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Evaluation of Cortisol, Malondialdehyde, Blood Glucose and Lipid Status on Haemoglobin Variants in Malaria Infected and Non- Malaria Infected Individuals

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Abstract The aim of this study was to determine the effect of haemoglobin variants on pre, post anti-malaria drug treatment in malaria infected and non-infected individuals. The study was conducted at Federal Medical Centre, Ido-Ekiti, Nigeria. Two hundred and two blood samples were collected twice from each malaria infected individuals; grouped as pre-treatment and post anti-malaria drug treatment. One hundred and two 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; haemoglobin electrophoresis was determined using cellulose acetate electrophoresis at alkaline pH. Cortisol was estimated using enzyme linked immunosorbent assay method. Data obtained was analyzed using SPSS version 16. The mean±SD of cortisol and MDA of HbAA in pre treatment and post treatment was significantly ($p<0.05$) higher compared to HbAS and HbAC. This present study showed that, malaria parasite is more intense in HbAA compared to HbAS and HbAC, however use of effective anti-malaria therapy reduced the burden of malaria intensity.

Keywords: *malaria parasite, stress and haemoglobin genotype*

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1. Introduction

The aimed of this study was to determine the effect of haemoglobin genotype variants on pre, post anti-malaria drug treatment in malaria infected and non-infected (control) individuals on stress index; cortisol, malondialdehyde (MDA), blood glucose and blood lipid profile. Severe *plasmodium falciparum* malaria has been associated with an increase in oxidative stress, in relation with disease severity [1,2,3]. Malaria infection has been found to be associated with lipid peroxidation (Malondialdehyde) accompanying reduction in antioxidant capacity of the infected patients especially *Plasmodium falciparum* infection. *Plasmodium* infected human erythrocytes are under increased oxidative stress exerted by the malaria parasite. Oxidative stress results from host immune reaction, as an acute phase response, and the intraerythrocytic parasite's metabolic processes [4]. Malaria parasite is capable of generating reactive oxygen species within the erythrocytes and the reactive oxygen species resulting from immune activation can further damage the uninfected erythrocytes. 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 hemoglobin (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 [5] the protective effect of HbAS, HbAC, and HbCC variants is attributed to the altered expression of *Plsamodium falciparum* erythrocyte membrane protein 1 (PfEMP1) and reduction in binding of these infected red blood cells (IRBCs) to endothelial mono-layers and blood monocytes possibly interfering trafficking and anchoring of PfEMP‑1, mediated by elevated levels of membrane-bound, oxidized, and denatured hemoglobin molecules (hemichromes) in HbS and HbC infected red blood cells [6,7]. Haemoglobin S (HbS) has become a stable polymorphism within malaria-endemic regions, associated with a limited life expectancy among homozygous individuals who suffer from sickle cell disease, and an extended life expectancy of heterozygous individuals who are more likely to evade malaria [8]. HbAS is widely known to confer significant protection from severe and uncomplicated malaria [9].

Several innate or immune mechanisms have been hypothesized to explain malaria-protective effects of HbS or HbC. Erythrocytes containing HbS or HbC may impede parasite growth and replication relative to normal red cells when subject to low oxygen tensions [5]. Protein targets of specific antibodies may be more rapidly exposed in HbS-containing red blood cells [10] resulting in an enhanced immune response to infection [5]. It is also possible that unknown innate protective processes may up-regulate the malaria-specific immune response or enhance nonspecific immunity to malaria [11].

2. Materials and Methods

2.1. Subjects and Study Design

This study was conducted at Federal Medical Centre, Ido-Ekiti, 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. Two hundred and two blood samples were collected (8 ml) twice from the same malaria infected individuals; grouped as pre-treatment (at presentation) and post anti-malaria drug treatment. One hundred and two blood samples from apparently healthy individuals negative to malaria infection were collected for control. 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.

2.2. Sample Collection

Eight millimeters (8ml) of blood sample was collected from each subject on the first day of visiting hospital as baseline sample after the patient has been clinically diagnosis for malaria infection, another 8 ml of blood sample was collected on the second or third day after taking anti-malaria drugs; 3 ml of blood sample was dispensed into plain bottles; serum was extracted to assay stress index hormone (cortisol) and malondialdehyde (MDA), 1ml of blood sample was dispensed into fluoride oxalate bottles to assay blood glucose level, 3ml of blood sample was collected into lithum heparin bottle to assay lipid profile and 1ml of blood sample was dispensed into di-potassium ethylenediaminetetracetic acid (K_2EDTA) vaccutainer bottles for malaria parasite detection on thick blood film. K_2EDTA sample was used to run haemoglobin electrophoresis genotype

2.3. Methodology

Thick blood film was made from EDTA blood sample and stained with Giemsa's staining technique for malaria parasite detection; observed under microscopy using x100 objective lenses, the procedure was described by Monica Cheesbrough [12]. 0.3 ml of blood sample was used to prepared hemolysate by centrifuge at 3000 g 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 (40 ul) 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. Cortisol was estimated using enzyme linked immunosorbent assay (ELISA) method by Monobind Inc. Lake Forest, CA 92630, USA. The procedure was as described by the manufacturer of the kit. Malondialdehyde (MDA) was estimated using thiobarbituric acid method by Tomotsu. Briefly; 0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1 ml of 0.6 % 2-thiobarbituric acid (TBA) was added to the mixture, shaken, and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of nbutyl- alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml of 1,1,3,3-tetraethoxypropane (TEP) were used. The results were expressed as nmol/ml plasma [14]. However, blood glucose was estimated using glucose oxidase method. The procedure was as described by the manufacturer of the kit (Randox); 0.01 ml of blood sample was pipette into a labeled clean test tube, 0.01 ml of reagent standard was pipette into another labeled clean test tube, 1.00 ml of glucose reagent was pipette into each of the test tube, water blank was used; contents in the test tube was mixed and incubated for 10 minutes at 37°C. Absorbance was read at 500 nm. Lipid profile majorly consist of high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides and cholesterol. Lipid profile was estimated using CHOD-PAP method; the procedures were as described by the manufacturer of the kit (randox); Low density lipoprotein was calculated by Freidewald formular.

3. 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.

4. Result

Table 1: show the mean ± SD of Biochemical parameters on haemoglobin genotype variants in pre anti-malaria drug treatment in malaria infected individuals. The biochemical parameters include cortisol (µg/dL), MDA (nmol/ml), glucose (mmol/L), HDL (mg/dL), LDL (mg/dL), Triglycerides (mg/dL) and total cholesterol (mg/dL). The mean ± SD of cortisol 23.11 ± 3.96 in Hb genotype AA was significantly ($P<0.05$) higher compared

to 21.39 ± 3.17 and 22.07 ± 2.06 In Hb genotype AS and AC respectively (F = 4.49, P= 0.01); the mean ± SD of MDA 20.14 ± 3.12 in Hb genotype AA was significantly (P<0.05) higher compared to 18.87 ± 2.71 and 18.88 ± 2.08 in Hb genotype AS and AC respectively(F = 4.35; P=0.01); the mean ± SD of glucose 4.97 ± 0.69 in Hb genotype AC was lower compared to 5.10 ± 0.75 and 5.20 ± 0.83 in Hb genotype AA and AS respectively. Comparison show no significant difference (P>0.05) (F =0.68, P=0.51); the mean ± SD of HDL 28.90 ± 4.55 in Hb genotype AA was lower compared to 29.89 ± 5.16 and 31.44 ± 4.10 in Hb genotype AS and AC respectively. The comparison show no significant difference (P>0.05) (F=2.75, P=0.07); the mean ± SD of LDL 43.22 ± 10.43 in Hb genotype AC was significantly (P<0.05) lower compared to 53.43 ± 15.22 and 56.22± 18.66 in Hb genotype AA and AS respectively (F = 4.54, P=0.01); the mean ± SD of Triglycerides 76.39 ± 0.36 in Hb genotype AC was lower compared to 78.33 ± 19.96 and 78.11 ± 20.75 in Hb genotype AA and AS respectively. Comparison show no significant difference (P>0.05) ((F=0.07, P=0.93); The mean ± SD of total cholesterol 89.83 ± 11.22 in Hb genotype AC was significantly (P<0.05) lower compared to 97.96 ± 15.05 and 101.78 ± 18.05 in Hb genotype AA and AS respectively (F=4.01, p=0.02). Multiple comparison between Hb genotype AA and AS show that the mean ± SD of cortisol and MDA in Hb genotype AA were significantly (P<0.05) higher compared to Hb genotype AS. However, the mean ± SD of glucose and HDL in Hb genotype AA were lower compared to Hb genotype AS. The comparison show no significant difference (P>0.05). The mean ± SD of Triglycerides in Hb genotype AA was higher compared to Hb genotype AS. The comparison show no significant difference (P>0.05). Also, the mean ± SD of total cholesterol in Hb genotype AA was significantly (P<0.05) lower compared to Hb genotype AS. Multiple comparison between Hb genotype AA and AC show that the mean ± SD of cortisol, MDA and glucose in Hb genotype AA were higher compared to Hb genotype AC. The comparison show no significant difference (P>0.05); however, the mean ± SD of HDL in Hb genotype AA was lower compared to Hb genotype AC. The comparison show no significant difference (P>0.05). Moreover, the mean ± SD of LDL in Hb genotype AA was significantly (P<0.05) higher compared to Hb genotype AC, also the mean ± SD of Triglycerides in Hb genotype AA was higher compared to Hb genotype AC. The comparisons show no significant difference. (P>0.05); the mean ± SD of total cholesterol in Hb genotype AA was significantly (P<0.05) higher compared to Hb genotype AC. Multiple comparison between Hb genotype AS and AC show that; the mean ± SD of Cortisol and MDA in Hb genotype AS were lower compared to Hb genotype AC. The comparison show no significant difference (P>0.05); also, the mean ± SD of glucose in Hb genotype AS was higher compared to Hb genotype AC. The comparison show no significant difference (P>0.05); the mean ± SD of HDL in Hb genotype AS was lower compared to Hb genotype AC. The comparison show no significant difference (P>0.05). However, the mean ± SD of LDL in Hb genotype AS was significantly (P<0.05) higher compared to Hb genotype AC. The mean ± SD of Triglycerides in Hb genotype AS was higher compared to Hb genotype AC, the comparison show no significant difference (P>0.05) also, the mean ± SD of total cholesterol in Hb genotype AS was significantly (P<0.05) higher in Hb AS compared to Hb genotype AC.

Table 1. Mean ±Sd Of Biochemical Parameters On Haemoglobin Genotype Variants In Pre Treatment Of Malaria Infected Sujects

GROUPS	CORTISOL µg/dL	MDA nmol/L	GLUCOSE mmol/L	HDL mg/dL	LDL mg/dL	TRIG mg/dL	TOTAL CHO mg/dL
Hb AA (N=130)	23.11+3.96	20.14+13.12	5.10+0.75	28.90+4.55	53.43+15.22	78.33+19.96	97.96+15.03
Hb AS (N=54)	21.39+3.17	18.87+2.71	5.20+0.83	29.89+5.16	56.22+18.66	78.11+20.75	101.78+18.05
Hb AC (N=18)	22.07+7.06	18.88+2.08	4.97+0.69	31.44+4.10	43.22+10.43	76.39+20.36	89.83+11.22
F (p-value)	4.49 (0.01*)	4.35 (0.01*)	0.68 (0.51)	2.75 (0.07)	4.54 (0.01*)	0.07 (0.93)	4.01 (0.02*)
Hb AA vs Hb AS p-value	0.01*	0.02*	0.73	0.44	0.59	0.99	0.36
Hb AA vs Hb AC p-value	0.20	0.67	0.73	0.06	0.00*	0.92	0.03*
Hb AS vs HbAC p-value	0.56	0.99	0.48	0.40	0.00*	0.95	0.00*

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

Table 2: show mean ± SD of biochemical parameters on haemoglobin genotype variants in post anti-malaria drug treatment in malaria infected individuals treatment. The mean ± SD of cortisol 17.69± 7.32 in Hb genotype AA was significantly (P<0.05) higher compared to 14.98± 4.38 and 15.24 ± 3.89 in Hb genotype AS and AC respectively. (F=3.93, P=0.02). The mean ± SD of MDA 16.26 ± 6.33 in Hb genotype AA was significantly (P<0.05) higher compared to 13.50±3.62 and 13.85±2.92 in Hb genotype AS and AC respectively (F=5.50; P=0.01); the mean ±SD of glucose 4.47 ± 0.95 in Hb genotype AA was higher compared to 4.21 ± 0.71 and 4.11 ± 0.60 in Hb genotype AS and AC respectively. The comparison show no significant difference (P>0.05) (F=2.50, P=0.09); the mean ± SD of HDL 27.33 ± 3.49 in Hb genotype AA was lower compared to 27.50 ± 3.45 and 28.33 ± 3.04 in Hb genotype AS and AC respectively, comparison show no significant difference (P>0.05) (F=0.67; P=0.51). The mean ± SD of LDL 51.89 ± 8.21 in Hb genotype AC was significantly (P<0.05) lower compared to 58.19 ± 11.61 and 61.67 ± 13.79 in Hb genotype AA and AS respectively.(F = 4.66; P=0.01); the mean ± SD of Triglycerides 87.04 ± 16.83 in Hb genotype AA was higher compared to 86.72 ± 19.24 and 86.28 ± 19.02 in Hb genotype AS and AC respectively. The comparison show no significant difference (P>0.05) (F=0.02, P=0.98); the mean ± SD of total cholesterol 97.56 ±9.00 in Hb genotype AC was significantly (P<0.05) lower compared to 102.88 ± 11.74 and 106.50 ± 13.84 in Hb genotype AA and AS respectively. (F=3.97, P=0.02). Multiple comparison between Hb genotype AA and AS show that the mean ± SD of cortisol and MDA in Hb genotype AA were significantly (P<0.05) higher compared to Hb genotype AS; also, the mean ± SD of glucose in Hb genotype AA was higher compared to Hb genotype AS. Comparison show no significant difference (P>0.05); the

mean ± SD of LDL and total cholesterol, HDL in Hb genotype AA were lower compared to Hb genotype AS; comparison show no significant difference (P>0.05). Multiple comparison between Hb genotype AA and AC show that the mean ± SD of cortisol, glucose, Triglycerides and total cholesterol in Hb genotype AA were higher compared to Hb genotype AC. The comparison show no significant difference (P>0.05); however, the mean ± SD of MDA and LDL in Hb genotype AA were significantly (P< 0.05) higher compared to Hb genotype AC. Also, mean ± SD of HDL in Hb genotype AA was lower compared to Hb genotype AC. The comparison show no significant difference (P>0.05). Multiple comparison between Hb genotype AS and AC show that the mean ± SD of cortisol, MDA, HDL in Hb genotype AS were lower compared to Hb genotype AC. The comparisons show no significant difference (P> 0.05); however, the mean ± SD of glucose Triglycerides and total cholesterol in Hb genotype AS were higher compared to Hb genotype AC. The comparisons show no significant difference (P> 0.05); also, the mean ± SD of LDL in Hb genotype AS was significantly higher compared to Hb genotype AC.

Table 2. Mean±Sd Of Biochemical Parameters On Haemoglobin Genotype Variants In Post Anti Malaria Drug Treatment In Malaria Infected Subjects

GROUPS	CORTISOL µg/dL	MDA nmol/L	GLUCOSE mmol/L	HDL mg/dL	LDL mg/dL	TRIG mg/dL	TOTAL CHO mg/dL
Hb AA (N=130)	17.69 +7.32	16.26 +6.33	4.47 +0.95	27.33 +3.49	58.19 +11.61	87.04 +16.83	102.88 +11.74
Hb AS (N=54)	14.98 +4.38	13.50 +3.62	4.21 +0.71	27.50 +3.45	61.67 +13.79	86.72 +19.24	106.50 +13.84
Hb AC (N=18)	15.24 +3.89	13.85 +2.92	4.11 +0.60	28.33 +3.04	51.89 +8.21	86.28 +19.02	97.56 +9.00
F (p-value)	3.93 (0.02*)	5.50 (0.01*)	2.50 (0.09)	0.67 (0.51)	4.66 (0.01*)	0.02 (0.98)	3.97 (0.02*)
Hb AA vs Hb AS p-value	0.01*	0.00*	0.12	0.95	0.24	0.99	0.22
Hb AA vs Hb AC p-value	0.09	0.02*	0.09	0.42	0.02*	0.99	0.08
Hb AS vs HbAC p-value	0.97	0.91	0.82	0.60	0.00*	1.00	0.08

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

Table 3. Mean ±Sd Of Biochemical Parameters On Haemoglobin Genotype Variants In Non-Malaria Infected Subjects (Control)

GROUPS	CORTISOL µg/dL	MDA nmol/L	GLUCOSE mmol/L	HDL mg/dL	LDL mg/dL	TRIG mg/dL	TOTAL CHO mg/dL
Hb AA (=79)	6.67+1.63	8.39+0.90	3.61+0.31	42.18+3.63	63.82+6.59	98.65+11.53	125.82+10.12
Hb AS (N=21)	6.27+1.62	8.38+0.82	3.60+0.37	41.14+3.41	58.33+6.52	89.14+9.46	116.86+7.78
Hb AC (N=02)	7.35+0.07	8.40+0.28	3.35+0.21	48.00+2.83	60.50+7.78	99.00+1.41	128.50+10.61
F (Pv-alue)	0.71 (0.49)	0.71 (0.93)	0.63 (0.53)	3.49 (0.03*)	5.87 (0.00*)	6.14 (0.00*)	7.30 (0.00*)
Hb AA vs Hb AS p-value	0.59	0.93	1.00	0.45	0.01*	0.00*	0.00*
Hb AA vs Hb AC p-value	0.00*	0.90	0.47	0.30	0.85	0.98	0.94
Hb AS vs HbAC p-value	0.02*	1.00	0.47	0.23	0.93	0.00*	0.52

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

Table 3: show the mean ± SD of biochemical parameters on haemoglobin genotype variants in control subjects. The mean ± SD of cortisol 7.35± 0.07 in Hb genotype AC was higher compared to 6.67 ± 1.63 and 6.27 ±1.62 in Hb genotype AA and AS respectively; the comparison show no significant difference (P>0.05) (F=0.71; P=0.49); the mean ± SD of MDA 8.40 ± 0.28 in Hb genotype AC was higher compared to 8.39 ± 0.90 and 8.38 ± 0.82 in Hb genotype AA and AS respectively. Comparison show no significant difference (P>0.05) (F=0.71; P=0.93); the mean ± SD of glucose 3.35 ± 0.21 in Hb genotype AC was lower compared to 3.61 ± 0.31 and 3.60 ± 3.35 in Hb genotype AA and AS respectively; comparison show no significant difference (P>0.05) (F=0.63, P=0.53); the mean ± SD of HDL 48.00 ± 2.83 in Hb genotype AC was significantly (P<0.05) higher compared to 42.18 ± 3.63 and 41.14 ± 3.41 respectively (F=3.49; p=0.03); the mean SD of LDL 63.82 ± 6.59 in Hb genotype AA was significantly (P<0.05) higher compared to 58.33 ± 6.52 and 60 50 ± 7.78 in Hb genotype AS and AC respectively (F = 5.87, P=0.00); the mean ± SD of triglyceride 99.00 ± 1.41 in Hb genotype AC was significantly (P< 0.05) higher compared to 98.65 ± 11.53 and 89.14 ±9.46 in Hb genotype AA and AS respectively (F = 6.14, P=0.00); the mean ± SD of total cholesterol 128.50 ± 10.61 in Hb genotype AC was significantly (P<0.05) higher compared to 125.82 ± 10.12 and 116.86 ± 7.78 in Hb genotype AA and AS respectively (F = 7.30, P=0.00). Multiple comparisons between Hb AA and AS show that mean ± SD of cortisol, MDA, glucose and HDL in Hb genotype AA were higher compared to Hb genotype AS. The comparisons show no significant difference (P>0.05). However, the mean ± SD of LDL, Triglycerides and total cholesterol were significantly (P<0.05) higher in Hb AA compared to Hb AS. Multiple comparison between Hb AA and AC show that mean ± SD of cortisol in Hb AA was significantly (P<0.05) lower compared to Hb genotype AC. Also, the mean ± SD of MDA, HDL, Triglycerides and total cholesterol in Hb genotype AA were lower compared to Hb genotype AC. The comparison show no significant difference (P>0.05); however, the mean ± SD of glucose and LDL in Hb genotype AA were higher compared to Hb genotype AC. The comparisons show no significant

difference (P>0.05). Multiple comparison between Hb genotype AS and AC show that; the mean ± SD of cortisol in Hb genotype AS was significantly lower compared to Hb genotype AC also, the mean ± SD of MDA, HDC, LDL and total cholesterol in Hb genotype AS were lower compared to Hb genotype AC, the comparison show no significant difference (P>0.05); however, mean ± SD of glucose in Hb genotype AS was higher compared to Hb genotype AC, the comparison show no significant difference. (P>0.05); also, the comparison show no significant difference. (P>0.05) also, the comparison show Triglycerides was significantly (P< 0.05) lower in Hb genotype AS compared to Hb genotype AC.

5. Dicussion

Statistical data analysis of cortisol and malondialdehyde estimated values on haemoglobin variants, in participants infected with *plasmodium falciparum* in pre anti-malaria drug treatment showed that Hb AA had significant higher mean value of cortisol and Malondialdehyde (MDA) compared to Hb AS and AC. This present study reflects the level of stress induced by malaria parasite infection among the subjects. Increases in cortisol level in this present study was supported by Rosana *et al*., [15] reported that increased cortisol levels in patients with *P. falciparum* malaria infection, indicating stimulation of the HPA axis in these patients. Similarly, Parker *et al.,* [16] stated that acute stimulation of the HPA axis by stress induced in malaria infection leads to the release of cortisol and other steroids produced and secreted by the adrenal glands resulted in increased cortisol concentrations throughout the stimulus, a reduction in the stimulation of the HPA axis occurs with clinical improvement. Tim *et al.,* [17] reported that there was significant rise in serum cortisol level of malaria patients compared to control and there was no a single patient with cortisol level less than the mean level of controls during the seven days period of treatment. However, Increase in malondialdehyde (MDA) in this present study support the fact that increased in malondialdehyde (MDA) in malaria patients indicates that there is increased production of reactive oxygen species in these patients. According to Amy *et al.,* [18], malaria parasite is capable of generating reactive oxygen species within the erythrocytes and this reactive oxygen species resulting from immune activation which further damages the uninfected erythrocytes. Kulkarni *et al.,* [19] reported that malaria activates the immune system of body thereby causing release of reactive oxygen species as an antimicrobial action. Highly Malondialdehyde (MDA) activity found in malaria positive patients is an indication of increased production of reactive oxygen species, Increased lipid peroxidation is also observed to be equally accountable for development of oxidative stress in malaria patients [20,21,22]. Increase in MDA level showed the extent of oxidative stress produced in *plasmodium falciparum* infection. In post treatment, level of cortisol, MDA and glucose were generally decrease, however, Hb. AA had higher mean value compared to AS and AC. Hb AS had lower mean value. This study showed the effect of anti-malaria during treatment which reflect in post treatment. decrease in cortisol level as observed in post treatment in this present was similar to the finding of Rosana *et al*., [15] reported that, Serum cortisol levels were measured on Days 0, 1 and 7, with a higher mean concentration being observed on Day 0 followed by a significant reduction on Day 1 and a highly significant decline on Day 7. In consistent with this study, Ringwald *et al.,* [23] reported that cortisol levels will be declining as the clinical condition improves and parasitemia decreases, probably due to a lower cytokine production. A decline in the levels of cortisol is found in maintained stress. Blood glucose level was slightly higher in Hb AA compared to AS and AC, this support the effect of high cortisol level produced during stress. Cortisol increase blood sugar through gluconeogenesis, similar to this study, Francis and Pete [24] reported that hypoglycaemic was found to be more common in HbAA subjects compared to HbAS. Hypoglycaemia was evident at malaria presentation and could be due to impaired hepatic gluconeogenesis and increased consumption of glucose in peripheral tissue as well as by parasite. Gluconeogenesis probably failed to compensate, in the presence of decreased glycogen flux of glucose, increasing the risk of hypoglycaemia in *falciparum* infected subjects as reported by Dekker *et al.,* [25] however, in post treatment, blood glucose level was gradually reduced as the cortisol level reduced. This was similar report of Goodyer, [26] stated that hypoglycemia occurs during the management of patients with malaria infection. There is no significant difference in the glucose level. Mean value of lipid profile (HDL, LDL, Triglyceride and Total Cholesterol) in pre treatment and post treatment were generally lower in Hb AA, AS and AC while compared to control subject. Mean value of HDL was observed lower in Hb AA compared to AS and AC in both pre and post treatment. However, LDL, triglycerides and total cholesterol were observed and significantly lower in Hb AC compared to Hb AA and AS in pre and post treatment. Multiple comparisons showed no significant difference among Hb AA, AS and AC in most of the comparison in pre treatment, post treatment and control subjects.

6. Conclusion

Malaria parasite infection is intense more in HbAA compared to HbAS and HbAC; however use of effective anti-malaria therapy reduced the burden of malaria parasite infection.

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