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Plasmodium falciparum hyperparasitaemia in Nigerian children: epidemiology, clinical characteristics, and therapeutic responses to oral artemisinin−based combination treatments

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ABSTRACT

Objective: To evaluate the epidemiology, clinical characteristics and response to oral artemisinin−based combination treatments (ACTs) of children with *Plasmodium falciparum* hyperparasitaemia (*Pf*HP). **Methods:** All children with febrile or history of febrile illness who were suspected to be malaria were evaluated for the presence of *Pf*HP and their parasitological and clinical characteristics at presentation and follow−up for four weeks were recorded during a 3−year period. Patients were treated with oral artemisinin−baesd combination drugs. **Results:** *Pf*HP was present in 3% (97/3 338) of parasite−positive children, and with no seasonal variation. The proportion of children with *Pf*HP increased significantly over the years (P=0.001). Compared with non−hyperparasitaemic children, hyperparasitaemic children were younger, had significantly shorter duration of illness, and higher core temperature on presentation (P=0.04, 0.04, <0.000 1, respectively). Parasite clearance and half−lives of parasitaemia were similar in both groups of children following treatment with artemether−lumefantrine or artesunate−amodiaquine but half−life of parasitaemia increased significantly as parasite clearance time increased (P<0.000 1). The proportions of children in which there was no change in haematocrit following treatment with these drugs were similar (65% *vs* 76%, P=0.09), but fall in haematocrit/1 000 parasites cleared from peripheral blood was 10−fold higher in patients without hyperparasitaemia suggesting that artemether−lumefantrine or artesunate−amodiaquine may conserve haematocrit in children with hyperparasitaemia. Recrudescent infections were significantly more common in hyperparasitaemic children (P=0.014). **Conclusions:** *Pf*HP is common in young malarious Nigerian children, and severe malaria which is in the absence of other features responds promptly to oral ACTs.

1. Introduction

Plasmodium falciparum hyperparasitaemia (*Pf*HP), defined as peripheral asexual parasitaemia >250 000/μL or >4% parasitized red blood cells, is depending on the epidemiologic setting. A severe malaria is associated with a relatively longer parasite clearance time and is a major risk for recrudescent infections because of high parasite burden particularly in children[1−4].

In Nigerian children, *Pf*HP may not be accompanied by other features of severe malaria[5,6]. However, little is known about the epidemiology of *Pf*HP in many endemic areas and it is not clear whether season influences *Pf*HP. It is desirable that this should be evaluated as it may influence community management of the disease.

Anaemia, an inevitable complication of falciparum malaria in children living in endemic areas, is ascribed to destruction of infected and non−infected red cells[7−10]. In children with *Pf*HP, the risk of developing anaemia after treatment with antimalarial drugs is high as both infected and non−infected red cells may be expected to be destroyed. However, it is unclear if artmesinin−based combination drugs can modify this risk following treatment of hyperparasitaemic African children compared with non−hyperparastaemic ones.

In order to address the above issues, we have evaluated the epidemiology, clinical characteristics, and responses to orally administered artemisinin−based combination treatments (ACTs) of children with hyperparasitaemia and

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compared these with children without hyperparasitaemia in a malaria endemic area of Southwestern Nigeria. In addition, we evaluated the fall in haematocrit per 1000 parasites cleared from peripheral blood in order to determine whether there is 'haematocrit conservation' in children with hyperparasitaemia. It may explain why the fall in haematocrit in children with hyperparasitaemia may be less than that predicted if all infected red cells were lost after treatment with ACTs.

2. Materials and methods

2.1. Study area

The epidemiology of malaria in Ibadan, Southwest Nigeria, has been described elsewhere[5]. Briefly, hyperendemic malaria transmission occurs all year round but is more intense during the rainy season from April to October in this area. *Plasmodium falciparum* (*P. falciparum*) is the predominant species accounting for 99% of all infections. Children are more affected than adults, and apparently asymptomatic infections occur in older school children and adults.

2.2. Study procedure

2.2.1. Epidemiology and clinical characteristics of hyperparasitaemia in children

Between January 2008 and December 2010, all children with febrile illnesses who were suspected to be or diagnosed as malaria were enrolled in the study. Thick and thin blood smears, obtained from a finger prick blood, were Giemsa–stained for parasite identification and quantification as previously described[11]. Demographic and clinical parameters including age, gender, body weight, height, core temperature were recorded and physical examination was performed on all subjects. The data were stored in the database. There were 6 807 children aged 0.5–17.0 years in the database.

2.2.2. In vivo study of drug efficacy in children with or without hyperparasitaemia

Patients were eligible to participate the drug study if they were ≤12 years of age, had symptoms compatible with acute uncomplicated malaria such as anorexia, vomiting, or abdominal discomfort with or without diarrhea, with *P. falciparum* parasitaemia >2 000 asexual forms/μL, a body (axillary) temperature >37.4 ℃ or history of fever in the 24–48 h preceding presentation, absence of other concomitant illness, no history of antimalarial drug use in the two weeks preceding presentation. Written informed consent was given by parents or guardians. Patients with severe malaria, other than hyperparasitaemia defined as asexual parasite density >250 000/μL blood or >4% parasitized erythrocytes[1,2], severe

malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and allergy to study drugs were excluded from the study. The study protocol was reviewed and approved by the Ethics Committee of the Ministry of Health, Ibadan. The disease history, taken by the attending physician, was recorded by asking patients or their parents or guardians when the present symptomatic period started, and the patients were followed by a full physical examination by the same physician.

2.2.3. Drug regimens

Artemether–lumefantrine (Coartem®; Novartis, Basel, Switzerland) was given according to the patient' body weight. Patients weighing 5–14 kg received one tablet, those weighing 14–24 kg received 2 tablets, those weighing 25–34 kg received 3 tablets, and those weighing more than 34 kg received 4 tablets at presentation (0 h), 8 h later, and at 24, 36, 48 and 60 h after the first dose. Each tablet of artemether–lumefantrine contains 20 mg of artemether and 120 mg of lumefantrine. Co–formulation of artesunate and amodiaquine (Coarsucam®; Sanofi Aventis, Casablanca, Morocco) was given as follows. Children weighing >9 – <18 kg or aged 1–5 years received 0.5 tablet, children weighing >18 – 36 kg or aged 6–13 years received 1 tablets, children weighing >36 kg or aged >14 years received 2 tablets. Each tablet of artesunate and amodiaquine co–formulation contains 270 mg amodiaquine base and 100 mg artesunate co–formulated in a bi–layer. Co–package of artesunate and amodiaquine (Dart®, Swiss Pharma, Lagos, Nigeria) was given according to age or body weight as follows. 1–5 years or 5–15 kg received 1 tablet, 6–10 years or 16–24 kg received 2 tablets, 11–15 years or 25–34 kg received 3 tablets. Each tablet contains 153 mg of amodiaquine base and 50 mg of artesunate in the co–packed unit. All artesunate–amodiaquine doses were given daily for 3 days. If necessary, patients were provided with antipyretics (paracetamol tablets, 10–15 mg/kg every 8 h for 24 h).

The day of enrollment was regarded as day 0 (or 0 h). Follow–up with clinical and parasitological evaluation was carried before treatment (day 0, 0 h) and at 1, 2, 4, 8, 16, and 24 h in 191 after treatment began, and on days 2–7, 14, 21, 28, 35 and 42 in 191 children. In all other children follow–up was done on days 1–7, 14, 21, 28, 35, and 42. Follow–up consisted of enquiry about the patient's well–being, presence or absence of initial presenting symptoms, presence of additional symptoms, measurement of body temperature, heart and respiratory rates, and a blood smear for the quantification of parasitaemia.

Side effects were defined as symptoms and signs that first occurred or became worse after treatment was started. Any new events occurring during treatment were also considered as side effects.

Thick and thin blood films prepared from a finger prick were stained with Geimsa and examined by light microscopy under an oil–immersion objective at 1 000× magnification as previously described[11–14]. Capillary blood collected before

and during follow–up, was used to measure packed cell volume (PCV) or haematocrit. Haematocrits were measured using a microhaematocrit tube and microcentrifuge (Hawksley, Lancing, UK). Routine haematocrit was done on days 0–7, 14, 21, 28, 35 and 42. Anaemia was defined as a haematocrit <30%. Blood was spotted on filter papers on days 0, 3, 7, 14, 21, 28, 35 and 42 and at the time of treatment failure for parasite genotyping.

2.3. Outcome measures

Response to drug treatment was assessed using criteria as previously described[12–14]. Parasite clearance time was defined as the time after drug administration until there was no patent parasitaemia for at least 48 h. Fever clearance time was defined as the time after drug administration until body temperature fell to or below 37.4 ℃ and remained below this value for at least 48 h. Recurrent infection was defined as the presence of asexual forms of *P. falciparum* from days 5–42. Recrudescent infections were differentiated from new infections using 3 loci genotyping as previously described[15,16]. Cure rates were defined as the percentages of patients whose asexual parasitaemia cleared from peripheral blood and who were free of patent asexual parasitaemia on days 28, 35 and 42 of follow–up.

2.4. Laboratory evaluation

2.4.1. Fall in haematocrit per 1 000 parasites cleared from peripheral blood

Fall in haematocrit per one thousand parasites cleared from peripheral blood (FIH/1 000 parasites cpb) was calculated as there were differences in haematocrit values at baseline (pre–treatment, day 0) and the first 1 (day 1) or 2 (day 2) days after treatment. The differences in baseline (pre–treatment) parasitaemia and the parasitaemia on the first 1 or 2 days after treatment were due to denominator and expressing it per 1 000 parasites cleared from peripheral blood. The unit of quantification is % if haematocrit values were used or g/dL if haemoglobin values were used. Haematocrit values may be converted to haemoglobin values by dividing by 3.

2.4.2. Haematocrit conservation ratio

Haematocrit conservation ratio was defined as the ratio of fall in haematocrit per 1 000 parasites cleared from peripheral blood at a particular level of parasitaemia and below divided by the corresponding value of fall in haematocrit for parasitaemias above this level. In the present study, this ratio was the mean fall in haematocrit of hyperparasitaemia: non–hyperparasitaemia.

2.5. Kinetics of parasitaemia

In 191 children enrolled between 2008 and 2009, follow–up

with clinical and parasitological evaluation was done at the following times in all patients: 0, 1, 2, 4, 8, 16, 24 h and daily on days 2–7, 14, 21, 28, 35 and 42. The kinetics of the time course of the parasitaemia was estimated by using a non–compartmental model as previously described[17].

2.6. Statistical analysis

Data were analyzed using version 6 of the Epi–Info software and the statistical programme SPSS for Windows version 10.01[18,19]. Variables considered in the analysis were related to the densities of *P. falciparum* gametocytes and trophozoites. Proportions were compared by calculating χ^2 with Yates' correction or by Fisher exact test. Normally distributed, continuous data were compared by Student's *t*–test, and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann–Whitney U–tests or the Kruskal–Wallis tests or by Wilcoxon ranked sum test. The cumulative risk of parasite recrudescence was calculated by survival using the Kaplan–Meier method. Changes in prevalence parasite rate over the years were analyzed by χ^2 test for trend. All tests of significance were two–tailed. *P*–values of <0.05 were taken to indicate significant differences. Data were (double)–entered serially using the patients' codes and were only analyzed at the end of the study.

3. Results

3.1. Parasite rates and the proportion of children who had hyperparasitaemia

During the study period of 2008–2010, 6 807 children with illnesses diagnosed as malaria were screened for *P. falciparum* parasitaemia. Parasitaemia was present in 3 338 children of which 97 (3%) had hyperparasitaemia. Hyperparasitaemia was significantly more common in children aged 2–<5 and 5–10 years compared with children <2 years (χ^2=11.1, df=2, *P*=0.003) (Table 1). Hyperparasitaemia was also significantly more frequent in children aged 2–<5 and 5–10 years compared with children >10 years (χ^2=10.88, df=2, *P*=0.004) (Table 1). There was no change in overall parasite rates during the study period: 912 of 1 956 (46.6%), 1 469 of 2 853 (51.5%), and 957 of 1 998 (47.9%) in 2008, 2009, and 2010, respectively (χ^2=0.6, *P*=0.43). There was a significant increase in the proportion of parasitaemic children in which there was hyperparasitaemia during the same period: 11 of 912 (1.2%), 50 of 1 469 (3.4%), and 36 of 957 (3.8%), respectively (χ^2=10.6, *P*=0.001). Figure 1 showed the monthly distribution of parasite rates and the proportion of parasitaemic children in which hyperparasitaemia was found. Overall during the rainy season (April to October) parasite rate 2 540 of 5 035 (50.4%) was significantly higher than during the dry season (November to March) 798 of 1

772 (45%) (χ^2=15.4, *P*=0.000 1). However, the proportions of parasitaemic children in which hyperparasitaemia was found during the two seasons were similar: 79 of 2 540 (3.1%) in the rainy season and 18 of 798 (2.2%) in dry season (χ^2=1.28, *P*=0.25). The distribution of individual levels of parasitaemia in the 97 children who had hyperparasitaemia on presentation was shown in Figure 2.

3.2. Clinical characteristics of children with hyperparasitaemia

The clinical and other characteristics of the children with hyperparasitaemia were summarized in Table 2. Compared

with non−hyperparasitaemic children at presentation, hyperparasitaemic children were younger, had significantly shorter duration of illness and higher core temperature on presentation (*P*=0.04, 0.04, <0.000 1, respectively).

Table 1
Age distribution of children with *P. falciparum* hyperparasitaemia [*n* (%)].

Age group (years)	No. screened	No. positive	No. with hyperparasitaemia
0 − <1	191	40 (20.9)	0
1 − <2	463	131 (28.3)	1
2 − <3	553	199 (35.9)	6
3 − <4	642	266 (41.4)	9
4 − <5	537	272 (50.6)	14
5 − 10	2 791	1 558 (56.0)	56
>10	1 548	781 (50.5)	11

Table 2
Demographic and clinical characteristics of children with hyperparasitaemia and without hyperparasitaemia (Mean±SD).

Variable	Hyperparasitaemia	No hyperparasitaemia
No. of patients	97	3 241
Male/female	45/52	1 688/1 558
Age (year)	6.6±3.2*	7.4±4.0 (*n*=3 241)*
Range	0.87−13.00	0.93−13.00
No. < 5 years	30	908
No. ⩽ 2 years	4	298
Weight (kg)	19.2±7.4 (*n*=89)	21.8±10.3 (*n*=3 083)
Range	8−50	8−71
Duration of illness (d)	2.8±1.3 (*n*=88)*	3.1±1.9 (*n*=2 995)*
Range	1−21	1−21
No. with >3 d	10*	1 610*
Haematocrit (%)	31.2±5.8 (*n*=70)	32.3±5.4 (*n*=1 724)
Range	10−45	10−51
No. < 30%	24	437
Temperature (℃)	38.2±1.3 (*n*=88)**	37.7±1.3 (*n*=3 087)**
Range	36.2−40.5	35.8−40.3
No. > 37.4 ℃	63**	1 638**
No. > 40 ℃	3	77
No. < 36 ℃	1	164

*P<0.05, **P<0.01.

3.3. Therapeutic responses of children with and without hperparasitaemia

Thirty five of 97 children with hyperparasitaemia were treated with artemether−lumefantrine or amodiaquine −artesunate and were not randomized to receive these drugs. These patients' parasitaemia and fever were cleared within 2 days of treatment. The therapeutic responses of 124 consecutive children with or without hyperparasitaemia who were randomly assigned to treatment with artemether−lumefantrine or artesunate−amodiaquine were summarized in Table 3. All patients recovered clinically. Fever but not parasite clearance was significantly faster in children with hyperparasitaemia. No patient had parasitaemia on day 3. Adverse events reported within the first week of the study with a frequency ⩾ 4 consisted of abdominal pain, 5 and 4 children with hyperparasitaemia and non−hyperparasitaemia, respectively and cough in 5 children without hyperparasitaemia.

3.4. Kinetics of parasitaemia and the relationship between enrollment parasite density and half life of parasitaemia

Parasite kinetic data were available in 191 children, 29 with hyperparasitaemia and 162 without hyperparasitaemia. Decline of parasitaemia was mono−exponential with similar half lives of parasitaemia of (0.99±0.10) (sem), range (0.65−2.09) h and (1.09±0.04) (sem), range (0.49−2.27) h, (*P*=0.38) (Figure 3 and Table 4). These data were combined in order to examine the relationship between enrollment parasite density and the terminal elimination half−life of parasitaemia. Overall, elimination half−life of parasitaemia was significantly lower in children in which parasitaemia cleared by day 1 (24 h) (*n*=152) compared with those in which parasitaemia cleared after day 1 (*n*=40) (0.81; 95% *CI* 0.76−0.85) h *vs* (2.05; 95% *CI* 1.98−2.11) h, *P* <0.000 1). Area under the curve of parasitaemia versus time (AUC) and the clearance of parasitaemia (CLp) were significantly higher in hyperparasitaemic compared with non−hyperparasitaemic children (*P*=0.023, 0.015, respectively) (Table 5).

Table 3
Therapeutic responses of children with or without hyperparasitaemia to artemether−lumefantrine or artesunate−amodiaquine (Mean±SD).

Variable	Hyperparasitaemia	No hyperparasitaemia
No. of patients	62	62
Male/female	36/26	35/27
Age (year)	7.1±3.0	6.2±3.0
Range	0.87−13.00	0.93−13.00
No. < 5 years	25	24
Weight (kg)	18.5±72.0	17.4±5.5
Range	7−46	8−34
Haematocrit (%)	32.8±4.5	32.6±3.9
Range	21−41	25−44
No. with < 30%	9 (n=44)	10 (n=51)
Temperature (℃)	38.2±1.2	38.3±1.1
Range	36.1− 41.0	35.8−40.3
Duration of illness (d)	2.7±1.2	2.7±1.2
Range	1−7	1−7
GMPD (/μ L of blood)	428 315**	71 934**
Range	251 142−2 124 000	2 024−242 330
GMGD (/μ L of blood)	34 (n=4)	12 (n=1)
Range	6−168	−
Fever clearance time (d)	1.1±0.3 (n=39)*	1.3±0.6 (n=82)*
Range	1−2	1−3
95% CI	1.0−1.2	1.1−1.5
No. of patient with parasitaemia on day 1	16	15
Parasite clearance time (d)	1.1±0.6	1.1±0.5
Range	0.33−3.00	0.33−2.00
95% CI	1.04−1.34	0.97−1.24
Day and responses (S/RI/RII*†)		
28	60/2/0/	60/2/0/
42	55/7/0	56/6/0
ACPR ∱	55	56
LPF	7	6
ETF	0	0
LCF	0	0
PCR−corrected cure rate (%)	93.5	93.5

Cure rates on D28 and 42 for hyperparasitaemia were 100% and 95.2%, respectively. For no hyperparasitaemia, the corresponding values were 100% and 91.9%, respectively.
*PCR−uncorrected; †No RII or RIII response in hyperparasitaemia or no hyperparasitaemia groups; ∱ Adequate clinical and parasitological response (on D42); LPF = late parasitological failure; LCF = late clinical failure; ETF = early treatment failure.
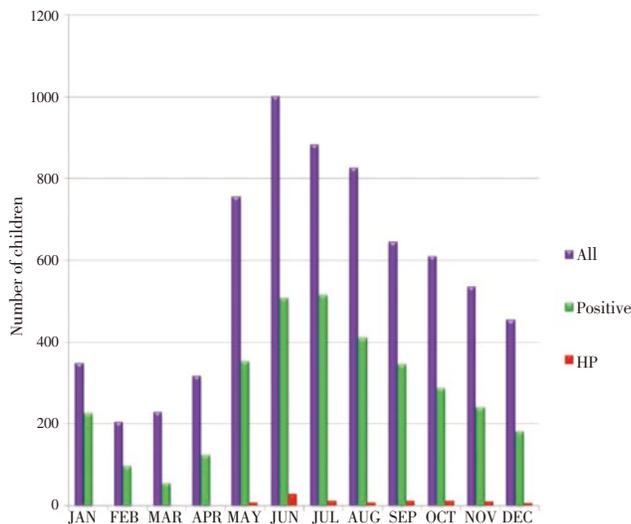*$P<0.05$, **$P<0.01$.

Figure 1. Monthly distribution of parasite positive children and the proportion in which hyperparasitaemia (HP) were found in an endemic area over a three year−period of 2008−2010.
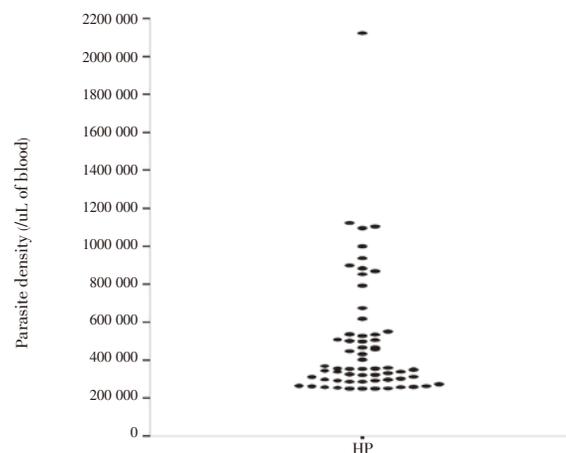

Figure 2. Distribution of individual parasitaemia in children with hyperparasitaemia.

3.5. Anaemia in children with or without hyperparasitaemia

Table 4
Demographic and clinical characteristics of children enrolled in kinetics of parasitaemia study (Mean±SD).
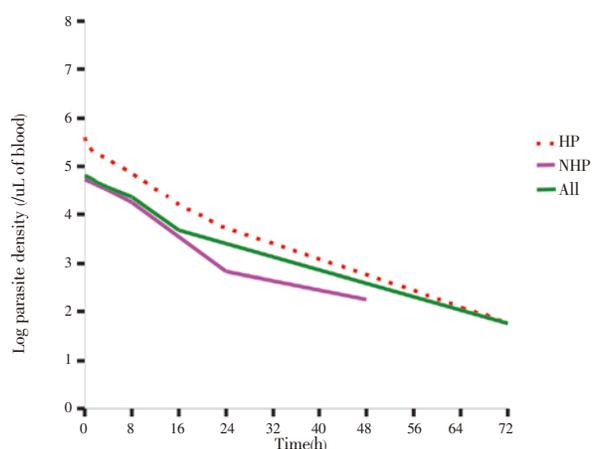
Variable	Hyperparasitaemia	No hyperparasitaemia
No. of patients	29	162
Male/female	14/15	80/85
Age (year)	6.7±2.9 (*n*=26)	7.0±2.9 (*n*=159)
Range	2−12	0.7−13.0
No. < 5 years	8	45
Weight (kg)	19.1±6.1 (*n*=26)	19.5±6.3 (*n*=159)
Range	10−36	6−46
Duration of illness (d)	2.6±1.1	2.7±1.2
Range	1−7	1−14
Haematocrit (%)	32.8±4.0 (*n*=22)	32.6±3.9 (*n*=121)
Range	22−40	17−44
No. with < 30%	1	22
Temperature (℃)	38.3±1.2	38.5±1.1
Range	36.3−40.2	36.0−41.0
No. with >40 ℃	3	12
GMPD (/μ L of blood)	461 400*	63 424*
Range	251 142−1 125 000	2 024−249 990

*P<0.01.

Table 5
Parameters of the disposition of parasitaemia in children with or without hyperparasitaemia following oral administration of artemether−lumefantrine or artesunate−amodiaquine (Mean±SEM).

Variable	Hyperparasitaemia (*n*=29)	No hyperparasitaemia (*n*=162)
$t_{1/2}$ (h)	0.99±0.10	1.09±0.04
Range	0.65−2.09	0.49−2.27
AUC (μ L/h)	74*	60*
Range	33−219	23−177
CLp (μ L/h)	0.13±0.03*	0.09±0.005*
Range	0.026−0.740	0.007−0.690

*P<0.05.

Haematocrit <30% was present in 24 of 70 and 824 of 3 663 children with and without hyperparasitaemia, respectively. The difference between the two proportions was significant (χ^2=4.36, P=0.03). Severe anaemia (haematocrit <15%) was found in 1 and 17 children, respectively.

Figure 3. Semi−log plots of parasitaemia versus time in all children (green line), children with hyperparasitaemia (HP, red broken line) and children without hyperparasitaemia (NHP, purple line) following oral administration of artemether−lumefantrine or artesunate−amodiaquine.

3.6. Fall in haematocrit per 1 000 parasites cleared from peripheral blood

Data for the evaluation of changes in haematocrit were available in 360 children. Fall in haematocrit (FIH) between day 0 and day 3 was present in 13 of 20 and 147 of 193 with and without hyperparasitaemia, respectively. The difference between the two proportions was not significant (χ^2=2.80, P=0.09). Mean FIH/1 000 parasites cpb in children were insignificantly higher in children with NHP [(0.010±0.003) *vs* (0.100±0.015)] (P=0.058). However, the relative change in the mean value was ten−fold higher in children with HP compare with children NHP suggesting a haematocrit conservation of 10 folds.

3.7. Recrudescence of parasitaemia in children with or without hyperparasitaemia

Molecular analysis data were available in 495 children enrolled between 2008 and 2010. In this sub−group, 43 children had hyperparasitaemia and 452 had no hyperparasitaemia at enrollment. Recrudescent infections determined by parasite genotyping occurred in 12 children,

4 (9.3%) with hyperparasitaemia and 8 (1.7%) without hyperparasitaemia (P=0.014). Kaplan–Meier plot of the cumulative probability of recrudescence showed that recrudescence was significantly more likely to occur in children with hyperparasitaemia compared with those without hyperparasitaemia (log rank statistic=9.1, P=0.003) (Figure 4).
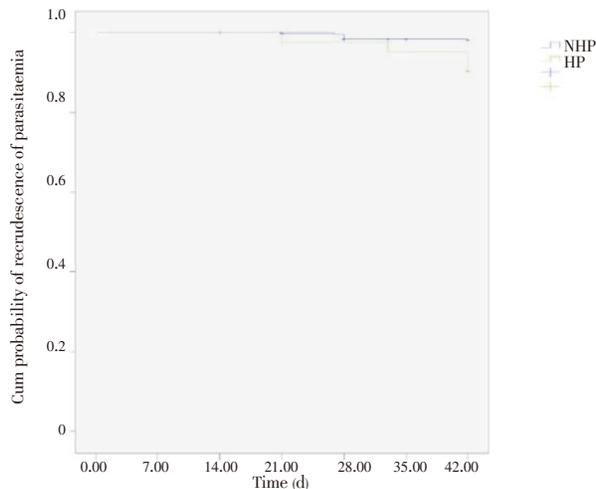

Figure 4. Kaplan–Meier plot of cumulative probability of recrudescence of parasitaemia in children with hyperparasitaemia (HP) or without hyperparasitaemia (NHP).
Log rank statistics=9.1, P=0.003.

4. Discussion

Hyperparasitaemia, a criterion of severe malaria, is considered as an evidence of a large sequestered parasite biomass, but little is known of its epidemiology even in malaria endemic countries[1,2,4]. In the present study, hyperparasitaemia comprised 3% of all falciparum infections, and as expected, was more frequently encountered in children aged 2–10 years suggesting that children with or without relative lack of antimalarial immunity are at considerable risk of hyperparasitaemia. This may partly explain the great variability in the severity of infection or of parasitaemia in both immune and non–immune individuals. When compared with the previous study in the early 1990s, the proportion of infections with hyperparasitaemia appeared to be significantly lower than 16% previously recorded in the same study area[5]. The reasons for the difference are not immediately apparent from the results presented. With approximately 10% of the children with hyperparasitaemia having over 10% parasitaemia, and with little or no other features of severe malaria, it is plausible to conclude that many children from this endemic area tolerate considerable degree of hyperparasitaemia[6]. To the best of our knowledge, this is the largest study of the epidemiology of hyperparasitaemia in children from an endemic area.

With significant differences in mean parasite rates of 38% and 43%, respectively in the dry and rainy seasons in children clinically diagnosed as malaria, there was clearly seasonal variation in parasite rates in keeping with previous findings from this endemic area[5] although overall rates of 49% is a little lower than the 57% reported 20 years ago[5]. The similarity in overall parasite rates over a 20–year period of 1990–2010, suggests stable transmission in the area and may imply that the recent introduction and increasing use of ACTs for malaria treatment has little or no impact on malaria transmission in the area[20]. It is intriguing that there was no seasonal variation in the proportion of parasitaemic children who had hyperparasitaemia. The reasons for the latter and for the significant increase in the proportion of parasitaemia that were hyperparasitaemia over the years are unclear. The absence of seasonal variation and increasing trend suggest that hyperparasitaemic children can be expected to be seen all year round in this endemic area. If hyperparasitaemia is associated with increased probability of recrudescence[4], recrudescence after antimalarial treatment (and subsequent transmission of gametocytes arising from these infections) can be expected to occur all year round and to increase in the future.

Compared with non–hyperparasitaemic children, hyperparasitaemic children were younger, had significantly high core temperature and longer duration of illness < 4 days. Except for duration of illness, these factors are similar to those associated with delay in parasite clearance and antimalarial drug treatment failure in children from this and other endemic areas[21–25].

All of the children responded promptly to artemether–lumefantrine or artesunate–amodiaquine with rapid clearance of parasitaemia in approximately 75% of hyperparasitaemic and non–hyperparasitaemic children within 24 h. However, parasite clearance times were similar suggesting that, in the absence of other symptoms and signs of severe malaria, oral ACTs may be used in children with hyperparasitaemia in this endemic area. With the absence of parasitaemia in all children on day 3, a positive result is a good predictor of subsequent treatment failure[3]. The possibility of subsequent treatment failure appeared low in the cohort of children. Additionally, the similar frequencies of reported adverse events suggested that hyperparasitaemic children had no proclivity to develop adverse events following ACTs. The reported adverse events, however, were difficult to distinguish from those of malaria infection.

The parasitaemia half life of one hour provides a baseline for which future changes in parasite population *in vivo* and *in vitro* susceptibility profiles from this endemic area may be measured or compared, and may be relevant in the evolution of drug resistance to the adopted ACTs. With an overall similar parasitaemia half life of one hour in patients with and without hyperparasitaemia, it would appear unlikely that genuine differences occur in the parasitaemia kinetics following ACTs administration in children with differing range of parasitaemia. However, overall parasitaemia half–life of one hour would suggest that genuine difference occur in parasite disposition kinetics in children from this endemic area and in patients from Vietnam (8 h)[26]

or Thailand (3 h)[27], where the half lives of parasitaemia are considerably longer, than in the present study. These differences may be related to different sampling times, pharmacokinetic models, population variations in drug handling and regional differences in susceptibilities in *P. falciparum* isolates to artemisinins and the partner drugs[3], in a region where recent data suggest that artemisinin resistance has developed[28−40].

It would appear that enrollment parasitaemias significantly influenced the half life of parasitaemia. Thus high parasitaemias were associated with a longer half−life of parasitaemia. The implications of this finding are unclear. In the present study, relating the fall in haematocrit to 1 000 parasites cleared from peripheral blood within a time frame of 24−48 h permitted estimation of falls in a standardized way in children treated with rapidly acting schizontocidal antimalarials. Falls in haematocrit at hyperparasitaemia levels were much lower than at levels below hyperparasitaemia suggesting much conservation of haematocrit or red blood cells in patients with hyperparasitaemia treated with artemisinin−based combination drugs. This finding supports, in a qualified manner, that from more sophisticated studies which quantified RESA−positive parasite−negative red blood cells and showing that rapid malaria clearance after treatment with artemisinin derivatives results from the extraction of drug−affected parasites from host erythrocytes−presumably by the spleen[41]. Thus, the relatively high haematocrit conservation ratio observed in the present study may partly explain why the fall in haematocrit after treatment of hyperparasitaemia is often less than that predicted from loss of parasitized red blood cell in patients treated with artemisinin−based combination drugs.

Children with hyperparasitaemia had higher risk of recrudescence following treatment compared with children without hyperparasitaemia suggesting that children with heavy parasite burden are identified and treated adequately to avoid recrudescence. In conclusion, *P. falciparum* hyperparasitaemia is common in Nigerian children aged 2−10 years, and in the absence of other features of severe malaria, and responds promptly to oral ACTs. It is also associated with increased risk of recrudescence after ACTs.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] World Health Organization. Severe and complicated malaria. *Trans R Soc Trop Med Hyg* 1990; **84**(Suppl 1): 1−65.

[2] World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000; **94**(Suppl 1): 1−90.

[3] Stepniewska K, Ashley E, Lee SJ, Anstey N, Barnes KI, Binh TQ, et al. *In vivo* parasitological measures of artemisinin susceptibility. *J Infect Dis* 2010; **201**: 570−579.

[4] White NJ, Pontavornpinyo W, Maude RJ, Saralamba S, Aguas R, Stepniewska K, et al. Hyperparasitemia and low dosing are an important source of anti−malarial drug resistance. *Malaria J* 2009; **8**: 253.

[5] Salako LA, Ajayi FO, Sowunmi A, Walker O. Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 1990; **84**: 435−445.

[6] Sowunmi A, Walker O, Salako LA. Hyperparasitemia: not a reliable indicator of severity or poor prognosis in falciparum malaria in children in endemic African countries. *Ann Trop Paediatr* 1992; **12**: 155−158.

[7] Crawley J. Reducing the burden of anaemia in infants and young children in malaria−endemic countries of Africa. *Am J Trop Med Hyg* 2004; **71**(Suppl 2): 25−34.

[8] Ehrhardt S, Burchard GD, Mantel C, Cramer JP, Kaiser S, Kubo M, et al. Malaria, anaemia, and malnutrition in African children− defining interventional priorities. *J Infect Dis* 2006; **94**: 108−114.

[9] Davis TME, Krishna S, Looareesuwan S, Supanaranond W, Pukrittayakamee S, Attatasoonthorn K, et al. Erythrocyte sequestration and anaemia in severe falciparum malaria: analysis of acute changes in venous haematocrit using a simple mathematical model. *J Clin Invest* 1991; **86**: 793−800.

[10] Jakeman CN, Saul A, Hogarth WL, Collins WE. Anaemia of acute malaria infections in non−immune patients primarily results from the destruction of uninfected erythrocytes. *Parasitology* 1999; **119**: 127−133.

[11] Sowunmi A, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA, Folarin OA, et al. Therapeutic efficacy and effects of artemether− lumefantrine and amodiaquine−sulfalene−pyrimethamine on gametocyte carriage in children with uncomplicated *Plasmodium falciparum* malaria in southwestern Nigeria. *Am J Trop Med Hyg* 2007; **77**: 235−241.

[12] Sowunmi A, Balogun T, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA. Activities of amodiaquine, artesunate, and artesunate−amodiaquine against asexual− and sexual−stage parasites in falciparum malaria in children. *Antimicrob Agent Chemother* 2007; **51**: 1694−1699.

[13] Michael OS, Gbotosho GO, Folarin OA, Okuboyejo T, Sowunmi A, Oduola AMJ, et al. Early variation in *Plasmodium falciparum* dynamics in Nigerian children after treatment with two artemisinin−based combinations: implications on delayed parasite clearance. *Malaria J* 2010; **9**: 335.

[14] Gbotosho GO, Sowunmi A, Okuboyejo TM, Happi CT, Folarin OO, Michael SO, et al. Therapeutic efficacy and effects of artemether− lumefantrine and artesunate−amodiaquine co−formulated or co−packaged, on malaria−associated anemia in children with

uncomplicated *Plasmodium falciparum* malaria in southwest Nigeria. *Am J Trop Med Hyg* 2011; **84**: 813–819.

[15] Happi CT, Gbotosho GO, Folarin OA, Bolaji OM, Sowunmi A, Kyle DE, et al. Association between mutations in *Plasmodium falciparum* chloroquine resistance transporter and *P. falciparum* multidrug resistance 1 genes and *in vivo* amodiaquine resistance in *P. falciparum* malaria–infected children in Nigeria. *Am J Trop Med Hyg* 2006; **75**: 155–161.

[16] Happi CT, Gbotosho GO, Folarin OA, Sowunmi A, Hudson T, O'Neil M, et al. Selection of *Plasmodium falciparum* multi–drug resistance gene 1 alleles in asexual stages and gametocytes by artemether–lumefantrine in Nigerian children with uncomplicated falciparum malaria. *Antimicrob Agents Chemother* 2009; **53**: 888–895.

[17] Gbotosho GO, Sowunmi A, Happi CT, Okuboyejo TM. Therapeutic efficacies of artemisinin–based combination therapies in Nigerian children with uncomplicated falciparum malaria during five years of adoption as first–line treatments. *Am J Trop Med Hyg* 2011.

[18] WHO. *A word processing database and statistics program for public health on IBM–compatible microcomputers*. Atlanta: Centers for Disease Control and Prevention; 1994.

[19] Djimde AA, Doumbo OK, Traore O, Guindo AB, Kayentao K, Diourte Y, et al. *SPSS for windows release 10.01 (standard version)*. Chicago: SPSS Inc.; 1999.

[20] Mokuolu OA, Okoro EO, Ayetoro SO, Adewara AA. Effect of artemisinin–based treatment policy on consumption pattern of antimalarials. *Am J Trop Med Hyg* 2007; **76**: 7–11.

[21] Hamer DH, Macleod WB, Addo–Yobo E, Duggan CP, Estrella B, Fawzi WW, et al. Age, temperature, and parasitaemia predict chloroquine treatment failure and anaemia in children with uncomplicated *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 2003; **97**: 422–428.

[22] Dorsey G, Gasasira AF, Machekano R, Kamya MR, Staedke SG, Hubbard A. The impact of age, temperature, and parasite density on treatment outcomes from antimalarial trials in Kampala, Uganda. *Am J Trop Med Hyg* 2004; **71**: 531–536.

[23] Sowunmi A, Fateye BA, Adedeji AA, Fehintola FA, Gbotosho GO, Happi TC, et al. Predictors of the failure of treatment with chloroquine in children with acute, uncomplicated, *Plasmodium falciparum* malaria, in an area with high and increasing incidences of chloroquine resistance. *Ann Trop Med Parasitol* 2005; **99**: 535–544.

[24] Sowunmi A, Fateye BA, Adedeji AA, Gbotosho GO, Happi TC, Bamgboye AE, et al. Predictors of the failure of treatment with pyrimethamine–sulfadoxine in children with uncomplicated falciparum malaria. *Acta Tropica* 2006; **98**: 6–14.

[25] Sowunmi A, Adewoye EO, Gbotosho GO, Happi CT, Sijuade AO, Folarin OA, et al. Factors contributing to delay in parasite clearance in uncomplicated falciparum malaria in children. *Malaria J* 2010; **9**: 53.

[26] de Vries JP, Bich NN, Thien HV, Hung LN, Anh TK, Kager PA, et al. Combinations of artemisinin and quinine for uncomplicated falciaprum malaria: efficacy and pharmacodynamics. *Antimicrob Agents Chemother* 2000; **44**: 1302–1308.

[27] Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo AP, et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open–label randomized trial. *Lancet Infect Dis* 2010; **10**: 673–683.

[28] Wongsrichanalai C, Meshnick SR. Declining artesunate–mefloquine efficacy against falciparum malaria on the Cambodia–Thailand border. *Emerg Infect Dis* 2008; **14**: 716–719.

[29] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin–resistant malaria in western Cambodia. *New Engl J Med* 2008; **359**: 2619–2620.

[30] Noedl H, Se Y, Sriwichai S, Schaecher K, Teja–Isavadharm P, Smith B, et al. Artemisinin resistance in Cambodia: a clinical trial designed to address an emerging problem in Southeast Asia. *Clin Infect Dis* 2010; **51**: e82–e89.

[31] Zerihun T, Degarege A, Erko B. Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pac J Trop Biomed* 2011; **1**(4): 289–294.

[32] Fan ZG, Zhang LM, Yan GG, Wu Q, Gan XF, Zhong SF, et al. Bioinformatics analysis for structure and function of CPR of *Plasmodium falciparum*. *Asian Pac J Trop Med* 2011; **4**(2): 85–87.

[33] Ibrahim EA, Kheir MM, Elhardello OA, Almahi WA, Ali NI, Elbashir MI, et al. Cortisol and uncomplicated *Plasmodium falciparum* malaria in an area of unstable malaria transmission in eastern Sudan. *Asian Pac J Trop Med* 2011; **4**(2): 146–147.

[34] Khan HM, Shujatullah F, Ashfaq M, Raza A. Changing trends in prevalence of different *Plasmodium* species with dominance of *Plasmodium falciparum* malaria infection in Aligarh (India). *Asian Pac J Trop Med* 2011; **4**(1): 64–66.

[35] Tangpukdee N, Wai KM, Muangnoicharoen S, Kano S, Phophak N, Tiemprasert J, et al. Indicators of fatal outcome in severe *Plasmodium falciparum* malaria: a study in a tertiary–care hospital in Thailand. *Asian Pac J Trop Med* 2010; **3**(11): 855–859.

[36] Wisedpanichkij R, Chaicharoenkul W, Mahamad P, Prompradit P, Na–Bangchang K. Polymorphisms of the oxidant enzymes glutathione S–transferase and glutathione reductase and their association with resistance of *Plasmodium falciparum* isolates to antimalarial drugs. *Asian Pac J Trop Med* 2010; **3**(9): 673–677.

[37] Nmorsi OPG, Isaac C, Ohaneme BA, Obiazi HAK. Pro–inflammatory cytokines profiles in Nigerian pregnant women infected with *Plasmodium falciparum* malaria. *Asian Pac J Trop Med* 2010; **3**(9): 731–733.

[38] Mauro P, Valentina G, Paolo A. Higher production of tumor necrosis factor alpha in hemozoin–fed–human adherent monocytes is dependent on lipidic component of malarial pigment: new evidences on cytokine regulation in *Plasmodium falciparum* malaria. *Asian Pac J Trop Med* 2010; **3**(2): 85–89.

[39] Nmorsi OPG, Isaac C, Ukwandu NCD, Ohaneme BA. Pro–and anti–inflammatory cytokines profiles among Nigerian children infected with *Plasmodium falciparum* malaria. *Asian Pac J Trop Med* 2010; **3**(1): 41–44.

[40] Lim P, Alker AP, Khim N, Shah NK, Incardona S, Doung S, et al. Pfmdr 1 copy number and artemisinin derivatives combination therapy failure in falciparum malaria in Cambodia. *Malaria J* 2009; **8**: 11.

[41] Chotivanich K, Udomsangpetch R, Dondorp A, Williams T, Angus B, Simpson JA, et al. The mechanism of parasite clearance after antimalarial treatment of *Plasmodium falciparum* malaria. *J Infect Dis* 2000; **182**: 629–633.