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Comparison of Parasitologic Efficacy of Chloroquine, Doxycycline and Sulfadoxine-pyrmethamine in Asymptomatic Carriers of *Plasmodium falciparum* in Abraka, Southern Nigeria

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INTRODUCTION

In malaria, *Plasmodium* protozoa injected by female anopheles mosquito multiply in red blood cells causing haemolysis, stasis and cytokine release (Hope *et. al.,* 1998). It is the most important parasitic disease of humans with transmission occurring in 103 countries affecting more than 2 billion people (40% of the earth's population) with 300-500 million cases annually causing 1-3 million deaths (Martin-Rabada and Bouza, 2004; White and Bergman, 2001). With the cost of the recommended artemisinin based combination therapy being 5-10 dollars per complete regimen, this puts the annual cost of treatment of this disease globally at 1.5-5 billion dollars.

Estimates of malaria parasite density are used in determination of post therapy parasite clearance which is an index of parasitologic efficacy of antimalarial drugs. This provides information on desirability of use of any given antimalarial drug, considering its efficacy, or resistance of the Plasmodium parasite to it. According to World Health Organisation (WHO) scheme, a normal/ expected parasitologic response involves a reduction of pre-treatment parasite density/count to 25% of original count (Kakkilaya,2006)

According to the WHO report on malaria profile in Nigeria, transmission is all year round in southern Nigeria but seasonal in northern Nigeria.

According to Leighton and Foster (1993), 7-19% of household income of Nigerian homes are spent on malaria treatment or prevention. Funding for malaria control in Nigeria rose from USD $17 million in 2005 to USD $60 million in 2007, provided by the government, Global fund and World Bank. This is estimated to be insufficient to reach national targets for prevention and cure (WHO, 2008[a]).

Southern Nigeria being an area of stable malaria transmission, it would be expected that the clinical disease preferentially affect children while there is a protective partial immunity in adults (Van den Enden, 2009). This will of course be characterized by asymptomatic parasitaemia in adults, as well as spontaneous parasite clearance. Some studies have reported this spontaneous clearance in some endemic areas (Nguyen-Dinh *et. al.*, 1988; Franks *et. al.*, 2001).

According to Aguas *et al.,* (2008), asymptomatic carriers of malaria parasites constitute a significant reservoir indispensable in any program to eradicate this pernicious disease.

Currently sulfadoxine-pyrimethamine (SP) is used as part of the ACT therapy in intermittent preventive therapy (IPT) in pregnant women in Nigeria, whereas doxycycline has no place even though it is known to have anti-malaria effects. Chloroquine was used for malaria chemotherapy and IPT until its use was discontinued due to high prevalence of resistance (FmOH 2005, Ukwuoma, 2005). A reversal of widespread resistance to chloroquine has been reported in Malawi. (Laufer et al, 2006)

This study is aimed at calculating and comparing the parasite clearance rate of two chemo-prophylactic agents as an indication of anti-malaria sensitivity and resistance in the study population.

MATERIAL AND METHODS

Study area

Abraka is a semi-urban town located in the Niger-Delta region of Nigeria between latitude 5 degrees, 30 minutes and latitude 6 degrees north of the equator and longitude 6 degrees and 6 degrees, 30 minutes from the Greenwich meridian. The height above sea level is between 50-200 metres. Abraka is covered by rainforest vegetation and receives not less than 4000mm of rain annually with the rainy season starting as early as late January-early February and dry season between late November and early December. The indigenes are Urhobo by tribe and it is host to the main campus of the multi campus state university; Delta State University.the inhabitants who are mainly students hail from other parts of southern Nigeria. The fresh water River Ethiope runs through the town very close to the university hostels. This study was carried out between june and august 2008.

Sampling

A random sample of students of College of Health Sciences Delta State University, Abraka were studied. However due to difficulty in obtaining informed consent from asymptomatic students willing to have their blood taken for the study I was just able to obtain 93 asymptomatic (apparently healthy) subjects.

Criteria for inclusion

Volunteers with no history of fever in the previous two weeks and not on any anti-malaria treatment and not on any antibiotic nor having any objection to being a part of this research having being informed of the purpose, procedure, risks and benefits (informed consent) were recruited for the study. The volunteers were mainly medical and nursing students.

Ethical Permission

Ethical permission was sought and obtained from the Director of Health Services, Delta State University, Abraka.

Procedure

A questionnaire was administered at first contact, the first page of which is for informed consent. Test periods for asymptomatic subjects were categorized into week 0 (1st contact), week 1 (7 days later) and week 2 (14 days from 1st contact). Chemoprophylaxis was administered at week 1.

A drop of blood was used to make a thin film and three drops of blood was used to make a thick film of diameter about 2.0cm. A slide card was used to standardize the area to be covered by the thick film. Both the thick and thin film was made on the same slide. The thin blood film was air dried and fixed using absolute methanol and stained with Giemsa stain. The thick film shall was air dried and stained using Giemsa stain.

Stained slides were examined using x100 objective, x10 eyepiece and oil immersion. The thick film were used for counting malaria parasites against 200 white blood cells. The thin film were used to identify the species. We identified only *Plasmodium falciparum* species. The proportion of samples having malaria parasites were calculated as parasite rate. Using an estimated white blood cell count of 7000/µl, parasite density was calculated.

Parasite density and rate was repeated at week 1 and week 2. 93 asymptomatic subjects were evaluated at week 0 but only 74 returned at week 1, then asymptomatic subjects were divided into groups, all groups were told they were given

anti-malaria drugs but not which.

Group A : Received 4 tablets stat dose each of 200mg chloroquine equivalent to 600mg base (nivaquine by Rhone-Poulenc Rorer)

Group B : Received 100 mg capsules of doxycycline (by Hovid) b.i.d for 5 days

Group C : Received 3 tablets stat dose of sulfadoxine-pyrimethamine(SP) Laridox by Ipca

Group D : Received 3 tablets stat dose of 100mg vitamin C by Emzor. (control group)

RESULTS

7th Day post-chemoprophylaxis parasite clearance in asymptomatic subjects, % efficiency of clearance.

Post-therapy parasite clearance was calculated using the formula by Rebecca *et. al.,* (2008)

$$\frac{\text{Pre-therapy density -post-therapy density}}{\text{Pre-therapy density}} \times 100$$

Comparing the parasitologic clearance at 7th day post treatment of the groups , we calculated

$$\frac{\text{\% efficiency of clearance} \times \text{\% of subject with} > 75\% \text{ clearance}}{100}$$

DISCUSSION

Effect of Chemoprophylactic Agents Studied on Parasite Density

Chloroquine is the most successful 4-aminoquionoline and acts on sensitive strains of

Table 1: Distribution of genotype and parasite density at week 0 among asymptomatic subjects

Parasite Density Range (/mm³)	Male		Female		Total	
	AA	AS	AA	AS	No	%
0-1	1	-	6	-	7	7.5
1-100	-	-	2	-	2	2.2
101-1000	16	-	11	-	27	29.0
1001-10000	17	2	31	4	54	58.1
>10000	-	-	1	2	3	3.2
Total	34	2	51	6	93	100

Table 2: Distribution of genotype and parasite density at week 1 among asymptomatic subjects

Parasite Density Range (/mm³)	Male		Female		Total	
	AA	AS	AA	AS	No	%
0-1	1	-	3	-	4	5.4
1-100	1	-	-	-	1	1.4
101-1000	7	-	6	-	13	17.6
1001-10000	12	-	19	4	35	47.3
>10000	8	-	13	-	21	28.4
Total	29	0	41	4	74	100

Plasmodium falciparum by concentrating within the food vacuole and prevent the detoxifying conversion of heme to haemozoin. Widespread resistance led to the abandonment of chloroquine in the Nigerian anti-malaria policy since 2005 (FMoH, 2005; Ukuoma, 2005) . Residual drug levels are drivers for selecting resistant strains of *Plasmodium falciparum* and encourage the spread of chloroquine resistant malignant tertian malaria. Another issue which helped encourage the abandonment of chloroquine is the accumulation in tissues especially melanin containing tissue like skin and retina, where it cause pruritus and retinopathy respectively. It also has a long half-life such that can last 30-60 days so it can remain in the system at sub-therapeutic levels such that next attack of malaria would select resistant strains (Tracy and Webster, 2001).

The average post-chemoprophylaxis parasite clearance of 71.8% is rather high but does not exceed the WHO recommended cut off of 75%. Considering the ≥ 75% clearance in 63.6% of subjects, this represents an increase in the efficacy of chloroquine. Some other studies have documented a reversal in the widespread resistance which led to its abandonment. This reversal has also been reported in Malawi (Laufer *et. al*., 2006).

Table 3: Effect of chloroquine on parasite density in asymptomatic subjects (Test Group A)

Parasite Density (/mm³)					
Male			Female		
Before Rx	After Rx	% Clearance	Before Rx	After Rx	% Clearance
19200	4600	76.0	18669	4200	77.5
29169	88	99.7	23100	12474	46.0
1064	539	49.3	7314	1686	76.9
1069	4200	-292.9	14000	3080	78.0
			18670	4250	76.2
			13689	5325	38.9
			7160	1701	23.8

Table 4: effect of doxycycline on parasite density in asymptomatic subjects (Test Group B)

Parasite Density (/mm³)					
Male			Female		
Before Rx	After Rx	% Clearance	Before Rx	After Rx	% Clearance
36169	1841	94.9	1666	168	89.9
21770	2880	86.8	40922	14732	64.0
1071	1382	-29.0	10136	1309	87.1
			1660	565	66.0
			1685	192	88.6
			41900	5621	88.6
			1715	91	94.7
			1721	106	93.8

Table 5: Effect of Sulfadoxine-Pyrimethamine (SP) on Parasite Density in asymptomatic subjects (Test Group C)

Parasite Density (/mm³)					
Male			Female		
Before Rx	After Rx	% Clearance	Before Rx	After Rx	% Clearance
70	132	-88.6	91000	1029	98.9
17885	2387	86.6	420	0	100
2086	945	54.7	19250	1421	92.6
371	791	-113.2	2420	3050	92.6
707	63	91.1	9160	1021	-26.0
1036	1260	-21.6	19350	1486	88.8
392	761	-94.1	70000	98	92.3
716	68	90.5	29750	427	99.9
17960	2345	86.9	8519	588	98.6
			2240	3500	-56.3
			8516	600	92.9

Table 6: Effect of Placebo (Vitamin C) on Parasite Density in asymptomatic subjects (Test Group D)

Parasite Density (/mm³)					
Male			Female		
Before Rx	After Rx	% Clearance	Before Rx	After Rx	% Clearance
13210	68790	-420.7	12628	20790	64.6
13314	69502	-422.0	1869	1428	23.6
1344	1064	20.8	210	0	100
560	3276	-485.0	1860	1521	18.2
1820	10920	-500.0	1342	2066	-54.0
			252	1321	-424.2
			290	200	31

Table 7: Comparing sensitivity of test cases to anti-malaria drugs

Name of Drug	Av. %Clearance	% Sensitive cases	% Efficacy (raw)	% Efficacy (corrected)
Chloroquine	71.8	63.6	45.7	60.8
Doxycycline	74.9	72.3	54.2	69.3
SP	43.4	65.0	28.2	43.3
Vitamin C (Control group)	-181.4	8.3	-15.1	0.0

This rising sensitivity to chloroquine is good news but one that must be handled with caution so as to curtail the selection pressure in favour of resistant strains. A combination of an artemisinin based drug with chloroquine is a likely way to handle this rising sensitivity.

Doxycycline is an antibiotic in the class of tetracycline which posseses anti-malaria activity. It is completely absorbed from the gastrointestinal tract and this is not affected by food or milk as with tetracycline. It is also primarily excreted in faeces and can be used in patients with renal insufficiency (Baird *et. al.*, 2005). Doxycycline is recommended as an alternative to mefloquine in anti-malaria prophylaxis and as an adjunct drug with quinine in malaria treatment (Hope *et. al.*, 1998; White and Breman, 2001). It is however not used as such in Nigeria as the national malaria treatment policy discourages malaria chemoprophylaxis in areas of stable transmission (which is much the whole country) and recommends only proguanil in cases like sickle cell anaemia (FMoH, 2005). The high average post chemoprophylaxis clearance of about 75% and the >75% clearance in 72.3% of test subjects indicate that this drug which is little used could be a cheaper and effective combination with artemisinin based drugs in the combat against malaria.

Doxycycline however cannot be used in children or pregnant women because of the possibility of staining bones and teeth. It can also cause photosensitivity and predispose to moniliasis in cases of long term use (White and Breman, 2001).

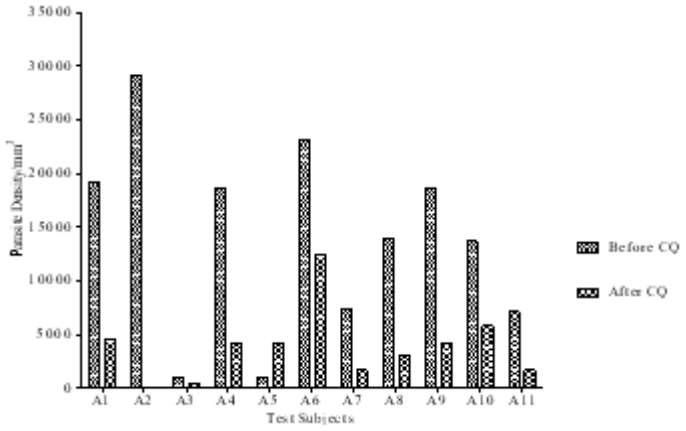

Fig. 1: Effect of Chloroquine on parasite density at 7th day post chemoprophylaxis

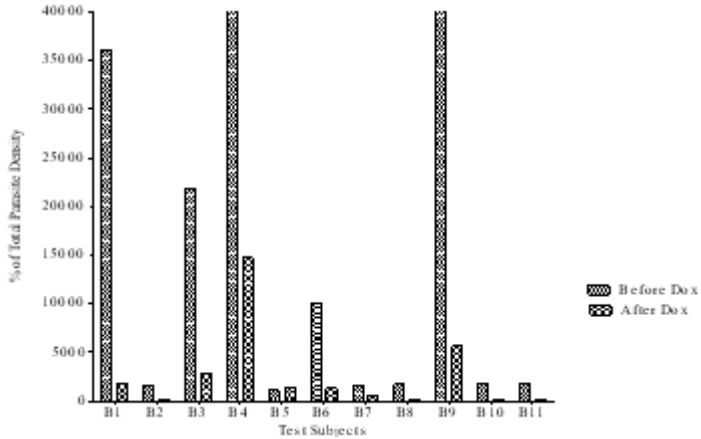

Fig. 2: Effect of Doxycycline on parasite density at 7th day post chemoprophylaxis

It is hereby recommended that doxycycline be studied and included in chemoprophylactic/ therapeutic regimen for malaria in Nigerian adults as this antibiotic has a fairly long half life (18hours), is relatively cheap with a simple dosing procedure, and it demonstrates about 75% clearance of parasites in majority of patients.

Sulfadoxine-Pyrimethamine (SP)

This anti-folate combination of sulfadoxine and pyrimethamine has been used extensively for prophylaxis and suppression of human malarias, especially those caused by chloroquine resistant strains. It inhibits two consecutive steps in the formation of folic acid from para-aminobenzoic acid (PABA) in Plasmodia at concentrations far lower than those required to produce comparable inhibition of mammalian enzymes (Tracy and Webster, 2001; Baird *et. al.*, 2005). It is slow acting inhibiting cell division in schizonts and in the liver forms. It has a half life of at least 7 days (4 days for pyrimethamine and 7 days for sulfadoxine); this

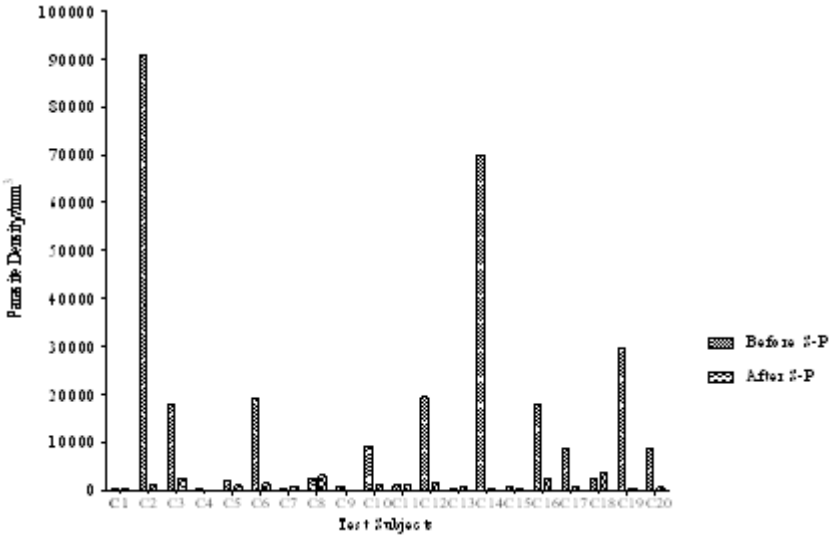

Fig. 3: Effect of Sulfadoxine-Pyrimethamine (S-P) on parasite density at 7th day post chemoprophylaxis

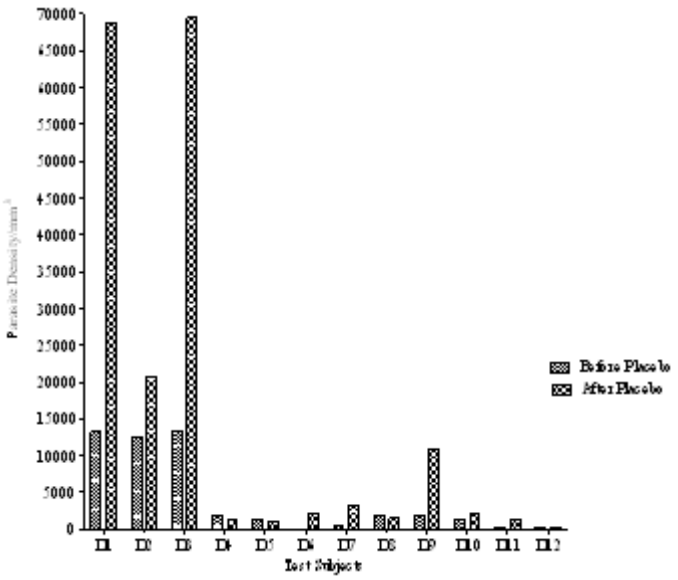

Fig. 4: Effect of placebo (vitamin C) on parasite density at 7th day post chemoprophylaxis

prolonged elimination encourages the selection of resistant parasites and this resistance has been limiting its use. In combination with artesunate it is part of the recommended ACT regimen with special recommendation for intermittent preventive therapy (IPT) in pregnant women (WHO, 2002[b]).

An average percentage post chemoprophylaxis clearance of43.4% was recorded and clearance of ≥ 75% in 65% of the subjects. This low efficiency in clearance of parasites depicts the picture of rising resistance to SP in this region and this would limit the effectiveness of the drug in both chemoprophylaxis and chemotherapy. Increasing resistance to SP has also been reported, with treatment failure occurring in 81.6% of subjects and an efficacy of 21% in Malawi (Laufer *et. al.*, 2006). SP resistant to *Plasmodium falciparum* malaria is due to mutations which are enhanced by the pressure of residual levels of SP which has been known to last up to two weeks in the blood (Tracy and Webster, 2001). These mutations are also driven by the presence of residual levels of other long acting sulphonamide preparations especially co-trimoxazole which is often prescribed for suspected bacterial infections (Khalil *et. al.*, 2003).

With a % efficiency of 28.2%, it is here suggested that a restriction of the use of both SP and co-trimoxazole in order to halt this trend of resistance and probably occasion a reversal of resistance is desirable.

Control

A nutritional supplement (vitamin C) was used as placebo and all subjects were informed they were taking anti-malaria drugs but were not told which so as to see the effect of psychological stimulation and non-use of anti-malaria drugs. The average % post therapy clearance of -181.4% means that on the average parasite density increased by 281.4%. The decimatory effects of the immune system on parasite multiplication and probably the psycho-neuro-immune stimulation of placebo shows a definite effect in a small percentage of the population(8.3% at week 2 vs 5.4% at week 1) but the resultant effect in most subjects is a geometric increase in parasite density.

Comparison of the Parasitologic Efficiency of Anti-Malaria Drugs Studied

Comparing the efficiency of parasite clearance reveals a similar efficiency in Doxycycline and a low efficiency with SP alone. This suggests the proposal to include doxycyline in our anti-malaria regimen. This cheap antibiotic can be an effective combination with an artemisinin based drug for the effective control of falciparum malaria. More studies are needed to confirm this and develop protocols for its effective deployment in Nigeria. Doxycycline is already being used as a second line prophylactic agent in developed countries like United States of America (White and Breman, 2001), second to mefloquine which has been withdrawn from Nigeria due to the widespread adverse effects experienced by Nigerians who used the drug. Its use in non-pregnant Nigerian adults is here recommended as short term therapy or short term chemoprophylaxis.

Chloroquine showed a rising efficiency and this is likely a benefit being reaped by the restriction of its use by the federal ministry of health for the past four years. This reversal in chloroquine resistance is important and should be harnessed by not using it again as mono-therapy, rather as a combination drug and in a way that will avoid indiscriminate use which occasioned the emergence of resistance in the first place.

CONCLUSION

Young asymptomatic adults constitute the reservoir of infection which maintains transmission to the high risk groups and control activities cannot be effective until it involves especially, this large population which provide a bank of gametocytes to effect transmission.

A rising resistance to SP and relatively rising sensitivity to chloroquine was found. Doxycycline showed an efficiency of parasite clearance similar to that of the ACT. A restriction of the use of SP in both monotherapy and combination therapy is hereby recommended, and a cautious reconsideration of chloroquine only as possible part of combination therapy. It is also suggested that doxycycline be considered for inclusion as part of short term prophylaxis or therapy in combination with other effective fast acting anti-malaria drugs like artesunate.

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