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# Effect of Haemoglobin Genotype Variants on Pre, Post Anti-Malaria Drug Treatment in *Plasmodium Falciparum* Malaria Infected and Non-Infected Individuals on Blood Cell Line Parameters in Ido-Ekiti

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**Abstract:** ***The aim of this study is to determine the effect of haemoglobin genotype variants on pre, post anti-malaria drug treatment in plasmodium falciparum malaria infected and non-infected individuals on blood cell line parameters. The study was conducted at Federal Medical Centre, Ido-Ekiti, Nigeria. Malaria infected adult individuals; presented with signs and symptoms of malaria infection was used for the study. Two hundred and two (202) blood samples were collected twice from each of malaria infected individuals; grouped as pre-treatment and post anti-malaria drug treatment. One hundred and two (102) blood samples from apparently healthy individuals were collected as control. Thick blood film was made and stained with Giemsa's staining technique for malaria parasite detection and malaria parasite count, the procedure was described by Monica Cheesbrough, blood cell line parameters were analysed using haematology analyser. Haemoglobin genotype variants were determined using cellulose acetate electrophoresis at alkaline pH between 8.4-8.6 as described by Dacie and Lewis. Data obtained was analysed using SPSS version 16. The result of this present study showed that, the mean±SD of malaria parasite count, total white blood cell count, relative and absolute neutrophil count, absolute lymphocyte, absolute monocyte count, of Hb AA were significantly (p<0.05) higher compared to HbAS and HbAC in pre treatment and post anti-malaria drug treatment. The study showed that, HbAS and HbAC has genetic resistance against malaria infection compared to HbAA, abnormal haemoglobin may not allow for optimal development of* **Plasmodium** ***in deep organs where oxygen pressure is reduced.***

**Keywords:** malaria parasite, haemoglobin genotype and blood cells

## 1. Introduction

The aim of this study is to determine the effect of haemoglobin genotype variants on pre, post anti-malaria drug treatment in *plasmodium falciparum* malaria infected and non-infected individuals on blood cell line parameters. Malaria is a major cause of morbidity and mortality in developing countries, accounting for an estimated 300 to 500 million morbid episodes and 2 to 3 million death per year worldwide (1, 2, 3). More than 90% of these deaths occur in sub-Saharan Africa, most of them are due to *plasmodium falciparum* (4). The genetic resistance to malaria infection, particularly *p. falciparum* malaria, associated with the hemoglobinopathies, the mechanisms of genetic resistance to *P. falciparum* malaria at the erythrocytic stage may involve one or more of the following: inhibition of merozoite entry into the red cell (5), impairment in intracellular growth of the parasite, prevention of the erythrocyte lysis that occurs at the end of parasite maturation, which leads to release of merozoites into the bloodstream, enhanced phagocytosis of parasite-infected red cells (6), reduced cyto-adherence of infected erythrocytes to endothelial cells, uninfected red blood cells, platelets or antigen-presenting cells, enhanced immune responses to malaria infection. Erythrocytes containing HbS or HbC may impede parasite growth and replication relative to normal haemoglobin red cells when subjected to low oxygen tensions (7). Protein targets of specific antibodies may be more rapidly exposed in HbS-containing red blood cells (6) resulting in an enhanced immune response to infection (7, 8). It is also possible that unknown innate protective processes may up-regulate the malaria-specific immune response (9) or enhance nonspecific immunity to malaria (10). Mean parasite density was markedly lower in children AS relative to AA genotype normal haemoglobin, parasite density was however higher among AC relative to AA genotype, suggesting potential mechanistic variation among protection afforded by abnormal genotypes in early childhood, a similar protective advantage of HbAC has been less consistently supported. Parasite growth within the erythrocyte causes dramatic alterations of host cell which on one hand facilitates nutrients acquisition from extracellular environment and on other hand contributes to the symptoms of severe malaria. *Plasmodium* parasites degrade haemoglobin (Hb) for nutritional needs; however, disorders of hemoglobin structure (HbS, HbC, HbE) and production due to deletions or point mutations in the non-coding portion of the globin genes causing inadequate synthesis of the α- and β -globin chains (α - and β -thalassaemias respectively) have been shown to protect against death from malaria. Although, HbAS is strongly protective against all forms of clinical malaria, HbC variants appear to be protective against relatively specific cerebral malaria, and both are associated with reduced parasite densities and increased phagocytosis of infected erythrocytes (11)

## 2. Materials And Methods

### 2.1 Subjects, Study Design and Sample Collection

This study was conducted at Federal Medical Centre, Ido-Ekiti, Ekiti State Nigeria. Subjects were *Plasmodium falciparum* malaria infected adult individuals; presented with signs and symptoms of malaria infection. This was confirmed using malaria rapid kit test and microscopy detection of malaria parasite. 202 blood samples were collected (4ml) twice from the same malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria drug treatment. 102 blood samples from apparently healthy individuals negative to malaria infection was collected for control; both *Plasmodium falciparum* malaria infected subjects and controls were within the age 15-64 years of both sex. Patient's consent was sort for through an informed consent form; also ethical approval was obtained from the hospital. Structured questionnaire was used to obtained demographic characteristic and other relevant information for the study.

## 3. Methodology

Thick blood film was made from EDTA blood sample and stained with Giemsa's staining technique for malaria parasite detection and malaria parasite count; observed under microscopy using x100 objective lenses. Malaria parasite counts were estimated by counting malaria parasites against leukocytes (200), and multiply by the patient's own leukocyte count (total white blood cell count); the procedure was described by Monica Cheesbrough, (12). Blood cell parameters which include absolute platelet count, mean platelet volume, platelet distribution width, relative and absolute differential white blood cell count and total white blood cell count were analysed using haematology analyser (sysmex automated haematology analyser model kx-21n, manufactured by sysmex co-operation kobe, Japan). 0.3ml of blood sample was used to prepared hemolysate by centrifuge at 3000g for ten minutes with Hittich universal bench centrifuge, model 1200. Plasma was aspirated off while the precipitate (blood cell layer) was resuspended in equal volume of normal saline (0.85% NaCl) for washing. The washing was repeated three times and finally resuspended in equal volume of normal saline. The red blood cell suspension (40ul) was mixed with equal volume of distilled water to lyse the blood cell. The resulting haemoglobin lysate (the lysate) was used for haemoglobin genotype determination (13). The method described by Brown was used for haemoglobin electrophoresis. A small quantity of hemolysate of venous blood from each of the subject was placed on a cellulose acetate membrane and carefully introduced into the electrophoretic tank containing tris-EDTA-borate buffer at pH 8.6. Electrophoretic separation was then allowed to operate for 15 to 20 minutes at an electromotive force (e.m.f) of 160v. The results were read immediately. Hemolysate from blood samples of known haemoglobin (AA, AS, AC, SC, CC and SS) were run as controls.

## 4. Statistical Analysis

Data obtained were analysed for mean and standard deviation; significant test was done by ANOVA. Level of significance was considered as <0.05.

## 5. Result

**Table 1:** show the comparison of mean ± SD in blood cell parameters on haemoglobin genotype variants in pre treatment. Blood cell parameters include malaria parasite count (per ul), absolute platelet count ($XI0^9$), platelets distribution width (%), mean platelet volume (pL), white blood cell count ($XI0^9$), relative neutrophil count(%) , relative lymphocyte count(%), relative monocyte count(%), relative eosinophil count(%), absolute neutrophil ($XI0^9$), absolute lymphocyte ($XI0^9$) and absolute monocyte($XI0^9$). The mean±SD of malaria parasite count 2795.10±378.60 in haemoglobin genotype AA, was significantly ($p<0.05$) higher compared to 2311.10 ±295.37 and 2374.60 ±279.74 in haemoglobin genotype AS and AC respectively ($F=41.56$; $p=0.00$). The mean ± SD of absolute platelet count 171.61 ± 50.88 in haemoglobin genotype AA was higher compared to 162.57 ± 48.51 and 165.22 ± 49.40 in haemoglobin genotype AS and AC respectively, the comparison show no statistical significant difference ($p>0.05$) ($F = 0.67$, $p=0.52$). The mean of ± SD of platelet distribution width (PDW) 13.45±2.51 in haemoglobin AA was observed lower compared to 13.83 ±2.16 and 13.98 ±2.71 in haemoglobin genotype AS and AC respectively the comparison show no statistical significant difference ($p>0.05$) ($F=0.69$; $p=0.50$). The mean ± SD of mean platelet volume (MPV) 9.79 ± 0.81 in haemoglobin genotype AA was observed higher compared to 9.78±0.78 and 9.59±0.75 in haemoglobin genotype AS and AC respectively; the comparison show no statistical significant difference ($p>0.05$) ($F =0.69$; $p=0.59$). The mean ± SD of total white blood cell count 7.52 ± 1.37 in haemoglobin genotype AA was significantly ($p<0.05$) higher compared to 5.09 ± 1.01 and 5.47 ± 0.87 in haemoglobin genotype AS and AC respectively ($F= 81.59$ $p=0.00$). The mean ± SD of relative neutrophil count 59.81 ± 9.28 in haemoglobin genotype AA was significantly ($p<0.05$) higher compared to 51.44 ± 10.29 and 54.89 ± 5.79 in haemoglobin genotype AS and AC respectively ($F = 15.93$; $P=0.00$). The mean ± SD of relative lymphocyte count 35.77 ± 8.79 in haemoglobin genotype AA was significantly ($p<0.05$) lower compared to 43.17 ± 9.31 and 41.61 ± 6.30 in haemoglobin genotype AS and AC respectively ($F=15.08$; $P=0.00$). The mean ± SD of relative monocyte count 2.94±1.39 in haemoglobin genotype AC was observed lower compared to 3.65 ± 2.34 and 3.96± 1.97 in haemoglobin genotype AA and AS respectively, the comparison show no statistical significant difference ($p>0.05$) ($F=1.49$; $p=0.23$). The mean ± SD of relative eosinophil count 0.67 ± 0.97 in haemoglobin genotype AC was significantly ($p<0.05$) lower compared to 0.79 ± 0.99 and 1.44 ± 1.99 in haemoglobin AA and AS respectively ($F = 5.04$; $p = 0.01$). The mean ± SD of absolute neutrophil count 4.53 ±1.20 in haemoglobin genotype AA was significantly ($p<0.05$) higher compared to 2.63 ± 0.78 and 3.03 ±0.72 in haemoglobin AS and AC respectively (66.29; $P=0.00$). The mean ± SD of absolute lymphocyte count 2.66 ± 0.72 in haemoglobin genotype AA was significantly ($p<0.05$) higher compared to 2.19 ± 0.64

and 2.25 ± 0.33 in haemoglobin genotype AS and AC respectively (F = 10.48; P=0.00). The mean ± SD of absolute monocyte count 0.27 ± 0.20 in haemoglobin genotype AA was significantly (p<0.05) higher compared to 0.20 ± 0.10 and 0.16 ±0.08 in haemoglobin genotype AS and AC respectively (F = 5.66; P = 0.00). Multiple comparison between haemoglobin genotype AA and AS show that mean ± SD of MPC, in haemoglobin genotype AA was significantly (p<0.05) higher compared to haemoglobin genotype AS; mean ± SD of absolute platelet count and MPV in Hb genotype AA were higher compared to Hb genotype AS also mean ± SD of PDW in haemoglobin genotype AA was lower compared to Hb genotype AS although the p-value show no statistical significant (p>0.05) difference. However, mean ± SD of TWBC and relative neutrophil count were significantly (p<0.05) higher compared to Hb genotype AS while mean±SD of relative lymphocyte count in, haemoglobin genotype AA was significantly (p<0.05) lower compared to Hb genotype AS. Also, mean ±SD of relative monocyte and relative eosinophil in Hb genotype AA were lower compared to Hb genotype AS; although the p-value show no statistical significant (p>0.05). Moreover, mean±SD of absolute neutrophil count, absolute lymphocyte and absolute monocyte in Hb genotype AA were significantly (p<0.05) higher compared to Hb genotype AS. However, multiple comparison between Hb genotype AA and AC show that mean ± SD of MPC in Hb genotype AA was significantly (p<0.05) higher compared to Hb genotype AC, mean ± SD of absolute platelet count and MPV in Hb genotype AA were higher compared to Hb genotype AC also, mean ± SD of PDW in Hb genotype AA was lower compared to Hb genotype AC, although the P- value show no statistical significant (p>0.05). However, mean ± SD of total WBC and relative neutrophil count were significantly (p<0.05) higher compared to Hb genotype AC while mean ± SD of relative lymphocyte in haemoglobin AA was significantly (p<0.05) lower compared to Hb genotype AC. Also, mean ± SD of relative monocyte and relative eosinophil in Hb genotype AA were lower compared to Hb genotype AC, P- value show no statistical significant (p>0.05). Moreover, mean ± SD of absolute neutrophil count, absolute lymphocyte and absolute monocyte in Hb genotype AA were significantly (p<0.05) higher compared to Hb genotype AC. Multiple comparison between haemoglobin genotype AS and AC show that mean ±SD of MPC, absolute platelet count and PDW in haemoglobin genotype AS were lower compared to Hb genotype AC, the P-value show no statistical significant (p>0.05). Mean ± SD of mean platelet volume, in Hb genotype AS was higher compared to Hb genotype AC. Mean ± SD total WBC, relative neutrophil count in Hb genotype AS were lower compared to Hb genotype AC. However, mean ±SD of relative lymphocyte, relative monocyte, relative eosinophil and absolute monocyte in Hb genotype AS were higher compared to Hb genotype AC; mean ±SD of absolute neutrophil and absolute lymphocyte in Hb genotype AS were lower compared to Hb genotype AC, the comparisons show no statistical significant (p>0.05).

**Table 2** show the comparison of mean ± SD in blood cell parameters on haemoglobin genotype variants in post anti-malaria drug treatment. The mean ± SD of MPC 2477.30 ± 594.81 in haemoglobin genotype AA was significantly higher (p<0.05) compared to 1983.50 161.26 and 2026 ± 165.03 in Hb genotype AS and AC respectively (F=22.71; P=0.00). The mean ± SD of absolute platelet count 184.65 ± 59.58 in haemoglobin AA was higher compared to 170.26± 52 85 and 173.11 ± 53.03 in Hb genotype AS and AC respectively, comparisons show no statistical significant difference (p>0.05) (F=1.33; P=0.27). The mean ±SD of platelet distribution width and mean platelet volume 13.59 ±2.51 and 9.54 ± 0.67 respectively in Hb genotype AA were lower compared to mean ± SD of PDW and MPV 13.93 ± 2.25 and 9.71 ± 0.63 in Hb genotype AS and 14.18 ± 2.70 and 9.79 ± 0.57 in Hb genotype AC with (F = 0.67, P=0.51) and (F = 2.19; P= 0.12) respectively; the comparisons show no statistical significant difference (p>0.05). The mean ± SD of WBC and relative neutrophil 6.75 ± 1.79 and 55.04 ± 8.24 respectively in Hb genotype AA were significantly higher (P<0.05) compared to mean ± SD of WBC and relative neutrophil 4.15 ± 0.93 and 48.41 ± 8.39 in Hb genotype AS and 4.43 ± 0.88 and 50.94 ± 5.42 in Hb genotype AC with (F = 62.73, P=0.00) and (F= 13.44, P=0.00) respectively. The mean ± SD of relative lymphocyte count 42.78 ± 7.78 in Hb genotype AA was significantly (P<0.05) lower compared to mean ± SD relative lymphocyte 48.80 ± 7.49 and 47.39 ± 5.45 in Hb genotype AS and AC respectively(F=13.31, P=0.00). The mean ± SD of relative monocyte count 1.33 ± 0.97 in Hb genotype AC was lower compared to mean ± SD of relative monocyte 1.79 ± 1.54 and 2.02 ± 1.57 in Hb genotype AA and AS respectively; comparison show no statistical significant (p>0.05) (F=1.42, P=0.25). The mean ± SD of relative eosinophil 0.33 ± 0.49 in Hb genotype AC was significantly (P<0.05) lower compared to 0.38 ±0.68 and 0.80 ±1.29 in Hb genotype AA and AS respectively (F = 4.57, P=0.01). The mean ± SD of absolute neutrophil, absolute lymphocyte and absolute monocyte 3.73 ± 1.22, 2.87 ± 0.87and 0.13 ± 0.14 respectively in Hb genotype AA were significantly (P<0.05) higher compared to absolute neutrophil, absolute lymphocyte and absolute monocyte 2.02 ± 0.61, 2.02 ± 0.51 and 0.08 ± 0,07 respectively in Hb genotype AS and 2.29 ± 0.64, 2.07 ± 0.33 and 0.06 ± 0.04 respectively in Hb genotype AC with (F= 57.68 P = 0.00) (F = 28.99; P = 0.00) and (F = 3.96; P=0.02) in absolute neutrophil, lymphocyte and monocyte respectively. Multiple comparison between Hb genotype AA and AS show that, mean ± SD of MPC in haemoglobin genotype AA was significantly (P<0.05) higher compared to AS, mean ± SD of platelet count in Hb genotype AA was higher compared to AS; it show no significant difference (p>0.05), the mean ± SD of PDW and MPV in Hb genotype AA were lower compared to Hb genotype AS, there is no significant difference (p > 0.05). However, the mean ± SD of WBC, relative neutrophil, in Hb genotype AA were significantly (P< 0.05) higher compared to Hb genotype AS, mean ± SD of relative lymphocyte in Hb genotype AA was significantly (P<0.05) lower compared to Hb genotype AS. Furthermore, mean SD relative monocyte and relative eosinophil in Hb genotype AA were lower compared to Hb genotype AS. There is significant difference (P > 0.05). Moreover, mean ± SD of absolute neutrophil, absolute lymphocyte and absolute monocyte in Hb genotype AA were significantly (P < 0.05) higher compared to Hb genotype AS. Multiple comparisons between Hb genotype AA and AC show that mean ± SD of MPC in Hb genotype AA was significantly (P < 0.05) higher

compared to Hb genotype AC; mean ± SD of absolute platelet count in Hb genotype AA was higher compared to Hb genotype AC. There is no significant difference (P> 0.05) in the comparison, mean ± SD of PDW and MPV in Hb genotype AA were lower compared to Hb genotype AC, it show no significant difference (P > 0.05). However, mean ± SD WBC and relative neutrophil in Hb genotype AC; mean ± SD of relative lymphocyte in Hb genotype AA was significantly (P > 0.05) lower compared to Hb AC. Moreover, mean ± SD of relative monocyte and eosinophil in Hb genotype AA were higher compared to Hb genotype AC. There is no significant difference in the comparison (P > 0.05). However, mean ± SD of absolute neutrophil, absolute lymphocyte and absolute monocyte in Hb genotype AA were significantly (P < 0.05) higher compared to Hb genotype AC. Multiple comparison between Hb genotype AS and AC show that mean ± SD of MPC, absolute platelet count, PDW, MPV, total WBC and relative neutrophil count in Hb AS were lower compared to Hb genotype AC; there is no significant difference (P> 0.05) in the comparison. However, mean±SD of relative lymphocyte, relative monocyte and relative eosinophil in Hb genotype AS were higher compared to Hb genotype AC there is no significant difference (P > 0.05). Moreover, mean±SD of absolute neutrophil, absolute lymphocyte and absolute monocyte in Hb genotype AS were lower compared to Hb genotype AC. There is no significant difference (P > 0.05).

**Table 3**: show comparison of mean ± SD in blood cell parameters on haemoglobin genotype variants in control subjects. The mean ± SD of absolute platelet 271.00 ± 25.46 in Hb genotype AC was lower compared to 284.53 ± 46.27 and 291.24 ± 55.68 in Hb genotype AA and AS respectively (F = 0.26; P = 0.78). The mean ± SD of PDW 11.99 ± 1.61 in Hb genotype AA was lower compared to 12.01 ± 2.26 and 12.60 ± 1.69 in Hb genotype AS and AC respectively (F=0.12; P=0.89). The mean ± SD of MPV 9.25 ± 0.49 in Hb genotype AC was lower compared to 9.52 ± 0.22 and 9.52 ± 0.31 in Hb genotype AA and AS respectively(F = 1.71; P=0.31). The mean ±SD of total WBC 4.43±0.32 in Hb genotype AA was higher compared to 4.41 ±0.39 and 4.5 ± 0.35 in Hb genotype AS and AC respectively (F = 0.08; P=0.92). The mean ± SD of relative neutrophil count 57.19 ± 4.48 in Hb genotype AA was lower compared to 57.57 ± 3.19 and 58.00 ± 7.07 in Hb genotype AS and AC respectively (F = 0.08, P= 0.93). The mean ± SD of relative lymphocyte 42.00 ± 7.07 in Hb genotype AC was higher compared to 41.62 ± 4.76 and 41.29 ± 2.90 in Hb genotype AA and AS respectively, the comparison show no statistical difference (p>0.05). (F = 0.06, P = 0.95). The mean ±SD of relative monocyte and eosinophil 0.97 ±1.07 and 0.33±0.71 respectively in Hb genotype AA were lower compared to mean ± SD of relative monocyte and eosinophil 0.86 ± 0.96 and 0.29 ± 0.56 respectively in Hb genotype AS with respect to (F=0.91, P = 0.41) and (0.25, P= 0.78) in relative monocyte and eosinophil respectively; the comparison show no significant difference (p>0.05). The mean ± SD of absolute neutrophil 2.53 ± 0.51 in Hb genotype AC was lower compared to 2.54 ± 0.31 and 2.54 ± 0.22 in Hb genotype AA and AS respectively (F = 0.00; P= 0.99). The mean ± SD of absolute lymphocyte 1.84 ± 0.23 in Hb genotype AA was higher compared to 1.82 ± 0.23 and 1.82 ± 0.16 in Hb genotype AS and AC respectively (F = 0.06, P= 0.95). The mean ± SD of absolute monocyte 0.04 ± 0.05 and 0.04 ± 0.05 in Hb genotype AA and AS respectively is the same mean value; hence, the comparison show no significant difference (p>0.05). (F = 0.86; P=0.43). However multiple comparisons between Hb genotype AA and AS show that, there is no significant difference (P>0.05) in any of the parameters compared. The mean ± SD of absolute platelets and PDW in Hb genotype AA were lower compared to Hb genotype AS; the mean ± SD of MPV in Hb genotype AA and AS was the same; the mean ± SD of WBC in Hb genotype AA was higher compared to Hb genotype AS. However, mean ±SD of relative neutrophil in Hb genotype AA was lower compared to Hb genotype AS. The mean ± SD of relative lymphocyte in Hb genotype AA was higher compared to Hb genotype AS. The mean ±SD of relative monocyte and eosinophil in Hb genotype AA were higher compared to Hb genotype AS. The mean ±SD absolute neutrophil and absolute monocyte in Hb genotype AA and AS were of the same mean value; however mean ± SD of absolute lymphocyte in Hb genotype AA was higher compared to Hb genotype AS. Multiple comparisons of Hb AA and AC show that, the mean ± SD of absolute platelet count, in Hb AA was higher compared to Hb genotype AC. Comparisons show no significant difference (P>0.05); the mean ± SD of PDW in Hb AA was lower compared to Hb genotype AC, comparison show no significant difference (P>0.05); the mean ± SD of MPV and WBC in Hb genotype AA were higher compared to Hb genotype AC, comparisons show no significant difference (P>0.05); however, mean ± SD of relative neutrophil and relative lymphocyte in Hb genotype AA was lower compared to Hb genotype AC, the comparisons show no significant difference (P>0.05). The mean ± SD of relative monocyte, eosinophil and absolute monocyte in Hb genotype AA has nothing to compare with in Hb genotype AC; however mean ± SD of absolute neutrophil and lymphocyte in Hb genotype AA were higher compared to Hb genotype AC. The comparison shows no significant difference (P> 0.05). Multiple comparison of Hb AS and AC show that the mean±SD of platelet in Hb genotype AS was higher compared to the genotype AC, the comparison show no significant difference (P> 0.05). The mean ± SD of PDW in Hb genotype AS was lower comparison to Hb genotype AC; the comparison show no significant difference (P>0.05); the mean ± SD of MPV and WBC in Hb genotype AS were higher compared to Hb AC; the comparison show no significant difference (P> 0.05). However, the mean ± SD of relative neutrophil and relative lymphocyte in Hb genotype AS were lower compared to Hb genotype AC, the comparisons show no significant difference (P>0.05); the mean ± SD of relative monocyte, relative eosinophil and absolute monocyte in Hb genotype AS has no mean±SD value compared to Hb genotype AC. Hence, the mean ± SD of absolute neutrophil in Hb genotype AS were higher compared to Hb genotype AC, comparison show no significant difference (P>0.05); absolute lymphocyte in Hb genotype AS and AC has the same mean± SD value.

## 6. Discussion

Out of 202 *plasmodium falciparum* malaria patients in this study, 130 were haemoglobin genotype AA, 54 were haemoglobin genotype AS, and 18 were haemoglobin

genotype AC, while in control group, 79 were haemoglobin genotype AA, 21 were haemoglobin genotype AS and 02 were haemoglobin genotype AC. Prevalence of haemoglobin genotype AA and haemoglobin genotype AS in this study was similar to the report of Francis and Pete (14), stated that of the four hundred (400) subjects screened for haemoglobin genotype in malaria infected patients, 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, similarly, Edith *et al.,* (15) reported the frequencies of Hb genotypes in *plasmodium falciparum* infected patients as 73.2% AA; 15.0% AC; 8.2% AS; 2.2% CC; 1.1% CS and 0.2% SS. This study showed Hb AS had lower malaria parasite count compared to Hb AC and Hb AA in pre-treatment and post-treatment. Hb AA had higher malaria parasite count; result in this present study showed that Hb AS and AC had genetic resistance to malaria attack compared to Hb AA. This was supported by Francis and Pete (14), reported that heterozygote individuals (HbAS) were more resistant to *plasmodium falciparum* malaria than normal dominant homozygous (HbAA) individuals. This present study support the facts that AS and AC genotype was associated with lower incidence of clinical malaria relative to AA genotype which has been widely reported. According to Rihet *et al.,*; Williams *et al.,* ; Verra *et al.*, and Kreuels *et al.,* (16, 17a, 17b,18 and 19) they reported that HbAS is widely known to confer significant protection from severe and uncomplicated malaria. Similar protection afforded by haemoglobin C (HbC) was more recently demonstrated although findings are less conclusive. Clinical studies performed in Nigeria and Mali has found no protection (20, 21), while other Malian study and Burkina study indicated an association between HbAC and clinical malaria (22, 23). However, there was significant decrease in malaria parasite count mean value of Hb AA, AS and AC in post treatment, Hb AS still had significantly lower mean malaria parasite count compared to Hb AC and AA. This was due to effect of anti-malaria drug used during treatment. In pre- treatment, absolute platelet count of Hb AS was lower compared to Hb AC while Hb AA had higher mean value. In post treatment, there was generally increase in absolute platelet count although no significant difference was observed, in both pre treatment and post treatment. Hb AA still had higher absolute platelet mean value compared with Hb AS and AC. low platelet in this present study support the facts that platelet could form 'clumps' with *Plasmodium*-infected erythrocytes, hence thrombocytopenia may be helpful as a sensitive but not specific marker of active infection. However, low amount of platelets may not only be a marker of parasite burden but may be protective against severe disease (24, 25). Control subject in this present study of haemoglobin variants Hb AA, AS and AC had absolute platelet count within the normal range although there is no significant difference. Platelet distribution width was lower in Hb AA compared to Hb AS and AC while mean platelet volume was higher in Hb AA compared to Hb AS and Hb AC in pre treatment. However, in post treatment, platelet distribution width and mean platelet volume were low in Hb AA compared to Hb AS and Hb AC although there is no significant difference in pre treatment and post treatment. There was general increase in immune response to malaria attack in Hb AA compared to Hb AS and AC. Hb AA had slightly high total white blood cell count, relative and absolute neutrophil count, absolute lymphocyte and monocyte were slightly higher in Hb AA compared to Hb AS and Hb AC in pre treatment. Moreover, there was general decrease in total and differential count except slight lymphocytosis that was observed in post treatment compared to pre treatment, it was generally observed that, immune response started returned to normal while compared the mean value in post treatment with control subject. This decrease was due to the effect of anti-malaria drug used during treatments. There is significant difference in total and differential leukocyte count in pre treatment and post treatment. The findings in this present study was similar to the report of Francis and Pete (14), stated that white blood cell and granulocyte counts were higher in Hb AA subjects compared with other haemoglobin variants and on the contrary they reported that, lymphocyte count was higher in Hb AA compared with other haemoglobin variants during the progress of malaria infection which could be associated with severe or acute malaria. However, in Hb AS and Hb AC, there is genetic immune resistance to malaria infection which reflected in this present study as supported by Francis and Pete (14), who reported that heterozygote individuals (HbAS) were more resistant to *falciparum malaria* than normal dominant homozygous (HbAA) individuals and Hb AA subjects suffered malaria more frequently, had significantly higher parasite density than other haemoglobin variants which is similar to this present study. Multiple comparisons among Hb genotype AA, AS and AC showed significant difference in most of the comparison in pre treatment and post treatment, but there is no significant difference in most of the comparison in control.

## 7. Conclusion

Mean parasite density was markedly lower in HbAS relative to HbAA genotype normal haemoglobin, Parasite density was however higher among AC relative to AA genotype, suggesting potential mechanistic variation among protection afforded by abnormal genotypes. Indeed, abnormal haemoglobin may not allow for optimal development of *Plasmodium* in deep organs where oxygen pressure is reduced.

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**Table 1:** Blood Cell Parameters on Haemoglobin Genotype Variant in Pre Anti-Malaria Drug Treatment Of Malaria Infected Sujects

| Groups | MPC µ/L | PLT $X10^{9/L}$ | PDW fl | MPV pL | WBC $X10^{9/L}$ | NEU % | LYMP % | MONO % | EOSIN % | NUE $X10^{9/L}$ | LYM $X10^{9/L}$ | MONO $X10^{9/L}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hb AA (N=130) | 2795.10 ±378.60 | 171.61 ±50.88 | 13.45 ±2.51 | 9.79 ±0.81 | 7.52± 1.37 | 59.81± 9.28 | 35.77± 8.79 | 3.65± 2.34 | 0.79± 0.99 | 4.53± 1.20 | 2.66± 0.72 | 0.27± 0.20 |
| Hb AS (N=54) | 2311.10 ±295.37 | 162.57 ±48.51 | 13.83 ±2.16 | 9.78 ±0.78 | 5.09± 1.01 | 51.44± 10.29 | 43.17± 9.31 | 3.96± 1.97 | 1.44± 1.99 | 2.63± 0.78 | 2.19± 0.64 | 0.20± 0.10 |
| Hb AC (N=18) | 2374.60 ± 279.74 | 165.22 ±49.40 | 13.98 ±2.71 | 9.59 ±0.75 | 5.47± 0.87 | 54.89± 5.79 | 41.61± 6.30 | 2.94± 1.39 | 0.67± 0.97 | 3.03± 0.72 | 2.25± 0.33 | 0.16± 0.08 |
| F (P-value) | 41.56 (0.00*) | 0.67 (0.52) | 0.96 (0.50) | 0.52 (0.59) | 81.59 (0.00*) | 15.93 (0.00*) | 15.08 (0.00*) | 1.49 (0.23) | 5.04 (0.01*) | 66.29 (0.00*) | 10.48 (0.00*) | 5.66 (0.00*) |
| Hb AA vs Hb AS p-value | 0.00* | 0.49 | 0.57 | 0.99 | 0.00* | 0.00* | 0.00* | 0.62 | 0.06 | 0.00* | 0.00* | 0.01* |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hb AA vs Hb AC p-value | 0.00* | 0.87 | 0.72 | 0.54 | 0.00* | 0.01* | 0.01* | 0.18 | 0.87 | 0.00* | 0.00* | 0.00* |
| Hb AS vs HbAC p-value | 0.69 | 0.98 | 0.97 | 0.63 | 0.31 | 0.19 | 0.71 | 0.05* | 0.08 | 0.13 | 0.89 | 0.16 |

P<0.05 significance, P>0.05 no Significant, F (P-value) = mean ± SD of parameters compared using ANOVA

**Table 2:** Blood Cell Parameters On Haemoglobin Genotype Variants In Post Anti Malaria Drug Treatment In Malaria Infected Subjects

| Groups | MPC µ/L | PLT X10$^{9/L}$ | PDW fl | MPV pL | WBC X10$^{9/L}$ | NEU % | LYMP % | MONO % | EOSIN % | NUE X10$^{9/L}$ | LYM X10$^{9/L}$ | MONO X10$^{9/L}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hb AA (N=130) | 2477.30±594.81 | 184.65±59.58 | 13.59±2.51 | 9.54±0.67 | 6.75±1.79 | 55.04±8.24 | 42.78±7.78 | 1.79±1.54 | 0.38±0.68 | 3.73±1.22 | 2.87±0.87 | 0.13±0.14 |
| Hb AS (N=54) | 1983.50±161.26 | 170.26±52.85 | 13.93±2.28 | 9.71±0.63 | 4.15±0.93 | 48.41±8.39 | 48.80±7.49 | 2.02±1.57 | 0.80±1.29 | 2.02±0.61 | 2.02±0.51 | 0.08±0.07 |
| Hb AC (N=18) | 2026±165.03 | 173.11±53.03 | 14.18±2.70 | 9.79±0.57 | 4.43±0.88 | 50.94±5.42 | 47.39±5.45 | 1.33±0.97 | 0.33±0.49 | 2.29±0.64 | 2.07±0.33 | 0.06±0.04 |
| F (P-value) | 22.71 (0.00*) | 1.33 (0.27) | 0.61 (0.51) | 2.19 (0.12) | 62.73 (0.00*) | 13.44 (0.00*) | 13.31 (0.00*) | 1.42 (0.25) | 4.57 (0.01*) | 57.68 (0.00*) | 28.99 (0.00*) | 3.96 (0.02*) |
| Hb AA vs Hb AS p-value | 0.00* | 0.24 | 0.65 | 0.21 | 0.00* | 0.00* | 0.00* | 0.65 | 0.08 | 0.00* | 0.00* | 0.02* |
| Hb AA vs Hb AC p-value | 0.00* | 0.68 | 0.67 | 0.22 | 0.00* | 0.02* | 0.01* | 0.21 | 0.92 | 0.00* | 0.00* | 0.00* |
| Hb AS vs HbAC p-value | 0.61 | 0.98 | 0.94 | 0.88 | 0.48 | 0.31 | 0.67 | 0.08 | 0.08 | 0.28 | 0.85 | 0.21 |

P<0.05 Significance, P>0.05 no Significant, F (P-value) = mean ± SD of parameters compared using ANOVA

**Table 3:** Mean ±Sd Of Blood Cells Parameters On Haemoglobin Genotype Variants In Non-Malaria Infected Subjects (Control)

| Groups | MPC µ/L | Platelet X10$^{9/L}$ | PDW fl | MPV pL | WBC X10$^{9/L}$ | NEU % | LYMP % | MONO % | EOSIN % | NEU X10$^{9/L}$ | LYM X10$^{9/L}$ | MONO X10$^{9/L}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hb AA (N=79) | - | 284.53±46.27 | 11.99±1.61 | 9.52±0.22 | 4.43±0.32 | 57.19±4.98 | 41.62±4.76 | 0.97±1.07 | 0.33±0.71 | 2.54±0.31 | 1.84±0.23 | 0.04±0.05 |
| Hb AS (N=21) | - | 291.24±55.68 | 12.01±2.26 | 9.52±0.31 | 4.41±0.39 | 57.57±3.19 | 41.29±2.90 | 0.86±0.96 | 0.29±0.56 | 2.54±0.22 | 1.82±0.23 | 0.04±0.05 |
| Hb AC (N=02) | - | 271.00±25.46 | 12.60±1.67 | 9.25±0.49 | 4.35±0.35 | 58.00±7.07 | 4.2.00±7.07 | - | - | 2.53±0.51 | 1.82±0.16 | - |
| F (p-value) | - | 0.26 (0.78) | 0.12 (0.89) | 1.17 (0.31) | 0.08 (0.92) | 0.08 (0.93) | 0.06 (0.95) | 0.91 (0.41) | 0.25 (0.78) | 0.00 (0.99) | 0.06 (0.95) | 0.86 (0.43) |
| Hb AA vs Hb AS p-value | - | 0.87 | 1.00 | 1.00 | 0.97 | 0.91 | 0.91 | 0.88 | 0.95 | 1.00 | 0.95 | 0.93 |
| Hb AA vs Hb AC p-value | - | 0.79 | 0.89 | 0.78 | 0.95 | 0.99 | 0.99 | 0.00* | 0.00* | 1.00 | 0.98 | 0.00* |
| Hb AS vs HbAC p-value | - | 0.68 | 0.90 | 0.78 | 0.93 | 1.00 | 0.98 | 0.00* | 0.07 | 1.00 | 1.00 | 0.00* |

P<0.05 Significance, P>0.05 no Significant, F (P-value) = mean ± SD of parameters compared using ANOVA