COMPARATIVE STUDIES ON THE EFFICIENCY AND TOLERABILITY OF FOUR ANTIMALARIALS IN MALARIAL PATIENTS OF DIFFERENT HAEMOGLOBIN GENOTYPES (AA, AS, AND SS)

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ABSTRACT

Comparative studies on the efficiency and tolerability of four antimalarials, quinine dihydrochloride, sulfadoxine/pyrimethamine, halofantrine and artesunate were carried out in 480 volunteer malaria patients of different HB - genotypes (AA, AS, and SS). Patients with HB - genotype AA were 350 (73%). Those with HB - genotype AS were 102 (21.2%) and those with HB - genotype SS were 28 (5.8%), those with HB genotype AA were randomly divided into four groups of 84, 86, 90 and 90 patients each. Those with HB genotype AS were randomly divided into four groups of 25, 25, 26, 26 patients each and those with HB genotype SS were divided into four groups of 6, 7, 7 and 8-patients each. First group received sulfadoxine/pyrimethamine, 3 tablets of 500mg/25mg orally at once. Second group received quinine dihydrochloride 200mg or 8mg/kg 3 times daily for 6 days. Third group received halofantrine 160mg tablets (start) and 80mg 6 hourly for 3 days orally. Fourth group received artasunate 100mg tablets (start) and 50mg 12 hourly for 5 days orally. On day 7 efficacy of these antimalarials was evaluated and compared according to different heamoglobin genotypes AA, AS, and SS. Artesunate had the highest efficacy of 98% in AA, 93.3% in AS and 100% in SS. The efficacy of artesunate is not significantly different (P>0.05) in AA, AS and SS. Halofantrine ranked second in efficacy with 97.8% in AA, 92.0 % in AS and 85.7% in SS. Efficacy of halofantrine is not significantly different in these HB - genotypes (P>0.05), AA, AS and SS. Sulfadoxine/pyrimethamine ranked third in efficacy with 95.3% in AA, 84.0% in AS and 100% in SS. The efficacy is not significantly different (P>0.05). While quinine dihydrochloride ranked fourth in efficacy in AA with 93.0%, 87.7% in AS and 100% in SS. Efficacy is not significantly different (P>0.05) in these HB - genotypes. The adverse effects of these drugs were found to be mild to moderate as well as reversible immediately after drug withdrawal. Also these antimalarials under investigation were well tolerated when administered to these groups of patients.

Keywords: *Antimalarials, genotypes, malaria*

INTRODUCTION

There are few studies on efficacy and tolerability of antimalarials in malarial patients of different haemoglobin genotypes: AA, AS and SS. Malaria is a serious global health challenge. It continues to be one of the most important and devastating infective diseases in developing areas of the world[1]. Malaria is a disease of global importance and remains an overwhelming global problem and accounts for up to 500 million febrile illnesses and millions of deaths annually[2]. Malaria is a serious blood dependent disease caused by *plasmodium falciparum*. During the acute phase of *P. falciparum* malaria, destruction of parasitized and healthy erythrocytes, release of parasites and erythrocyte materials into the circulation and secondary host reaction

occur[3]. In the malaria endemic areas, the susceptibility of HB-S gene carriers to malaria parasites appears to be less than in Hb-A gene carriers[3]. *P. Falciparum* malaria attack was explored and data showed that paradoxically sickle cell trait is less hospitable to *P. falciparum* than AA gene which has more malaria parasites. It has also been reported that in all areas that have a past or present history of malaria endemicity, the HB SS gene frequency is high while the non-malaria regions have a much lower frequency[4]. There are many antimalaria drugs used in treating patients with malaria. These antimalaria drugs include chloroquine, quinine dihydrochloride, sulfadoxine/pyrimethamine combination, mefloquine, primaquine, amodiaquine, proguanil, halofantrine, lumen fantrine, chlorproguanil, dapsone, artesunate, artemeter etc[5]. These antimalarials, their tolerability and efficacy have not been documented with respect to different haemoglobin genotypes: AA, AS and SS and because there are differences in genetic constitution of different individuals with respect to their genotypes, malaria parasites may have different sensitivity to these antimalarials in different genotypes. The aim of this present study is to compare the efficacy and tolerability profile of four antimalarials, sulfadoxine/pyrimethamine combination, quinine dihydrochloride, halofantrine and artesunate in malarial patients of different genotypes.

MATERIALS AND METHODS

Patients: The studies were carried out at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. Malaria Patients attending General Out Patient Department (GOPD) of the hospital were used. A total of 480 Nigerian febrile patients were evaluated between March, 2008 and October, 2009. Males 260 (54%) and females 220 (46%). Patients with microscopically diagnosed *P Falciparum* malaria were considered for entry if they had parasitologic evidence for uncomplicated malaria[6]. Patients who fulfilled the inclusion criteria were randomly selected during their presentation and sequentially entered into groups.

Ethical committee approval of Nnamdi Azikiwe University Teaching Hospital was obtained for the study with human subjects. Also, written and oral consent was obtained from patients or their relatives.

Inclusion Criteria

Nigerians alone: Only those who have been in Nigeria for more than 6 months were included.

Sex:	Males and Females
Age:	6 years to 30 years
Genotypes:	HBAA, HBAS, HBSS
Malarial patients:	Uncomplicated malaria
High fever temperature:	38°-40° Headache

Exclusion Criteria

Non-Nigerians, complicated malaria, recent treatment with antimalarials over the previous 2 weeks, tonsillitis and fever caused by bacteria or viral infections were excluded. Nigerians who have not been within this study area or not 6 months or those who did not fall within the age bracket of 6 to 30 years also were not included.

Clinical Procedures

A full clinical examination was undertaken by a general practitioner on the day of presentation. Body temperature, body weight, pulse rate, respiration rate and blood pressure were measured. The patients were evaluated for the evalution of the signs and symptoms and any new events elicited during treatment daily for 7 days were recorded. Patients who were outpatients were monitored for compliance by

visiting them at homes.

Laboratory Procedures

Blood samples were collected from each patient for malaria parasite detection. The left thumb of each patient was pierced with sterile lancet after cleaning the area with cotton wool soaked in methylated spirit. The cleaned part was allowed to stay for few seconds for spirit to dry so that the red blood cells would not be distorted by toxic methylated spirit. The subsequent drop in each patient was collected on a grease free clean slide and smears were made. The species differentiation was obtained from thin smears. A parasite count was obtained using thick blood films counted as the number of parasites per cell that is percentage parasitaemia, parasite clearances was determined using thick blood film on the day of presentation, then followed up, 12 hourly to day 4 and for 7 days. The thick films were considered negative if no parasites were seen in 100 oil immersion fields on thick smears. Giemsa staining method was used. The patients were also screened any of genotypes AA, AS, and SS as they came for several days and then grouped. 350 (73%) patients were in genotype group AA, 102 (21%) patients were in genotype AS and 28 (5.8%) patients were in genotype group SS. They had uncomplicated P. falciparum malaria.

Protocol

350 patients - males (180) and females (170) of HB AA group were randomly divided into four groups as:

1.	Group A	84 patients
2.	Group B	86 patients
3.	Group C	90 patients
4.	Group D	90 patients

Again, 102 patients (50) males and (52) females of HB AS group were randomly divided into four groups as:

1.	Group A	25 patients
2.	Group B	25 patients
3.	Group C	26 patients
4.	Group D	26 patients

Lastly, 28 patients (13) males and (15) females and HBSS group were randomly divided into four groups as:

1.	Group A	6 patients
2.	Group B	7 patients
3.	Group C	7 patients
4.	Group D	8 patients

Drug Administration

Patients of HBAA group that were randomly divided into four groups received the following therapeutic regimes:

1. Group A, 84 patients - each was given 3 tablets of sulfadoxine/pyrimethamine 500mg/25mg orally with a full glass of water on the first day (Hans-Elembeke, Germany source of the drug).
2. Group B, 86 patients - each was given orally with water 600mg tablets of quinine dihydrochloride or 8 mg/kg body weight daily for 6 days (Roch Nig. Ltd, source of the drug).
3. Group C, 90 patients - each was given orally with water 160mg tablets of halofantrine on the first day (start) and 50mg every 6 hourly for 3 days (Han-Elembeke, Germany, source of the drug).
4. Group D, 90 patients - each was given orally with water 100mg tablets of artesunate on the first day (start) and 50mg twice daily for 5days (Kumming, China, source of the drug).

All drugs were obtained from reputable retail pharmaceutical outfits in Nigeria.

The same regimens were applied to the genotypes HB AS groups and HB SS groups. All drugs were administrated by a trained nurse with clean water.

Blood samples were collected from each patient in each group on the day of

presentation and then every 12 hours on follow-up to day 4 and daily for 7 days for determination of parasitaemia reduction levels using Giemsa staining method as mentioned above.

Evaluation Criteria for Treatment Efficacy
Efficacy Assessment
The following laboratory and clinical end points were used to measure response to treatment objectively: fever clearance (the initial body temperature before the commencement of therapy until *body* temperature decreased to 37^{oc} and remained so at least 2 days), percentage cure rate (the percentage of patients who had recovery with complete initial disappearance of parasitaemia within 7 days), laboratory *measures* include parasite clearance (the initial parasite load before the initiation of therapy until the first negative blood film that remained negative for 2 days), and percentage failure rate (the percentage of patients who had initial treatment with antimalaria but their parasitaemia remained after 7 days period).

STATISTICAL ANALYSIS OF DATA
The comparison of data within the groups was carried out by using chi-square test and one-way analysis of variance for the comparison between the groups.

Result
The efficacy and tolerability of antimalarial drugs, quinine dihydrochloride, sulfadoxine/pyrimethamine, halofantrine and artesunate were investigated and compared in 480 malaria patients of different genotypes (AA, AS, and SS). The fever clearance/temperature reduction was recorded among the antimalarials used and the percentage clearances of parasitaemia after treatment are also shown on the tables 1-3.

Artesunate has the greatest efficacy of 98.9% and the lowest failure rate of 1.1% and followed by halofantrine with 97.8% and the 2.2% failure rate. Sulfadoxine/phrimethamine has efficacies of 95.2% with 4.8% failure rate and quinine dihydrochloride has 93.0% and failure rate of 7.0% in AA patients. Their efficacy in patients with HB-genotype AA are not significantly different (P>0.05). in patients with HB-genotype AS; artesunate has the highest efficacy of 93,3^% with failure rate of 6.7% followed by halofantrine with efficacy of 92.0% and failure rate of 8,09; sulfadoxine/pyrimethamine showed cure rate of 84.0% with failure rate of 16.0% and quinine dihydrochloride has cure rate of 87.7% and failure rate of 12.3%. The cure rates of these drugs are not significantly different when applied to patients with HB-genotype AS (P>0.05). In patients with HB-genotype SS, artesunate, sulfadoxine/pyrimethamine and quinine dihydrochloride have equal cure rate of 100% while halofantrine has cure rate of 85.7% with 14.3% failure rate. These drugs are not

Table 1: Age, *weight*, percentage parasitaemis, drugs and percentage cure and failure rates in patients with genotype AA (350)

Drugs	Sulfadoxine /Pyrimethamine	Quinine	Halofantrine	Artesunate
No. of patients	84	86	90	90
No. of males	46 (54.8%)	46 (53.5%)	52(57.8%)	46(51.1%)
No. of females	38 (45.5%)	40 (46.5%)	38(42.2%)	44(48.9%)
Ave. age	- -	20 ± 3.2 yrs	18 ±4.8 yrs	21 ±4.6 yrs
Ave. weight	44 ±1.8 kg	46 ±1.2 kg	39 ± 1.4kg	43 ± 1.5 kg
Initial temp. (fever	40.2 ^{oc}	39.8^{oc}	40.0^{oc}	40. 0^{oc}
Pre-treatment Parasitaemia	27.8 ± 7%	2.7.5 ± 6%	27 ± 8%	27.7 ± 6%
Reduction in fever	37. 1^{oc}	37.6^{oc}	36.8 ^{oc}	36.7^{oc}
No. of cure rate	80 (95.2%)	80 (93%)	88 (97.8%)	89 (98.9%)
No. of failure rate	44 (4.8%)	6 (7%)	2 (2.2%)	1. (1.1%)
Parasitaemia on day 7 (failure rate)	14.8%	21.5%	7.2%	4.0%

- (+ SEM)

Table 1 shows the efficacy of different antimalarials used in 350 malaria patients of genotype AA. Artesunate has the greatest efficacy of 98.9% followed by halofantrine with 97.2% and sulfadoxine/pyrimethamine with 95.2% and quinine dihydrochloride with 93.0% as the least drug. The cure rates of these drugs are not significantly different when applied to patients with HB-genotype AA (P>0.05).

Table 2: Efficacy of different antimalarials used in 102 malaria patients with HB-genotype AS

Drugs	Sulfadoxine /Pyrimethamine	Quinine	Halofantrine	Artesunate
No. of patients	25	26	25	26
No. of males	15(61.5%)	16(61.5%)	13(52%)	12(46%)
No. of females	10(38.5%)	12(52%)	14(53.9%)	14 (53.9%)
Ave. age	19.2 ± 1.2 yrs	19.4 ±3 yrs	19.8 ±6 yrs	20.2 + 4yrs
Ave. weight	10 ±1.2 kg	12 ± 3 kg	12 ± 3 kg	12 ± 5yrs
Initial temp. (fever	40.2^{oc}	39.8^{oc}	40.0^{oc}	40.0^{oc}
Pre-treatment (Parasitaemia)	22 ± 6%	22 ± 5%	21 ± 6%	22 ± 7%
Temp. reduction (fever)	37.2^{oc}	36.8^{oc}	36.4^{oc}	36.2^{oc}
No. of cure rate	21 (84%)	23 (87.7%)	23 (93%)	24 (93.3%)
No. of failure rate	4(16%)	3(12.3%)	2 (8%)	2 (6.7%)
Parasitaemia on day 7 (failure rate group)	11.4%	8%	5%	3.6%

- (± SEM)

Table 2 shows the efficacy of different antimalarials used in 102 malaria patients with HB-genotype AS. Artesunate has the highest efficacy of 93.3% followed by halofantrine 92% and quinine dihydrochloride 87.7% and quinine dihydrochloride with 84.0%. The cure rate or efficacy of these drugs is not significantly different from each other which applied to patients with HB- genotype AS (p>0.05).

Table 3: Efficacy of sulfadoxine/pyrimethamine, quinine and artesunate used in 28 malaria patients of HB-genotypes SS (sickle-cell disease)

Drugs	Sulfadoxine /pyrimethamine	Quinine	Halofantrine	Artesunate
No. of patients	6	7	7	8
No. of males	4 (66.7%)	4(57.1%)	3 (42.9%)	3 (37.5%)
No. of females	2 (33.3%)	3 (42.9%)	4(57.1%)	5 (62.5%)
Ave. age	-	19.3 ± 1.2yrs	19.6 ± 5yrs	19.4 ±4.2yrs
Ave. weight	34. 1 ± 2 kg	25 ± 5.5 kg	28 ± 2.2 kg	32 ± 4 kg
Initial temp, (fever	39.8^{oc}	40.1^{oc}	39.7^{oc}	40.0^{oc}
Pre-treatment (Parasitaemia)	20.8 ± 6%	20.2 ± 5%	20.3 ± 6%	20.8 ± 5%
Reduction Temp, in (fever	37.1^{oc}	36.8^{oc}	36.5^{oc}	36.8^{oc}
No. of cure rate	6(100%)	7 (100%)	6(85.7%)	8 (100%)
No. of failure rate	Nil (0%)	Nil (0%)	1 (85.7%)	Nil (0%)
Parasitaemia on day 7 (failure rate group)	Nil	Nil	18.2%	Nil

- (+ SEM)

Table 3 shows the efficacy of sulfadoxine/pyrimethamine, quinine and artesunate used in 28 malarial patients of HB-genotype SS (sickle cell disease). Artesunate, sulfadoxine/pyrimethamine and quinine have equal cure rates of 100% while halofantrine has 85.7%.

Discussion

The efficacy and tolerability of four antimalarials, quinine-dihydrochloride, sulfadoxine/pyrimethamine, halofantrine and artesunate were studied in malarial patients of different HB-genotypes AA, AS and SS. Artesunate has the highest efficacy and lowest failure rate and well tolerated as mild adverse effects were only reported. Halofantrine ranked second in efficacy and failure rate and sulfadoxine/pyrimethamine ranked third in efficacy and failure rate while quinine dihydroride ranked last in both efficacy and failure rate. In these drugs studied, their efficacies are not significantly different in AA HB-genotype patients treated ($P > 0.05$). This is due to the fact that the first two drugs especially artesunate; resistance has not been eleveloped because it has not been used extensively, unlike quinine dihydrochoride and sulfadoxine/pyrimethamine[7]. Resistance has not been reported in artesunate and mino resistance halofantrine. Artesunate is the drug of choice in the treatment of uncomplicated *Falciparum* malaria which compares favourably among other antimalarials studies. Mild to moderate side effects were recorded with these drugs which include nausea, vomiting weakness and generalized body pain which stopped once the treatment terminated. Artesunate ranked best in terms of efficacy and tolerability among the other antimalarials studied and, therefore, should be recommended, for the treatment of uncomplicated malaria in both AS and SS HB-genotypes. Although sickle-cell trait patients do not habour much malaria parasites as the parasitized sickled-cell is removed preferentially from circulation and, therefore, parasites could not complete their life cycle[5] and[6] had shown that the advanced stages of development of *P. falciparum* were not seen in the peripheral blood. [9]reported that under low oxygen tension, the cells would be giving up oxygen to both the tissue and the parasites, thus the erythrocytes of sickle-cell carriers with *falciparum* malaria would have a tendency to sickle in all the organs of the body except the lungs and be more easily phagocytized with the consequent interruption of the life cycle. These confer relative advantage to those patients with sickle-cell disease and might be the reason why artesunate, sulfadoxine/pyrimethamine and quinine dihydrochloride recorded 100% free parasitaemia in patients with HB-genotype SS.

CONCLUSION

We have been able to establish that these antimalarial drugs exhibited good efficacy and tolerability in the treatment of *P. falciparum* malaria especially in patients with HB-genotype SS group with uncomplicated *falciparum* attack.

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