PREVALENCE OF SICKLE HAEMOGLOBIN AND GLUCOSE–6–PHOSPHATE DEHYDROGENASE DEFICIENCY GENES IN THE POPULATIONS OF NORTH WEST AND SOUTH WEST PROVINCES, CAMEROON

UZOEGWU, Peter Nzeemndu and AWAH, Francis Mbuh
Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

Corresponding Author: Uzoegwu, P. N. Department of Biochemistry, Tropical Disease Research Laboratory, University of Nigeria, Nsukka, Email: peteuzoegwu@yahoo.com

ABSTRACT

Hereditary disorders of erythrocytes are common in many areas of the world, including Cameroon. Limited knowledge on the consequences of high incidences of sickle haemoglobin (HbS) and glucose-6-phosphate dehydrogenase (G6PD) deficiency genes in the Cameroons might have been responsible for the haemoglobin genotype mismatched marriages among the sickle heterozygotes and drug-induced anaemia among the G6PD deficient individuals ignorantly treated with oxidant drugs having high redox potential. The situation therefore, informed the random screening of the populace of the North West and South West populations of Cameroon for these genes with a view not only to reveal their current incidences and level of interaction but also to educate the people on the consequences of these genetic defects. Our results revealed the total incidences of 32.20 % sickle and 11.61 % G6PD deficiency genes. The percentage frequency of the sickle cell gene was higher in the South western (18.80 %) than in the North West (14.51 %) populations. The percentage incidence of G6PD deficiency was 9.21 % and 1.20 % for males and females respectively in the North West and 10.85 % and 1.46 % for males and females respectively in the South West. The interaction was not significant (P > 0.01) between G6PD deficiency and HbS for the North West and South West populations. These genetic defects must have reached polymorphic levels due to natural selection through survival advantage against death from malaria and consanguineous marriages.

Keywords: Sickle cell gene, G6PD Deficiency gene, Prevalence, Cameroon

INTRODUCTION

Human beings have interacted with malaria parasites for very long and thus the parasite has had ample time to adapt and evolve with the human host (Troye–Bloomberg *et al.,* 1999). 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. Haemoglobinopathies and glucose-6-phosphate dehydrogenase (G6PD) deficiency are among the most common single gene disorders, which affect red blood cell (RBC) stability and integrity (Kar *et al.,* 1990). More than 700 abnormal haemoglobins have been described world wide and more than 200 million people world-wide have RBC enzyme abnormality (Arya, 1995). These genetic lesions are major causes of morbidity and mortality around the world (Angastiniostis, 1995). Sickle haemoglobin and G6PD deficiency are genetically independent, their loci being located on chromosome 11 for sickle and chromosome X for G6PD deficiency genes.

Glucose-6-phosphate dehydrogenase [G6PD, EC 1:1:1:49; D–Glucose–6–phosphate: NADP Oxidoreductase (G6PD)] is a key enzyme in the pentose phosphate pathway (PPP) that is essential for adequate supply of phosphorylated nicotinamide-adenine dinucleotide (NADPH), which protects RBCs from oxidative stress. Reduced NADPH is needed to maintain glutathione (GSH), which in turn keeps the sulfhydryl groups of haemoglobin and other RBC proteins in a reduced active form. This activity enables the RBCs to withstand lysis from oxidant damage, instituted particularly during viral/bacterial or protozoa infections, or following exposure to oxidant drugs with high redox potential such as antimalarials (primaquine and pamaquine), sulfnamide, sulfamethoxazole and other drugs and chemicals and consumption of certain food stuff (fava beans) (Beutler, 1959; Lui *et al*., 1994; Cheesbrough, 2000).

Biochemical characterization has led to the identification of about 442 distinct G6PD variants, of which 299 were characterized and about 100 variants found to be polymorphic in various human populations (Beutler, 1990). Many of which have no haematological consequences. Commonly, however, intermittent episodes of haemolytic anaemia with or without chronic haemolysis may be associated with G6PD deficiency (Beutler *et al.,* 1996). Variants including the common G6PD-B (Gd^B) (wild-type), G6PD-A^+ (Gd^A) (non-deficient type) and G6PD-A^- (Gd^{A-})(deficient type) are observed in people living in tropical and sub-tropical areas (Beutler, 1994). Molecular basis of G6PD showed that both G6PD-A^+ and G6PD-A^- differ from G6PD-B by a variation at nucleotide 367 (A→G), while G6PD-A^- had an additional mutation at nucleotide 202 (G→A). G6PD A^+ is the most common variant found in 20% blacks Africans while G6PD-A^- variant is seen in 11% black Americans (Beutler, 1994).

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 position in the β-globin product (Koch *et al.,* 2000). Sickle gene is widely distributed in malarial belts of Africa, but its frequency varies widely in different West African populations (Allison, 2002). The prevalence of sickle cell disease (SCD) in Africa ranges from 1 – 10 % while the sickle heterozygous state range between 15 – 40.5 % in malaria endemic areas (American Academy of Family Physicians, 1994, Uzoegwu and Onwurah, 2003). The high frequency of HbS and G6PD deficiency genes in some malaria endemic areas parallels the historical incidence of malaria (Allison, 1954; Allison and Clyde, 1961). Sickle haemoglobin and G6PD deficiency are common in Central and West Africa, probably due to their advantage over malaria. Although, there has been published report on the incidences and interaction of the sickle and G6PD deficiency genes in many West African countries, little or no such reports exist for the North and South Western populations of Cameroon. This study therefore was aimed at providing such information, considering the consequences of these genes in the population.

MATERIALS AND METHODS

Study Area: Latitudinally, the North West (NW) province metropolitan area lies between 5º56´N and 5º58´N of the equator, and longitudinally, it falls within 10°9´E and 10º1´E, of the Greenwich meridian. The South West (SW) province lies between longitude 9°75'E and 10°E and Latitude 4°4' N and 5°75' N. Their populations make up the Southern Cameroons, which in 1961 left the Federal Republic of Nigeria to join the then Eastern Cameroon after a referendum and have a history and present of malaria endemicity (Figure 1).

Subjects: A total of 12,470 volunteer indigenes of the two provinces aged one to seventy (1 – 70) years, who sought treatment in the Provincial Hospitals and Health Centers, some secondary school students and many community volunteers in the neighbourhoods, were randomly screened for haemoglobin genotype while 6,540 were screened for G6PD deficiency. No blood transfusion had been administered to any of these subjects for at least three months before the tests. A questionnaire was designed to obtain a subject's name, age, sex, residence, consanguinity of parents and family history of blood diseases were recorded.

Blood Collection: Blood samples (5 ml) were collected by venipuncture into sample tubes containing EDTA (1.0 ± 0.15 mg/ml of blood) as anticoagulant and then rocked gently to mix and used for both haemoglobin genotype and G6PD deficiency determinations.

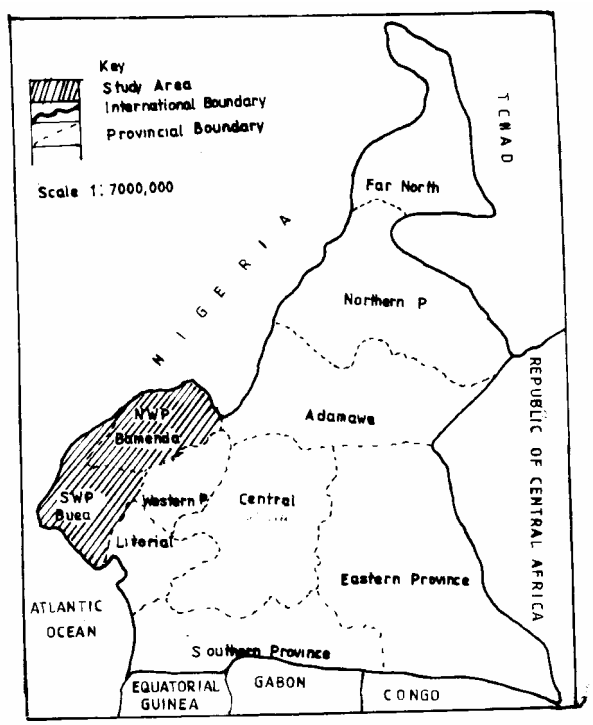

FIG.1: A MAP OF CAMEROON INDICATING THE STUDY AREAS SOUTH WEST AND NORTH WEST PROVINCES

Preparation of Haemoglobin Lysate and Haemoglobin Genotype Determination: Uncoagulated whole blood was centrifuged at 3,000 x g for ten minutes to separate the red blood cells from the 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) and then re-centrifuged at the same speed. The packed cells were re-suspended in an equal volume of normal saline. About 20 μl of the suspended cells was then mixed with 80 μl of distilled water in a 1:4 dilution to lyse the cells and release the haemoglobin. The solution was gently shaken for two minutes and then centrifuged at 3,000 x g for 20 minutes. The resulting supernatant, haemoglobin lysate, was used for the genotype test while the precipitate was discarded. The Hb lysate was stored in the refrigerator (2 – 4 ^{o}C) until used within four days. Alternately, 20 μl of uncoagulated whole blood was mixed with 60 μl of distilled water and the resulting haemoglobin lysate spotted directly for haemoglobin genotype determination. Haemoglobin genotypes were determined by cellulose acetate membrane electrophoresis (CAME) of Evans (1971) as modified by Uzoegwu and Onwurah (2003).

Determination of G6PD Deficiency: The reaction mixture contained glucose-6-phosphate (0.01M), $NADP^+$ (0.01 M), saponins (0.02 M), phosphate buffer, pH 7.4 and distilled water. The activity of G6PD was determined by fluorescent spot test as described by Beutler *et al.* (1996). Fluorescence was produced during the reduction of $NADP^+$ to NADPH.

This reaction is coupled with oxidation of glucose-6-phosphate to 6-phosphogluconolactone and catalysed by G6PD. Specimen with G6PD activity of <20 % of normal (severe deficiency) do not fluoresce as the small amount of NADPH formed is reoxidised by glutathione present in the reagent. Presence of fluorescence indicated normal cells while weak fluorescence indicated slight deficiency.

Data Analysis: The prevalences of sickle haemoglobin and G6PD deficiency were estimated from the carrier frequency using Hardy-Weinberg equation. Difference in gender distribution was tested using student's t–test, while ANOVA and Fischer's exact test were used for analysis of age distribution and interaction between the two genetic defects.

RESULTS

Sickle Cell Disease and G6PD Deficiency Awareness: The levels of awareness about SCD and G6PD deficiency in the studied populations, gleaned from the questionnaire answers and post-lecture questions, were extremely low [North West population (12.6 % : 0.3 %) and South West population (12 % : 0.1 %)]. The North West population therefore was more knowledgeable about SCD and G6PD deficiency genes than South West population.

Incidences of Sickle Cell Disease: The haemoglobin genotype distribution in 4,042 and 8,428 volunteers screened for haemoglobin shows that the North West population exhibited lower incidence of both sickle and HbC genes (26.15 % and 0.15 % respectively) while the South West manifested higher sickle and HbC genes of 35.32 % and 0.21 % respectively (Table 1).

Frequencies of Haemoglobin Gene Mutation: Table 2 shows the haemoglobin gene frequencies calculated according to the method of Burn (1976) by applying the Hardy-Weinberg equation. The ratio of the percentage gene frequencies of male and female is 0.9 : 1. The estimated birth incidence of children with HbSS, SC and CC were 2.1×10^{-3} (2.1/1000 live births), 2.0×10^{-4} (0.2/1000 live births) and 5.4×10^{-7} (0.00054/1000 live births) and 8.1×10^{-3} (8.1/1000 live births), 1.9×10^{-4} (0.19/1000 live births) and 2.5×10^{-7} (0.00025/1000 live births) for the North West and South West populations respectively (Table 3).

Prevalence of G6PD Deficiency: G6PD deficiency was classified as completely deficient homozygotes and slightly deficient heterozygotes. G6PD deficiency was detected in 252 NW subjects (10.4 %) and 507 SW subject (12.4 %) with male : female percentages of 9.2 % : 2.4 % and 10.8 % : 3.0 % respectively for the NW and SW populations respectively. Of the females, 0.7 % had severe enzyme deficiency (homozygotes) and 0.5 % had moderate enzyme activity in the NW population while 1.2 % had severe enzyme deficiency (homozygotes) and 0.3 % had moderate enzyme activity in the SW population (Table 5).

Interaction of G6PD Deficiency with Different Haemoglobin Genotype: Co-inheritance of G6PD deficiency and HbS genes was detected in 3 (3.79 %) and 5 (2.89 %) of NW and SW subjects respectively (Table 6). The interaction of the two genetic defects was calculated and found not to be significant ($P > 0.01$) for the NW and SW populations respectively.

DISCUSSION

Limited information is available on the current distribution of sickle and G6PD deficiency genes in most West African populations. This study provided the much needed information on the subject matter. The availability of adequate information on the subject matter could invariably help respective government health ministries to plan for adequate health care provision. In Cameroon, it was discovered, that the frequencies of occurrence of sickle and glucose-6-phosphate dehydrogenase deficiency genes were high. Consequently, the abnormalities manifest diverse adverse effects on the populations studied. The overall high frequency of sickle (32.20 %) and G6PD deficiency (11.61 %) genes revealed by this study was not surprising in view of common consanguineous marriages contracted in the study area as well as the high malaria endemicity usually associated with high frequencies of sickle gene (Allison, 2004). High HbS gene has been reported to be confined to populations living in malarious areas while low frequencies were seen in tribes living in areas with low malaria transmission (Allison, 1954). For instance, in Africa, high HbS gene frequencies are confined to the malaria belt north of South Africa as well as south of the Sahara while low frequencies occur in the non-malarious highlands in East Africa and parts of West Africa (Allison, 2002). The overall frequency of these genetic defects could not be compared with those of the other eight provinces of Cameroon, since no such data were available.

Haemoglobin C gene is known to be polymorphic in West Africa attaining heterogeneous frequencies approaching 20% in northern Ghana and Burkina Faso (Modiano *et al.,* 2001). The rarity of this gene in Cameroon as revealed in this study could be corroborated by the fact that its frequency declined in all directions from northern Ghana and Burkina Faso (Allison, 2002) to 0.49% in Nigerian population (Uzoegwu, 2006).

The high frequency of G6PD deficiency (11.61 %) in malaria endemic populations of Cameroon corroborates the role malaria play in the distribution of G6PD genes in most malaria endemic areas in the world (El-Hazmi and Warsy, 1994). The percentage gene frequencies for G6PD deficiency of hemizygous males were computed to be 9.21 % and 10.85 % for the NW and SW populations respectively. Although the electrophoretic mobility was not carried out to ascertain the G6PD variant, the common African variant G6PD A^{-} (Beutler, 1994) was assumed

Table 1: Haemoglobin Genotype Distribution in North and South Western Cameroon

Sex	AA		AS		AC		SS		SC		CC		Total
	M	F	M	F	M	F	M	F	M	F	M	F	
North West population	1,390	1,609	385	517	2	2	59	76	2	0	0	0	
Total incidence (%)	2,999		902		4		135		2		0		4,042
	74.20		22.32		0.10		3.33		0.05		0.0		
South West population	2,679	2,760	1,309	1,321	7	5	181	160	4	1	1	0	
Total incidence (%)	5,439		2,630		12		341		5		1		8,428
	64.53		31.21		0.14		4.05		0.06		0.01		
Sex total	4,069	4,369	1,694	1,838	9	7	240	236	6	1	1	0	12,470
Incidence (%)	32.60	35.04	13.60	14.70	0.07	0.06	1.93	1.90	0.05	0.01	0.01	-	100
Group total	8,438		3,532		16		476		7		1		12,470
Total incidence (%)	67.67		28.32		0.13		3.82		0.06		0.01		100

Table 2: Gene Frequencies in the NW and SW Populations

Haemoglobin Genotype	Gene Frequency and Percentage (%)	
	North West Population	South West Population
A	0.8541 (85.41%)	0.8116 (81.16%)
S	0.1451 (14.51%)	0.1880 (18.80%)
C	0.0007 (0.07%)	0.0005 (0.05%)
Total	1 (100%)	1 (100%)

Table 3: Population Probabilities for Haemoglobinopathies in the NW and SW Populations

Haemoglobin Genotype	Gene Frequency	Population Probability	
		North West Population	South West Population
AA	p^2	0.7295	0.6587
AS	2 pq	0.2479	0.3052
AC	2 pt	1.3×10^{-3}	0.0353
SS	q^2	2.1×10^{-3}	8.1×10^{-3}
SC	2 qt	2.0×10^{-4}	1.9×10^{-4}
CC	t^2	5.4×10^{-7}	2.5×10^{-7}

Table 5: Prevalence and Gene Frequency of G6PD Deficiency in NW and SW Populations of Cameroon

Sex	North West Population (%)	Gene Frequency (%)	South West Population (%)	Gene Frequency	Total	Gene Frequency
Males	9.21 % (223/2420)	0.0921	10.85% (447/4120)	0.1085	10.24% (670/6540)	0.1024
Females	1.20 % (29/2420)	0.0120	1.46% (60/4120)	0.0146	1.36% (89/6540)	0.0136
Severe	0.74 % (18/2420)	0.0074	1.2% (49/4120)	0.0119	1.02% (67/6540)	0.0102
Moderate	0.45% (11/2420)	0.0045	0.3% (11/4120)	0.0027	0.34% (22/6540)	0.0034
Total	10.41 % (252/2420)	0.1041	12.30 (507/4120)	0.1230	11.61% (759/6540)	0.1161

Table 6: Interaction of G6PD Deficiency with Different Haemoglobin Genotype

Hb Genotype	North West population			South West population		
		G6PD deficient			G6PD deficient	
	Nº Investigated	Nº	%	Nº Investigated	Nº	%
HbA	173	22	12.72	334	51	15.27
HbS	79	3	3.79	173	5	2.89
HbC	-	-	-	-	-	-

to be the variant in Cameroon. A greater proportion of male subjects were deficient compared to their female counterparts probably due to a higher inactivation of normal X-chromosome in heterozygous females. Since G6PD deficiency is sex linked, severe enzyme deficiency occurred in hemizygous males and homozygous females while heterozygous females have normal or moderately lower enzyme level (Beutler, 1990).

Plasmodial parasite densities were reported to be lower in G6PD deficient Tanzanian children than in children in other resident areas where chemoprophylaxis was not used against plasmodial parasites (Allison and Clyde, 1961). Furthermore, it was observed that G6PD deficient cells do not efficiently support malaria parasite growth in culture and that high frequency of G6PD deficiency are confined to malarious parts of the world (Allison,

2002). These observations are further supported with the high G6PD deficiency frequency as observed in this study. The significantly lower frequencies of sickle gene in the North West as compared to that in the South West populations of Cameroon may be attributed to differences in the environmental factors and malarial endemicity, rather than in the affinities defined by tribal (Foy *et al.,* 1954) and linguistic (Lehmann and Raper, 1949) factors or even blood group antigens (Allison, 1954) in the two populations. Similar occurrence of sickle differences was reported in the population of central Greece, which had 16.0 % malaria frequency (Choramis *et al.,* 1952) as compared to 32.0 % observed in the Chalkhide peninsular of northern Greece (Deliyannis and Tavlarakis, 1955). These areas were notorious for malaria before it (malaria) was controlled. But however, the former had lower malaria than the later populations (Allison, 2002). The higher incidence of sickle cell anaemia (4.05 %) manifested in the south western population of Cameroon, when compared with that of the north western population (3.33 %) could therefore implicate higher incidence of sickle cell trait in South West (31.21 %) then North Western (22.32 %) provincial populations as observed in this investigation. Higher frequency of sickle heterozygotes is capable of generating more haemoglobin incompatible marriages particularly in populations with limited knowledge of SCD and its control. A risk of the reproduction of children with sickle cell anaemia could be high from such mismatched marriages. Our results on the frequencies of the haemoglobinopathies in this study confirmed the rarity of HbC gene in Cameroon.

The population probabilities for HbAA, AS, AC, SS, SC and CC as shown in Table 7 indicated that one in every four hundred persons may be sickle cell anaemia patients while one in every five thousand persons could have HbSC disease and one in every two million could suffer HbCC disease in the NW population. In the SW population one in two hundred can suffer SCD, one in every six thousand persons could have HbSC disease and one in every four million could suffer HbCC disease. However, high rate of ignorance, consanguinity and other forms of intermarriages can disturb the Hardy-Weinberg equilibrium. Since this study was hospital-based, conducted on supposed malaria patients and subjects with minor illnesses, there may therefore be a slight difference when compared to the study in a general population devoid of any restrictions. Both genes were found to interact even though they are located on separate genes, in consonance with the reports of some scientists (Obaid *et al.,* 2001; Hassan *et al.,* 2003). Their interaction could possibly influence the survival of the carriers by protecting them against malaria. It is important for African clinicians to be aware of RBC disorders in their population. Known oxidative drugs linked to clinically important haemolysis in G6PD deficient subjects should be avoided unless G6PD deficiency has been ruled out. By keeping to this principle, unnecessary complications and unfruitful diagnostic evaluations may be avoided.

Recommendations: As effective management of SCD involves blood transfusion and iron chelating agents, may be too expensive for most developing countries particularly now that HIV infection is much higher. It is therefore cogent to assert that health education; knowledge of ones genotype through effective genotype screening should form a critical part of management. For the prospective control of sickle cell disease, proper enlightenment and genetic counseling on the disease are very essential. Health educators must create the awareness of the disease and methods of control and management in the population. Compulsory screening will probably inform people of their genotypes. SCD being an autosomal recessive genetic disorder, one of the important preventive measures is to avoid inbreeding between sickle heterozygotes. Education on the implication of glucose-6-phosphate dehydrogenase deficiency and the subsequent screening will ensure reduction in the problems associated with this enzyme deficiency, particularly in malaria endemic country like Cameroon.

ACKNOWLEDGEMENTS

We thank the Director of the Bamenda Provincial Hospital, Dr Wafuan, J. N. for allowing us access to the hospital facilities, and all the laboratory staff for their marvelous cooperation. We are greatly indebted to Rev. Sr. (Dr) Eunice Atsu and the other Sisters of Handmaid of Holy Child Jesus Congregation, stationed at Girls Secondary School, Okoyong, Mamfe for their wonderful sponsorship of the survey in Mamfe.

REFERENCES

ALLISON, A. C. (1954). The Distribution of the Sickle Cell Trait in East Africa and Elsewhere, and its Apparent Relationship to the Incidence of Subtertian Malaria. *Transactions of Royal Society of Tropical Medicine and Hygiene, 81:* 312 – 318.

ALLISON, A. C. (2002). The Discovery of Resistance to Malaria of Sickle Cell Heterozygotes. *Biochemistry and Molecular Biology Education*, *30:* 279 – 287.

ALLISON, A. C. (2004). Two Lessons from the Interface of Genetics and Medicine. *Genetics,* 166: 1591 – 1599.

ALLISON, A. C. and CLYDE, D. F. (1961). Malaria in African children with deficient erythrocyte G6PD. *British Medical Journal*, *5236:* 1346 – 1349.

ANGASTINIOITIS, M. MODELL, B., ENGLEZOS, P. and BOULYENKOV, V. (1995). Prevention and control of haemoglobinopathies. *Bulletin of World Health Organization,* 73: 375 – 386.

ARYA, R., LAYTON, D. M. and BELLINGHAM, A. J. (1995). Hereditary Red Cell Enzymopathies. *Blood Review*, 9: 165 – 175.

BEUTLER, E. (1959). The Haemolytic Effect of Premaquine and Related Compounds: A Review. *Blood*, *14:* 103 – 139.

BEUTLER, E. (1990). The Genetics of G6PD Deficiency. *Semin Haematology*, 27: 137 – 164.

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

BEUTLER, E., VULLIAMY, T. and LUZZATTO, L. (1996). Haematologically Important Mutations: G6PD, *Blood Cells and Molecular Diseases*, *22:* 49 – 56.

BURN, G. W. (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.

CHORAMIS, C., ZERVOS, N., CONSTANTANIDES, B., ZANNOS, L. and SKLAVOUNOVA, S. (1952). Sickle Cell Trait in the Lake Copais Region of Central Greece. *Schweitzer Medicine Wonchenchr*, *82:* 709 – 712.

DELIYANNIS, G. A. and TAVLARAKIS, N. (1955). Sickling Phenomenon in Northern Greece. *British Medical Journal, 4934:* 299 – 301.

EL-HAZMI, M. A. F. and WARSY, A. S. (1994). The Frequency of Glucose-6-Phosphate Dehydrogenase Phenotypes and Sickle Cell Genes in Al-Qatif Oasis. *Annals of Saudi Medicine,* 14(6): 491 – 494.

EVANS, D. I. K. (1971). Haemoglobin Electrophoresis on Cellulose Acetate Using Whole Blood Samples. *Journal of Clinical Pathology, 24: 8*77 – 878.

FOY, H., KONDI, A., TIMMS, G. L., BRASS, W. and BUSHRA, F. (1954). The Variability of Sickle Cell Rates in Tribes of Kenya and Southern Sudan. *British Medical Journal*, 4857: 294 – 297.

HASSAN, M. K., TAHA, I. J. Y., AL-NAAMA, L. M., WIDAD, N. M. and JASIM, S. N. (2003). Frequency of Haemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Barsa. *Eastern Mediterranean Health Journal*, *9(1/2):* 132 – 147.

KAR, B. C, AGRAWAL, K. C. and PANDA, A. (1990). Sickle Cell Haemoglobin, G6PD Deficiency and Malaria in Western Orissa. *Journal of the Association of Physicians of India*, *38(8):* 555 – 557.

KOCH, A. A., OLNEY, R. S. and YANG, Q. (2000). Sickle Haemoglobin Allele and Sickle Cell Disease. *American Journal of Epidemiology, 9:* 839 – 845.

LEHMANN, H. and RAPER, A. B. (1949). Distribution of Sickle Cell Trait in Uganda and its Ethnological Significance. *Nature*, 164: 494 – 495.

LUI, T. Z, LIN, T. F., HUNG, I. J, WEI, J. S. and CHIU, D. T. Y, (1994). Enhanced Susceptibility of Erythrocytes Deficient in Glucose-6-Phosphate Dehydrogenase in Alloxan, GSH-induced decrease in Red Cell Deformability. *Life Sciences. 55:* l55 – 60.

MODIANO, D., LUONI, G., SIRIMA, B. S., LANGRANCOTTI, A., PETRARCA, V., CRUCIANI, F., SIMPORE, J., CIMINELLI, B. M., FOGLIETTA, E., GRISANTI, P., BIANCO, I., MODIANO, G. and 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. *Transactions of Royal Society of Tropical Medicine and Hygiene*, *95:* 149 – 152.

OBAID, A. D., HASSAN, M. K. and AL-NAAMA, L. M. (2001). Sickle Cell and Glucose-6-Phosphate Dehydrogenase Deficiency Genes in Abu Al-Khasib District of Southern Iraq. *Medical Journal of Barsa University, 19:* 12 – 18.

TROYE–BLOOMBERG, M., PERLMANN, P., MINCHEVA, N. L. and PERLMANN, L. (1999). Immune Regulation of Protection and Pathogenesis in *P. falciparum* Malaria. *Parasitologia*, *41(1 – 3):* 131 – 138.

UZOEGWU, P. N. and ONWURAH, A. E. (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. *Journal of Biological Research and Biotechnology*, *1(1):* 97 – 144.

UZOEGWU, P. N. (2006). The Possible Role of Diet in Mitigating Oxidative Stress Induction during Plasmodial Infection. *Bio-Research*, 4(2): 201 – 206.