Comparative Biochemical Profiles in Children Treated with Artemether-lumefantrine and Artesunate-amodiaquine for Acute Uncomplicated *falciparum* Malaria in Jos, North – Central Nigeria.

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Abstract:
Background: *Acute clinical episode of uncomplicated malaria induces several pathophysiological biochemical disturbances in its host. We examined and compared these biochemical alterations in children pre- and post-treatment with ACTs up to day 14 and how the ACTs affect these biochemical parameters during recovery.*
Aim: *To determine and compare the alterations in selected biochemical indices pre- and post-treatment of uncomplicated P. falciparum malaria with artemether-lumefantrine (AL) and artesunate-amodiaquine (AA) in under-five children up toDay 14 day.*
Method: *Data on 111 children aged 6 to 60 months who were enrolled into a drug therapeutic efficacy testing (DTET) of AL (20/120mg) with AA (25mg/67.5mg or 50/135mg) in the treatment of uncomplicated falciparum malaria, were analysed. Inclusion criteria were: history of fever in the last 24hrs and /or measured axillary temperature ≥ 37.5ºC, P. falciparum infection with parasitaemia≥ 1000 to ≤ 250,000 parasites/µL, HIV seronegative status, and a written informed consent from parents/ guardians.Enrolees were randomized into one of two treatment arms, and their biochemical indices (ALT, AST, Bilirubin and creatinine) were measured longitudinally on D0, D7 and D14 and analyzed.*
Results:
Of649 subjects screened for parasitaemia , 282 (43.5%) were febrile (temperature ≥ 37.5ºC),of which 252 (38.8%) had P. falciparumparasitaemia. Parasite counts varied from 1000 - 200,000 asexual forms/µL. The mean age (months) of study population were AL (38.9 ± 16.90)and AA (37.7 ± 16.76) arms respectively, (p= 0.72). Thirty- one (55.4%) and 25 (44.6%) were males and females in the ALstudy arm respectively while 32 (58.2%) and 23 (41.8%) were males and females respectively in the AA study arm, (p=0.77). There were comparable elevations in mean serum levels of AST, ALT,creatinine and total Bilirubin from D0 to peaks by D7 with gradual decline to patients' normal at D14 post-treatment in AL and AA treatment arms. The difference was not significant. ACTs chemotherapy appeared to have no significant relationship to biochemical disturbances in the study subjects.
Conclusions: *Uncomplicated paediatric Plasmodium falciparum malaria induces transient alterations in biochemical indices in Jos, North Central Nigeria. Our findings agreed with the literature. Therefore, malaria infection should be considered as a differential diagnosis in febrile children with alterations in selected biochemical indices.*
Key words: *Children, uncomplicated Plasmodium falciparum malaria, biochemical indices, ACTs.*

I. Introduction

Changes in biochemical indices in acute and convalescent paediatric uncomplicated Plasmodium *falciparum* malaria has not been fully studied especially in children residing in most malaria endemic countries of the world. The usefulness of such knowledge increases clinical diagnosis and appreciation of biochemical toxicities of the antimalarials.[1,2]

Acute clinical episode of malaria induces several pathophysiological biochemical disturbances in its host.[2,3,4] Many studies have examined the changes in the biochemical alterations in uncomplicated malaria

infection; few however, have examined and compared these biochemical alterations in children pre- and post-treatment with ACTs up to day14 and how the ACTs affect these biochemical parameters during recovery.

To the best of our knowledge, there is paucity of data on the biochemical profiles of children pre and post treatment of paediatric uncomplicated falciparum malariawith ACTs and how these ACTs affect these biochemicalindices during convalescence.This study was therefore, conducted, to compare and evaluatethe changes in biochemical indices in children pre and post treated with artemether-lumefantrine (AL) and artesunate-amodiaquine (AA) for uncomplicated Plasmodium falciparum malaria.

II.   Methods

Data on age, sex, weight, length/height, presenting symptoms, parasitaemia and biochemical parameters (alanine transaminase (ALT), aspartate transaminase (AST), creatinine and total serum bilirubin (estimated using **Cobas C111** auto-analyzer)on 114 children aged 6 months – 60 months treated for uncomplicated Plasmodium *falciparum* malaria were extracted from drug therapeutic efficacy (DTET), safety and tolerability study. Inclusion criteria were: history of fever in the last 24hrs and /or measured axillary temperature $\geq 37.5^oC$, P. *falciparum* infection with parasitaemia≥ 1000 to $\leq 250,000$ parasites/μL, HIV seronegative status, and a written informed consent from parents/ guardians including readiness to comply with the follow-up visits by the parents but excluded subjects with signs of severe malaria or other common childhood diseases associated with fever for example; otitis media, tonsillitis.

Urinary tract infection was excluded by the use of combi-11 urinalysis strip test Kitts for presence of nitrite and leukocyte esterase) in a clean catch midstream urine specimen. Severe malnutrition – defined by z score < -3 SD or bilateral oedema.[6]Patients with history of allergy to ACTs or refusal to give consent were also excluded.

HIV infection was excluded by Abbott Determine HIV-1 and 2 Whole Blood Assay Kit (**Abbott Laboratory, USA**). Those who where positive were to be confirmed by Western Blotting principle using Qualicode[TM]HIV-1and 2 Kitts.

Parasite genotyping Blood spot on filter paper for parasite genotyping was obtained on Day 0 for all subjects then on any subsequent day patient was found to have parasitaemia. Polymorphic markers, Merozoites Surface Protein-1(MSP-1), MSP-2 and Glutamate-Rich Protein (GLURP) in P. *falciparum* isolates were used to examine the complexity of parasite populations and discriminate between recrudescent and re-infections in the study participants.

Antimalarial chemotherapy Enrollees who met the inclusion criteria were randomized into the two treatment arms to receive weight-dependent study medications artemether-lumefantrine (20/120mg) or a 3 day course of artesunate-amodiaquine (25mg/67.5mg or 50mg/135mg) according to the manufacturer's instructions. Clinical, parasitological and biochemical evaluations were carried out on Days 0, 7 and 14.

Analysis was restricted to 111 children who completed the study at day 28 (D28) follow-up visit. The number of patients treated with artemether-lumefantrine (AL) was 56 vs55 in the artesunate-amodiaquine(AA) group. Parasite densities (μL) were computed using the actual baseline leukocyte counts at enrollment (D0).

Site of the study This study was conducted in General hospital Barki-Ladi, Plateau State,located 53 km south of Jos, the state capitalwith a population of 175, 267 (2006 National Census).

Ethical consideration Ethical/approval was received by the Institutional Health Research Ethic Committee (IHREC) of the Jos University Teaching Hospital, Jos Nigeria.

Time of the study The study was conducted between 27[th]September and 16[th]December, 2010.

Data management All patients' specific details and laboratory parameters were recorded in a case record form (CRF). The information was then entered into the computer using SPSS software versions 15.0 and 17.0. All patients' codes were double entered serially to avoid transcriptional errors and were only analyzed at the end of the study. Frequency tables and percentages were used to determine the means of all parameters used. Means were used to get the difference in averages between the two drugs (AL and AA) according to all parameter used. Student`s t-test was used to calculate the significant difference between the two drugs. Standard deviation and standard error were also calculated. Levene`s test for equality of variances was used to get the F and P- values. All tests of significance were two-tailed. P-value < 0.05 was taken to indicate significant difference.

III.   Results

Baseline characteristics of study patients

Of 649 subjects screened 282 (43.5%) were febrile (temperature $\geq 37.5^oC$). Two hundred and fifty-two (38.8%) had positive peripheral blood film for P *falciparum*mono-infection while 397 (61.2%) children had negative peripheral blood film,There was no mixed infection. Of the 252 patients with parasitaemia, 114 (45.2 %) fulfilled the enrollment criteria and were randomized into one of two treatment arms to receive either artemether-lumefantrine (AL)orartesunate-amodiaquine (AA). In each treatment arm, there were 57 participants. Of the 114 enrollees,111 (97.4%) completed the study (Figure 1). One subject was lost to follow-up because he

relocated from the study area to his home state for reasons not connected to the study and was followed-up for safety using mobile telephone. One subject was excluded on account of the development of signs of severe malaria and one subject was excluded from analysis owing to missing packed cell volume and parasite count data.All these three patients were excluded from data analysis. The subject with severe malaria was successfully treated with rate-controlled intravenous infusion of quinine. The study profile of all participants is as shown in Figure 1 below:

Age and sex Distribution The mean age of study population in the AL and AA arms were 38.9 ± 16.90 and 37.7 ± 16.76 respectively, (p = 0.72). Thirty one (55.4%) and 25 (44.6%) were males and females in the AL study arm respectively while 32 (58.2%) and 23 (41.8%) were males and females respectively in the AA study arm, (p = 0.77).
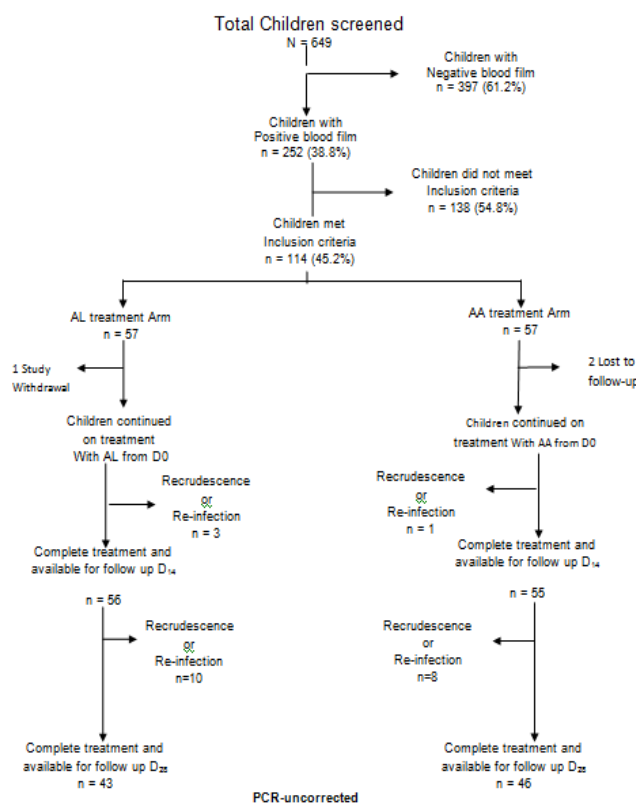

Figure 1: The profile of study subjects.

The baseline presenting symptoms were compared with the study medications in the two treatment arms; there was no significant difference, Table 1.

Table 1: Baseline Presenting Symptoms of Patients studied.

Baseline symptoms	All subjects N=111(%)	Subjects on AL treatment arm n=56 (%)	Subjects on AA treatment arm n=55 (%)	X^2	P - value
Abdominal pain	27(24.3)	17(30.4)	10(18.2)	2.235	0.135
Vomiting	23(20.7)	10(17.9)	13(23.6)	0.564	0.453
Cough	22(19.8)	12(21.4)	10(18.2)	0.841	0.668
Diarrhoea	18(16.2)	9(16.4)	9(16.4)	0.002	0.967
Headaches	9(8.1)	6(10.7)	3(5.5)	1.030	0.310
Poor appetite	4(3.6)	2(3.6)	2(3.6)	0.000	0.985
Mean axillary Temp (^{o}C)	37.8 ± 0.14	37.8 ± 0.98	37.9 ± 1.12	0.865	0.684
<37.5^{o}C	31(27.9)	16 (28.6)	15 (27.3)		
≥37.5^{o}C	80(72.1)	39 (69.8)	41(74.5)		
Hepatomegaly	14(12.6)	8(57.1)	8(57.1)	0.370	0.543
Splenomegaly	20(18.0)	10(50.50)	10(50.50)	0.002	0.965
Spleen Rate (%)	20 (18.0)	9 (50.0)	11 (50.0)	0.002	0.660

*Temp = Temperature, AL = Artemether-lumefantrine, AA = Artesunate-lumefantrine, Pre-enrollment parasite count (Day 0)

Pre-enrollment parasite count (Day 0) When subjects were grouped according to range-specific parasitaemia and according to study arms (AL versus AA), there was no statistical difference in the range of parasitaemia and the number of study participants in different ranges, Table 2

Table 2: Day 0 Parasite Counts of Patients studied

Laboratory Feature	All subjects N=111	AL Treatment arm n=56(%)	AA Treatment arm n=55(%)	X^2	P-value
Mean Parasite Density at D0(µl)	27,884.2 ± 23,797.0	27.697.6± 26,025.6	27,884.2 ± 23,797.0	0.49	0.92
1000 -<5000	13(11.7)	7(6.3)	6(5.4)	0.07	0.79
5000 - <50000	84(75.7)	42(37.8)	42(37.8)	0.03	0.87
50000 - <100000	11(9.9)	5(4.5)	6(5.4)	0.12	0.73
100000 - <200000	3(2.7)	2(1.8)	1(0.9)	0.32	0.57
>200000	0(0.0)	0(0.0)	0(0.0)		

*AL = Artemether-lumefantrine, AA = Artesunate-amodiaquine,µl = microliter

IV.   Polymerase chain reaction (PCR) results

Matched sample pairs collected before and after treatment from patients who were successfully analyzed at all the three loci MSP-1, MSP-2 and GLURP. Genotyping of these samples showed that the allelic families of MSP-1, MSP-2 and GLURP were often represented in parasite DNA derived from a single patient, indicating a polyclonal infection. Only P *falciparum* malaria mono-infection was identified.

Table 3: Biochemical Characteristics of Subjects studied on D0, D7 and D14

Parameter	D0		D7		D14		t-test	p-value
	AL	AA	AL	AA	AL	AA		
ALT (IU/L)	20.8±18.7	24.1±26.9	29.7±19.5	35.3±23.6	27.3±12.6	26.5±19.5	1.921	0.057
AST (IU/L)	26.5±24.4	27.4±26.7	33.7±21.6	34.2±21.6	28.0±17.8	33.0±19.2	0.764	0.447
Total bilirubin (mg/dl)	0.8±0.9	0.8±0.9	0.4±0.4	0.4±0.5	0.4±0.6	0.4±0.7	-0.083	0.934
Creatinine (µmol/dl)	36.8±35.9	36.2±31.8	49.6±40.7	44.9±38.8	35.2±27.9	36.9±32.6	0.287	0.775

* *µmol/dl = micromol per deciliter, IU/L = international unit per liter, mg/dl = milligram per deciliter. AL = Artemether-lumefantrine, AA = Artesunate-amodiaquine, D = day*
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V.   Discussion

At enrolment, renal impairment was noticed in both treatment arms. Functional renal impairment in the form of transient decrease in endogenous clearance of creatinine was reflected in this study by the mild elevation of serum creatinine levels.[5]This decrease was not significant. Serum creatinine levels normalized in both treatment arms within the first week following initiation of antimalarial chemotherapy. The finding in this study is consistent with the findings of other authors in Ogume Delta State, Nigeria.[5, 6] The precise mechanisms of mild renal impairment in P. *falciparum* malaria are multifactorial. [6]

No records of clinical hyperbilirubinaemiawere noticed in the study participants in AL and AA treatment arms during the study. However, at enrollment, mild and transient increases in serum bilirubin were noticedin both treatment arms. The serum bilirubin levels fell to the nadir by the D7 then rose gradually to the patients' normal values by the D14 following antimalarial chemotherapy and clearance of malaria parasites in the peripheral blood.[8] These changes however, did not reach statistically significant levels. The etiopathogenesis of hyperbilirunaemia in acute malaria is multi-factorial: intravascular haemolysis of both parasitized and unparasitized erythrocytes. [8]These acute but transient alterations are thought more as acute phase reactants. [9]

Transient disturbances in hepatic transaminases (ALT and AST) were noted during the study. Alterations in ALT and AST levels appeared comparable at enrollment in both treatment arms (AL and AA). Hepatic transaminitis were remarkable at D7follow-up visits in AL and AA treatment arms. The transaminases

levels normalized at D14. No study participants had values greater than mean $\pm$ 2SD in both treatment arms. The reasons for these biochemical disturbances are not completely clear. They are thought tobe frequent accompaniment of acute uncomplicated P. *falciparum* malaria.[9]The rapid normalization of these hepatic enzymes within the second week following treatment and parasite clearance wereconsidered more as acute phase response. The findings in this study agree with those found elsewhere, who for many years worked on the fields of malaria.[9, 10]

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Disclosures

The authors have no conflicts of interest to disclose.

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