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The Effect of Glucose 6-Phosphate Dehydrogenase Deficiency (G6PDd) and Haemoglobin Variants on Malaria in Port Harcourt, Rivers State, Nigeria

Authors
Susanna O. Akwuebu*[1], Evelyn M. Eze[1], Zacchaeus A. Jeremiah[2]
[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria
[2]Dept. of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
*Corresponding Author
Susanna O. Akwuebu
Email**:** *osiro_akwuebu@yahoo.com*, Phone: +23487054795302

Abstract
The burden of the disease due to malaria is believed to share the same geographical distribution that correlates with G6PD deficiency and sickle cell haemoglobin (HbSS) due to protective advantage against malaria parasites. This study was aimed at studying the interactions between G6PD and haemoglobin variants in sub-clinical malaria infected pregnant women. Five millilitres (5ml) of venous blood was collected aseptically into 1% diamine tetraacetic acid bottle and carefully mixed for the analysis. G6PD activity was measured quantitatively with 3000 BSA spectrophotometer using the Randox G6PD kit. Malaria parasite density was determined microscopically using thick and thin Giemsa stained blood smears. Determination of haemoglobin variants was done using cellulose acetate membrane electrophoresis with Tris-EDTA-borate buffer (pH 8.9). All results were analysed using measure of significance taken as p values, that is ($p>0.05$) was regarded as insignificance while $p<0.05$ was regarded as significant. A total of eight hundred and twenty-eight (828) participated in the study. Of these, five hundred and seven 507 (61.2%) were infected with malaria parasite and three hundred and twenty-one (321) (38.8%) served as controls (uninfected subjects). HbAA genotype took dominance in malaria parasite infestation, 379 (74.8%), then HbAS 126 (24.9%) and HbSS 2 (0.4%). The mean age of the study participant was 29.5±5.31 years. Also, five hundred and seventeen 517 (62.4%) were G6PD deficient out of the eight hundred and twenty eight participants. The mean value of G6PD levels for HbSS was significantly elevated at 12.20±0.30u/gHb compared to HbAS, 6.28±0.16u/gHb and HbAA, 6.60±0.07u/gHb. It is therefore concluded that co-inheritance of G6PD and HbS has no advantage over single inheritance of HbS variant.
Keywords: *G6PD deficiency, Haemoglobin variant, Plasmodium falciparum malaria, Port Harcourt, Rivers State.*

Introduction

The majority of malaria infections are associated with some degree of anaemia, the severity of which depend upon patients-specific characteristic (e.g., Age (young children and elderly people), pregnant women and people who are immune-suppressed), travellers to endemic regions as well as the type of *Plasmodium* species[1][2][3]. The prevalence of malaria infection among children under five in Nigeria is 25% - 30% causing over

300,000 Death per year[2] and 72.5% pregnant women infected[4].

Malaria during pregnancy is a major public health concern that kills both mothers and infants and mortality in malaria-endemic countries[5]. In malaria endemic area, it can be expected that 1-50% of pregnant women may carry malaria parasitaemia, especially in the placenta, without noticing it[6]. In sub-Saharan Africa, malaria in pregnancy is predominantly asymptomatic and yet a major cause of severe maternal anaemia and low birth weight babies[7][8]. Twenty-five million pregnant women are recently at risk for malaria, and, according to the World Health Organization (WHO), malaria accounts for over 10,000 maternal and 200,000 neonatal deaths per year[3]. The second trimester appears to bring the highest rate of infection, supporting the need for antepartum care as part of malarial prevention and treatment efforts[2][9].

The burden of disease due to malaria share the same geographical distribution that correlates with the alleles of genes encoding haemoglobin, red cell enzymes, and membrane proteins[10][11][12]. It was thought by some researchers that co-inheritance of both G6PD deficiency and HbS enhanced additional protection against the burden of malaria parasite[13][14]. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency worldwide, causing a spectrum of disease including neonatal hyperbilirubinemia, acute haemolysis, although some persons with this disease may be asymptomatic. G6PD encoded by the G6PD gene on chromosome X, is a housekeeping enzyme that acts to control oxidative damage in red blood cells. G6PD is involved in the generation of phosphorylated nicotinamide-adenine dinucleotide (NADPH) in the pentose phosphate pathway (hexose monophosphate shunt)[15]. NADPH is needed to provide glutathione (GSH) which maintains haemoglobin and other red cell proteins in a reduced active form[16].

Exposure to an oxidant drug and infection increases the need for NADPH and GSH. G6PD deficiency prevents this need from being met, resulting in oxidation of haemoglobin to methaemoglobin. This precipitates to form Heinz bodies which attach to the red cell membrane. Cells containing Heinz bodies are easily damaged and lyzed extravascularly and intravascularly. The common variant implicated in the sub Saharan region to protect against malaria parasite is the G6PD A- and Med mutation[17][18]. Plasmodium infected G6PD deficient cells are thought to impair parasite growth, as they serve as an unstable host for the parasite,[11][19]. Nevertheless, protection against malaria parasite by G6PD deficiency was not observed by[20][21][22][23].

The parasite depends on anaerobic glycolysis for energy in the form of adenosine triphosphate (ATP) which is obtained only from the glycolytic break down of glucose with the production of lactate[24][25][26]. As the parasite grows and multiplies within the red cells, the cations pump and cytosolic composition of the host cell is modified[27]. These new permeation pathways help the parasite for uptake of solutes from the extracellular medium and also in the disposal of metabolic wastes, and in the origin and maintenance of the cations pump. At the same time, the premature hemolysis of the highly permeabilized infected red cell is prevented by the excessive ingestion, digestion and detoxification of the host cell haemoglobin and its discharge out of the infected RBCs through the new permeation pathways, thereby preserving the osmotic stability of the infected cells[27].

Sickle haemoglobin (HbS) gene is formed when valine replaces glutamic acid in the β globin chain (6^{th} amino acid position). In areas where *falciparum* malaria is endemic, sickle cell trait carriers are said to have lower parasite densities and are more protected against death from severe malaria which is likely due to the expression of heme oxygenase-1 which has a significant antioxidant against the excessive superoxide production which may stimulate several adhesion molecule, ICAM-1, PfEMP-1[28-32], translocation of host micro-RNA into the parasite[33] and

endocytic vesicles in HbS that is deoxygenated, polymerizes and is poorly digested [34][10][35][36] The superoxide can also react with nitric oxide (NO) to form peroxynitrite. Peroxynitrite can oxidize small molecule antioxidants such as glutathione (GSH) and tetrahydrobiopterin (BP4) resulting in cytotoxicity[37]. The aim of this study was to assess the interactions between G6PD and haemoglobin variants in sub-clinical malaria infected pregnant women.

Materials and Methods

Study Area

The study was carried out in Port Harcourt, the capital city of Rivers State Nigeria. Port Harcourt is situated within geographical co-ordinates 4°′49′27″N 7°2′1″E. Port Harcourt features tropical wet climates with lengthy and heavy Rainy seasons and very short dry seasons. Only the months of December and January truly qualifies as dry season months in the city. The harmattan, which climatically influences many cities in West Africa, is less pronounced in Port Harcourt. Port Harcourt's heaviest precipitation occurs during September with an average of 367 mm of rain. December on average is the driest month of the year with an average rainfall of 20 mm. Temperatures throughout the year in the city are relatively constant, showing little variation throughout the course of the year. Average temperatures are typically between 25 °C-28 °C in the city. University of Port Harcourt Teaching Hospital, Rivers State, Nigeria; was used as the centre for the study. University of Port Harcourt Teaching Hospital, Choba Port Harcourt was established in 1980 with 500 bed spaces.

A total of eight hundred and twenty eight (828) subjects were randomly selected. This comprised of 507 infected with malaria parasite and three hundred and twenty one (321) non-infected with the malaria parasite (controls). These subject whose ages ranged from<21 to 36 years and above were sub-clinical pregnant women registered in the antennal clinic of University of Port Harcourt Teaching Hospital, UPTH. Consent was obtained from each participant prior to blood collection. Their demographical information was collected using a questionnaire.

Experimental Analyses

Determination ofG6PD

G6PD deficiency was determined quantitatively [38] using BSA-3000, a semi-automated biochemistry analyzer with Randox kit. The enzyme activity was determined by measurement of the rate of absorbance change at 340nm due to the reduction of NADP+. 200µl of blood was washed with 2ml 0.9% Nacl solution and centrifuged after each wash at 3000 rpm for 10minutes. Wash was repeated three times. The washed centrifuge erythrocytes were then suspended in 500µl of Digitonin solution and left to stand for 15mins at 4^0C and then centrifuged again. The supernatant was used for the assay within 2 hours. Briefly, 7.5µl of haemolysate prepared from the EDTA anticoagulated blood samples was added to 500µl of Buffer reagent followed by 15µl of nicotineamide adeninne dinucleotide (NADP) reagent. It was then mixed and incubated for 5 min at 37°C and then 7.5µl of substrate reagent was added. It was then mixed properly and aspirated into the analyzer. Results were displayed in mU.erythrocyte/ml and then converted to U/Hb with the formula below.

$$\text{G6PD U/Hb} = \frac{\text{mU.erythrocyte/ml X100}}{\text{Hb (g/dl) X 1000}}$$

Determination of Haemoglobin Genotypes

The haemoglobin genotypes was determined by cellulose acetate membrane electrophoresis (CAME) as describe by**[13].** Haemolysate was prepared from EDTA anticoagulated whole blood. The electrophoretic tank was prepared by filling the tank with 300ml approximately of TEB buffer (pH 8.9). Wicks were cut from Grade NO. 3 chromatography paper and were placed along the 22cm long bridges in the tank. The cellulose acetate membranes were cut in 40 X 100mm each and soaked (shiny side down) in TEB buffer for 5minutes. Five strips were blotted and placed on

the electrophoresis tanks but were not allowed to dry out before sample application. Voltage current was applied at 250v for 5 minutes to the membranes to equilibrate the membranes with the buffer. The current was then turned off and 8-10µl haemolysate (10g/µl) was applied on each membrane at the cathodal end by placing a small volume (10µl) of each diluted sample into a sample well. Then the applicator stick was dipped into the sample wells and the samples were applied to the cellulose acetate. The voltage was set at 250v working at constant current of 2mA for each strip. The electrophoresis was run for approximately 10-15 minutes until there was a clear area between the bands of control sample. The current was turned off and the separated Hb on the cellulose acetate membrane were read and noted on the working sheet. HbA migrate faster follow by HbS respectively.

Determination of Malaria Parasitemia

Light microscope of thick and thin Giemsa stained blood smears remains the standard method for diagnosing malaria[39][40]. Pre-cleaned frosted ends slides were labeled according to the code on the bottles. Using a micro pipette, 5µl of blood was collected from each bottle and placed on the labeled slide to prepare a thick film, and 2µlto prepare a thin film. The films were then air dried on a slide rack on a flat surface and protected from debris. The thin blood film was methanol fixed. This allowed the morphological identification of the parasite to the species level because of the fixed monolayer of RBC which was critical in accurate interpretation of the developmental stages that may indicate the possibility of a more severe clinical situation and the density of parasitaemiain RBCs. The thick blood film of correct thickness was made through which newsprint was barely visible. It was dried for 30 minutes and not fixed with methanol.

All films were stained with the slow (3% Giemsa stain working solution) of pH 7.0 for 30-minute method[40]. For a 3% working solution dilution, 3ml of stock Giemsa was added to 7ml of buffered water in a Coplin jar. The films were then washed briefly and air dried thereafter in a vertical position. The films were examined using X100 objective (oil immersion). The parasite density count of parasites/µl of blood was accomplished by enumerating the number of parasites in relation to absolute leukocytes/µl with the formula below.

$$\frac{\text{Parasite count X Absolute WBC Value}}{\text{Number of leucocytes (WBC) counted (200)}} = \text{Parasite/µl}$$

Ethical Issues

Ethical approval and permission letter was obtained from the ethical committee of University of Port Harcourt Teaching Hospital, Rivers State. Informed consent of the participants involved was also obtained.

Informed Consent

Informed consent was obtained from all participants

Eligibility criteria

Inclusion

Pregnant women in their different trimesters who were registered with the antenatal clinic of University of Port Harcourt Teaching Hospital irrespective of their gestational age with the support of ante-partum care as part of malaria prevention and treatment effort were included in the research after their informed consents were obtained.

Exclusion

Post-natal subjects were excluded in the research. Also pregnant women who registered in the antenatal clinic in University of Port Harcourt Teaching Hospital without the support of antepartum care as part of malaria prevention and treatment effort, and those who refused to sign the informed consents were excluded

Statistical Analysis

Data collected for this study were registered in the computer by creating a spreadsheet and subjected to statistical analysis using statistical package Statistical Analysis System (SAS) version 12, 2013. Descriptive statistics mean separation using one-way analysis of variance (ANOVA). The effect of G6PD deficiency was determined on parasite density. Comparisons were assessed using

mean and chi-square test. Quantitative data were presented as percentages, while continuous variables such as age are expressed as mean ±standard deviation and a level of significance set at $P < 0.05$.

Results

A total of eight hundred and twenty-eight (828) participated in the study. Of this, five hundred and seven (507) representing 61.2% were infected with malaria parasite while the remaining three hundred and twenty-one (321) representing 38.8% served as controls (uninfected subjects). The mean age of the study participant was 29.5±5.31 years. Majority of the pregnant women 480(59.9%) were in their third trimester, of which 289(57.0%) were infected with the malaria parasite. Four hundred and ninety-three 493(59.5%) of the study participants were multigravida and 298 (58.87%) were infected with malaria parasite and the primigravida, 195(60.8%) were more infected. Majority of the participants 612(73.9%) had haemoglobin electrophoretic pattern of AA. The abnormal haemoglobin variant of SS constituted 2(0.24%) in this study population. Those with AS were 214(25.8%).The demographic characteristics of the participants are shown in table 4.1.

Table 4.1 Demographic Characteristics of Study Population

Characteristic	N (%)	Infected N(%)	Uninfected (Control) N(%)
Overall	828 (100)	507(61.2)	321(38.8)
Age Group (Years)			
< 21	41 (5.0)	20(3.9)	21(6.5)
21-25	159 (19.2)	103(20.3)	56(17.5)
26-30	328 (39.6)	221(43.6)	107(33.3)
31-35	185 (22.3)	105(20.7)	80(24.9)
36+	115 (13.9)	58(11.4)	57(17.8)
Age (Mean ±SD)	29.5±5.31	29.2±4.97	29.9±5.79
Trimester			
1	56 (6.8)	34(6.7)	22(6.9)
2	292 (35.3)	184(36.3)	108(33.6)
3	480 (57.9)	289(57.0)	191(59.5)
Parity			
Primigravida	335 (40.5)	209(41.2)	126(39.3)
Multigravida	493 (59.5)	298(58.8)	195(60.8)
Genotype			
AA	612 (73.9)	379(74.8)	233(72.6)
AS	214 (25.8)	126(24.9)	88(27.4)
SS	2 (0.24)	2(0.4)	0(0.0)

Table 4.2; shows comparison of malaria infection and G6PD status among the subjects based on Genotype. A total of eight hundred and twenty-eight (828) participated in the study. Of this, five hundred and seven (507) representing 61.2% were infected with malaria parasite while the remaining three hundred and twenty-one (321) representing 38.8% served as controls (uninfected subjects). The prevalence of malaria infection in co-inheritance subjects with HbAA, HbAS and G6PD deficiency were 74.6% and 25.4% respectively. While malaria infection in co-inheritance subjects with HbAA, HbS and normal G6PD were 75.0% and 24.0% respectively. Also, the prevalence of malaria infection in single inheritance of haemoglobin variants was, HbAA: 74.8%, HbAS: 24.9% and HbSS: .4%. A total of five hundred and seventeen 517 (62.2%) subjects were G6PD deficient in the study out of eight hundred and twenty eight 828 (100%) participated subjects, and three hundred and eleven 311 (37.6%) subjects with normal G6PD levels. Majority of the G6PD deficient were observed among HbAA genotype 364 (70.4%), followed by HbAS 153 (29.6%). The difference in the parasite density based on different genotypes with G6PD deficient

and G6PD normal were statistically significant P<0.0001. But, the difference in the parasite density among different genotype was statistically difference been lower in HbSS 2 (0.4%), followed by HbAS 126 (24.9%) and HbAA 379 (74.8%).

Table 4.2: Comparison of Malaria Infection and G6PD Status among the Subjects Based on Genotype

Genotype	Total Genotype (N%)	Malaria Infected	G6PD Deficient	G6PD Deficient and Malaria Infected	Normal G6PD and Malaria infected
Overall	828 (100)	507 (61.2)	517 (62.4)	315 (38.0)	192 (23.2)
AA	612 (73.9)	379 (74.8)	364 (70.4)	235 (74.6)	144 (75.0)
AS	214 (25.8)	126 (24.9)	153 (29.6)	80 (25.4)	46 (24.0)
SS	2 (0.24)	2 (0.4)	0 (0.0)	0 (0.0)	0)0.0)
X^2 P-value		436.92 P<0.0001	86.11 P<0.0001	76.27 P<0.0001	50.55 P<0.0001

Table 4.3 shows the mean ± SEM of G6PD and malaria density according to the experimental group, age, trimester, parity and haemoglobin electrophoretic pattern. The overall mean parasite density of the infected subjects was 741.835±309.8/µl. The difference in the parasite density among different age groups were not statistically significant (P>0.05). Similarly, no statistical significant difference was observed when the mean values among different trimesters were compared as a well as the haemoglobin pattern of the subjects (P>0.05). The mean parasite density of 5147.78±356.79/µl in the primigravida group was significantly higher than 4131.02±294.11/µl observed in the multigravida group (P = 0.028).

Table 4.3: Mean ± SEM of G6PD and Malaria Density by Experimental Group, Age Group, Trimester, Parity and Genotype

Characteristic	N	Parasite Density (Parasites/µl)		G6PD (u/gHb)	
		Mean ± SEM	P-value	Mean ± SEM	P-value
Experimental Group					
Infected	507	741,835±309.8	-----	6.60±0.09	
Uninfected (Control)	321	0.0±0.0		6.41±0.11	0.1182^{ns}
Age Group (Years)					
< 21	41	4484.95±1107.50		$6.69±0.29^{ab}$	
21-25	159	4695.85±446.60		$6.65±0.15^{ab}$	
26-30	328	4454.06±347.80		$6.76±0.12^{a}$	
31-35	185	4543.42±544.10	0.997^{ns}	$6.12±0.14^{b}$	0.006**
36+	115	4600.99±638.90		$6.29±0.18^{ab}$	
Trimester					
1	56	5467.29±930.13		6.15±0.25	
2	292	4006.59±294.21		6.47±0.11	
3	480	4760.44±331.43	0.165^{ns}	6.60±0.10	0.234^{ns}
Parity					
Primigravida	335	$5147.78±356.79^{a}$		6.47±0.10	
Multigravida	493	$4131.02±294.11^{b}$	0.028*	6.57±0.09	506^{ns}
Genotype					
AA	612	4533.37±272.70		$6.60±0.07^{a}$	
AS	214	4573.54±409.10		$6.28±0.16^{a}$	
SS	2	3970.00±1095.00	0.989^{ns}	$12.20±0.30^{b}$	0.0001***

SEM: Standard error of mean;within each Characteristic, means ± SEM with different superscripts are significantly different at p<0.05.Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ns=Not Significant (p>0.05).

Table 4.4 shows parasite density was observed to correlate significantly and negatively with G6PD deficiency (r = -0.1442, P = 0.0011).

Table 4.4: Pair wise Correlations between Hematological Parameters among Infected Group

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Sign if Prob
G6PD (u/gHb)	Parasite Density (Parasites/µl)	-0.1442	507	-0.2284	-0.0578	0.0011*

Table 4.5: show the comparison of parasitized G6PD deficient subjects and parasitized G6PD normal subject against haemoglobin electrophoretic pattern. There was a statistical significant difference between parasitized G6PD deficient and parasitized G6PD normal subjects P< 0.0001

Table 4.5: Comparison between Parasitized G6PD Deficient subjects and Parasitized G6PD Normal subjects against Haemoglobin Electrophoretic Pattern

Genotype	Parasitized G6PD deficien	Parasitized G6PD normal	X^2	P value
AA	235 (74.6%)	144 (75.0%)	21.84	P < 0.0001
AS	80 (25.4%)	46 (24.0%)	16.80	P < 0.0001
SS	0 (0.0%)	2 (1.0%)	0	0

Discussion

This study was conducted to assess the interactions between genetic factors such as glucose-6-phosphate dehydrogenase deficiency and haemoglobin variants on malaria parasites in sub-clinical malarial infected pregnant women attending the antenatal care of University of Port Harcourt Teaching Hospital.

In this study, a prevalence of malaria burden in pregnant women was 61.2% as against the prevalence of 72.5%[4]. The slight difference may be due to the method of analysis used to estimate malaria parasitaemia. The plus sign is a semi-quantitative method that is less accurate and can be misinterpreted since the density of parasitaemia cannot be accurately quantified based on the volume of blood used, unlike the quantitative method of count against absolute TWBC[39]. This gives approximate density of parasites per microliter of blood. Also, the estimation of all the four *Plasmodium species* (*P. ovale, P. malaria, P. vivax and P. falciparum*) by[4] could be the reason for the difference in the prevalence. Moreso, the prevalence of HbAS (25.8) was lower in malaria endemic area (Port Harcourt) than HbAA (73.9) in this study, which has a relative protection against malaria parasite[4].

The difference in the parasite density among different genotypes was statistically different been lower in HbAS and then followed by HbAA. This finding agrees with that of[10-12]. This may be due to the point mutation at the 6th position of beta globin chain where valine is substituted for glutamic acid resulting in polymerization HbS cell[10] this leads to the generation of ROS .Generally, oxidative stress caused damage by inducing endothelial cell dysfunction, apoptosis and angiogenesis[31]. The uncoupling of the mitochondrial electron in HbS leads to excessive superoxide production which may stimulate several adhesion molecules such as ICAM-1[28][32] Heme oxygenase-1 (SOD-1) which has significant antioxidative and anti-inflammatory effects is increased in HbS[29] and thereby decreased the expressions of levels of hypoxia inducing factors such as MiRNA, vascular endothelial growth factor (VEGF) and neutralized free peroxynitrite, decreased the activation of NF-kB and the transcription of NF-kB-dependent molecules such as ICAM-1, PfEMP-1 and blood vessels from angiogenesis. The altered cytoskeletal properties of HbS-containing RBC have been suggested to impact parasite protein trafficking to the RBC surface due to short half-life compared with HbAA[34][35] thereby reducing cytoadhesion. Also, HbAS has an effect on the immune system. It was suggested that the HbAS variant favourably alters the immune response directly by increasing phagocytosis of infected RBCs or through the influence of inflammatory cytokines following endothelial activation as a result of altered cytoadhesion properties[30][36][12].

HbAA has increased levels of gamma globulin thereby preventing sequestration of malaria parasite properties[30][12][36]. Spleen clearance does not prevent malaria parasites, as more and more are continually pumped into the system through the exoerythrocytic phase in the liver to the erythrocytic phase in the blood stream. When the liver cells rupture, the merozoites are release into the bloodstream and invade red blood cells where they initiate a second phase of asexual multiplication (erythrocytic schizogony) resulting in the production of about 8-16 merozoites which invade new red blood cells causing fever, body weakness, vomiting, just to mention a few[41]. The spleen clearance only alleviates the complications posed by placenta sequestration on the baby.

Protection against malaria parasite by glucose-6-phosphate dehydrogenase (G6PD) deficiency was not observed in this study corroborated by the report of[20-23].This may be due to, the anaerobic glycolytic pathway of the parasite as a way of generating ATP defending against reactive oxygen species[24-26].

The parasite density based on different genotypes with G6PD deficient and G6PD normal were statistically significant compared with the single inheritance with HbS corroborated by[21] although, a controversial results were reported with lack of statistical significance [13][41]. Indicating that G6PD normal or deficient have no effect on malaria as both normal and deficient shows high levels of malaria parasite infection compared with the single inheritance of HbS

Conclusion

In conclusion, the incidence rate of malaria parasite in both G6PD deficient status and normal subjects against the various haemoglobin variants were observed to be significantly high. However, haemoglobin variants suffer malaria infection but at varying frequencies. HbAS subjects were observed to be relatively protected against malaria parasite compared to HbAA subjects. It is obvious from this study that G6PDhas no effect on malaria parasite. It is therefore concluded that co-inheritance of G6PD deficiency and haemoglobin variants (HbS), has no protective advantage against malaria parasite infection and also, the geographical correlation of G6PD deficiency is as a result of less iron uptake due to reduced transferrin recptor in matured haemoglobin.

References

1. Olasehinde, G.I., Ajayi, A.A., Taiwo, S.O., Adekeye, B.T. & Adeyeba, O.A. (2010). Prevalence and management of falciparum malaria among infants and children in Ota, Ogun State, Southwestern Nigeria. African Journal of Clinical Experimental Microbiology, 11, 159-163.
2. Oliver, O., Angela, I., Nneka, L.O. & Ngozi, O. (2014).Prevalence of malaria and possible parity in Abakaliki South Nigeria. European Journal of Experimental Biology, 4, 15-19.
3. World Health Organization. (2009). Global Malaria Programme: pregnant women and infants.
4. World Health Organization. (2015). Guidelines for the treatment of malaria,3rd ed, WHO, Geneva.
5. Nzeako, S.O., Nduka, F.O. & Origie, O.A. (2013). Prevalence of malaria in pregnant women attending antenatal care at University of Port Harcourt Primary Health Care centre Aluu, Port Harcourt, Rivers State Nigeria. International Journal of Scientific Research in Environment Sciences, 1, 268-272.
6. Steketee, R.W., Nahlen, B.L., Parise, M.E. & Menendez, C. (2001). The burden of malaria in pregnancy in malaria-endemic areas. American Journal of Tropical Medicine of Hygiene, 64, 28- 35.
7. Adefioye, O.A., Hassan, W.O. & Oyeniran, O.A.(2007). Prevalence of Malaria Parasite Infection among Pregnant Women in Osogbo, Southwest Nigeria. American-Europian Journal of Scientific Research, 2, 43-45.

8. Aribodor, D.N., Nwaorgu, O.C., Eneanya, C.I., Okoli, I., Pukkila-Worley, R. & Etaga, O.H. (2009).Association of low birth weight and placental malaria infection in Nigeria. The Journal of Infection in Developing Countries, 3, 620-623.
9. Schantz-Dunn, J. and Nour, N.M. (2009). Malaria and pregnancy: a global health perspective. Reviews of Obstetrics Gynecology, 2, 186-192.
10. Elliott, D.A., McIntosh, M.T., Hosgood, H.D., Chen, S., Zhang, G., Baevova, P & Joiner, K.A. (2008).Proceedings of the National Academy of Science**,** 105, 2463-2468.
11. Föller, M., Bobbala, D., Koka, S., Huber, S.M., Gulbins, E & Lang, F. (2009). Suicide for survival-death of infected erythrocytes as a host mechanism to survive malaria. Cellular Physiology and Biochemistry, 24, 133-140.
12. Taylor, S.M., Cerami, C., Fairhurst, R.M. (2013). Hemoglobinopathies: slicing the Gordian knot of Plasmodium falciparum malaria pathogenesis. PLoS Pathogens, 9, 1-2
13. Awah, F.M. & Uzoegwu, P.N. (2006). Influence of Sickle Heterozygous status & glucose-6-phosphate dehydrogenase deficiency on the clinic-haematolgoical profile of Plasmodium falciparum-infected children. Biochemistry, 18, 89-97.
14. Francis, M.A., Nwanedo, C.G., Salami, I.O., August, E.A., & Mbaike, N. (2012). A positive protective role of glucose-6-phosphate dehydrogenase deficiency and sickle haemoglobin genes against severe malaria in Madonna University, Elele community. Journal of Medicine and Medical Science, 3, 375-381
15. Prchal, J.T. & Gregg, X.T. (2005). Red cell Enzymes. Hematolgy/American Society of Hematology Education Book.
16. Cappellini, M.D. & Fiorelli, G. (2008). Glucose-6-phosphate dehydrogenase deficiency. Lancet Infectious Disease, 71, 64-74.
17. Mason, K.D., Carpinelli M.R., Fletcher J.I & Collinge J.E. (2007).“Programmed anuclear cell death deli its platelet life span”.Cell, 128, 1173-1186.
18. Shah, S.S., Macharria, A., Makale, J., Uyoga, S., Kivinen, K. & Craik, R. (2014). Genetic determinants of glucose-6-phosphate dehydrogenase activity in Kenya.BMC Medical Genetics
19. Vaidya, A.B. & Mather, M.W. (2009). Mitochondrial Evolution & Functions in Malaria Parasites. Annual Review of Microbiology, 63, 249-267.
20. Carter, N., Allan. P., Stephan, D. & John, N.W. (2011). Frequency of G6PD deficiency in malaria patients from six Africa countries enrolled in two randomized anti-malarial clinical trials. Malaria Journal, 10, 241-242.
21. Jeremiah, Z.A., Jeremiah, T.A. & Emelike, F.O. (2010).Frequencies of some human genetic markers and their association with Plasmodium falciparum malaria in the Niger Delta, Nigeria.Journal of Vector Borne Diseases, 47, 11-16.
22. Johnson, M.K., Clark, T.D., Njama-Meya, D., Rossenthal, P.J. & Parikh, S. (2009). Impact of the method of G6PD deficiency assessment on genetic association studies of malaria susceptibility.PLoS One, 4, 1-2
23. Toure, O., Konates, S., Sissoko, S., Niangaly, A., Barry, A., Sall, A.H., Diarra, E., Poudiougou, B., Supulveda, N., Campino, S., Rockett, K.A., Clark. T.G., Thera, M.A., Doumbo, O. &Collaboration with The Malaria GEN Consortium. (2012). Candidate polymorphisms and severe malaria in a Malian population. PLoS One, 7, 1-2
24. Alan, F.C.& Brendan, S.C. (2006). Invasion of Red Blood Cells by Mlaria Parasites. Cell, 124, 755-766.

25. Brain, M.G., David, A.F., Dennis, E.K., Stefan, H.I.K., Pedro, L.A., Frank, H.C. & Patrick, E.D. (2008). Malaria: progress, perils, and prospects for eradication. Journal of Clinical Investigation, 118, 1266- 1276.
26. Olivier, S., Maria, M.M., Kai, M., Miguel, P. (2008).Interactions of the malaria parasite and its mammalian host. Current Opinon in Microbiology, 11, 352-359.
27. Virgilio, L.L. & Teresa, H.G. (2003). Excess haemoglobin digestion and the osmotic stability of Plasmodium falciparum-infected red blood cells. Blood, 101, 4189-4194.
28. Amer, J., Ghoti, H., Rachmilewitz, E., Koren, A. & Levin, C. (2006).Red blood cells, platelets and polymorphonuclear neutrophils ofpatients with sickle cell diseaseexhibit oxidative stress that can be amelioratedby antioxidants. British Journal of Haematology,132, 108–113.
29. Belcher, J. D., Beckman, J. D., Balla, G., Balla, J.& Vercellotti, G. (2010).Heme degradation and vascular injury. Antioxidants and Redox Signaling, 12, 233-248.
30. Cholera, R., Brittain, N.J., Gillrie, M.R., Lopera-Mesa, T.M., Diakité, S.A., Arie, T., Krause, M.A., Guindo, A., Tubman, A., Fujioka, H., Diallo, D.A., Doumbo, O.K., Ho, M., Wellems, T.E & Fairhurst, R.M. (2008). Impaired cytoadherence of Plasmodium falciparum-infected erythrocytes containing sickle hemoglobin. Proceedings of the National Academy of Science. U.S.A. 105, 991-996.
31. Erica, N.C. & Vincent, P. (2011).Role of oxidative strees in the pathogenesis of sickle cell disease, 64, 1-2
32. Kato, G. J., Martyr, S., Blackwelder, W. C., Nichols, J. S. & Coles, W.A. (2005). Levels of soluble endothelium-derived adhesion moleculesin patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. British Journal of Haematology, 130, 943-953.
33. LaMonte, G., Philip, N., Reardon, J., Lacsina, J.R., Majoros, W., Chapman, L., Thornburg, C.D., M.J, Ohler, U., Nicchitta, C.V., Haystead, T. & Chi, J.T. (2012). Translocation of sickle cell erythrocyte micro RNAs into Plasmodium falciparum inhibits parasite translation and contributes to malaria resistance. Cell Host & Microbe, 12, 187-199.
34. Cyrklaf, F M., Sanchez, C.P., Kilian, N., Bisseye, C., Simpore, J., Frischknecht, F. & Lanzer, M. (2011).Hemoglobins S and C Interfere with Actin Remodeling in Plasmodium falciparum-infected Erythrocytes. Science, 334, 1283-1286.
35. Kilian, N., Srismith, S., Dittmer, M., Ouermi, D., Bisseye, C., Simpore, J., Cyrklaff, M., Sanchez, C.P., Lanzer, M. (2015). Hemoglobin S and C affect protein export in Plasmodium falciparum-infected erythrocytes. Biology Open, 4, 400-410.
36. Manjurano, A., Sepulveda, N., Nadjm, B., Mtove, G., Wangai, H., Maxwell, C., Olomi, R., Reyburn, H., Drakeley, C.J., Riley, E.M. & Clark, T.G. (2015). African glucose-6-phosphate dehydrogenase alleles associated with protection from severe malaria in heterozygous females in Tanzania. PLoS Genetics, 11, 1-2
37. World Health Organization.(2006). Guidelines for the Treatment of Malaria. Geneva: World Health Organization.
38. Shah, S.S., Diakite, S.A.S., Traore, K., Diakite, M., Kwiatkowski, D.P. & Rockett, K.A. (2012).A novel cytofluoro-metric assay for the detection and quantification of glucose-6-phosphate dehydrogenase deficiency. Scientific Report, 2, 29-30
39. Hammamil, N.G. & Garcia, A. (2013). Statistical properties of parasite density estimators in malaria.PLoS ONE, 3, 1-2.

40. World Health Organization. (2010).Basic Malaria Microscopy. Part 1 Learners Guide, 2nd (ed), 3-7
41. Francis, E.G. (2010).History of the discovery of the malaria parasites and their vectors. Parasites and Vectors, 3, 5-6.