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Full Length Research Paper

Haematological changes and recovery associated with treated and untreated *Plasmodium falciparum* infection in children in the Mount Cameroon Region

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Pre-hospital antimalarial treatment of febrile children remains a significant common practice among individuals in the Mount Cameroon region. To evaluate the effect of routinely administered monotherapy sulphadoxine pyrimethamine (SP), treatment using amodiaquine artesunate (AQAS) combination therapy and untreated malaria on haematological and parasitological parameters, 332 malaria positive subjects were assigned to three groups comprising 138 children treated with AQAS, 43 treated with SP and 151 untreated. The changes and recovery in red cell indices, white blood cell and differential and platelets counts were compared. The highest haematological recovery (39.1%) occurred in the AQAS treatment group. The majority (94%) of the untreated cases never achieved haematological recovery even though there was spontaneous clearance of parasites in some cases. Haematological insult was greatest in untreated children followed by those treated with SP, the 1.1 - 3 year age group whether or not they received treatment and in those with high parasitaemia. Delayed parasite clearance observed in the untreated and SP treatment group may be responsible for the occurrence of haematological insult. Treatment type and parasitological cure was associated with haematological recovery. Prompt use of effective arthemisinin combination therapy reduced the burden of malaria, hence the greater clinical and haematological benefits observed in our study.

Key words: Malaria, children, anaemia, haemoglobin, treatment, haematological recovery.

INTRODUCTION

Malaria remains an important health problem and anaemia is a common and sometimes serious complication of *Plasmodium falciparum* infection (Nussenblatt, 2002). As intraerythrocytic parasites of the blood, the blood, *Plasmodium* expectedly induces haematological alterations. Haematological abnormalities that have been reported to consistently accompany infection with malaria include anaemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (Facer, 1994; Perrin et al., 1982).

Although haematologic changes associated with malaria infection are well recognized, specific changes may vary with the level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors and malaria immunity (Price et al., 2001). In malaria holoendemic areas children are anaemic but the exact influence of falciparum malaria on haemoglobin (Hb) concentration remains to be determined. Cross-

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Abbreviations: SP, Sulphadoxine pyrimethamine; **AQAS,** amodiaquine artesunate; **ACT,** arthemisinin combination therapy; **CQ,** chloroquine; **Hb,** haemoglobin **; WBC,** white blood cell; **Hct**, haematocrit, **RBC,** red blood cell count; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular haemoglobin; **MCHC,** mean corpuscular haemoglobin concentration.

sectional data or data from hospitalized children with severe anaemia are unable to capture the relationship between falciparum malaria and anaemia and only longitudinal data may reveal whether a low Hb level is as a result of recent high parasite density or of long standing parasitaemia (Abdalla et al., 1980; Trape et al., 1994).

Pre-hospital antimalarial treatment of febrile children remains a significant common practice among individuals in this malaria endemic region as revealed by a questionnaire survey (Nkuo-Akenji et al., 2005a). Caregivers/parents for the most part begin treatment using mostly traditional herbs or drugs of questionable quality purchased from street vendors. The commonly used antimalarials included chloroquine(CQ), sulfadoxine pyrimethamine (SP) and amodiaquine (AQ). About 48.5% of children are taken to a health facility only when other treatment(s) failed (Nkuo-Akenji et al., 2005a).

Prompt treatment of malaria infections with effective, fast-acting antimalarial drugs rapidly reduces symptomatic high density parasitaemia and clears parasites from the blood, allowing erythrocyte numbers to be restored (Ekvall et al., 2001; Bjorkman, 2002). There has been a significant increase in the resistance of the malaria parasite to common antimalarial drugs in the past 20 years. CQ resistance for example varies from 25% in the Northern Sahel savannah regions to about 87% in the forest, South of Cameroon (Mbacham et al., 2005). The useful therapeutic lifespan of SP has been threatened by the rapid emergence and spread of resistance to the drug (WHO, 2005). Artemisinin - based combination therapy (ACTs) are useful, particularly artesunate and amodiaquine (AS/AQ), because they are readily available and affordable and parasites have remained sensitive to AS/AQ in most part of Africa (Bloland et al., 2000; Gasasira et al., 2003).

The World Health Organization (WHO) protocol for the evaluation of an antimalarial drug or drug combination now includes haematological recovery as an efficacy endpoint (WHO, 1996, 2002). Although the treatment of uncomplicated *P. falciparum* malaria aims at clearing the parasites, relieve the symptoms and permit haematological recovery, data on the impact of antimalarial treatment on haematological recovery are few. First insight into the dynamics of anaemia and haematological recovery in relation to ACT for uncomplicated *P. falciparum* malaria in African children indicate that treatment using artesunate plus SP produces suboptimal frequencies of parasitological cure and haematological recovery (Obonyo et al., 2007a). Further research is needed to assess the factors related to haematological recovery and the causes of anaemia in African children in order to exploit the underlying mechanisms. Therefore, the aims of the study were firstly to evaluate the efficacy of SP a routinely used monotherapy, amodiaquine artesunate a combination therapy and the impact of untreated malaria on haematological and parasitological parameters. Secondly to determine the changes if any and recovery in red cell count and indices, WBC, differential white cell and platelets counts in the different groups.

MATERIALS AND METHODS

Study site

Muea village is a semi-rural setting in South west Cameroon, situated in the rain forest ecozone at an altitude of 562 m above sea level on the Eastern flank of the active volcanic Mount Cameroon. Muea is about 29 km from the Atlantic Ocean, has an equatorial climate with relative humidity of above 80%, a temperature range of 18 - 28°C and an annual rainfall of about 4096 mm. The rainy season characterised by frequent light showers spans from March to October. The dry season runs from November to February. Muea has a heterogeneous and multi-ethnic population of approximately 9,000 inhabitants. Of these 3500 are children < 14 years old. Of the 848 households identified in Muea, about 78.9% of the heads of households are farmers who have no steady source of income and live from hand to mouth (Sumbele, 2009).

Study population

To be included in the study, children had to be 14 years old, *P. falciparum* parasitaemia positive and sickle test negative. Children weighing < 5 kg and those with severe malaria (unable to drink or breastfeed, vomiting more than twice in the preceding 24 h before presentation, recent history of convulsions, unconscious state or unable to sit or stand and other diseases requiring hospital admission) were excluded from the study. Children were enrolled into the study if a parent or guardian signed the informed consent form. Children left the study when informed consent was withdrawn, if they moved out of the study area or died. The study was approved by the Ministry of Public Health Cameroon. The Ethical Committee of the University of Buea issued the ethical clearance document. Additional authorisation was obtained from the local health committee and the village chief.

Study design

This longitudinal study was carried out from July 2004 to November 2006 to include the rainy season (April - September) which has been reported as the peak malaria transmission period in the Mount Cameroon Region (Nkuo-Akenji et al., 2005b). The investigations carried out included clinical and laboratory evaluations. Clinical and parasitological examinations were done at enrolment followed by treatment on Day Zero (D0). Follow up investigations were conducted on D 7, 14, 21, 28 and 42. At each follow- up visit, the children were assessed clinically and fresh samples of capillary blood obtained from them by finger-prick for the determination of Hb, haematocrit (Hct) and parasitological counts. Full haematological examination consisting of measurements of Hb, Hct, red blood cell (RBC), Platelets, WBC and differential counts was done on D0 and D42. Haematological recovery was defined as an Hb concentration of at least 11.0 g/dl on D42 in a patient found anaemic on D0 (Price et al., 2001; Obonyo, 2007a).

Malaria positive patients (332) with parasitaemia of at least 2000 asexual form of *P. falciparum* were assigned to three groups namely, those treated with AQAS (138 children), those treated with SP (43 children) and the untreated (151 children). A standard dose of AQAS, 4 mg/kg body weight (bw) of Artesunate and 10 mg /kg bw of amodiaquine was given once a day for 3 days. In the SP treatment arm, a single oral dose of sulfadoxine 500 mg and pyrimethamine 25 mg/kg bw (WHO, 1996, 2006) was administered. All doses were given under the supervision of a registered medical

officer and or guardian who had been properly instructed. Whenever a child was found to have an axillary temperature of 38.0°C, paracetamol was administered as an antipyretic. Children who failed to respond adequately to the study treatment received rescue treatment with a full dose of quinine (24 -30 mg/kg bw/day for 7 days) from the medical officer and were dropped from the study. Children in the untreated group who became febrile 3 days or more into the study period were treated and dropped from the study. However, all children in the untreated group who completed the study were given a single oral dose of SP after sample collection on day 42. Children with febrile illness due to other causes were excluded. All children were screened for helminth infection and a single-dose of 500 mg of mebendazole (SmithKline Beecham) was administered to each child found positive for soil-transmitted helminth (STH).

The clinical evaluation carried out by trained medical personnel from the Muea Health Centre consisted of measurement of weight, height, body temperature and palpation of the spleen. The symptoms and duration of symptoms before presentation were recorded. Axillary and core body temperature was measured using a digital thermometer. A child with a body temperature ≥ 37.5°C. was considered febrile.

Laboratory procedures

On D0 and D42, approximately 4 – 5 ml of blood sample was collected from the children by venipuncture into 5 ml sterile disposable syringes (Cathy Yougo) and dispensed into micro-containers or vacutainers containing ethylenediaminetetraacetate (EDTA) solution. Drops of whole blood were dispensed immediately on slides to prepare blood films. A portion of the blood was used to fill (¾ full) heparinized capillary tubes (Marienfeld, Germany) for measurement of Hct. Labelled blood and stool samples were then transported on ice in a cool box to the University of Buea malaria research laboratory for further analyses.

Thick and thin blood smears prepared in the field were stained with 2% Giemsa for 30 min in the laboratory. Parasite densities were determined from thick blood smears by counting the number of asexual parasites per 200 WBCs and calculating parasites/µl with reference to the data from the full blood count. Thin blood smears were used to determine the parasite species and differential white cell counts. Hb concentrations were measured in the field using a Stanbio STAT-Site ^{R} Test Kit (STAT-site M^{Hgb} Meter, stanbio Laboratory, Texas, USA) following the manufacturer's instructions. Hct was measured using the microhaematocrit centrifugation method (Cheesbrough, 2004). Labelled capillary tubes were spun in a microhaematocrit centrifuge (Hettich, Zentrifugen, Germany) for 5 min at 10,000 rpm to obtain constant packing of the cells. The Hct values were read from the scale of the microhaematocrit reader and the results expressed as percentages. WBC, RBC and platelet counts were determined using the improved Naubauer haemacytometer as described by Cheesbrough (2004) and Dacie and Lewis (1995). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from values of Hct, Hb conc. and RBC using formulae stated by Cheesbrough (2004). Microcytosis was defined as a MCV of less than 67 fl in children under 2 years and less than 73 fl in children above 2 years (Calis et al., 2008). Plasma Ft was assayed using the LISA 300 plus system (Labo Tech Medicale) and ferritin calibrator set (Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany) prepared from human sera. The values of the calibrator set were referenced to the WHO reference material 1st IS 80/602. Normal Ft concentration range 30 – 100 ng/L. Iron deficiency **(**ID) was defined as MCHC < 320 g/l, MCV < 78 fl, plasma ferritin concentration < 30 ng/L. (Nyakeriga et al., 2004). Stool samples were examined for helminths by the Kato–Katz thick smear technique (Cheesbrough, 2004).

Statistical analysis

Data was doubly entered and validated using SPSS version 11.5 and Epi -info soft ware. Analysis was done with SPSS version 12 (SPSS, Inc., Chicago, IL, USA). Parasite densities were normalized using log-transformation. The normally distributed continuous data were compared with Student's t-tests. Skewed data were explored using either, Kruskal–Wallis (KW) tests or Wilcoxon signed rank test. The association between two continuous variables was assessed using the Pearson's rank correlation coefficient (r). Proportions were compared, between treatment groups, using ^{2} tests. The level of statistical significance was set at $P < 0.05$

RESULTS

Of the 332 recruited for haematological assessment and recovery, 216 (65.1%: 114 in AQAS arm, 38 in the SP arm and 64 in the untreated arm) were completely followed up. In the AQAS treatment arm, 1 (0.7%) child died, 5 (3.6%) refused to continue participation in the study and 18 (13%) received rescue treatment with quinine and so dropped out of the study. In the SP arm, 6 (14%) children dropped out of the study due to a change in treatment. In the untreated arm, 64 (42.4%) of the children developed uncomplicated malaria during follow up, were treated and thus dropped from the study.

Baseline characteristics of the different groups

The prevalence of gametocytaemia, splenomegaly and STH infection in the study population was 31.0% (103), 25.9% (86) and 32.8% (109) respectively. An ID and iron deficiency anaemia (IDA) prevalence of 28.0% (90/332) and 27.1% (90/332) respectively was also observed. At enrolment the AQAS treatment group registered a significantly higher prevalence of fever when compared with the other groups (Table1).

Variation in temperature and prevalence of fever during follow-up

The overall mean (mean ± SD) temperature on D0 was 37.2 ± 0.6°C while on D42, it was 36.9 ± 0.6°C. There was a significant drop in temperature on D42 (Wilcoxon Z = 5.9, P = 0.001), with the highest drop occurring in the AQAS treatment group (D0 = 37.4 ± 0.8, D42 = 37.0 ± 0.6°C) when compared with the SP (D0 = 37.1 ± 0.5, D42 = 36.8 ± 0.5°C) and untreated groups (D0 = 37.0 ± 0.4, D42 = 37.0 ± 0.8°C). A significant negative correlation (r = - 0.2, P<0.01) between temperature and Hb concentration was observed in the different treatment groups with the highest increase in Hb concentration recorded alongside a decrease in temperature in the AQAS treatment arm.

Table 1. Baseline clinical characteristics of the untreated and treated children.

Parameter			Untreated group	Treated groups		All	Level of significance
				AQAS	SP		
Sex	Female	%	48.34	41.7	9.9	(45.5)	^{2}= 2.5 p = 0.3
		n	(73)	(63)	(15)	151	
	Male	%	43.1	41.4	15.5	(54.5)	
		n	(78)	(75)	(28)	181	
Febrile status	Temp 37.5°C	%	15.9 (24)	46.4 (64)	23.3 (10)	29.5 (98)	^{2} = 33.1 P = 0.0001
		n					
Microcytosis		%	64.9	71.0	69.8	68.1	^{2}=1.3
		N	(98)	(98)	(30)	(226)	p = 0.5
Anaemia status	Hb <11g/dl	%	74.2	85.5	81.4	79.8	^{2}=5.8
		n	(112)	(118)	(35)	(265)	P = 0.06
Mean	Hb g/dl		9.7 ± 1.7	9.2 ± 1.8	9.3 ± 1.8	9.4 ± 1.8	KW = 5.4 P = 0.07
Mean	WBC ×10^9 L		7.8 ± 3.8	7.07 ± 2.9	7.4 ± 2.7	7.7 ± 6.1	KW = 2.2 P=0.3

*Significant difference observed between AQAS and the SP and untreated groups.

From D7 to D42, there was no significant difference in the prevalence of fever when all three arms were compared. However, the untreated arm had the highest prevalence of fever on D7, D14 and D21 (21.2, 24.2, and 19.7% respectively), when compared with the two treatment arms. On D28, the SP treatment arm recorded a higher prevalence of fever (15.4%). On D42 a higher prevalence of fever (16.7%) was recorded for the AQAS group when compared with the prevalence for the untreated (6.3%) and the SP treatment group (10.8%).

Anaemia at enrolment and during follow up

At enrolment, the prevalence of anaemia as evaluated by Hb concentration was 79.8% (265/332). Of the 265 anaemic (Hb < 11g/dl) subjects, 21.5% (57) had mild anaemia (Hb between 10.1 – 10.9 g/dl), 66.4% (176) had moderate anaemia (Hb between 7.0 - 10.0 g/dl) and 12.1% (32) had severe anaemia (Hb < 7 g/dl). This difference in anaemia prevalence was not statistically significant. Microcytic anaemia (MCV < 76 fl, MCH < 27 pg and MCHC < 310 g/dl) was prevalent in 75.3% (250) of the cases. Although a drop in prevalence of anaemia was observed during follow up, the percentage of children found anaemic was not significantly different statistically in the treated and untreated arms on D7 and D14. On D21, the difference in the prevalence of anaemia was significant (^{2} = 11.9, P = 0.003) with the SP treatment arm recording the highest prevalence (59%) when compared with the AQAS treatment arm (33.3%) and untreated arm (54.5%). Between D0 and D42, the overall prevalence of anaemia fell from 79.8 to 49.8%. The highest drop in prevalence of anaemia occurred in the AQAS arm and the lowest in the SP treatment arm (Figure 1).This difference in prevalence was significant (^{2} = 6.7, P = 0.04).

Prevalence of persistent anaemia

The prevalence of persistent anaemia (Hb concentration that remained below 11 g/dl for the duration of the follow-up) was 9.7% (21). The untreated arm recorded the highest prevalence of persistent anaemia (17.2%) when compared with the AQAS arm (4.9%) and the SP treatment arm (7.9%). While the prevalence of mild anaemia increased during follow up, a drop in prevalence of moderate and severe anaemia was observed. The prevalence of persistent anaemia was highest in the 1.1 - 3 year age group (36.4%) and the 1 year group (22.7%). The 9.1 – 14 year age group had the lowest prevalence (9.1%). The difference between these two age groups in the prevalence of persistent anaemia was significant (^{2} = 26.7 P = 0.001) . The 5 age group had a higher prevalence of persistent anaemia (7.9%, 17) than the > 5 (3.0%) and the difference was significant (^{2} =4.66, P = 0.03).

Variation in haemoglobin and malaria parasite density

Although there was a general rise in Hb concentration in

Figure 1. Prevalence of anaemia at enrolment and end of study.

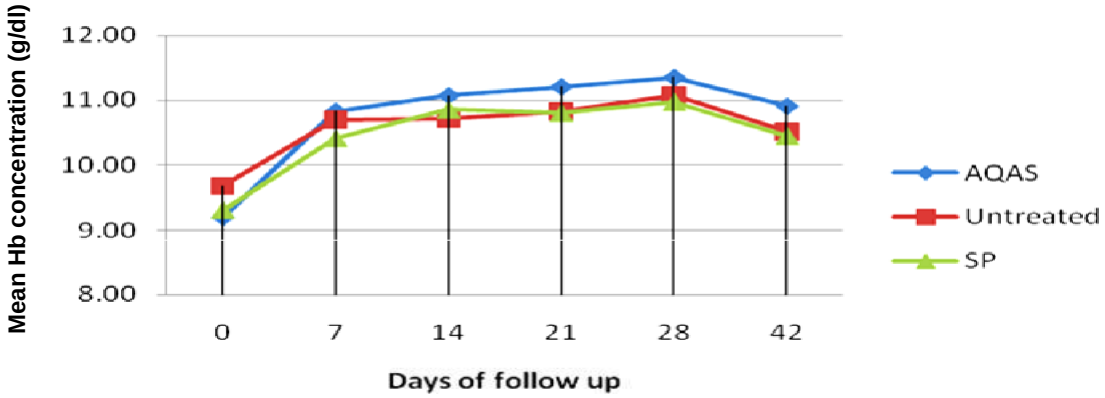

Figure 2. Changes in mean Hb concentration during follow up.

both treatment arms compared with the untreated arm from D0 to D28 (Figure 2), the AQAS arm recorded the highest mean increase in Hb concentration (D28 = 2.2 ± 1.9 g/dl, CI: 1.8 - 2.5). The difference in increase of mean Hb between D0 value and those for the different days of follow up was statistically significant. As parasites were cleared, an increase in mean Hb was observed in the AQAS and untreated arms. A statistically significant negative correlation existed between Hb and malaria parasite density on both D0 (r = -0.1, P = 0.02) and D42 (r = -0.4, P = 0.001) irrespective of treatment received.

Parasitological cure and anaemia

Of the 216 children who completed the follow up study, 198 (91.7%) achieved parasitological cure (complete clearance of parasitaemia after treatment and the absence of parasitaemia on day 28). A significant difference (^{2} = 4.28, P = 0.04) was observed in the prevalence of anaemia in parasitological cured children when compared with the prevalence observed for parasitological uncured children. The prevalence of anaemia was higher in the parasitological uncured children (Figure 3).

Changes in red cell indices

There was an overall increase in the mean MCHC and MCH from D0 to D42 while there was a decrease in mean MCV from D0 to D42. The mean MCHC (g/L), MCH (pg) and MCV (fl) in the study population on D0 was, 304.8 ± 58.7 g/l, 18.9 ± 7.8 pg and 64.5 ± 32.6 fl, while on D42, it was 323.2 ± 21.7 g/L, 20.0 ± 8.4 pg and 62.5 ± 26.9 fl respectively. The increase in MCHC and MCH on D42 was statistically significant (Table 2). At enrolment, there was no significant difference in the prevalence of microcytosis between the treated and untreated groups (Table 1). However, a drop in the prevalence of microcytosis was observed on D42 (49.7%) with the highest drop occurring in the untreated group (D42 = 31.6%) when compared with those treated with SP (D42 = 65.9%) and AQAS (D42 = 64.5%). This difference in prevalence was statistically significant (^{2} = 36.7, P = 0.0001).

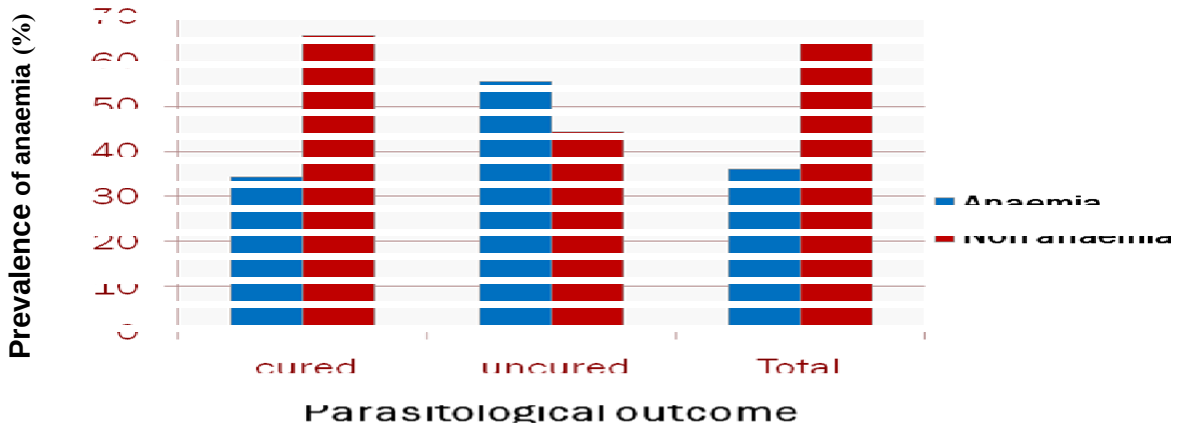

Figure 3. Prevalence of anaemia in the cured and uncured.

Table 2. A comparison of variation in mean (mean ± SD) red cell indices in the treated and untreated groups.

Red Cell Indices / Day		Untreated	Children given		Level of significance
			AQAS	SP	
MCHC(g/L)	D0	297.0± 46.7	313.2± 68.0	303.3 ± 59.11	Z=5.1
	D42	325.0± 22.5	321.6± 22.4	324.9 ±18.1	P = 0.001**
MCH(pg)	D0	18.4± 7.2	19.1± 8.0	19.8 ± 9.2	Z=2.4
	D42	18.9± 5.5	20.4± 9.9	20.4 ± 7.4	P = 0.02*
MCV(fl)	D0	62.6 ± 23.8	64.7 ± 35.8	69.5 ± 42.7	Z=0.5
	D42	60.3 ± 22.3	63.5 ± 30.2	63.1 ± 23.7	P=0.7

Z = Wilcoxon Signed Rank Test. ** P < 0.01 significant difference observed in the increase of D42 MCHC in the untreated group when compared with the AQAS and SP groups. *P < 0.05 significant difference observed in the increase of D42 MCH in the AQAS when compared with the SP and the untreated

Changes in WBC and differential counts over time

Total and differential white blood cell counts were assessed at the onset and compared with the treated and untreated groups at the end of the study. Children with untreated falciparum infection (D0 = 7.8 ± 3.8 × 10^9 /L; D42 = 7.2 ± 2.8 × 10^9 /L) and those treated with SP (D0 = 7.4 ± 2.7 × 10^9 /L; D42 = 7.2 ± 2.2 × 10^9 /L) showed a decrease in mean WBC counts contrary to an increase shown by those treated with AQAS (D0 = 7.1 ± 2.9 × 10^9 /L; D42 = 7.4 ± 2.6 × 10^9 /L). Although not statistically significant, mean neutrophil counts increased over time in the untreated group while a drop was observed for those treated. Conversely mean monocyte count decreased in the treated groups while an increase was observed in the untreated group.

Changes in platelet count following treatment

The mean platelet count before treatment was 236.2 ± 99.6 × 10^9 /L. A decrease in platelet count was observed on D42 (207.8 ± 97.7× 10^9 /L) following treatment. The decrease in mean was statistically significant. The highest decrease occurred in the AQAS treatment group (D0 = 231.3 ± 98.3 × 10^9 /L; D42 = 187.0 ± 81.8 × 10^9 /L). Unlike the AQAS and the untreated arm (D0 = 221.9 ± 88.0 × 10^9 /L; D42 = 183.3 ± 61.2 × 10^9 /L), the mean platelet count in the SP treatment arm (D0 = 285.3 ± 116.5 × 10^9/L; D42 = 314.5 ± 122.9 × 10^9/L) showed an increase on D42. This difference in mean was statistically significant (Z = 5.3, P = 0.001).

Haematological recovery

Of the 265 (79.8%) falciparum infected children who were anaemic on D0, only 73 (27.5%) achieved haematologic recovery on D42. The difference in the prevalence of haematologic recovery using various treatments was significant (2 = 44.4, P = 0.001) with the highest

recovery occurring in the AQAS treatment arm (39.1%, 54/138). The majority (94%, 142/151) of the untreated cases never achieved haematologic recovery.

DISCUSSION

Our study revealed that anaemia is a major public health problem and a common haematological state in the Mount Cameroon region. The high prevalence of gametocytaemia in the study population may be due to the observed low parasitaemia and high prevalence of anaemia. Studies by Price et al. (1999) and von Seidlein et al. (2001) reported a significant association of gametocyte carriage with low grade parasitaemia and anaemia. The low haemoglobin concentrations may have triggered gametocytogenesis (Nacher et al., 2001).

Haemoglobin concentrations fluctuate over time in different individuals. The negative association between temperature and Hb concentration observed may be due to certain immunologic responses such as the secretion of high levels of TNF - , a potent pyrogen. Chronic low-grade production of TNF- , in response to *P. falciparum* parasitaemia may induce dyserythropoiesis thus contributing to the pathogenesis of malarial anaemia (Tchinda et al., 2007).

The SP treatment and untreated groups maintaining the highest prevalence of anaemia highlights the need for effective and prompt antimalarial therapy to combat malarial anaemia. Artemisinin-based combinations are known to effect rapid fever and parasite clearance (Koram et al., 2005). The wide-spread use of more effective antimalarial would probably result in greater clinical and haematological benefits.

The significantly high prevalence of anaemia in parasitologically uncured children when compared with parasitologically cured children indicated that parasitological cure is necessary for haematological recovery. The delayed parasite clearance observed in the untreated and SP treatment groups may be responsible for the persistent anaemia. Delayed parasite clearance was highlighted by Price et al. (2001) as a significant independent risk factor for anaemia. Age (children < 24 months of age) was also identified as a risk factor for persistent anaemia by Price et al. (2001) and Obonyo *et al*. (2007b). The unpleasant effect of young age is probably related to a lack of acquired antimalarial immunity and reduced ability to clear parasites. Acquired immunity requires exposure to several infections and develops over several years.

In the untreated group, there was an increase in spontaneous clearance of parasite from D21 – D28 which was lacking in the SP treatment group. Residents of malaria-endemic areas sometimes spontaneously clear *P. falciparum* infection without treatment, implying an important role of host factors such as immunity (Djimde et al., 2003). SP has been reported (Warsame et al., 2009) to remain homogenously effective during a 14 day follow up when compared with AQ. Our results showed a treatment failure of 25.6% on D28. This confirms that the use of SP monotherapy is not beneficial to the patient. Although an official recommendation to change from the use of SP and CQ monotherapy to ACT was made by the government of Cameroon in 2005, the former drugs are commonly used in these communities because they are cheaper and available in local drug stores and can be purchased without prescription.

After the recovery period, the highest significant increase in MCHC and MCH occurred in the untreated and the AQAS group respectively. This is not unexpected as artemisinin drugs are reported to cause less antimalarial drug-related falls in haematocrit during treatment (Sowunmi et al., 2009).

Although 77.9% of the children had normal WBC counts, children with patent parasitaemia had lower WBC counts than those with none patent and those malaria negative. The increase in mean WBC count observed in the AQAS group following treatment, lends support to the finding that *P. falciparum* infection contributes to the localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis (McKenzie, 2005; Taha et al., 2007).

The majority of the children examined (72.2%) had normal lymphocyte count. While a significant negative correlation ($r = -0.3$, $P = 0.01$) existed between lymphocyte count and parasitaemia density following treatment, a positive correlation was observed with the Hb concentration ($r = 0.2$, $P = 0.02$). The malaria associated decrease in lymphocyte count observed in the untreated group is striking as lymphocytes, particularly T cells, play a major role in immunity to falciparum malaria by releasing proinflammatory cytokines such as TNF- , interferon- and other cytokines, and activating other inflammatory cells. However, excessive secretion of the proinflammatory cytokines contributes to disease severity (Biemba et al., 2000).

Although neutrophil count positively correlated ($r = 0.4$, $P = 0.001$) with parasitaemia density following treatment, no association was found with haemoglobin concentration. On the contrary, Ladhani et al. (2002) reported that neutrophil counts were not raised by hyperparasitaemia and severe anaemia. The positive association of neutrophil and parasitaemia may have been influenced by intercurrent bacterial infection which was not investigated. Monocytes are also important in developing immunity against falciparum malaria. The decrease in mean monocyte count following treatment and an increase in count in the untreated group is not unprecedented. Monocytes have been reported to act against the malaria parasite via several mechanisms, including antibody-dependent cellular inhibition of parasite growth (Tebo et al., 2001), phagocytosis of parasite- infected red blood cells, and release of cytokines, such as TNF- .

Of the 66 (29.5%) children who on D42 had thrombocytopaenia, 23 of them were malaria positive. Those who were malaria positive were 1.5 times (95% C.I.: 0.8 – 2.6) at odds of having abnormal (high or low) platelet count than those who were parasitaemia negative. Unlike Erhart et al. (2004) who showed platelet count to have a high predictive value for malaria infection than any other symptom in semi-immune patients, our finding showed no significant association between platelet count, parasitaemia density and Hb concentration before and after treatment. Platelet count may therefore be of limited diagnostic applicability in this setting due to its lack of predictive power.

Haematological insult was greatest in the untreated, those treated with SP, the 1.1 - 3 year age group and those having high parasitaemia. Results from our study showed the highest haematological recovery occurred in the AQAS treatment group (39.1%) while the majority (94%) of the untreated cases never achieved haematological recovery even though there was spontaneous clearance of the parasite. Our finding lends support to those of Obonyo et al. (2007a) who showed that haematological recovery was associated with mild anaemia at disease presentation and the achievement of parasitological cure. We further associated treatment type with haematological recovery. The age group < 5 were 1.97 times (95% C.I.: 1.2 – 3.4) more likely to recover from haematological insult than those > 5 years. Therefore, proper malaria control strategies targeted at the less immune age group (1.1 – 3 years) will likely reduce the burden of malaria resulting in greater clinical and haematological benefits.

There are implications of our findings for anaemia control efforts in malaria endemic areas: Prompt use of effective ACT targeted at the less immune age group will reduce the burden of malaria associated anaemia and haematological insults; the regular monitoring of local drug stores to ensure that only antimalarial drugs recommended by the government are being sold should form an integral part of the malaria and anaemia control policy.

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