (7)
Total	**400**	**9.00% (36)**	**4.25% (17)**

deficient of the homozygotes.

Determining Haemoglobin (Hb) Genotype

The haemoglobin genotype was determined by cellulose acetate membrane electrophoresis (CAME) as describe by Awah and Uzoegwu (2006). Briefly, haemoglobin lysates were spotted in triplicates at 0.5 cm of one end of well-dried cellulose acetate strips. Reference HbAS and HbAC haemolysates were spotted also as control. The spots were allowed to dry and the strips placed in an electrophoretic tank to form a bridge between the anode and cathode of the tank in such a way that haemoglobin bands migrate towards the anode. Both compartments of the tank contained the same working buffer–Tris-EDTA-Borate buffer (pH 8.9). A constant voltage of 150 V (2 mA) was applied through a Shandon power supply unit to achieve excellent separation within 5–10 minutes. After separation, the haemoglobin bands were visualized directly or otherwise stained for 2 minutes with 2% Ponseau S stain and then de-stained with distilled water. Pink spots of separated haemoglobin spots were observed upon white background of the cellulose acetate membrane. HbA migrated fastest followed by HbS then HbC respectively (Evans, 1971). The haemoglobin gene frequencies were calculated according to Burn's method (1976).

Malaria diagnosis

Subjects were tested for malaria parasite based on clinical malaria symptoms and confirmed by microscopic examination of Giemsa or Leishman stained thick and thin blood films. The presence of even one parasite per microscopic field indicated positivity for malaria parasite. The severity of parasitaemia was represented as MP+ (scanty), MP++ (moderately high) and MP+++ (very high or severe).

RESULTS AND DISCUSSION

The malaria hypothesis maintains that during pre-history, on average people without the G6PD deficiency and sickle cell gene died of malaria at a high frequency. The

results of the present study show a high prevalence of G6PD deficiency and sickle gene in the study population and seem to corroborate the malaria protection hypothesis.

Prevalence of G6PD Deficiency in Elele Community

G6PD deficiency was classified as completely deficient homozygous and slightly deficient heterozygous. Of the four (400) individuals screened for this enzyme deficiency, 347 (86.75%) had normal G6PD levels and 53 (13.25%) were G6PD deficient of which 36 (9.0%) were heterozygous and 17 (4.25%) were homozygous (Table 1). The high frequency of G6PD deficiency in the Madonna University, Elele community corroborates the role malaria play in the distribution of G6PD genes in most malaria endemic areas in the world (El-Hazmi and Warsy, 1994). Although the electrophoretic mobility was not carried out to ascertain the G6PD variant, the common African variant G6PD A^{-} (Beutler, 1994) was assumed. G6PD deficiency was associated with significant reduction in the risk of severe malaria for both G6PD deficient females and males in accordance with the reports of Uzoegwu and Awah (2006). G6PD deficient parasitised erythrocytes could therefore have been phagocytosised earlier thereby destroying the malaria parasite.

Haemoglobin Genotype Distribution and Frequency of Gene Mutation in Elele Community

Of the four hundred (400) subjects screened for haemoglobin genotype two hundred and sixty-eight (268) (67.0%) were dorminant homozygous (HbAA), one hundred and twenty-eight (128) (32.0%) were sickle heterozygous (HbAS), four (4) (1.0%) were recessive homozygous (HbSS), while none of the subjects had HbAC or HbSC genotype (Table 2). The haemoglobin gene frequencies of HbA, HbS and HbC in the Elele population were found to be 0.83, 0.17 and 0.00 respectively (Table 3). The incidences of sickle gene carriers and sickle cell anaemia were lower than that reported by previous studies in other Nigerian communities (Uzoegwu and Onwurah, 2003). This could be due to the fact that most of the subjects in study

Table 2. Incidence of Different Haemoglobin Genotypes in Elele Community

Haemoglobin Genotype	Number observed	% Incidence
AA	268	67.0
AS	128	32.0
AC	00	0.0
SS	04	1.0
SC	00	0.0
Total	**400**	**100%**

Table 3. Gene Frequencies

Haemoglobin Type	Gene Frequency	Percentage (%)
A	0.83	83.0
S	0.17	17.0
C	0.0	0.0
Total	**1**	**100**

Table 4. Annual Frequency of Malaria Attack

Hb Type	< 2 times	2 – 5 times	> 5 times
AA (n=268)	46.8%	32.1%	21.1%
AS (n=128)	70.9%	23.0%	6.1%
SS (n=4)	50%	50%	--

population are enlightened and genotype mismatch marriage might have been avoided. People with the two genes (HbSS) suffered from sickle cell disease (SCD) whereas heterozygote individuals (HbAS) were more resistant to falciparum malaria than normal dominant homozygous (HbAA) individuals. Balanced polymorphism is therefore at work because of the carrier advantage that allows the detrimental sickle cell allele to persist in the population. The rarity of HbC gene was confirmed in this study.

Annual Frequency of Malaria Attack in Different Haemoglobin Genotypes

Structural haemoglobin variants are said to contribute to malaria resistance in one way or the other. As per the data extracted from the questionnaires and hospital files of subjects, among the normal subjects (HbAA), 46.8% had no malaria attack at all or did suffer malaria attack less than two (< 2) times yearly, 32.1% suffered malaria attack 2–5 times per annum and 21.1% suffered malaria attack more than five (>5) time per annum. For the sickle heterozygotes (HbAS), 70.9% suffered no malaria at all or did suffer malaria less than two times per annum, 23.0% of subjects suffered malaria between 2–5 times

yearly and 6.1% subjects suffered malaria attack more than five times per annum. Also 50% of the sicklers (HbSS) suffered malaria less than twice per annum, while 50% of the sicklers suffered malaria attack 2–5 times per annum (Table 4). This indicates decreased susceptibility of HbAS subjects to *P. falciparum* malaria in accordance with the reports of Aidoo et al. (2002) and Uzoegwu and Awah (2006). The dominant heterozygotes were as likely to be infected with *P. falciparum* as those who lack this genotype but were less likely to have a high parasite density and considerably less likely to develop cerebral malaria and other severe malarial episodes. Although the precise mechanism for this protective effect is uncertain, there are plausible proposed mechanisms: haemoglobin variants could reduce parasite replication within red blood cells and enhance splenic clearance in parasitised erythrocytes (Shear et al., 1993). The proportion of HbS (25%–46%) in sickle cell trait also contributes in reducing malaria parasite density and the corresponding annual frequency of malaria attack (Uzoegwu and Onwurah, 2003; Uzoegwu and Awah, 2006). The rate of sickling of deoxygenated parasitised RBCs from heterozygous is shown to be two to eight times faster than non-parasitised cells at 50% O_2 saturation (Luzzatto et al., 1970). Haem accelerated sickling and early removal by spleen, therefore limiting the overall level of parasitaemia.

Table 5. G6PD Deficiency in Individuals with Different Hb Genotype

Hb Genotype / G6PD Status	Sex	Nº Observed	% Prevalence
HbAA + G6PD Def.	Male	24	6.00
	Female	17	4.25
HbAS + G6PD Def.	Male	9	2.25
	Female	3	0.75
HbSS + G6PD Def.	Male	0	0.00
	Female	0	0.00

Table 6. Severity of Malaria Attack in Various Hb Genotype / G6PD Deficiency States

Parasitaemia Severity	HbAA only (n=227)	HbAA + G6PD Def. (n=41)	HbAS only (n=116)	HbAS + G6PD Def. (n=12)	HbSS only (n=4)	HbSS + G6PD Def. (n=0)
MP+++	18	5	4	--	--	--
MP++	20	9	10	1	--	--
MP+	54	11	17	4	2	--
MP–	135	16	85	7	2	--

Frequency of G6PD Deficiency in Individuals with Different Hb Genotypes

Co-inheritance of both the G6PD deficiency and sickle gene could confer a better protection against malaria. The G6PD deficient subjects observed in this study were separated and correlated with their Hb genotypes (i.e. HbAA, HbAS, and HbSS) and further analyzed for their susceptibility to malaria infection and the severity of their malaria. G6PD deficiency was more common in the dominant homozygous (HbAA) subjects though the difference in the genotype groups was not statistically significant (p>0.05) in accordance with the findings of El-Hazmi et al. (1994) and Awah and Uzoegwu (2006). Of the 53 G6PD deficient subjects 41 (10.25%) were HbAA subjects while 12 individuals (3.0%) were HbAS. None of the recessive homozygote was G6PD deficient (Table 5). This therefore suggests that these malaria resistance mutations arose independently in the population in order the combat malaria. Both G6PD deficiency and sickle cell trait was found to co-exist in 3.0% of the population.

Malaria Severity in Subjects with Different Hb Genotype / G6PD Deficiency Status

The effect of G6PD deficiency on patients with sickle cell trait is controversial. Some reports claim that the combination is beneficial (Awah and Uzoegwu, 2006) while others have found no beneficial or adverse relationship (Steinberg et al., 1998). In this study, high malarial parasitaemia (MP+++) was significantly more common in subjects with genotype HbAA only, than those with genotype HbAA + G6PD deficiency. Overall, subjects with genotype HbAS was significantly associated with reduced risks of high-density parasitaemia than subject with genotype HbAA (Table 6). Subjects with HbAS only and HbAS + G6PD deficiency had significantly fewer episodes of severe malarial characteristics than subjects with HbAA only and HbAA + G6PD deficiency (Table 7). This affirms the fact that carriers of these single gene disorders are resistant to malaria as postulated by Kar et al. (1990) and confirmed by Awah and Uzoegwu (2006). G6PD deficiency was associated with significant reduction in the risk of severe malaria for both G6PD deficient female heterozygotes and male hemizygotes as reported by Allison (2002) and Awah and Uzoegwu (2006). The protective mechanism(s) however are still not clear. It could possibly be due to impaired parasite growth or more efficient phagocytosis of parasitised red cells at an early stage of maturation (Cooke and Hill, 2001). The plasmodium infected G6PD deficient cells are thought to either serve as an unsuitable host for the parasite or to behave as a "suicidal package" being rapidly sequestered in the spleen once it is infected (Luzzatto and Notaro, 2001).

Oxidative stress is also one of the main mechanisms for G6PD deficiency and malaria protection hypothesis. Increased oxidative stress in the G6PD deficient cells due to low levels of reduced glutathione, causes RBCs/parasites damage (Lui et al., 1994). Plasmodia also propagates oxidant stress by oxidizing erythrocytes reduced NADP+ thereby hindering the regeneration of

Table 7. Protective Effect of Sickle Gene and G6PD Deficiency Gene Against Malaria Morbidity

Clinical Characteristic	HbAA (n=227)	HbAA + G6PD Def. (n=41)	HbAS (n=116)	HbAS + G6PD Def. (n=12)	HbSS only (n=4)	HbSS + G6PD Def. (n=0)
Severe Fever Episodes	36.6%	14.8%	6.6	--	--	--
Cerebral Malaria Episodes	2.8%	1.4%	--	--	--	--
Respiratory Distress Episodes	7.7%	1.3%	0.8%	--	--	--
Severe Headache Episodes	34.4%	14.8%	2.5%	3.0%	--	--
Severe Diarrhea Episodes	13.5%	7.4%	4.5%	2.0%	--	--
Severe Vomiting Episodes	22.6%	10.4%	11.3%	1.1%	--	--
Severe Pains, Weakness and Dizziness Episodes	39.1%	15.7%	13.4%	4.2%	--	--
Sour Lips and Taste Episodes	30.1%	17.3%	13.4%	4.8%	--	--

reduced glutathione. Infected cells deficient in G6PD would therefore be expected to be impaired in their ability to counter these oxidant effects and to lyse prematurely.

CONCLUSION

The incidences of the G6PD deficiency and sickle cell genes in Madonna University, Elele community have been highlighted in this study. The findings confirm the fact that the inheritance of these single gene disorders has less effect on malaria infection per se, since both HbAS and G6PD deficient subjects did suffer malaria but at varying frequencies. HbAS was however, seen to be a more powerful malarial resistant factor than G6PD deficiency since normal subjects with HbAA + G6PD deficiency suffered more malaria attack, had higher parasite density and % parasitaemia than HbAS subjects. The mutations therefore were associated with protection against severe malarial episodes and high-density parasitaemia. Further research is however required to better understand the role of the G6PD deficiency and sickle genes on *P. falciparum* malaria.

REFERENCES

Aidoo M, Terlouw DJ, Koleza MS, McElroy PD, TerKuile FO, Kariuki S, Nahlen BL, Lal AA, Udbayakumar V (2002). Protective effects of sickle cell gene against malaria morbidity and mortality. Lancet. 359: 1311-1312

Allison AC (2002). The discovery of Resistance to Malaria of Sickle Cell Heterozygotes. Biochem. Mol. Biol. Educ. 30: 279-287

Awah FM Uzoegwu PN (2006). Influence of sickle heterozygous status and glucose-6-phosphate dehydrogenase deficiency on the clinico-haematolgoical profile of Plasmodium falciparum-infected children. Biokemistri. 18(2): 89-97.

Beutler E (1994). G6PD Deficiency: Clinical Manifestations, Genetics and Treatment. Blood. 84(11): 3613 – 3623.

Burn GW (1976). The Science of Genetics. 3rd Edition, Collier Macmillan Publications, London.

Cheesbrough M (2000). District laboratory Practice in Tropical Countries Part 2. University Press, Cambridge, pp 271-340.

Cook GS, Hill AVS (2001). Genetics of susceptibility to human infectious disease. Nat. Rev. Genetics. 2: 967-977.

El-Hazmi MAF, Warsy AS (1994). The Frequency of Glucose-6-Phosphate Dehydrogenase Phenotypes and Sickle Cell Genes in Al-Qatif Oasis. Annals of Saudi Med. 14(6): 491–494.

El-Hazmi MAF, Warsy AS, Bahakim HH, Al-Swailem A (1994). Glucose-6-Phosphate Dehydrogenase Deficiency and the Sickle Cell Gene in Makkah, Saudi Arabia. J. Trop. Pediatr. 40 (1): 12-16.

Evans DIK (1971). Haemoglobin Electrophoresis on Cellulose Acetate Using Whole Blood Samples. J. Clinical Pathol, 24: 877–878.

Flint J, Harding RM, Boyce AJ, Clegg JB (1998). The Population Genetics of the Haemoglobinopathies. London, England: Bailliere Tindall, W. B. Saunders, pp 1-51.

Kar BC, Agrawal KC, Panda A (1990). Sickle Cell Haemoglobin, G6PD Deficiency and Malaria in Western Orissa. J. Assoc. Physicians India, 38:555-557.

Koch AA, Olney RS, Yang Q (2000). Sickle Hemoglobin Allele and Sickle Cell Disease. Am. J. Epidemiol. 9: 839-845.

Lui TZ, Lin TF, Hung IJ, Wei JS, Chiu DTY (1994). Enhanced Susceptibility of Erythrocytes Deficient in Glucose-6-Phosphate Dehydrogenase in Alloxan, GSH-induced decrease in Red Cell Deformability. Life Sci. 55: l55-160.

Luzatto L, Nwachaku-Janett ES, Reddy S (1970). Increased sickling of parasitised erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. Lancet 1: 319

Luzzatto L, Notaro R (2001). Malaria. Protecting against bad air. Science. 293: 442-443

Luzzatto L (2006). Glucose 6-phosphate dehydrogenase deficiency: from genotype to phenotype. Haematologica. 91:1303–1306.

Mason PJ, Bautista JM, Gilsanz F (2007). G6PD deficiency: the genotype-phenotype association. Blood Rev. 21: 267-283.

Modiano D, Luoni G, Sirima BS, Langrancotti A, Petrarca V, Cruciani F, Simpore J, Ciminelli BM, Foglietta E, Grisanti P, Bianco I, Modiano G, Coluzzi M (2001). The Lower Susceptibility of Plasmodium falciparum Malaria to Fulani of Burkina Faso (West Africa) is Associated with Low Frequencies of Classic Malaria-Resistance Genes. Trans. Royal Soc. Trop. Med. Hyg. 95: 149–152.

Newton CR, Warn PA, Winstanley PA, Peshu N, Snow RW, Pasvol G, Marsh K (1997). Severe Anaemia in Children Living in a Malaria Endemic Area of Kenya. Trop. Med. Int. Health. 2:165

Shear HL, Roth EF, Fabry ME, Costantini FD, Pachnis A, Hood A, Nagel RL (1993). Transgenic mice expressing human sickle hemoglobin are partially resistant to rodent malaria. Blood. 81: 222

Steinberg MH, Lu ZH, Nagel RL, Venkataramani S, Milner PF, Huey L, Safaya S, Rieder RF (1998). Hematological Effect of Atypical and Cameroon Beta-Globin Gene Haplotypes in Adult Sickle Cell Anemia. Am. J.Hematol. 59:121-126.

Tsuji M, Elaine GR, Nussenzweig RS (2001). Progress towards a Malaria Vaccine: Efficient Indication of Protective Anti-Malaria Immunity. Biol. Chem. 382: 553-570.

Udomsak S, Srivucha K, Sombat T, Polsat W, Kobsiri C, Hla YM, Pannamas M, Nicholas JW, Victor R, Gary MB, Sornchai L (2003). Clinical Trial of Oral Artesunate with or without High Dose Primaquine for the Treatment of vivax malaria in Thailand. Am. J. Trop. Med. Hyg. 6:14-18.

Uzoegwu PN, Onwurah AE (2003). Correlation of Lipid Peroxidation Index with Sickle Haemoglobin Concentration in Malaria-Positive and Negative of AA, AS, and SS individuals from the University of Nigeria, Nsukka Community. J. Biol. Res. Biotechnol. 1(1): 97–144.

Uzoegwu PN, Awah FM (2006). Prevalence of sickle haemoglobin and glucose–6–phosphate dehydrogenase deficiency genes in the populations of north west and south west provinces, Cameroon. Animal Res. International. 3(3): 581-586.

Weatherall DJ, Clegg JB (2002). Genetic variability in response to infection: malaria and after. Genes Immun. 3: 331-337