EVALUATION OF PLASMA CREATININE AND UREA LEVELS AFTER ORAL ADMINISTRATION OF ARTEMISININ AND SOME ARTEMISININ COMBINATION THERAPY REGIMENS TO HEALTHY NIGERIAN MALES

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ABSTRACT: Studies were carried out on the levels of plasma creatinine and urea when Artemisinin Derivatives Artesunate (ART) and dihydroartemisinin (DHA) were administered as monotherapeutic agents and in combination with other antimalarials as artemisinin combination therapy (ACT) on Nigerian male volunteers. Artesunate as a monotherapeutic agent caused an average increase of 11.0% and 15.0% on 2nd and 4th days of the studies in the level of plasma creatinine respectively, while in combination with Amodiaquine (AMQ), Mefloquine (MFQ) and Sulfadoxin-pyrimethamine (SP) the levels of creatinine was double. On these same days, DHA as a monothearpeutic agent caused an average increase of 20% and 30% respectively in the level of creatinine. The addition of piperaquine (PIP) to DHA had no significant effect on the plasma level of creatinine. On the 4th day of measurement, artesunate alone caused an average increase of 37.68% in the level of plasma urea as blood urea nitrogen (BUN) while DHA alone caused an increase of 42.47%. The combination of ART with AMQ on the same day caused a significant increase of 49.87% ($P<0.05$) in the level plasma BUN while all other combinations had no significant effect. The addition of PIP to DHA had no effect of on the level of plasma BUN.

INTRODUCTION

Malaria is a dual-host hematoprotozoan parasitic infection transmitted by certain species of the infected anopheline mosquitoe. Transmission depends on climatic conditions that may affect the abundance and survival of mosquitoes. In many places, transmission is seasonal, with the peak during and just after the rainy season, (Coker *et al.*, 2001). *Plasmodium*, a unicellular eukaryotic cell of the protozoa phylum is the fatal parasite responsible for malaria disease. In the human body, the parasites multiply in the liver, red blood cells, the brain, lungs, kidneys, placenta and other tissues, (David and Peter, 2004; Adams *et al*., 2002; Alkawe, 1988).

Control measures of malaria include preventive (which involves control of the vector of the malaria parasite by indoor spraying with residual insecticides, sleeping under long lasting insecticide-impregnated nets (LLIN), the maintenance of good sanitary conditions), and treatment of the disease by the use of drugs, (Habluetzel *et al.*, 1999). Drug treatment of malaria has so far not been a complete success story because of the complexity of the life cycle of the protozoa (*Plasmodium*), both in human and in the mosquito vector, and the development of resistance by Plasmodium.

The emergence of resistance to chloroquine and other antimalarials has led to the search and development of newer drugs including artemisinin and its derivatives, (Sanjeev *et al*., 2004). Artemisinin and its derivatives have a short elimination half life and this confers the theoretical advantage that selection for drug resistant parasite is less likely, however to avoid the higher

risk of recrudescence when used in monotherapeutic regimen, artemisinins are used in combination with other antimalarials with relatively longer elimination half life. This combination is known as artemisinin combination therapy (ACT) and has been adopted as the front line drugs for treatment of uncomplicated malaria in Nigeria (Olliaro and Taylor, 2004, Davis *et al*., 2005). Creatinine which is derived from craetine and phosphocreatine is a major constituent of muscle. The rate of formation of creatinine for a given individual is fairly constant and is primarily determined by the individual's muscle mass. It is known that serum creatinine level is slightly higher in muscular subjects than non-muscular subjects (Marshall and Bangert, 2009).

The reference range for plasma creatinine in adult population is 60-120 µmol/L. When creatinine is released from the muscule into plasma, it is excreted almost exclusively by the kidney through glomerula filtration process. It is neither reabsorbed, secreted, synthesized nor metabolized by the kidney hence, the clearance of creatinine is equal to the glomerula filtration rate (GFR). A decrease in the GFR would result in an increase in the plasma creatinine concentration, thus, the determination of the plasma creatinine concentration is used in the clinical evaluation of patients with suspected renal disease, (Onyeneke *et al*., 2000; Marshal and Bangert, 2009).

The end product of protein metabolism is urea and it is produced solely by the liver. After production, it travels through the blood and is excreted by the kidneys. Because the blood urea nitrogen (BUN) is completely filtered at the glomerulus of the kidneys, than reabsorbed and tubularly secreted within the nephrons, the concentrations of BUN reflect renal function. Hydrational status, protein intake and some drug which affect renal blood flow will affect the BUN of individuals (Onyeneke *et al*., 2000; Marshall and Bangert, 2009). Artemisinins are considered to have high safety margins, (garner and Graves, 2005); however, they may be toxic under certain conditions, (Udobre *et al*., 2009). The present study is aimed at investigating the effects of artemisnin (ART and DHA) when administered as monotheraepeutic agents and in combination as ACTs on the kidney functions by accessing the levels of plasma creatinine and urea.

MATERIALS AND METHOD

Ethical Approval

The study was conducted between October and December, 2011 at the Health Centre of the University of Uyo, after approval by the ethics committee of the University.

Recruitment of Subjects

Fifty six male volunteer subjects were identified through personal contact from among students, and staff of the University of Uyo community. The age range was 30 ± 5.5 years, and body weight 65 ± 5.5kg. Written informed consent to participate in the study was sought and obtained from all volunteers. They were physically fit with no history of malaria infection or antimalarial treatment in the past two weeks before commencement of the studies. They were also screened and found free from any chronic liver and kidney diseases. The volunteers were randomly divided into twelve groups with eight persons per group. Vital signs: body temperature, body weight between 60kg and 85kg and blood pressure of each volunteer was recorded before drug administration.

Drugs/Chemicals

The drug products used in these studies were: Artesunate 100mg tablets (manufactured by Bliss GVS Pharma India); Larimal containing Artesunate 50mg and 153.1mg Amodiaquine bases (manufactured by Ipca Laboratories Ltd., Mumbo, India) ; Artequine tablets, each containing 200mg of Artesunate and 250mg of mefloquine base, (manufactured by Mepha Ltd. Switzerland); Amala plus tablets each containing 100mg of artesunate and 500mg sulfadoxine 25mg pyrimethamine,(manufactured by Elbe Pharma Ltd. India); Alaxine tablets each

containing 60mg of dihydroartemisinin from (Greenlife Pharmaceuticals Lagos, Nigeria); P-alaxine tablets each containing 40mg dihydroartemisinin and 320mg piperaquine and artecxin tablets each containing 35mg dihydroartemisinin, 320mg piperaquine and 90mg trimethoprim from(O'Neil Pharma and Healthcare Ltd. Oshodi – Apapa Lagos, Nigeria). All the drug products were obtained direct from the manufacturers representative here in Uyo and they were all less than one year from the date of manufacture. The chemicals were freshly prepared as explained in Randox creatinine and urea manual.

Study Design
An open single centre study was carried out involving all the subjects. After an overnight fast and a light breakfast without much protein at 8.00am, the subjects were divided into eight groups with seven volunteers in each group. All the subjects were to abstain from any other medication, alcohol and cigarette within the period of study. They were also to eat similar snacks and food within the period of the studies.

Administration of Drugs to Volunteers
Each member in the groups received adult does of the following drugs, swallowed with 500ml of water.

Group A: 2 tablets of artesunate.
Group B: 4 tablets of larimal.
Group C: Mefloquine tablets (200mg of artesunate and 250mg of mefloquine).
Group D: Amala plus tablets (200mg of artesunate and 1500mg sulfadoxine - 75mg pyrimethamine on first day, followed by 200mg artesunate daily for the next two days.
Group E: 2 tablets of alaxine.
Group F: 3 tablets of P – alaxine..
Group G: 4 tablets of artecxin (given in two divided doses).
Group H: Did not take any drug.

N/B: The regimen as stated was taken daily for three days, except group G which was for two days.

Collection of Blood and Preparation of Plasma
5.0ml of blood was collected from each volunteer subject before drug administration on the first day. After administration of the drugs, 5.0ml of blood was collected from each volunteer between 8.0am and 10.00am and on the 2^{nd}, 4^{th}, 7^{th} and 14^{th} days. The blood was placed in heparinized tubes and allowed to stand for five minutes to equilibrate at room temperature.

The fresh blood was transferred into clean centrifuge tubes and spun at 5,000 rpm for 20 minutes in a centrifuge machine (MSE England) to separate the plasma from the cells. The plasma was aspirated, and transferred into specimen bottles and stored at -15^{o}C for a period not exceeding 24 hours, before analysis.

Quantitative Determination of Creatinine and Urea
The prepared plasma samples obtained from the blood of volunteers were analyzed. The amount of creatinine present in heparinized plasma was determined using colorimetric method as explained in Randox manual.

The absorbance of the sample solution (A_{sample}) and standard solution ($A_{standard}$) were measured against air at 492nm using a 1.0cm light path cuvatte at a temperature of 30^{o}C. The amount of creatinine present was calculated as follows:

$$\text{Creatinine concentration } \mu\text{mol/L} = \frac{\Delta \text{ A sample}}{\Delta \text{ A standard}} \times \frac{\text{standard}}{\text{con } (\mu\text{mol/L})}$$

The amount of urea present in the plasma was measured photometrically by Berthelot reaction as described in Randox manual. The absorbance of the standard solution provided ($A_{standard}$) and that of the sample (A_{sample}) were measured at 546nm in a 1.0cm light path cuvatte at a temperature of 37^{o}C against sample blank. The urea concentration in each sample was calculated as shown below:

$$\text{Urea concentration (mmol/L)} = \frac{A_{sample}}{A_{standard}} \times \frac{\text{Standard}}{\text{Conc.}}$$

N/B 1.0mg of urea corresponds to 0.467mg of urea nitrogen.

Statistical Analysis

The values obtained were expressed as percentage increase (+) or decrease (-) of the mean value of the blank. The data obtained were expressed as mean ±SD. Student t-test was used to assess statistical significance, values of $p<0.05$, were considered to be significant.

RESULTS AND DISCUSSION

Since healthy volunteers were used for the studies, an increase or decrease in the levels of creatinine or urea were expressed as percentage increase or decrease relative to the mean of the blank samples in each group. The antimalarials; ART, DHA and ACT used were normal therapeutic doses as are used in the treatment of malaria. Administration of artesunate alone gave an average plasma creatinine increase of 12.84% on the 4th day of measurement, but when it was concomitantly administered with AMQ., MFQ and SP on the same day, the mean CREA level was doubled: 24.39%; 23.26% and 26.33% respectively, Table 1. These values suggested that the addition of AMQ, MFQ and SP to artesunate had a significant increased adverse effect on the renal function.

Table 1: Effect of artesunate alone and artesunate with amodiaquine, mefloquine and sulfadoxine-pyrimethamine on the mean plasma levels of creatinine (CREA)

Days of observation	ART Alone		ART with AMQ		ART with MFQ		ART with SP	
	CREA ($\mu mol.L^{-1}$)	%	CREA. ($\mu mol.L^{-1}$)	%	CREA. ($\mu mol.L^{-1}$)	%	CREA. ($\mu mol.L^{-1}$)	%
1.	79.87±093	-	84.42±1.24	-	92.11±0.61	-	68.92±2.11	-
2	88.53±1.16	10.93	99.04±0.93	17.32*	104.17±0.73	13.10	81.56±1.92	18.34
4	90.06±1.07	12.84*	105.01±0.65	24.39*	113.53±1.14	23.26*	87.06±0.91	26.33*
7	82.31±0.97	3.14	93.68±1.16	10.97	108.33±0.89	17.61	78.09±1.01	13.31
14	80.76±1.77	1.20	87.71±0.39	3.90	101.22±2.17	0.99	75.74±2.10	0.97

ART: Artesunate, AMQ: Amodiaquine, MFQ: Mefloquine, SP: Sulfadoxine-pyrimethamine, CREA: Creatinin, %: percentage change.The results are expressed as mean±SD. N = 7.
* Statistical significance ($p < 0.05$)

Table 2: Effect of dihydroartemisinin (DHA) alone and DHA in combination with piperaquine on the mean plasma levels of cretinine.

Days of observation	DHA ALONE		DHA with PIP		Control	
	CREA. ($\mu mol.L^{-1}$)	%	CREA. ($\mu mol.L^{-1}$)	%	CREA. ($\mu mol.L^{-1}$)	%
1.	91.37±1.33	-	74.93±1.87	-	87.693	-
2	111.76±1.87	22.32	87.31±0.91	26.3	88.17±1.88	1.69
4	119.59±2.03	30.89	99.24±13	32.45	89.48±0.79	2.05
7	99.59±2.03	8.44	86.19±0.96	15.04	88.51±2.0	0.99
14	94.10±1.63	2.99	76.04±1.48	1.475	88.78±1.93	1.24

DHA: Dihydroartemini, PIP: Piperauine CREA: creatinin, %: percentage change. The results and expressed as mean ±SD. n =7
* statistical significance of ($P<0.05$)

Table 3: Effect of artesunate alone and artesunate with amodiaquine, mefloquine and sulfadoxine – piprimethamine on the mean plasma levels of urea.

Days of observation	ART Alone		ART with AMQ		ART with MFQ		ART with SP	
	Urea ($mmol.L^1$)	%	Urea ($mmol.L^1$)	%	Urea ($mmol.L^1$)	%	Urea ($mmol.L^1$)	%
1.	3.45±0.37	-	3.50±0.11	-	3.60±0.15	-	3.71±0.11	-
2	3.95±0.67	14.50*	3.85±0.23	10.00	4.05±0.23	12.50	4.11±0.15	11.08
4	4.75±0.03	37.68*	5.20±0.05	48.57*	4.95±0.31	37.50*	4.90±0.10	32.43*
7	3.45±0.11	0.28	3.85±0.18	10.00*	3.75±0.03	4.16	3.83±0.21	3.51
14	4.12±0.31	19.42*	3.54±0.23	1.14	3.67±0.41	1.96	3.88±0.03	4.86

ART: Artesunate, AMQ: Amodiaquine, MFQ: Mefloquine, SP: Sulfadoxine-pyrimethamine, $mmol.L^{-1}$: millimole per liter, %: percentage change. The results are expressed as mean ± SD. N = 7.

* Statistical significance ($P<0.05$)

Table 4: Effect of dihydroartemisinin alone and dihydroartemisinin with piperaquine on the mean plasma level of urea

Days of observation	ART Alone		DHA with PIP		Control	
	Urea ($mmol.L^{-1}$)	%	Urea ($mmol.L^{-1}$)	%	Urea ($mmol.L^{-1}$)	%
1.	3.65±0.03	-	3.50±0.05	-	3.32±0.31	-
2	4.21±0.12	15.34*	3.85±0.13	10.00	3.40±0.01	0.30
4	5.20±0.10	42.47*	5.00±0.05	42.96	3.33±0.23	0.30
7	4.00±0.03	9.58	4.05±0.02	15.71*	3.34±0.78	1.4
14	3.70+0.16	1.37	3.65±0.22	3.71	3.383.70.02	1.81

DHA: Dihydorartemisinin,PIP :Piperaquine, $mmol.L^{-1}$: millionable per liter, % percent change.The result are expressed as mean ± SD. n= 7.*Statistical significance ($P < 0.05$)

Administration of DHA alone and in combination with piperaquine had the same mean plasma creatinine increase 30.89% and 32.45% respectively on the forth day of measurement, Table 2. This suggested that the addition of PIP to DHA has no significant effect on kidney functions.

Administration of ART as a monotherapeutic agent caused an increase of 37.68% in the mean plasma level of BUN on the 4^{th} day of measurement. On this same day, the concomitant administration with MFQ and SP had no significant effect, Table 3.

DHA alone and in combination with PIP had the same effect on the mean plasma level of BUN, Table 4.

Artemisinin and its derivatives as monotherapeutic agents and in combination as ACT are very effective antimalarials. Their use in treatment of malaria in normal subjects is quite in order, but when administering them to a population with kidney problems, there should be close monitoring of renal functions to prevent worsening the health conditions of these patients.

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