

***In Vivo* Comparative Evaluation of Effects of Artemeter-Lumefantrine, Sulphadoxine-Pyrimetamine and Halofantrine on G6PD Activities, Haemoglobin Concentration and Malaria Parasite Clearance Rate in Malaria Infected Adults**

Ukwah BN^{1*}, Kanu SC², Elom MO¹, Nweze E³, Nwovu AI⁴, Imakwu CA⁵, Akpan JI⁶, Ezeonu CT⁶, Ndie EC⁷,
Azi SO¹, Obi IA⁷, Ewenighi CO³

¹Medical Microbiology/Parasitology Unit, Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria.

²Department of Biochemistry, Renaissance University, Ugbo-Akwa, Enugu, Nigeria.

³Chemical Pathology Unit, Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria. ⁴Haematology Unit, Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria. ⁵Parasitology Unit, Department of Zoology, University of Nigeria, Nsukka.

⁶Department of Pharmacology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki. ⁷Department of Nursing Science, Ebonyi State University, Abakaliki, Nigeria

*Corresponding Author: Boniface N. Ukwah. Email: bnukwah@gmail.com

Abstract

Background: The use of antimalaria combination therapy especially regimens containing an artemisin-based compound has been recommended as a good first-line treatment for malaria by WHO. However, limited reports exist on the effects of the ACT and other antimalarial drugs on some vital biological parameters such as G6PD activity and haemoglobin concentration. In this study, we investigated the effects of Artemeter-Lumefantrine, sulphadoxine-pyrimetamine combination therapies and Halofantrine monotherapy on G6PD activity, Haemoglobin level and parasite clearance rate in malaria-infected adults treated with the drugs in Enugu, Nigeria. **Methodology:** Forty malaria-infected adults aged between 20 and 30 years were used. The subjects were divided into four groups (A, B, C and D). The groups A, B and C were given Artemeter-Lumefantrine, sulphadoxine-pyrimetamine and halofantrine respectively, while group D was a control group (malaria-positive control). Blood samples of the subjects were collected through venepuncture at baseline (Day 0) and after treatment on Day 4, for comparative analysis of G6PD activity, haemoglobin concentration and parasite clearance for each group. **Results:** The result of this study showed that sulphadoxine-pyrimetamine significantly ($p < 0.05$) lowered haemoglobin concentration as compared with Halofantrine, Artemeter-lumefantrine and control. The haemoglobin concentration of the last three groups did not show any significant difference ($p > 0.05$) between each other. The G6PD activity of the group treated with sulphadoxine-pyrimetamine was significantly ($p < 0.05$) higher as compared with that treated with Artemeter-lumefantrine but non-significant ($p > 0.05$) as compared with halofantrine and control. Parasite clearance rate was significantly ($p < 0.05$) highest with the Halofantrine group (76%, $p < 0.05$) while Sulphadoxine-pyrimetamine had the lowest (52%) parasite clearance. **Conclusion:** This study therefore indicated that antimalaria drugs as well as malaria parasite could cause a reduction in haemoglobin concentration with sulphadoxine-pyrimetamine causing significant ($p < 0.05$) increase in G-6-PD activity.

Keywords: Antimalarials, Artemether-Lumefantrine, G6PD, sulphadoxine-pyrimetamine, Parasite clearance, Halofantrine, Haemoglobin concentration

1. Introduction

Malaria affects half of the world's population and is the third leading causes of death for children less than five years worldwide [WHO, 2011]. Africa including Nigeria is one of the countries most affected by malaria and accounts for 25 per cent of global malaria cases (WHO, 1996; WHO, 2011). It is estimated that about 97% of Nigeria's population are at risk of malaria. According to data from Carter Centre for Malaria Control Programme, over 300,000 Nigerians—mostly children—die from malaria attack each year [WHO, 2011].

Several reports of increasing incidence of drug resistant malaria parasites is of serious concern and which is responsible for the increasing cases of malaria in Africa [Roll Back Malaria Partnership Secretariat, 2010; Korenrop *et al.*, 2004]. In an attempt to improve malaria treatment efficacy and reduce malaria parasite resistance to antimalarial drugs, the World Health Organization has advocated the use of Artemisinin-based combination therapy (ACT) and other combination therapy for the treatment of malaria [Olurische *et al.*, 2007]. Scientific reports have shown that ACTs are preferred because artemisin compounds have rapid parasite and fever clearance effects and reduce gametocyte rate with the potential to reduce transmission (Meremikwu, *et al.*, 2012). Artemether (artemesin-base) and lumenfantrine are blood schizontocides. Artemether is rapidly metabolized into an active metabolite dihydroartemisinin (DHA) (Medilexicon, 2013). The anti-malarial activity of artemether and DHA has been attributed to endoperoxide moiety (Medilexicon, 2013; Pandey *et al.*, 1999) Hoppe *et al.*, 2004). The exact mechanism by which lumefantrine, exerts its anti-malarial effect is not well defined (Medilexicon, 2013). However, available data suggests lumefantrine inhibits the formation of hemozoin by forming a complex with hemozoin (Medilexicon, 2013). Halofantrine presently is used as an antimalarial monotherapy in the form of halofantrine-hydrochloride (Glaxosmithkline, 2006; Truste, 2013). Halofantrine like ACT and sulphadoxine-pyremethamine, is a blood schizontocide and acts against the asexual erythrocytic stage of malaria parasite but its mechanisms of action is not yet known (Truste, 2013; Shah *et al.*, 2006). However, the lack of alternative drugs to artemisinin derivatives and their short half-lives and susceptibility to recrudescence, when given as monotherapy are the major drawbacks of the artemisinin derivatives [Dondorp, *et al.*, 2009; Douglas *et al.*, 2010; Phyo, *et al.*, 2012].

There are poor reports on the *in vivo* effects of ACT, and sulphadoxine-pyrimetamine combination therapies as compared to halofantrine monotherapy on some vital biological parameters such as G6PD and haemoglobin concentration as well as their comparative efficacies in malaria parasite clearance. Few studies, which demonstrated the haematocrit and G6PD effects of ACT, sulphadoxine-pyrimetamine and halofantrine to the best of my knowledge as at the time of this study, were conducted *in vitro* on infected RBC. Although *in vitro* studies can be used to evaluate what happens in humans; they exhibit natural differences in susceptibility to malarial infection. In this study, we therefore investigated the effects of Artemether-lumefantrine, sulphadoxine-pyrimetamine combination therapies and Halofantrine monotherapy on G6PD activity, Haemoglobin level in malaria parasite infected adult humans. The comparative efficacies of these antimalarial drugs on parasite clearance were also evaluated as the three drugs are blood schizontocides (Shah *et al.*, 2006; Truste, 2006; MediLexicon, 2013).

2. STUDY DESIGN AND METHODOLOGY

2.1 Subjects

Forty human subjects within the ages of 20-30 years (mean age of 25years) with uncomplicated malaria were selected and used for the study. They were divided into four groups (A, B, C and D) comprising ten individuals in each group. Individuals with any form of G6PD deficiency (G6PD value of $<6 \mu\text{gHb}$), diabetics and pregnant women were excluded from the study. Informed consents of the subjects were obtained prior to their participation in the study and they were assured of their safety and privacy throughout the study. The subjects were free to withdraw from the study at any time they wished.

2.2 Drug treatment

Group A was given Combination Therapy consisting of Artemether/Lumefantrine supplied as coartem (Novartis) (20mg/120mg) respectively (four tablets were administered twice daily for three days). Group B was given Combination Therapy consisting of sulfadoxine/Pyrimetamine supplied as malareich (Medreich pharmaceutical company) (500 mg/25 mg) respectively, administered as 3 tablets in a single dose. The group C was given Halofantrine (250 mg) supplied as halfan (Glaxosmithkline Pharmaceutical Company) containing 233 mg of halofantrine base and 17 mg hydrochloride. Two tablets of this were administered 6 hourly making a total of 6 tablets per day. The group D (control) was not given any drug during the study.

2.3 Blood sample collection

Before the commencement of treatment (Day 0), blood samples were collected from subjects in each group for the determination of parasite density (parasitaemia), haemoglobin concentration and G6PD enzyme activity at baseline. The drug treatment commenced on (Day 1) and lasted for three days. On (Day 4) another blood sample was collected from each subject in the four groups for the analysis of G6PD activity, haemoglobin concentration and parasite clearance rate. Five millilitres (5 ml) of blood was collected from each subject (before and after treatment) and poured into EDTA (ethylene di-amino tetra-acetic acid). Sample in the EDTA bottle was used for the analysis of G6PD enzyme activity, haemoglobin concentration and Parasite density. The blood was collected through venepuncture.

2.4 Laboratory procedures

G6PD enzyme activity was determined using spectrophotometric method of Horecker and Komberg, (Randox Laboratories Ltd, United Kingdom). Normal range of G6PD activity in erythrocyte as prescribed by Randox G6PD Kit (2010) is 6.97- 20.5 U/gHb. Baseline values outside this range were excluded. Haemoglobin concentration was determined by Cyanmethaemoglobin method as described by Ochei and KolHatkar (2008) and measured colourimetrically at 540 nm. Parasitaemia assessment was done using a thick film stained with giemsa. A minimum of 200 WBC were counted per slide of a sample. Malaria parasite density was determined by dividing the number of parasites counted by the number of WBCs $\times 8000/1 =$ parasites/ml of blood (WHO, 1996; WHO, 2010). Blood films were considered negative if no parasites were seen in 100 oil-immersion fields in a thick blood film.

2.5 Statistical analysis

Data was expressed as mean \pm standard deviation. Comparative analysis involving four treatment groups and other variables were done using two-way ANOVA. Statistical significance was set at $P < 0.05$. All statistics were carried out using GenStat Discovery Edition.

3. Results

Data obtained in this study showed that sulfadoxine-Pyrimetamine (sulpha/pyrem) significantly ($p < 0.05$) lowered haemoglobin concentration as compared with artemether-lumefantrine (ART/LUME), halofantrine and control. The haemoglobin concentration of ART/Lume, halofantrine and control groups differed non-significantly ($p > 0.05$) between each other (Figure 1). All the groups except the Art/lume group, showed non-significant ($p > 0.05$) decrease in haemoglobin concentration after three days of treatments (Day 4) as compared with the baseline haemoglobin level. In other words, the ART/Lume group showed a statistically non-significant increase ($p > 0.05$) in haemoglobin concentration after treatment (Figures 1, Tables 2, 3, 4 and 5). The G6PD activity of the group treated with sulpha/pyrem was significantly ($p < 0.05$) higher than the group A treated with ART/Lume, but non-significant ($p > 0.05$) as compared with the group B treated with halofantrine and control. Of all the groups, only the sulpha/pyrem group had significant ($p < 0.05$) increase in G6PD activity after the treatment (Figure 2, Tables 2, 3, 4 and 5).

All the antimalarial drugs significantly ($p < 0.05$) cleared the malaria parasites after 3 days of treatment as compared with the baseline and control. The control had a non-significant ($p > 0.05$) increase in the number of parasites per millilitre of blood as compared with the initial number. Halofantrine showed the highest percentage (76%) of parasitic clearance, then Artemether-lumefantrine (72%) with sulfadoxine-Pyrimetamine having the least percentage (52%) clearance (Figure 3, Tables 1, 2, 3, 4, and 5). Comparing the efficacies of the three antimalarial drugs used in this study in malaria parasite clearance, the result showed that there is no statistically significant ($p > 0.05$) difference between the efficacies of Artemether-lumefantrine and Halofantrine but those two drugs differed significantly ($p < 0.05$) from the efficacy of sulfadoxine-Pyrimethamine (Figure 3).

4.0 Discussion

Malaria is a systemic disease caused by infection of the red blood cells (RBCs) with intracellular protozoan parasites of the genus *Plasmodium*. The parasites are inoculated into the human host by a feeding female Anopheles mosquito (WHO, 1996). Clinical and scientific reports have shown that malaria parasites have developed increased resistance to antimalarial drugs. Ensnare of the increase in resistance by these parasites, antimalaria combination therapy especially artemesin-based combination therapy has been recommended (WHO, 2011). Our findings in this study on the efficacy of some antimalarial drugs (Both combination therapy and monotherapy) showed that halofantrine monotherapy (76%) was the most effective in parasite clearance, and then followed by ACT (Artemeter-lumenfantrine) combination therapy (72%). Sulphadoxine-pyremethamine combination therapy (52%) was the least effective in parasite clearance (Table 1). However, data analysis using two-way ANOVA revealed that the parasite clearance rates of the ACT and halofantrine as seen in this study were not significantly different from each other, but both were significantly ($p < 0.05$) more effective in parasite clearance as compared with sulphadoxine-pyremethamine. This finding indicates that not all combination therapy is very effective in malaria treatment rather some antimalarial monotherapy could be more effective as observed in this study (Table 1). The high parasite clearance rate (76%) of halofantrine as observed in this study is consistent with the report of Glaxosmithkline, (2006), which showed that halofantrine had parasite clearance rate of 90% and 99% in two separate studies. The low parasite clearance rate of sulphadoxine-pyremethamine is attributable to possible resistance to the drug. Our findings in this study agree with the report of Shah *et al.*, (2006) that sulphadoxine-pyremethamine failed in treatment of malaria parasite infections but artemether-lumenfantrine was effective in malaria treatment.

Malaria parasites residing in the host erythrocytes, endocytose large amounts of the surrounding RBC cytoplasm through an invagination of the parasite plasma membrane called cytostome (Hoppe *et al.*, 2004). The

endocytosed host RBC cytoplasm consist almost entirely of haemoglobin and can be digested in the food vacuole (Hoppe *et al.*, 2004). The findings of this study showed that control group (untreated malaria positive subjects) had a non-significant ($p>0.05$) decrease in haemoglobin concentration after 3 days of monitoring (Figure 1 and Table 5). The decrease in haemoglobin concentration is consistent with the report by Hoppe *et al.*, (2004). The non-significant decrease in haemoglobin concentration of this group is attributable to the uncompleted state of the malaria parasite infections during the study. Of the three groups administered different antimalarial drugs, only the group treated with artemesin-based combination therapy (Artemether-lumefantrine) showed an increase in haemoglobin concentration after 3 days of treatment. The increase in haemoglobin concentration of the group treated with Artemether-lumefantrine is consistent with the reports of Hoppe *et al.*, (2004) and Pandey *et al.*, (1999) and attributable to the endoperoxide activity of artemesin-based combination therapy which has been reported to inhibit haemoglobin degradation in malaria parasite infected RBC. The decrease in haemoglobin concentration observed in the groups treated with sulphadoxine-pyremethamine and halofantrine is attributable to their inability to inhibit endocytosis of host RBC cytoplasm. The non-significant nature of the drop in haemoglobin level among those groups treated sulpho/pyrem and halofantrine is possibly due to the significant ($p<0.05$) reduction in parasitemia after the 3 days of treatment. The higher percentage (1.57%) drop in haemoglobin level in the group treated with sulphadoxine-pyremethamine as compared with the group treated with halofantrine (0.65%) drop in haemoglobin level, could be attributed to the lower rate (52%) of parasite clearance by the sulpho/pyrem as compared with that observed in the group treated with halofantrine (76%) (Table 1). Also, these two drugs may have possibly stimulated endocytosis of the host RBC by the parasites leading to decrease in host haemoglobin. Hoppe *et al.*, (2004) carried out an *in vitro* study with chloroquine and observed that the drugs increased endocytosis of host RBC by malaria parasites with reduction of host haemoglobin concentration. Therefore our finding in this present study with respect to halofantrine and sulphadoxine-pyremethamine agrees with the report of the above study. The above inference is because the more parasite present in host RBC, the more endocytosis of host RBC cytoplasm and consequent degradation of host haemoglobin (Pandey *et al.*, 1999).

Some antimalarial drugs including artemesin based combination drugs have been reported to lower blood glucose level (Ewenighi *et al.*, 2013; Olayemi *et al.*, 2012). The enzyme, glucose IV phosphate dehydrogenase is known to play a key role in glucose metabolism and protects red blood cells from oxidative injury of drugs and other oxidants (Ewenighi *et al.*, 2013; Abdoulaye *et al.*, 2010). Our findings in this present study showed a significant ($p<0.05$) increase in G6PD activity in the groups treated sulphadoxine-pyremethamine combination therapy but no significant ($p>0.05$) increase in the control group and groups treated with ART/lume combination and Halofantrine monotherapies, before and after treatment (Figure 2; Tables 2, 3, 4 and 5). The findings indicate that sulphadoxine-pyremethamine combination therapy have haemolytic adverse effects on the patients with G6PD sufficient individuals and could cause anaemia in such peoples if not well managed like in G6PD deficient individuals as reported by Shah *et al.*, (2006). The haemolytic effects of sulphadoxine-pyremethamine could cause oxidative stress in the host and is attributable to the significant ($p<0.05$) increase in G6PD activity observed in the group treated with the drug. The increase in G6PD activity is possibly to protect the red cells against such oxidative injury as G6PD is known to plays key roles in protecting host RBC (Abdoulaye *et al.*, 2010). The lack of significant difference in G6PD activity in the groups treated with ART/lume and halofantrine agrees with the reports of Ewenighi *et al.*, (2013) that ACT and amodiaquine monotherapy have no adverse haemolytic effects on patients.

5 Conclusions

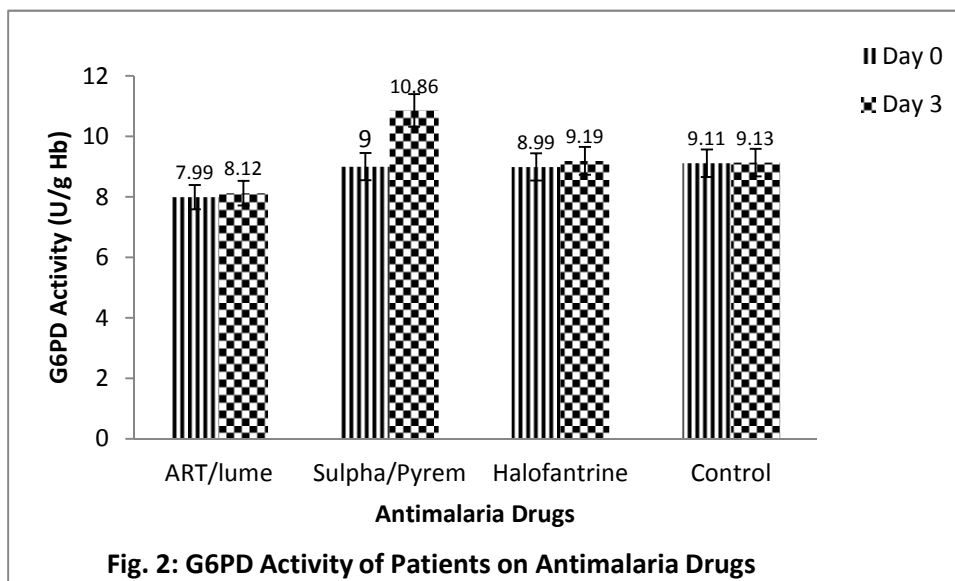
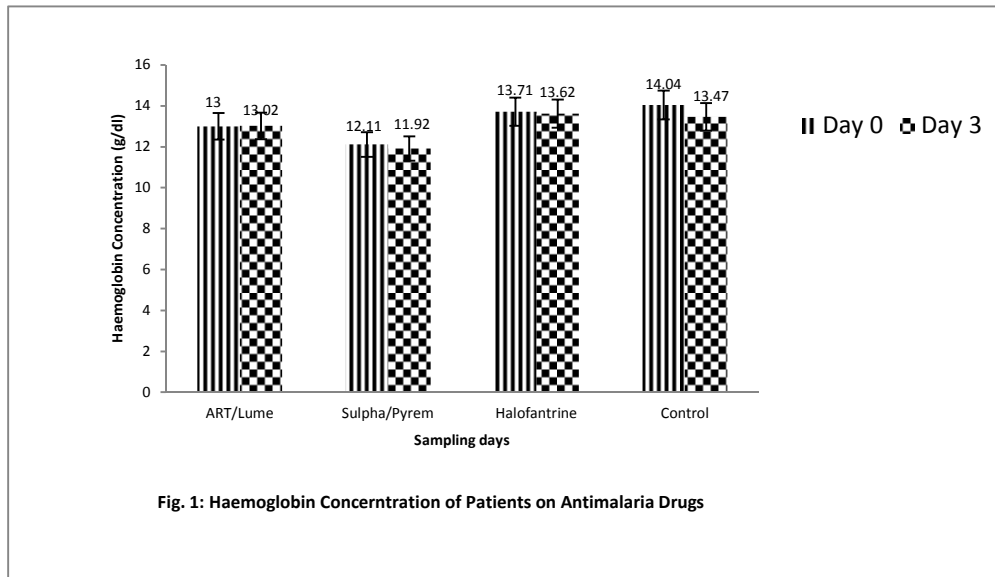
The findings of this study indicated that antimalarial drugs as well as malaria parasite possess the potential to reduce haemoglobin concentration and increase in G6PD activity which is significant with sulphadoxine-pyrimetamine. All the drugs used significantly ($p<0.05$) cleared malaria parasites after 3 days of treatment, with halofantrine being the most effective. We therefore recommend that further studies on these drugs be extended to 28 days or minimum of 14 days.

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Tables and Figures



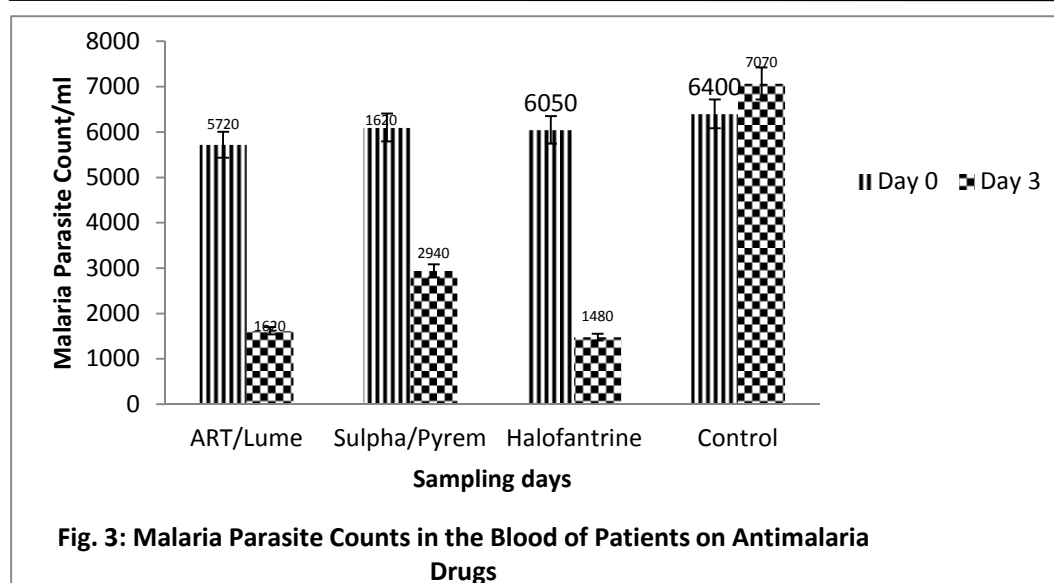


Table 1: The percentage malaria parasite clearance in patients treated with Artemether/lumefantrine, Sulfadoxine/Pyrimethamine and Halofantrine drugs

Drugs	Before Treatment	After Treatment	Mean Difference	% Clearance
Artemether-lumefantrine	5720±539	1620±421	4100	72%
Sulfadoxine-Pyrimethamine	6100±810	2940±1218	3160	52%
Halofantrine	6050±1066	1480±625	4570	76%
Control	6400±775	7070±879	670	10% increase

Table 2: G6PD activity, Haemoglobin concentration and malaria parasite density before and after treatment in subjects treated with Artemether/lumefantrine drug

Variables	Before Treatment	After Treatment	Mean Difference	P-value (Significance)
G6PD Activity (µ/gHb)	7.99±1.9	8.12±1.9	0.13	p>0.05
Haemoglobin (g/dl)	13.00±1.55	13.02±1.51	0.02	p>0.05
Parasites/ml of blood	5720±539	1620±421	4100	P<0.05

Table 3: G6PD activity, Haemoglobin concentration and malaria parasite density before and after treatment in subjects treated with Sulfadoxine/Pyrimethamine drug

Variables	Before Treatment	After Treatment	Mean Difference	P-value (Significance)
G6PD Activity (μ /gHb)	9.00 \pm 1.1	10.86 \pm 1.5	1.86	P<0.05
Haemoglobin (g/dl)	12.11 \pm 1.41	11.92 \pm 1.47	0.19	p>0.05
Parasites/ml of blood	6100 \pm 810	2940 \pm 1218	3160	P<0.05

Table 4: G6PD activity, Haemoglobin concentration and malaria parasite density before and after treatment in subjects treated with Halofantrine drug

Variables	Before Treatment	After Treatment	Mean Difference	P-value (Significance)
G6PD Activity (μ /gHb)	8.99 \pm 1.7	9.18 \pm 1.6	0.19	p>0.05
Haemoglobin (g/dl)	13.71 \pm 1.47	13.62 \pm 1.41	0.09	p>0.05
Parasites/ml of blood	6050 \pm 1066	1480 \pm 625	4570	P<0.05

Table 5: G6PD activity, Haemoglobin concentration and malaria parasite density of Malaria Patients without treatment after 3 days of initial Laboratory Diagnosis (Control group)

Variables	Initial Diagnosis	After 3 days	Mean Difference	P-value (Significance)
G6PD Activity (μ /gHb)	9.11	9.13	0.02	p>0.05
Haemoglobin (g/dl)	14.04 \pm 1.54	13.47 \pm 1.49	0.57	p>0.05
Parasites/ml of blood	6400 \pm 775	7070 \pm 879	670	P>0.05

Key: *P>0.05 = Non-significant; P<0.05 = Significant