RELATIVE EFFICACY OF ARTESUNATE OR AMODIAQUINE COMBINATION THERAPIES WITH SULPHODOXINE-PYRAMETAMINE IN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN BORNO STATE, NIGERIA

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Abstract*- The study was carried out in the malaria holo-endemic settlements around Lake-Alau, Borno State, Nigeria between July to December, 2011. The aim was to compare the efficacy of Artesunate + Sulphadoxine-Pyrimethamine (AT+SP) and Amodiaquine + Sulphadoxine-Pyrimethamine (AQ+SP) in the treatment of uncomplicated Plasmodium falciparum malaria in children. A total of 313 children (6-59 months) were screened, using standard protocols for therapeutic efficacy studies on fever and parasitaemia and the results were evaluated using regression analysis. The results obtained indicated that 79.6% of the children were cleared of fever by AT+SP as against 78.3% by AQ+SP on the first 24 hours after treatment and achieved a daily rate of temperature clearance of 0.35 0C and 0.29 0C and daily reduction in febrile children of 22.04% and 21.99% with the respective drugs, within the early follow-up days (0 - 3). The remaining febrile were relieved by day 28 and temperatures normalized to 36.0 0C and 36.7 0C with the respective drugs. The results further revealed that both AT+SP and AQ+SP cleared the bulk of the parasites from 21,738 to 30/µl and 19,953 to 66/µl, respectively within the first three days of follow-up; with proportionate success rates of 85.1, 95.2, 99.0, 99.5, 99.9 and 99.9% and 78.7, 93.4, 98.4, 99.4, 99.6 and 99.7% on days 1, 2, 3, 7, 14 and 28, respectively. These translates to mean parasite clearance time of 28.45 hours and 29.92 hours, with concomitant mean fever clearance time of 29.36 hours and 31.65 hours in AT+SP and AQ+SP, respectively. Regression analysis further revealed that each µl of the blood parasites cleared gave a temperature relief of 0.2071 0C and 0.1714 0C with the respective drugs. These results clearly put the efficacy of AT+SP in fever clearance ahead of AQ+SP in both early and late follow-up days. Based on rapid parasite and fever clearance accompanied by faster PCV (%) recovery and higher frequency of adequate clinical and parasitological response (ACPR) of AT + SP (91.4%) it could be adjudged to be superior to AQ+ SP (84.5%). Therefore, the Artesunate based combination therapy (AT + SP) is recommended for control of P. falciparum malaria in children.

***IndexTerms*—Relative-efficacy-malaria-Lake-alau**

I. INTRODUCTION

Malaria infects 59 million people in Nigeria annually out of which 24 million are children (WHO, 2003). Children less than 5 years of age suffer 2 to 4 episodes of malaria annually resulting in 30% childhood mortality, 25% under-5 mortality and 11% maternal mortality (NMCP, 1996). Resistance to mono-therapies has been documented for over a decade in Northern Nigeria (Molta, 1995) and more recently as a major setback to the "Roll Back Malaria" programme in Nigeria (FMOH, 2005).

Mono-therapies have become ineffective in the treatment of malaria while the parasitological failure rate exceeds 38.7% (Umotong*et al.*, 1991; Molta, 1995; Adagu*et al.*, 1995). In response to increasing levels of resistance to anti-malarial drugs, the World Health Organization strongly recommends that all countries experiencing resistance to conventional mono-therapies should revert to combination therapies. Combination therapy of rapid resolution drugs, Artesunate and Amodiaquine with longer acting drugs (Sulphadoxine-Pyrimethamine) offers the best alternative for effective prevention of resistance due to plasmodium species infections.

II. MATERIALS AND METHODS

Study Site

The study was conducted at Lake-Alau, a holoendemic area for malaria with transmission throughout the year. The peri-urban outpatient primary Health Center at Lake-Alau, Kayamla village in Konduga Local Government Area of Borno State, Nigeria caters for 63 village settlements with a combined population of 114,224 heads (National Population Commission, 1991).

Recruitment Procedure

Ethical clearance was sought through Borno State Ministry of Health from Konduga Local Government Council Authority and the letters of consent was served to the respective village and the district heads through the Konduga Local Government Authority. Standard World Health Organization (2003) recruitment procedure for evaluating anti-malarial drugs in children of age (6 - 59 months) was adopted. These includes, clinically apparent uncomplicated malaria, mono-infection and absence of severe malnutrition and measured axillary temperature (≥ 37.5 °C), parasite density (2,000 - 200,000 /µl) and packed cell volume (> 15%) was followed.

Randomization and treatment allocation

Patients were assigned study numbers at enrolment and were then randomly assigned to either AT + SP or AQ +SP treatment groups. A total of 500 children suffering from malaria were screened, out of which 400 children were selected and assigned randomly using a table of random numbers into two groups of 200 children each. The study ended up with 313 children that finally satisfied the inclusion criteria (WHO, 2003) with 161 and 152 patients in the AQ + SP and AT + SP treatment groups, respectively.

Experimental Procedure

Physical parameters

The age of each child was determined by interviews with the parents or from birth certificates and the body weight (kg) of each child was measured on a bathroom balance at enrolment. Body temperatures were equally determined using the digitalized electronic clinical thermometer in degrees centigrade (^{0}C) on days 1, 2, 3, 4, 7, 14 and 28 and children with body temperature ≥ 37.5 °C (fever) were enrolled for the study.

Haematological study

Blood for the assessment parasite density was sampled on days 0, 1, 2, 3, 4, 7, 14 and 28 by finger pricking, while the venipuncture sample was used for the assessment of Packed Cell Volume on days 0, 3, 7, 14 and 28 as described by WHO (1996; WHO,2006).

Parasite density count (per µl): WHO (1991) modified methods by Gilles (1993) and Cheesbrough (1998) were employed for the fixation, staining of blood films and morphological identification. Two slides of thick and thin blood films were considered for each patient. The first thick blood film was stained with 10% Giemsa (pH 7.1 – 7.2) for 10 - 15 minutes for rapid parasite appraisal for patient inclusion. The second slide was stained for 30 - 45 minutes with 3% Giemsa for the assessment of parasite density using Research Microscope (®) (x100). Asexual stages of the parasites were counted within 200 leukocytes field; in an event of parasite count below 10, counting was extended to 500 leucocytes. The parasite density was computed as the number of asexual parasites per ml of blood by assuming a mean normal leukocyte count of 8000/µl of blood.

Packed cell volume (PCV %): The EDTA anti-coagulated blood sample was centrifuged (Hawksley®) at 12000 (rpm) for 5 minutes and the PCV value was then read-off a hand held microhaematocrit reader and the values were expressed as percentages (Cheesbrough, 1998).

Assessment of Drug Efficacy

Drug efficacy was distinctly classified into four treatment outcomes as outlined by WHO (2003) thus:

a. Early treatment failure (ETF) for higher parasitaemia on day 2 than first day, parasitaemia on day 3 with axillary temperature ≥37.5°C and day 3 parasitaemia>25% of enrolment.
b. Late clinical failure (LCF) for auxiliary temperature ≥37.5°C on days 4 - 28 in the presence of parasitaemia.
c. Late parasitological failure (LPF) for presence of parasitaemia on days 7 - 28 and auxiliary temperature of < 37.5°C.
d. Adequate clinical and parasitological response (ACPR) for absence of parasitaemia on day 14 or 28, irrespective of temperature.

3.6 Data management and analysis

Data collected were subjected to descriptive statistics using the analytical software Staistix Version 8.0 (Microsoft, 2003). Charts were drawn using Microsoft Excel (2003) and the regression equations.

III. RESULTS

Baseline Characteristics of Patients at Enrolment

Table 1 depicts that the study cut across gender males (47.6%) and females (52.4%) of diverse age (43.3±14.4 months) and body weight (18.4±8.5 kg). Body weight and age were highly ($P<0.01$) correlated ($r = 0.4258$, $df = 311$). The result equally shows highly ($P<0.01$) variable parasite count (20820±5277.7⁄µl) and PCV (27.0±5.0%) among patients at enrolment, however, their body temperature clustered around the mean (38.15±0.47 ^{0}C).

Effects of the Drugs on the Parasite

Fig. 1 indicates rapid parasite clearance during early follow-up (days 0-3) and a more stable clearance during late follow-up (days 7-28 after drug administration) for both drugs. However, all regression parameters indicate faster parasite clearance in AT+SP than AQ+SP during both follow-up phases. The high coefficient of determination (r^2) for AQ+SP (76.06 - 97.24%) in both early and late clearance phases indicates that the drug is more time inclined than AT+SP (71.40 -77.03%); these suggest that parasite clearance was faster in AT+SP compared to AQ+SP during both phases. The intercept serves as measure of initial parasite density, which suggests relatively higher parasitaemia in children to which AT+SP was administered (23249⁄µl) than in those that eventually received AQ+SP treatment (21901⁄µl) at enrolment (Fig. 1a). Therefore, the result revealed rapid resolution of parasitaemia in Artesunate than Amodiaquine, in that in spite of the higher pre-treatment mean parasitaemia in AT+SP patients, the trend was reversed during the late phase in which lower parasite density (129.33⁄µl) was observed than 135.67⁄µl of blood in the AQ+SP (Fig. 1a). The regression coefficient serves as quantitative assay of the rate of parasite clearance, thus in the early phase, the daily rate of clearance was 6678.1⁄µl in AT+SP, equivalent to 30.99% compared to 6177.5⁄µl (30.71%) in AQ+SP (Fig. 1a, b). AT+SP cleared

37.0/µl of the residual parasites daily compared to 24.0/µl by AQ+SP, equivalent to 0.18% and 0.15% parasite clearance, respectively. In general therefore, the result also suggests faster parasite clearance time (PCT) in AT+SP than AQ+SP.

Effects of the Drugs on Fever

Fig. 2 similarly revealed faster fever (temperature) clearance in AT+SP than AQ+SP as deduced from the coefficient of determination (r^2) values of 65.6% and 76.64%, respectively, during the early follow-up (0-4 days). Mean auxillary temperature at enrolment was higher in children treated with AT+SP (38.15^{0}C) than AQ+SP (37.97 ^{0}C) but the trend was reversed in the later phase, implying faster fever clearance for AT+SP within the four initial days (Fig. 2a). However, during the late phase, the r^2-values for the two drugs were at par. Temperature cleared at the rate of 0.35 ^{0}C for AT+SP as against 0.29 ^{0}C for AQ+SP in the first four days, 0.50 ^{0}C and 0.05 ^{0}C during later follow-up (7 - 28 days), respectively. Fig. 2b shows that the percentage of febrile children dropped daily by 22.04% in AT+SP patients compared to 21.99% in AQ+SP patients in the early phase of treatment (0 - 4 days). These results clearly put the efficacy of AT+SP in fever clearance ahead of AQ+SP in both early and late follow-up days.

Effects of the Drugs on Packed Cell Volume (PCV)

Fig. 3 shows that parasites affected PCV by 98.41% in AT+SP patients and 93.51% in AQ+SP patients. Despite a much higher PCV level observed in children treated with AT+SP (25.69%) than AQ+SP (25.87%) at enrolment, the speed of recovery was relatively faster in AT+SP (0.0214%) than AQ+SP (0.0180%).

Drug Therapeutic Efficacy

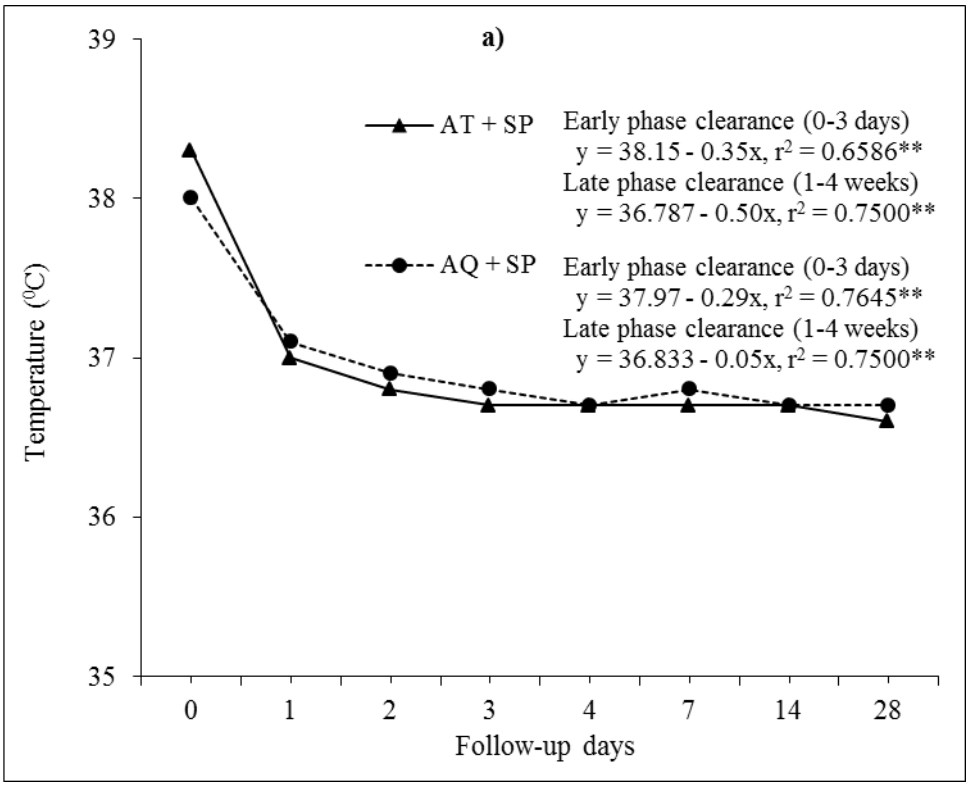
The result in Table 2 did not show cases of early treatment failure (ETF) in either of the drugs, as the case was also for the late clinical failure (LCF) in AT+SP, however, there was slight (0.06%) indication of late clinical failure for AQ+SP. The cases of late parasitological failure (LPF) in AT+SP (8.6%) was relatively lower compared to 14.9% in AQ+SP. Total treatment failure (TTF) was relatively higher for AQ+SP (14.96%) than AT+SP (8.6%). The result for adequate clinical and parasitological response (ACPR) was also in favour of AT+SP with 91.4% compared to 84.5% for AQ+SP.

Table 1 Baseline characteristics of patients at enrollment

Parameter	Baseline data
1. No. enrolled (N)	313
2. Gender (No. /%)	
Male	149 (47.6)
Female	164 (52.4)
3. Age (months)	
Mean ± SD	43.3 ± 14.4
Range	8 - 59
4. Body weight (kg)	
Mean ± SD	18.4 ± 8.5
Range	3.0 - 50.0
5. Temperature (^{0}C)	
Mean ± SD	38.15 ± 0.47
Range	37.0 - 39.6
6. Haematological	
a. Parasite count (µl)	
Mean ± SD	20820 ± 5277.7
Range	2304 - 36800
b. Haematocrit (PCV %)	
Mean ± SD	27.0 ± 0.5
Range	14.0 – 48.0

Table II. Therapeutic efficacy of Artesunate + Sulphadoxine-pyrimethamine versus Amodiaquine + Sulphadoxine-pyrimethamine in *P. falcifarum* infected children

Treatment outcome	Number (%) of patients	
	AT + SP (n = 152)	AQ + SP (n = 161)
Early treatment failure (ETF)	0 (0)	0 (0)
Late clinical failure (LCF)	0 (0)	1 (0.6)
Late parasitological failure (LPF)	13 (8.6)	24 (14.9)
Adequate clinical and parasitological response (ACPR)	139 (91.4)	136 (84.5)



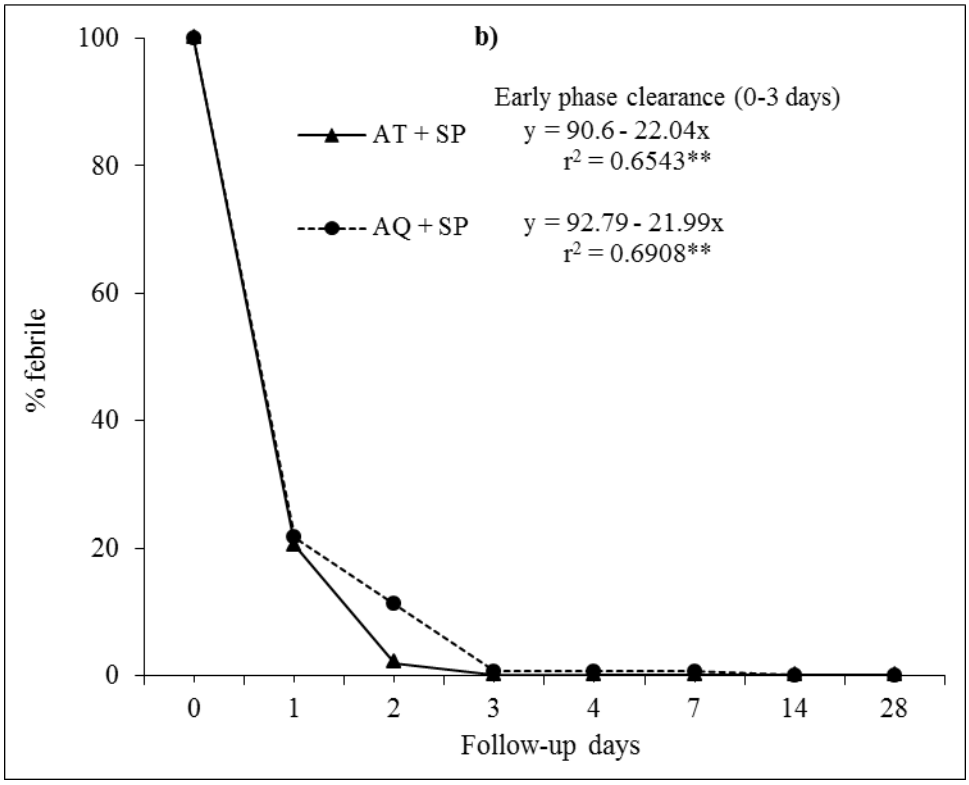
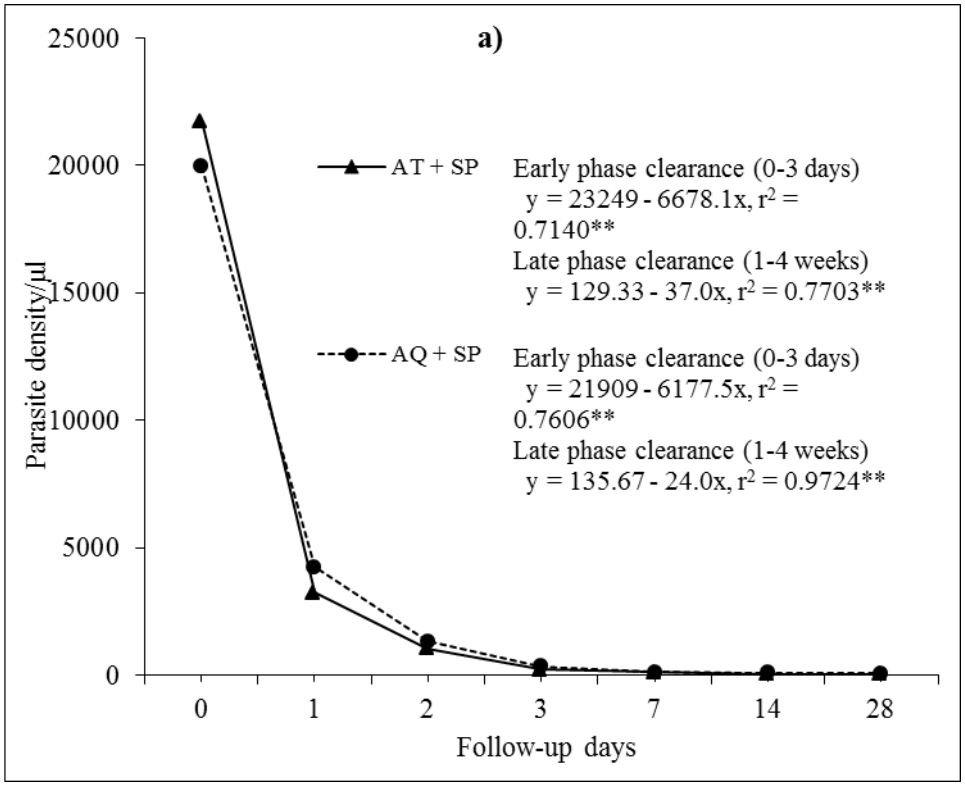


Fig. 1: Rate of a) temperature and b) fever clearance for each of the administered drug (AT+SP and AQ+SP) among children with *P. falcifarum*



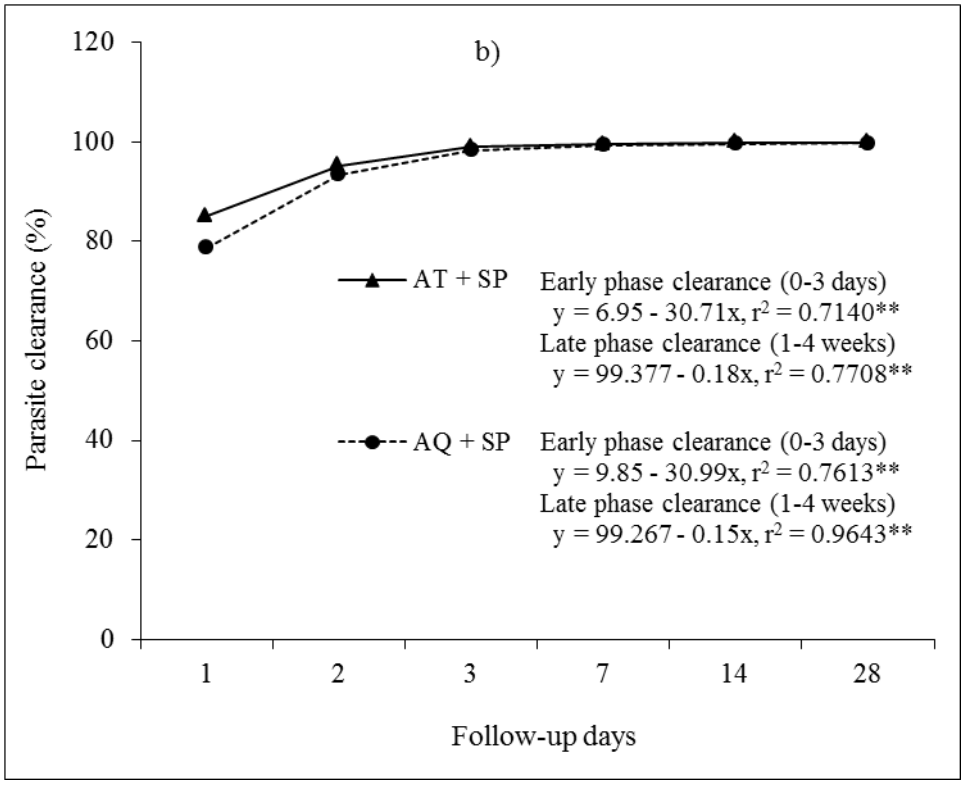
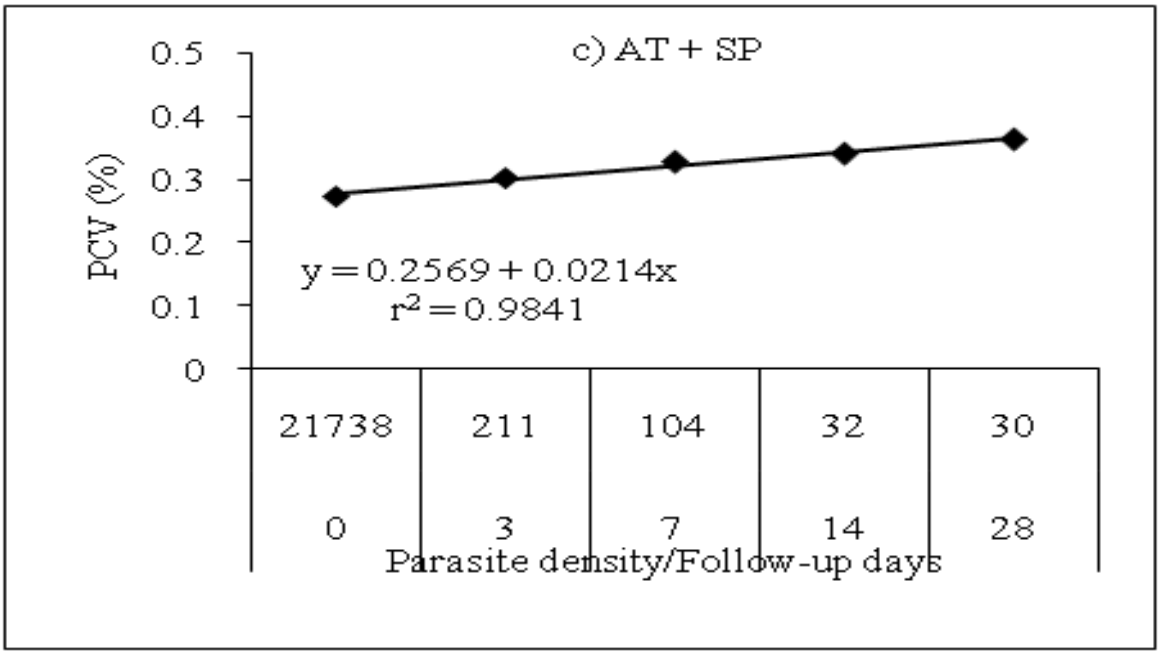


Fig. 2: Parasitamia and rate of clearance a) parasite density b) percentage parasite clearance in each of the administered drug (AT+SP and AQ+SP) among children with *P. falcifarum*



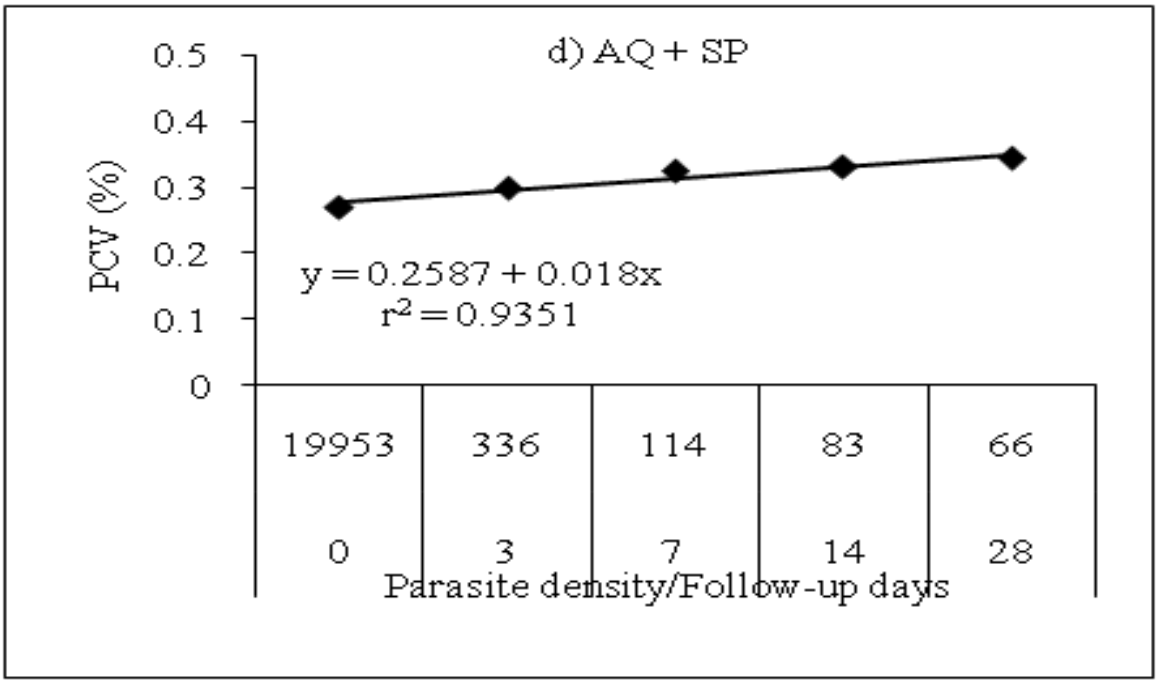


Fig. 3: Effects of parasitamia on PCV in *P. falcifarum* infected children treated with a) AT+SP and b) AQ+SP during follow-up period

IV. DISCUSSION

Parasite biomass and temperature at different follow-up periods serves as a strong indicator of drug performance. The present result revealed rapid fever clearance and parasitological cure with both drugs, however, the high parasite density at enrolment coupled with faster clearance rate by AT+SP during both early and late phases suggest its higher efficacy over AQ+SP. Previous reports also rated the Artesunate arm as the most rapid of all available antimalarial drugs in the initial reduction of parasitaemia (Nosten *et al.*, 2003; Bukirwa *et al.*, 2006; Nahum *et al.*, 2007). The faster speed of Artesunate arm in parasite clearance could be attributed to its active and potent effects on the early ring stages of plasmodium and early-stage gametocytes shortly after drug administration (Reichmann *et al.*, 1989). Pharmokinetic studies revealed that Artesunate is rapidly and quantitatively converted *in vivo* to the potent active metabolite dihydroartemisinin, which also rapidly and substantially reduces the parasite biomass followed by rapid resolution of clinical symptoms and reduction of gametocyte carriage (Bloland *et al.*, 2003). However, in spite of the sharp parasite clearance rate, both Artesunate and Amodiaquine arms have a very short half-life and are rapidly eliminated (Nosten*et al.*, 2003), which suggests that the combination therapy relied on the Sulphadoxine-Pyrimethermine component at the later phase. Reports suggest that Sulphadoxine-Pyrimethamine further improved parasitological clearance (Gasasira *et al.*, 2003; Zongo*et al.*, 2007; Adjei*et al.*, 2008), but Watkins and Mosobo (1993) pointed out that antifolate arm exert little or no effect on the parasites during the first 24 hours of their life cycle, while Hyde (1990) reported that even in the late phase it affects only the actively dividing forms of *Plasmodium* species (Schizonts).

The present study did not register early treatment failure (ETF) with either of the drugs or late clinical failure (LCF) in AT+SP. Reported cases of ETF in respect of the drugs are equally low ranging 0-1.7% (Zongo *et al.*, 2005; Blair *et al.*, 2006; Bonnet *et al.*, 2007; Mukhtar *et al.*, 2007; Ménard *et al.*, 2007; Nahum *et al.*, 2007; Sowunmi *et al.*, 2007). Similarly, reported LCF did not exceed 5.0% (Gasasira *et al.*, 2003; Zongo *et al.*, 2005; Blair *et al.*, 2006; Faye *et al.*, 2007; Nahum *et al.*, 2007; Ménard *et al.*, 2007; Mukhtar *et al.*, 2007; Sowunmi*et al.*, 2007). The present result relatively revealed lower frequency of late parasitological failure (LPF) in AT+SP (8.6%) than AQ+SP (14.9%). Values obtained in the present study fall within the reported ranges of 0 - 10.4% for AT + SP (Nahum *et al.*, 2007, Mukhtar *et al.*, 2007) and 0 - 16% for AQ + SP (Talisuna *et al.*, 2004; Zongo *et al.*, 2005; Molta *et al.*, 2006; Tagbor*et al.*, 2006; Faye *et al.*, 2007; Ménard*et al.*, 2007; Sowunmi *et al.*, 2007). TTF was found to be relatively higher for AQ+SP (14.96%) than AT+SP (8.6%), the values in other reports were lower with 1.0 - 3.4% occurrence for AT + SP (Blair *et al.*, 2006, Bonnet *et al.*, 2007) and 2.2% in AQ + SP (Blair *et al.*, 2006). The result on adequate clinical and parasitological response (ACPR) was in favour of AT+SP with 91.4% compared to 84.5% for AQ+SP. Most reports indicated 91.2 - 99.1% ACPR for AT + SP (Dorsey *et al.*, 2002, Hamour, 2005, van den Broek *et al.*, 2005, Blair *et al.*, 2006, Bonnet *et al.*, 2007, Nahum *et al.*,

2007, Mukhtar *et al.,* 2007) and 95.8 - 99.0% ACPR for AQ + SP (Dorsey *et al.,* 2002, Zongo *et al.,* 2005, Molta *et al.,* 2006, Faye *et al.,* 2007, Ménard *et al.,* 2007, Bell *et al.,* 2008). Although the present result falls within most reported values for AT + SP, it is in departure with the range for AQ + SP, showing lower success. Other reports also revealed lower efficacy with AT + SP of 70.0 – 86.0% (Sowunmi *et al.,* 2007, Bell *et al.,* 2008).

In conclusion, all the drugs were highly efficacious and safe, against *Plasmodium falciparum* and had very low frequencies of total treatment failures (TTF). Therefore, based on the higher efficacy (ACPR) of AT + SP it could be adjudged superior to AQ + SP. Literature generally show that Artesunate have a broad stage specificity and can be used to treat severe as well as uncomplicated malaria and exerts severe effects against gametocytes thus it has the potential to reduce transmission (Price *et al.,* 1996; White, 2004; Baired, 2005). The high parasite density at enrolment coupled with faster clearance rate by AT+SP during both early and late phases in this study suggest the higher efficacy of AT+SP over AQ+SP.

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