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Research Article

Clinical Efficacy of Selected Antimalarials with and Without Concomitant Administration of Antibacterials

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Background: There is a high rate of malaria treatment failure even with the use of the artemisinin combination therapy (ACT). Studies have revealed that some bacteria infections present the same symptoms as uncomplicated and severe malaria. In most outpatient clinics, prescriptions are based only on the symptoms presented by the patients.

Objectives: To determine the prevalence of concomitant bacterial infection in malaria parasitaemia, evaluate the type of bacteria and assess the effect of the concomitant administration of five classes of antibacterial and antimalarials on the symptoms presented by the patients.

Methodology: Malaria parasitaemia was determined by thick and thin blood smears stained with Giemsa**.** Blood samples were cultured in MacConkey, chocolate and blood agar respectively using oxoid signal system after the manufacturers' instructions and microbial load was determined by pour plate method.

Results: Out of the 210 symptomatic cases 170 (80.95%) were found infected with malaria out of which 96 (56.47%) had bacterial co-infection and 74(45.53%) had malaria mono-infection. Of the 50 non-symptomatic cases 6 (12.00%) were found infected with malaria parasite among whom 2(33.33%) had bacterial co-infection. 64% of the symptomatic patients with malaria mono-infection who took dihydroartemisinin (DHA) as monotherapeutic or combination therapy (ACT) respectively improved clinically within 24 hours after initiation of treatment. 88% of the patients with concomitant infection who took either, DHA or ACT in combination with various antibacterial improved clinically within 24 hours. After 72 hours (three day), 75% of patients with concomitant infection who used only antimalarial DHA or ACT still had mild to severe headache and fever while 91% of those in this group who used antimalarials concurrently with various antibacterials were free of all the clinical symptoms presented pre-treatment. On the 7th day post treatment, 95% of patients with concomitant infection who used anti-malarial for three days and then antibacterial from the 4th day and 94% of those in this group who used antimalarial concurrently with antibacterial from the commencement of treatment had all their clinical symptoms resolved

Discussion: From the results of this study, we conclude that some bacterial infection present the same clinical symptoms as malaria, as such proper laboratory check should be conducted before administration of antimalarials and that concomitant use of antimalarials with broad spectrum antibacterials is more effective in malaria chemotherapy.

Key words: Clinical efficacy; Antimalarials; Antibacterials; Concomitant Administration

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1. Introduction

Malaria is an acute febrile illness. Symptoms appear about seven days or more (usually 10 to 15 days) after the infective mosquito bite. The earliest symptoms of uncomplicated malaria are non-specific: fever, headache, chills, vomiting, rigors and joint pains, and are difficult to recognize as malaria disease. If not treated within 24 hour *Plasmodium falciparum* disease can progress to severe illness often leading to death. Children in endemic areas with severe malaria disease frequently develop one or more of the following syndromic presentation: severe anaemia, respiratory distress in relation to metabolic acidosis or cerebral malaria. In adults, multi-organs involvement is also frequent, (Adepoju-Bello and Ogbeche, 2003; David and Peter, 2004; Etim *et al*., 2012).

Early diagnosis and treatment of malaria reduces the disease and prevent death. 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 (LLINs), the maintenance of good sanitary conditions and treatment of the disease by the use of drug (Habluetzel *et al*., 1999; Coker *et al.,*2001; Udobre *et al.,*2013)

Concomitant infection refers to the combined infection of two or more parasites of different species or group within a single host. Some symptoms of bacterial infections are similar to those of malaria infection, (Ukagai *et al.*, 2006). When some bacterial infection combines with malaria infection, it can lead to gravious consequences (Usip and Amadi, 2012)

The disease malaria is a major health and developmental challenge for many of the poorest countries in the world. Recent studies have shown the existence of concomitant bacterial infections in severe malaria patients, which complicated the manifestation of malaria, thereby confusing diagnosis and chemotherapy especially where they are relatively unknown. Gram negative bacteria have been implicated as important cause of septicemia in some cases of *P. falciparum* malaria infection (Usip and Amadi, 2012). Septicemia presents fever, chill, anxiety and rapid breathing which is similar to malaria symptoms (Tietz, 1982; Marshall and Bangert, 2009).

Falciparum malaria is the most prevalent malaria in Nigeria but very little information on concomitant bacterial infection in severe and uncomplicated malaria is known. The present study is aimed at investigating the prevalence of concomitant bacterial infection in malaria parasitaemia, evaluate the type of bacteria and determine the clinical efficacy or otherwise of the concomitant administration of antimalarials with antibacterials in malaria parasitaemia.

2. Methods

2.1 Recruitment of Subjects

Patients were recruited after satisfying standard criteria of the study which include infection with *Plasmodium falciparum* with a density between 250 and 50,000 asexual parasites/mm^3 of whole blood, a concomitant infection of *P. falciparum* and a significant level of a pathogenic bacterium or bacteria with low, moderate or heavy density (Reginald and Gayle, 2001). The patients also had no history of intolerance to any of the drugs used in the study, they were able to take oral medication and gave a written consent to participate in the study after explanation of what the study will involve.

Three hundred and twelve symptomatic patients were identified by a physician consulting in the University of Uyo Health Centre, and fifty non-symptomatic volunteers were identified by personal contact from among staff and students of the University of Uyo community.

Patients with severe malaria who were anemic as identified by the determination of mean corpuscular haemoglobin count (MCHC), or not able to take oral drugs and those who were contraindicated to any of the drugs used in the studies were excluded. Vital signs: body temperature, body weight not less than 55kg, and blood pressure of each participant was recorded before drug administration.

2.2 Drugs, chemicals and reagents

The antimalarials used were Alaxin, (each tablet containing 60mg of dihydroartemisinin) and P-alaxin (an ACT with each tablet containing 40mg of dihydroartemisinin and 320mg of piperaquine phosphate), both manufactured by Bliss Pharm. India. The antibacterial used, their class, active ingredient, manufacturers and marketers are shown on **Table 1**. All drug products were obtained direct from the manufacturer's representative here in Uyo, and they were less than one year from the date of manufacture. The chemicals: giemsa, MacConkey, chocolate and blood agar were freshly prepared after the manufacturer's instructions.

2.3 Study Design

An open single centre study was carried out involving all the subjects. The World Health Organization *in vivo* seven days standard field test consisting of the administration of antimalarials over three days with a seven days observation period was used (WHO, 1973). Patients receiving drugs randomly fell into twelve groups and the thirteenth group were asymptomatic volunteers who were not given any drugs and served as a control group.

2.4 Administration of Drugs to patients

After consulting with the physician and obtaining laboratory test results, the symptomatic patients were given drugs based on their symptoms, plasmodium density and bacteria sensitivity. The dosing regimen was as recommended by the manufacturers and is in agreement with World Health Organization (WHO) guidelines for the treatment of uncomplicated and severe malaria (WHO, 2010). The dosing information is in **Table 2**. Two patients from each group with concomitant malaria and bacterial infection (**Table 4**), were given antimalarials only for three days, followed by various antibacterials from the fourth day.

Table 1: Antibacterials used in the study

S/N	Trade Name	Class	Active Ingredient(s)	Manufacturer	Marketer
1	Augmentin	Penicillin	Amoxicillin 500g and Clavulanic acid 125g	Smithkline Beecham, West Sussex, UK.	Smithkline Beecham, Nigeria
2	Cefunate	Cephalosporin	Cefuroxime Axetile 500mg	Bharet Pharm. India	Evans Medical Plc, Nigera
3	Ciprotab	Fluoroquinolone	Ciprofloxacin 500mg	Geltec Pvt Kamataka, India	Fidson Healthcare Plc, Nigeria
4	Clarimax	Macrolide	Clarithromycyin	Swiss Pharm Pvt, India	Feccox Pharmacy Ltd, Kano, Nigeria
5	Vatromax	Macrolide	Azithromycin 500mg	Jiangsu Qianjin Pharm. China	Vixa Pharmaceuticals, Nigeria

Table 2: Administration of Drugs to Volunteers

Group	Antimalarial used	Antibacterial used	Number of Patients
A	Alaxin	-	30
B	P-Alaxin	-	44
C	Alaxin	Augmentin	13
D	Alaxin	Cefunate	5
E	Alaxin	Ciptrotab	17
F	Alaxin	Clarimax	3
G	Alaxin	Vatromax	11
H	P-Alaxin	Augmentin	8
I	P-Alaxin	Cefunate	4
J	P-Alaxin	Ciprotab	7
K	P-Alaxin	Clarimax	12
L	P-Alaxin	Vatromax	16
M	-	-	50

2.5 Collection of Blood, Culture and Sensitivity Test

5ml of blood was collected from the cubital fossa vains of each patient after recruitment into sterilized EDTA bottles, allowed to stand for 5 minutes to equilibrate at room temperature and were stored at -20^{o}C until analyzed for parasite density. Patients enrolled for the study were examined on follow up days (3 and 7) post-therapy by experienced clinicians. Their body temperature and the response to symptoms they presented at their first visit were documented. 5ml of blood was collected from the patients on follow up days for parasite counts.

2.6 Quantitative Determination of Parasites

Malaria parasitaemia was established by thick and thin blood smears stained with Giemsa and was categorized into low, moderate and high or heavy infections. Majority of patients (83%) were in the moderate category with *P. falciparum* density between 250 and 50,000 asexual parasites/mm^3. *Plasmodium* density was estimated by counting the number of asexual parasites per 100 leucocytes assuming a mean leucocytes counts to be 6000mm^3 of whole blood. Parasite density was calculates as:

$$\text{Parasite density (PD)} = \frac{\text{Parasite count x 6000}}{\text{Leucocytes count}}$$

The microbial load of the blood drawn aseptically from each patient was determined by serial dilution and pour plate method (John *et al.,* 1998). Quantitative whole blood culture (QBC) was determined by measuring 1.0ml of blood and mixing it with 10.0ml of molten (50^{o}C) Columbia agar in a petri dish, this was rotated gently to disperse the inoculums in the medium and allowed to set and then incubated at 37 ^{o}C for 7 days. A minimum blood-to-broth ratio of 1:10 was maintained. After incubation colonies were counted and recorded as CFU per milliliter. The number of bacteria per milliliter of blood (QBC) was estimated from the number of CFU on each pour plate. The quantitative value was

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estimated from total volume of blood cultured (Bennett *et al*., 1986, John *et al*., 1998).

Various pathogenic bacteria were isolated, characterized and identified according to their colonial morphology (Cowan, 1985), gram stain reaction and motility (Fawole and Oso 1988), indole production, urease and coagulase (Collins and Lyne, 1976), methyl red, citrate and catalase (Cruickshank 1975).

The isolates from district colonies were further subjected to specific biochemical test sugar fermentation to test their ability to utilize different sugars. The sugars used were dextrose, glucose, lactose, mannitol and sucrose using the method described by Harrigan and McCance, 1976.

2.7 Statistical analysis

The data obtained were expressed as mean ± standard deviation (SD) students t-test was used to assess statistical significance, values of $P<0.05$ were considered to be significant.

2.8 Ethical considerations

The study was conducted between April and June 2013 at the Health Centre of the University of Uyo, after approval by the Ethics Committee of the University. The ethics approval number is **UU/HC/EC/vol.2/274** of March 6th 2013.

3. Results and Discussion

The results of the study revealed a high level of malaria infection among patients visiting the health centre each day. The doctor saw 512 patients within the period of the studies among which 312 accepted to participate in the exercise, but at the end 210 came back for check-up and gave blood samples and responded to question presented on the 3rd and 7th day. Out of the 210 patients who participated to the end of the studies, 170 (80.95%) had malaria and the most prevalent species was *P. falciparum*. Ninety-six patients representing (56.47%) of those with malaria infection had concomitant bacterial infection with *staphylococcus* species being the most prevalent (22.05%) followed by *Salmonella typhi* species (20.18%) and the least was *Micrococcus* species (3.11%) (**Table 3**).

The existence of concomitant bacterial infection in malaria patients complicates the manifestation of malaria disease thereby confusing physicians of proper diagnosis and chemotherapy especially where laboratory test is not conducted to establish the organisms present for proper prescription.

Among the 50 volunteers who were asymptomatic, 6 (12%) had low level of malaria parasite with two of them having concomitant infection of *Salmonella typhi*. The level of their parasite increased gradually within the week (**Figure 1**). This result suggests that in highly malaria endemic zones, there is need for monthly prophylactic treatment even if there is no symptom of malaria present.

Among the symptoms presented by the patients fever (body temperature above 42 °C) was most prevalent 205 (97.62%) followed by headaches 198 (94.29%) (**Figure 2**). Within 24 hours of administration of the various drugs, 64% of the patients who used DHA alone, 69% of those who used ACT, and 88% of those who used DHA or ACT in combination with various antibacterial were relieved of all the symptoms they presented at the commencement of their treatment. On the third day (72hours) post-therapy, 70% of the patients with malaria mono-infection who used DHA alone and 78% of those who used ACT had all their clinical symptoms resolved. On this same day, 15 out of 20 (75%) of patients who had malaria and bacteria co-infection (**Table 4**) and used only anti-malarias still had most of the symptoms they presented at the commencement of treatment, however, 91% of patients in this group who used anti-malarial concurrently with various anti-bacterial had all their clinical symptoms resolved (**Figure 1**). On the 7th day post treatment, 95% of patients with concomitant infection (**Table 4**) who used anti-malarial for three days and then antibacterial from the 4th day and 94% of those in this group who used antimalarial concurrently with antibacterial from the first day of treatment had all their clinical symptoms resolved, and showed a negative laboratory finding upon blood smear and culture (**Figure 1**).

These improvement is significant ($P < 0.05$) and goes a long way to confirm that concomitant antimalarials and antibacterial is very useful in the treatment of both uncomplicated and severe malaria. (Alaeibe *et al*., 1998).

There is daily report of high rate of malaria treatment failure which in most cases results in a large number of dead each year (WHO 2010). Most of the treatment failures are attributed to fake drug, self medication, incomplete regimen, resistance of plasmodium species to the drugs used among other factors, without considering the presence of other organisms which may present the same symptoms as malaria.

4. Conclusion

From the result of these studies, we conclude that malaria is a serious health problem in the tropics including Nigeria considering that about 81% of patients visiting a particular clinic were infected with the parasite. Due to poor sanitary conditions and sources of drinking water, there is high level of concomitant bacterial and malaria infection, and some bacterial infection present the same clinical symptoms as malaria infection as such treatment based on symptoms presented only should be discouraged.

Rather, specific laboratory diagnosis of malaria and differential diagnosis of other bacterial infection should be carried out before embarking on treatment for effective management of malaria and other related diseases in our health care system.

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Table 3: Colonial morphology, characterization and prevalence of bacteria isolates from 96 blood samples

Isolate	Morphology	Shape	Gram Stain								Sugar Fermentation					Bacteria Isolate	No.	%
			Gram reaction	Catalase	Coagulase	Methyl red	Motility	Citrate	Urease	Indole	Dextrose	Glucose	Lactose	Mannitol	Sucrose			
1.	Circular, smooth, raised milky, glistering.	Cocci in cluster	+	+	+	+	-	+	+	+	A	A	A	A	A	*Staphylococcus spp*	71	22.05
2.	Rough, filamentous, creamy flat, pungent	Long rod	-	-	+	+	+	+	+	-	A	AG	AG	A	AG	*Salmonella typhi spp.*	65	20.18
3.	Rough flat, filamentous, creamy, pungent	Cocci in chain	+	-	+	+	-	-	+	+	AG	AG	AG	AG	AG	*Streptococcus spp*	58	18.01
4.	Circular smooth, milky, raised, pungent	Rod	-	-	-	+	+	-	-	+	AG	A	A	A	A	*Escherichia coli*	4.7	14.60
5.	Irregular, creamy, shinning, translucent, pungent	Short rod	+	+	-	+	+	+	-	-	AG	AG	AG	AG	A	*Baccilus spp*	30	9.32
6.	Smooth, circular entire, pungent	Long rod	-	+	-	+	+	+	-	-	A	A	A	A	A	*Klebsiella spp*	23	7.14
7.	Green, rough, raised, filamentous, shinning	Rod	-	-	+	+	+	+	+	-	-	A	-	-	-	*Pseudomonas spp*	18	5.59
8.	Circular, cream, tiny glistering, raised, pungent	Short rod	-	-	-	+	-	+	+	+	A	A	A	-	A	*Micrococcus spp*	10	3.11
	Total																322	100

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Table 4: Distribution of different categories of infection among the different treatment groups

Treatment group	Drug administered	Malaria mono-infection No. (%)	Malaria mixed infection with							
			***Staphylococcus* spp** No. (%)	***Salmonella*** No. (%)	***Streptococcus* spp** No. (%)	***E. coli*** No. (%)	***Baccilus* spp** No. (%)	***Klebsiella* spp** No. (%)	***Pseudomonas* spp** No. (%)	***Micrococcus* spp** No. (%)
A	AL	30 (40.5)								
B	P – AL	44 (59.5)								
C	AL + AUG		8 (34.7)	5 (25.0)						
D	AL + CEF							3 (42.9)		2 (100)
E	AL + CIP		4 (17.3)	8 (40.0)		4 (28.6)			1 (25.0)	
F	AL + CLA				3 (18.8)					
G	AL + VAT				4 (25.0)		5 (50)		2 (50.0)	
H	P – AL + AUG		8 (34.7)							
I	P – AL + CEF							4 (57)		
J	P – AL + CIP			7 (35.0)						
K	P – AL + CLA		3 (13.3)		3 (18.8)	6 (42.8)				
L	P – AL + VAT				6 (37.5)	4 (28.6)	5 (50)		1 (25.0)	
TOTAL		**74**	**23**	**20**	**16**	**14**	**10**	**7**	**4**	**2**

AL – Alaxine; P–AL - P – Alaxine; AUG – Augmentin; CEF – Cefunate; CIP – Ciprotab; CLA – Clerimax; VAT - Vatromax
Malaria mono infection = 74; Mixed infection = 96; Other ailments and bacteria = 40

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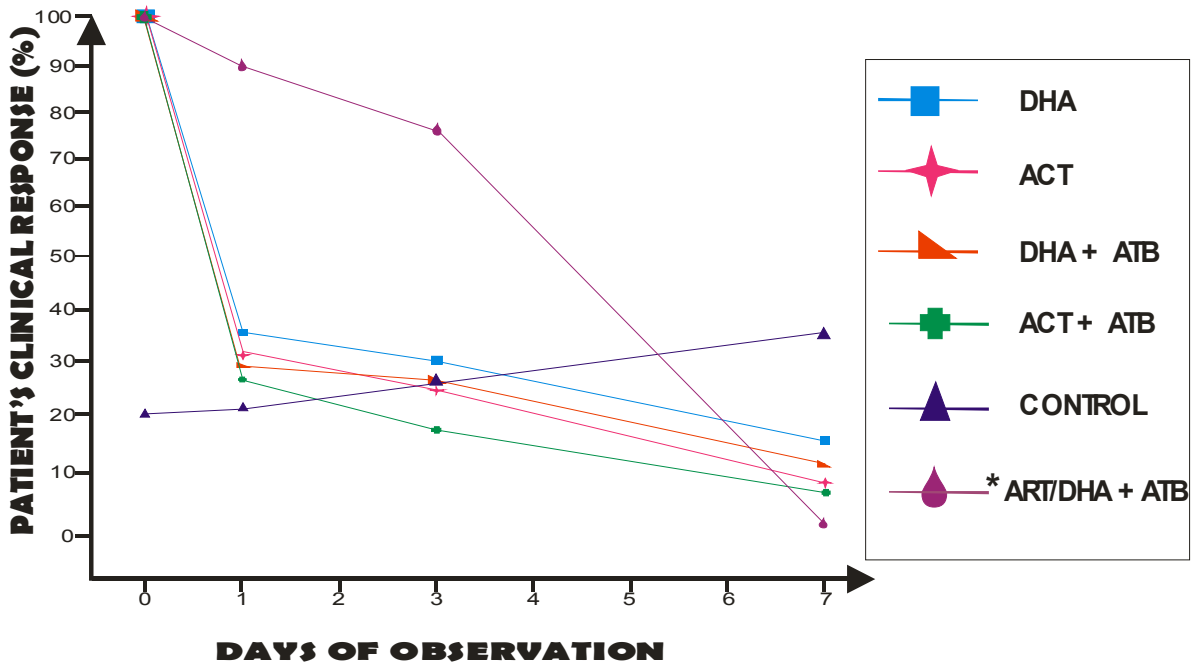

DHA = Dihydroartemisinin
ACT = Artemisinin Combination Therapy
ATB = Antibacterial
**ART/DHA + ATB = Patients with concomitant infection who used antimalarial only for three days then antibacterial from 4th day*

Figure 1: Clinical response using various drugs

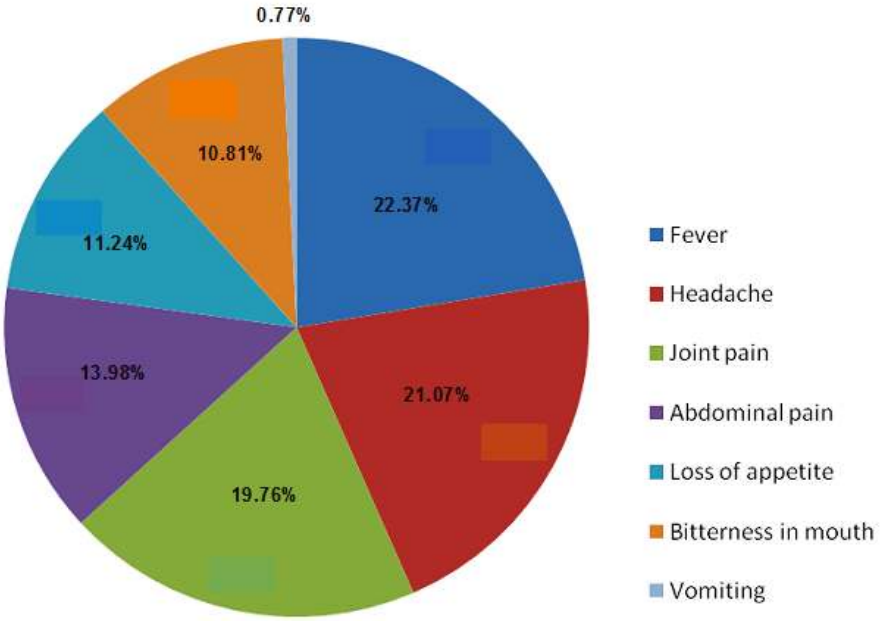

Figure 2: Symptoms presented by patients

Conflict of Interest declaration

The authors declare no conflict of interest

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