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EFFECT OF COARTEM ON METHAEMOGLOBIN, OXYHAEMOGLOBIN AND PACKED CELL VOLUME AMONG RIVERS STATE UNIVERSITY STUDENTS IN NIGERIA

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ABSTRACT

Malaria as a disease sickens and kills human through several pathological mechanisms that is understood to varying degrees. The reason for treatment is to prevent death or long-term deficits or complications from malaria parasitaemia. The aim of this study was to investigate the effect of coartem on methaemoglobin, oxyhaemoglobin and packed cell volume. Fifty-eight blood samples were collected from forty (40) subjects recruited from Rivers State University (RSU). Twenty (20) subjects were apparently healthy, and were used as control subjects (without malaria); twenty (20) subjects were those with malaria parasite (blood samples were collected from twenty (20) of them before medication, while blood samples were collected from eighteen (18) of them after they were given coartem antimalaria medication). Packed cell volume, methaemoglobin and oxyhaemoglobin concentration were analyzed in these subjects. Results revealed concentration of oxyhaemoglobin in control group to be 10.46 ± 1.51g/dl, malaria positive group before medication had 17.21 ± 3.45g/dl while group after medication had 11.21 ± 1.65g/dl, indicating statistical significance at $p<0.05$ (control vs after medication group showed no statistical significance; control vs malaria group before medication; and after medication group vs malaria group before medication showed statistical significance at $p<0.05$). Packed cell volume of control group was 45.45 ± 1.51%, malaria group before medication was 39.25 ± 5.99% while group after medication was 11.21 ± 1.65%, with statistical significance at $p<0.05$ (control vs after medication group was not significant, whereas control vs malaria group before medication; and after medication vs malaria group before medication was statistically significant at $p<0.05$). Methaemoglobin percentage in control group was 0.87 ± 0.19%, malaria group before medication was 3.23 ± 2.14%, while after medication group had 3.80 ± 2.31%. These results was significant at $p<0.05$ (control vs after medication group was significant at $p<0.05$, whereas control vs malaria group before medication; and after medication vs malaria group before medication; was not significant at $p<0.05$). This study has therefore indicated that malaria parasitaemia causes a reduction in packed cell volume and increases the methaemoglobin and oxyhaemoglobin levels of subjects with malaria, and that the administration of coartem as an antimalarial possesses little effect in restoring these differences.

KEYWORDS: Coartem, Methaemoglobin, Oxyhaemoglobin, Packed Cell Volume, Rivers State University.

INTRODUCTION

Malaria is a disease that sickens and kills those infected with the parasite that causes it through several pathological mechanisms that are understood to some extent. In addition to using antimalarial drug for treatments, adjunctive and supportive care measures (e.g., intravenous fluids, blood transfusions, supplemental oxygen, anti-seizure medications) may be required in cases of severe manifestations of the disease.[1] Malaria is also a major public health problem and cause of much suffering and premature death in the poorer areas of tropical Africa, Asia and Latin America. In many endemic areas it is becoming increasingly difficult to control because of the resistance of the parasite to antimalarial drugs and the failure of vector control measures.[2] The reason for treatment against malaria is to prevent death or long-term deficits and complications from malaria, to cut short the morbidity of an acute episode of illness or reduce the morbidity rate, and to clear the infection entirely so that it does not reoccur. Fever, sweating, and chills triggered by the release of plasmodia into the bloodstream from the mature blood schizonts, are the most common symptoms that heralds the onset of a clinical case of uncomplicated falciparum malaria. Without any form of treatment or an active immune response as a result of previous malaria infections, the number of parasites will increase with every 2-day cycle of reproduction. A mature infection

may involve up to 10^{12} circulating plasmodia in circulation.[3]

Upon the establishment of the infection, vast majority of plasmodia will be in some stage of asexual maturation leading to another round of multiplication within the patient's bloodstream. However, some parasites are transformed into sexual stages (gametocytes) that, once ingested by mosquitoes, have the ability to perpetuate the transmission cycle. Because each stage of the malarial life cycle exhibits unique biochemical and other characteristics (i.e., exhibit different proteins or locates in different sites within the body), a drug may kill one stage but have little or no effect on another stage. In other words, in each life-cycle stage, the parasite manifests unique biological properties that can offer a target for the action of one or more antimalarial drugs.[4]

Antimalarial drugs are drugs that are used for the treatment and prevention of malaria infection.[5] Most antimalarial drugs mode of action is to target the erythrocytic stage of malaria infection, the phase of infection that causes the symptomatic illness. The extent of pre-erythrocytic (which is the hepatic stage) activity for most antimalarial drugs is not well characterized. Treatment of the acute blood stage infection is necessary and very important for malaria caused by all malaria species.[6]

Haemoglobin is a respiratory pigment found in red blood corpuscles. Haemoglobin is a conjugated protein that is synthesized inside an immature erythrocyte in the red bone marrow. It consists of two components haem and globin. Haem, an iron and porphyrin compound is 4% and Globin (amino acids) is 96%. Haemoglobin gives red colour or pigment to the blood.[7]

Haemoglobin's function as an oxygen carrier is so overwhelmingly important that it has obscured some other functions haemoglobin plays in human physiology. The heme iron is carried in the ferrous state, a reduced form that can be oxidized to the ferric form (methaemoglobin), analogous to the cytochrome system. It is coupled to redox cycles in the cell, and is recycled itself. This allows for the generation of two types of cyclic pathways. In the first, driven by the NAD-cytochrome b5 reductase, haemoglobin and methaemoglobin are cycled. In the second, a cell redox cycle system is driven by the oxidation of haemoglobin, with methaemoglobin as the product.[6]

Methaemoglobin cannot bind oxygen, unlike oxyhaemoglobin. It is bluish chocolate-brown in colour and when in high concentration, it can result to methaemoglobinaemia which can be seen in some cases as bluish pigmentation underneath the skin of the individual. In human blood a trace amount of methaemoglobin is normally produced spontaneously, but when present in excess the blood becomes abnormally dark bluish brown. Coartem is the most available drug use in treating malaria in Rivers State as the Government under its free malaria treatment programme makes the drug available for free or at low cost, it is therefore necessary to see the effect of this drug on oxygen delivery hence the need for methaemogobin estimation, since methaemoglobin cannot bind to oxygen advantageously for normal haemoglobin physiology in human.

Oxyhaemoglobin is formed during normal physiological respiration when oxygen binds to the heme component of haemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs.[8] The oxygen then travels through the blood stream to be dropped off at cells where it is utilized as a terminal electron acceptor in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH.

The packed cell volume (PCV), also referred to as haematocrit is used to screen for anaemia (which is an outcome in cases of severe malaria parasitaemia) when it is not possible to measure haemoglobin accurately and mains electricity is available to operate a microhaematocrit centrifuge.[9] An increase in haematocrit value indicates an increase in red cell production which occurs as a result of oxygen supply and due to dehydration. [10]

This research is aimed at evaluating the effects of coartem on the methemoglobin level, oxyhaemoglobin concentration and packed cell volume of Rivers State University students taking the antimalarial drug, with following specific objectives: (i) To investigate the effect of coartem on methaemoglobin, oxyhaemoglobin, and packed cell volume of students in Rivers State University diagnosed with malaria. (ii) To compare the level of methaemoglobin, oxyhaemoglobin and packed cell volume in those without malaria, those with malaria but not on any antimalarial and those with malaria but treated with coartem.

MATERIALS AND METHODS

Research Design

This is a comparative-case control and longitudinal study. Fifty-eight blood samples were collected from forty (40) subjects recruited from Rivers State University (RSU). Twenty (20) subjects were apparently healthy, and were used as control subjects (without malaria); twenty (20) subjects were those with malaria parasite (blood samples were collected from twenty (20) of them before medication, while blood samples were collected from eighteen (18) of them after they were given coartem antimalaria medication).

Informed Consent

Those that participated in the study voluntarily gave their approval upon clearance by the Ethics Committee of the Department of Medical Laboratory Science, Rivers State

University and Prof Nimi Briggs Hospital, Rivers State University.

Specimen Collection, Preservation and Transportation

Venous blood sample was collected with the use of vacutainer from each participant, of which 3.0ml of blood was added into a glass bottle containing 0.5ml of 1.2mg/ml dipotassium ethylene diamine tetra-acetic acid (EDTA). The samples were preserved by placing it on crushed ice with cotton wool placed over it in a thermo cool container and then transported to the haematology laboratory, Rivers State University where they were analysed.

Sample Analysis/ Methodology

Malaria parasites were dictected with the gold standard method (thick blood film) as described by Cheesbrough.[2] Samples for packed cell volume (PCV) were analysed using the microhaematocrit method as described by Cheesbrough.[9] Methaemoglobin and oxyhaemoglobin were analysed by spectrophotometric method as described by Lewis and Roper; Evelyn and Malloy.[11,12]

Determination of Methaemoglobin

Principles: Methaemoglobin (Hi) has a maximum absorption at 630nm. When cyanide is added, this absorption band disappears and the resulting change in absorbance is directly proportional to the concentration of Hi. Total Haemoglobin in the sample is then measured after complete conversion to HiCN by the addition of ferricyanide – cyanide, reagent. The conversion will measure oxyhaemoglobin and methaemoglobin but not sulphaemoglobin. Thus, the presence of a large amount of sulphaemoglobin will result in an erroneously low measurement of total Haemoglobin. Turbidity of the haemolysate can be overcome by the addition of a non-ionic detergent.

Calculation: Methaemoglobin (%) = (D1-D2/D3-D4) X 100

Reagents: Phosphate buffer: 0.1mol/L, PH 6.8; Potassium cyanide: 50g/L; Potassium ferricyanide: 50g/L; Non Ionic Detergent: 10g/L.

Procedure: Lyse 0.2ml (200ml) of blood in a solution containing 4ml of buffer and 6ml of detergent solution. Divide the Lysate into 2 equal volumes (A and B). Measure the absorbance of A with a spectrophotometer at 630nm wavelength (D1). Add I drop of potassium cyanide solution and measure the absorbance again, after mixing (D2).Add I drop of potassium ferricyanide solution to B, and after 5 minutes, measure the absorbance at the same wavelength (D3). Then add I drop of potassium cyanide solution to B and after mixing make a final reading (D4).

N/B: All the measurements are made against a blank containing buffer and detergent in the same proportion as present in the sample.

Determination of Oxyhaemoglobin

Principle: The haemoglobin is converted to oxyhaemoglobin by the action of the ammoniated water.

Procedures: Prepare the ammoniated water fresh by adding 0.04ml of ammonia to 100ml of distilled water. Pipette 4ml of ammoniated water to the test tube. Mix the sample well and add 20mml (0.02ml) to the test tube and stopper it with rubber bung. Mix well by inversion. Read the standard solution and the test solution in the colorimeter using light-path of 1cm and wavelength of 540nm or yellow green filter against the ammoniated water.

Determination of Packed cell volume (Microhaematocrit Method)

Principle: Anticoagulated blood is centrifuged in a sealed capillary tube, and then packed cell volume is determined by a special haematocrit reader.

Procedures: Draw the blood sample into appropriate capillary tube with capillary action. Fill the tube about $3/4^{th}$ length with blood. Seal another end of the tube with plasticin or wax or sealant. Place two haematocrit tubes in the groove of the centrifuge exactly opposite each other. Centrifuge at 1500 rpm for 5-7mins. Remove the capillary tube from the centrifuge and read with a microhaematocrit reader.

Statistical Analysis

Data were analysed using Graph-pad prism 5.0 for descriptive statistics where the mean and standard deviations were obtained. Analysis of variance (ANOVA) was used to make comparisons among the three (3) groups of study and student t-test to make inferences between the study groups. Tukey multiple comparison test was used to check for statistical inference in-between groups, and p-values of <0.05 were considered to be statistically significant.

RESULTS

Demographic Details of Participants in the Study

A total of fifty-eight (58) samples were collected from the participants or subjects. Twenty (20) samples were collected as control consisting of eleven (11) females and nine (9) males; with age ranging from nineteen to twenty eight (19-28) who were diagnosed to be malaria negative, twenty (20) samples were collected those that have malaria parasite but were not yet on drug and they consist of ten (10) males and (10) females age matched same as control. Whereas eighteen (18) sample were collected from those with malaria after treatment with coartem, consisting of ten (10) females and Eight (8) males, age matched same as control, details of these are shown in Table 1.

Table 1: Demographic Details of Participants.

Sr. No.	Parameters	Frequency
1a	Total number of Subjects	40
1b	Total number of Samples	58
2	Control Subjects/Samples	20
3	Subjects /Samples with Malaria before taking Coartem	20
4a	Subjects/Samples with Malaria after taking Coartem	18
4b	Total number of males	19
4c	Total number of females	21

Comparison of methaemoglobin levels in the study group

Methaemoglobin levels among the various study groups were analysed using analysis of variance (ANOVA). Mean ± standard deviation of the different groups and p-value are represented in Table 2, $p<0.05$ was considered statistically significant. Also a Tukey Multiple comparison test was done between the 3 groups and the statistical inferences are also shown in Table 2.

Table 2: Mean ± Standard Deviation of Methaemoglobin in the Study Group

Sr. No	Parameters	Control	BTC	ATC	p-value	Remark	TMC Test
1.	MetHb (%)	0.87 ± 0.19	3.23 ± 2.14	3.80 ± 2.31	0.0102	(S)	C vs BTC **(NS)** C vs ATC **(S)** BTC vs ATC **(NS)**
2.	Range (Min-Max)	1.00 – 4.00	1.00 – 7.60	1.10 – 8.50			

Key: C=control; BTC=before taking coartem; ATC=after taking coartem; TMC=Tukey Multiple Comparison; NS=non-significant; S=significant

Comparison of Oxyhaemoglobin levels in the study groups

Oxyhaemoglobin levels among the various study groups were analysed using analysis of variance (ANOVA). Mean ± standard deviation of the different groups and their p-value are represented in Table 3, p-value of $p<0.05$ was considered statistically significant. Furthermore, a Tukey Multiple comparison test was done between the 3 groups and the statistical inference is shown in Table 3

Table 3: Mean ± Standard Deviation of Oxyhaemoglobin in the Study Group

Sr. No.	Parameters	Control	BTC	ATC	p-value	Remark	TMC Test
1.	OxyHb(g/dl)	10.46 ± 1.51	17.21 ± 3.45	11.21 ± 1.65	0.0001	(S)	C vs BTC **(S)** C vs ATC **(NS)** BTC vs ATC **(S)**
2.	Range (Min-Max)	7.40 – 13.20	6.10 – 13.00	11.70 – 24.50			

Key: C=control; BTC=before taking coartem; ATC=after taking coartem; TMC=Tukey Multiple Comparison; NS=non-significant; S=significant

Comparison of packed cell volume in the study groups

Packed cell volume levels among the various study groups were analysed using analysis of variance (ANOVA) to show the Mean ± standard deviation of The different groups and the p-value as shown in Table 4, p-value of <0.05 was considered statistically significant. Tukey Multiple comparison test was done between the groups and the statistical inference is shown in Table 4

Table 4: Mean ± Standard Deviation Table for Packed Cell Volume in the Study Group

Sr. No.	Parameters	Control	BTC	ATC	p-value	Remark	TMC Test
1.	PCV (%)	45.45 ± 1.51	35.67 ± 2.70	39.25 ± 5.99	0.0105	(S)	C vs BTC **(S)** C vs ATC **(NS)** BTC vs ATC **(S)**
2.	Range (Min-Max)	29.0 – 42.0	31.0 – 39.0	28.0 – 57.0			

Key: C=control; BTC=before taking coartem; ATC=after taking coartem; TMC=Tukey Multiple Comparison; NS=non-significant; S=significant

DISCUSSION

The determination of erythrocyte methaemoglobin concentration as a toxic outcome in chemical poisoning as a result of drug consumption based on pathologic conditions showed reliability and reproducibility in clinical diagnosis. [13, 14] Coartem was the malaria drug used in this study which is the predominant drug used by most persons diagnosed with malaria parasites because of its affordability and availability. This present study reported significant ($p<0.05$) elevation of methaemoglobin concentrations of human participants infected with malaria. Upon comparison within the groups (those without malaria as control, those with malaria after treatment with coartem and those with malaria before treatment with coartem), there was statistically significant difference only in control group of subject against those with malaria after medication or treatment with coartem. This observation was in agreement with the reports of [14] that demonstrated high level of methaemoglobin in subjects with severe malaria parasitaemia and suggested routine estimation of methaemoglobin in malaria for clinical evaluation of patients. Furthermore, Anstey and colleagues [15] documented elevated methaemoglobin concentration of Tanzanian children with severe and uncomplicated malaria after treated with antimalarials. In the same vein, the present report was in concordance with observations of Bradberry and coleagues.[13] where they showed that cultures with high levels of parasitaemia contained between 3 to 10 times more methaemoglobin than those with low levels of parasitemia. Akomopong and colleagues.[16] found isolated malarial parasites contained between 20 - 42% of methaemoglobin concentration. In contrast, uninfected red blood cells presented between 0.5 - 1.0% of methaemoglobin. Therefore, these reports suggest malaria parasite induced raised levels of erythrocyte methaemoglobin irrespective of the time of medication. In addition, these authors noted that increased methaemoglobin in malarial infection after medication was a reflection of rapid oxidation of haemoglobin ingested by the parasites.

Antimalarials affects inherited abnormalities of haemoglobin structure that give rise to methaemoglobinemia, known collectively as the haemoglobin M disorders, which are rare autosomal-dominant defects caused by point mutations that alter a single amino acid in the structure of normal globin. It is worthwhile to recall that raised oxidant levels compounded by compromised activity of erythrocyte redox enzymes further exacerbate the tendency towards spontaneous oxidation of haemoglobin molecule in parasitized red blood cells.[16]

Packed cell volume which is the proportion of red blood cell in the blood is considered as an integral part of an individual full or complete blood count result because of its capabilities of oxygen delivery.[17] and the major role of the red cell is to transfer oxygen from the lungs to the body tissues. Packed cell volume or the haematocrit serves as an indicator of health conditions such as polycythaemia vera and anaemia.[10] It was observed that there was a decrease in the packed cell volume observed in malaria patients before medication with respect to their degree of parasite activity in their blood physiology, duration of illness and age. This may have been the reason for anaemia to ensue**.** This is in line with,[18] who reported that malaria causes decrease in bone marrow alterations, packed cell volume, mean cell volume (in severe cases) and this is the common cause of anaemia in malaria endemic regions. According to studies conducted by Pamploma and colleagues,[19] their result showed that there was a significant difference between packed cell volume values of control and positive test because malaria parasite caused excessive destruction and reduced production of red cell. Anaemia in acute malaria have been demonstrated to be due to decrease in the rate of production of red blood cells, malaria drug administered increases the destruction of parasitized red blood cells and accelerates the removal of both parasitized and non- parasitized red blood cells, ineffective erythropoiesis is due to increase circulating TNF- (Tumour necrotising factor). After medication there was an increase in the packed cell volume but this was not significant, due to the fact that the parasite has been cleared and the bone marrow has started its normal physiological response in erythrocyte production.

Also, this increase might be as a result of the antimalarial working directly or indirectly on the bone marrow to aid effective erythropoiesis by producing more red cells to compensate the destroyed red cells by the malaria parasite. In the Tukey multiple comparisons there was statistically significant difference for control versus before medication and after medication vs before medication. This might be as a result of the malaria parasite acting on the bone marrow of the individual thereby affecting the erythropoietic function by not being able to produce more red blood cell than expected.[14]

It was also observe that there was an increase in the oxyhaemoglobin level observed in malaria patients before medication. The increase might be as a result of increased physiological respiration allowing oxygen binds to the heme component of the protein haemoglobin in red blood cells. [13] Increased level of oxyhaemoglobin causes a shift in the blood pH. After medication there was a slight increase in the oxyhaemoglobin level but this was not significant. This increase might be as a result of the antimalarial working directly to remove more carbon dioxide from the system thereby stabilizing the pH.[8] A high pH, low CO_2, or low 2, 3 DPG favours the relaxed form, which can better bind oxygen. The partial pressure of the system also affects O_2 affinity where, at high partial pressures of oxygen (such as those present in the alveoli), the relaxed state which leads to high oxygen affinity is favoured. Inversely, at low partial pressures (such as those present in respiring tissues), the (low affinity) a tense state is favoured.[7] Additionally, the binding of oxygen to the iron (ferrous state) heme

pulls the iron into the plane of the porphyrin ring, causing a slight conformational shift. The shift encourages oxygen to bind to the three remaining heme units within haemoglobin (thus, oxygen binding is cooperative).

CONCLUSION

This study has shown that malaria treatment using Coartem increases the level of methaemoglobin significantly. Oxyhaemoglobin level and packed cell volumes were not altered significantly from the range in control subject. It is therefore necessary to take into cognisance that coartem medication can increase methaemoglobin level and hence the need to monitor its levels so as to ensure oxygenation of red blood cells and oxygen delivery to tissues.

Antimalarial drugs play a major role in trying to compensate the significant difference in blood cells. Antimalarial are effective against malaria parasitaemia and it is recommended for use due to its positive effect after when administered to malaria patients. It is therefore necessary that methaemoglobin levels be checked after medication with Coartem and probably by extension, other antimalarials.

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