ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 10 | October - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CHANGING PATTERN OF HAEMOGLOBIN LEVEL FOLLOWING ANTI-MALARIAL TREATMENT AMONG MALARIA CASES ATTENDING A TERTIARY CARE HOSPITAL IN KOLKATA

Tropical Medicine

Subra Sekhar Nath	Department of Microbiology, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.
Mehebubar Rahman	Department of Tropical Medicine, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.
Sudeshna Mallik	Department of Tropical Medicine, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.
Netai Pramanik*	Department of Tropical Medicine, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India. *Corresponding Author
Dilip Kumar Bera	Department of Microbiology, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.
Pabitra Saha	Department of Zoology, P. R. Thakur Govt. College, Ganti, Thakurnagar, North 24 Parganas, West Bengal, India.
Banya Chakraborty	Department of Microbiology, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.
Subhasish Kamal Guha	Director, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India.

ABSTRACT

Anaemia is one of the major causes of severe and complicated malaria. Malaria associated anaemia are due to decreased production of RBCs and lysis of infected and uninfected erythrocytes. The role of anti-malarial treatment in correcting anaemia are not studied extensively. The present work was undertaken to study the changing pattern of haemoglobin level following anti-malarial therapy. A total of 201 microscopically positive mono-infected with *P. vivax* (103) and *P falciparum* (98) patients were recruited and treated with antimalarial drugs and followed up on day 3, 14, and 28 to study the changing pattern of haemoglobin level. Among the *P. falciparum* positive patients mean haemoglobin level on Day 0 and day 28 was 13.17 g/dl and 13.31 g/dl whereas among *P. vivax* cases mean haemoglobin level was 13.28 g/dl and 13.29g/dl, respectively. Among the *P. falciparum* cases (n = 98), 4.08%, 16.33% and 79.59% was classified as moderate anaemia, mild anaemia and normal, respectively. Similarly, among the *P. vivax* cases (n = 103), 1.94%, 17.47% and 80.58% had moderate anaemia, mild anaemia and normal, respectively. Mean haemoglobin level was declined on day 3 which gradually increased to its initial level by day 28 among both *P. falciparum* and *P. vivax* cases. Similar study in other malaria endemic areas will be helpful for better understanding the changing pattern of haemoglobin level among malaria patients.

KEYWORDS

P. *vivax, P. falciparum*, anaemia, haemoglobin, packed cell volume

INTRODUCTION:

Morbidity and mortality due to malaria still remains a great challenge, in spite of recent progress in roll back malaria programme. The burden of disease remains high with an estimated 229 million clinical cases and 409000 malaria-related deaths [1]. Severe and complicated malaria is a serious clinical manifestation which was initially associated with *P. falciparum* but *P. vivax* is also found to be associated with it [2]. Anaemia is a common symptom among malaria infected individuals. There are two different causes of malaria associated anaemia: due to decreased production of red cell (Central) and haemolysis of infected and uninfected erythrocytes (peripheral causes) [3, 4].

Due to development and wide spread distribution of resistant strains to different antimalarial components, artemisinin-based combination therapy (ACT) is the treatment of choice for uncomplicated falciparum malaria throughout the World [5]. ACT is generally well-tolerated and safe, but its potential concern remains with anaemia particularly for artemisinin derivatives [6]. Among malaria infected patients, delayed haemolytic anaemia has been documented following artesunate monotherapy [7], but no such event was recorded in case of treatment with ACT in uncomplicated falciparum malaria [8-12].

Chloroquine remains the drug of choice for the treatment of *P. vivax* malaria in areas where the parasite is sensitive to it. Chloroquine is a rapidly acting erythrocytic schizontocidal agent against all species of plasmodia, controls most attacks in 1-2 days with disappearance of parasites from peripheral blood. Chloroquine has a proven anti-inflammatory property which reduce the inflammation-induced iron delocalisation. So, it enhances speedy erythropoietic recovery after malaria infection [13].

Early treatment of malaria cases can prevent the impending reduction in haemoglobin level and helps in speedy haematological recovery [14]. Despite the overall benefits of anti-malarial treatment, haemoglobin typically falls slightly following initiation of an anti-malarial drug reaching a base between days three to 7 after treatment [14]. Treatment with artemisinin derivatives helps to an extremely rapid reduction in malaria parasite biomass but they also temporarily reduce red blood cell production [15]. Previously reported that treatment of vivax malaria patients with artesunate + pyronaridine was associated with a greater mean reduction in haemoglobin at days 3and 7 [17]. Complete removal of blood stage malaria parasites after blood schizontocidal treatment allows faster haematological recovery i.e., pre-infection haemoglobin concentrations are generally achieved in approximately 4–5 weeks following effective treatment [14]. The present study was designed to study the changing pattern of haemoglobin level following anti-malaria treatment among malaria positive patients in Kolkata, West Bengal.

MATERIALS AND METHODS

Study site and design:

The study was conducted from June, 2017 to April, 2018 at the Malaria Clinic, Department of Protozoology, Calcutta School of Tropical Medicine, Kolkata, India, where the annual parasite index (API) was 3.72 in 2016. In Kolkata, malaria transmission is seasonal (July-December) and *P. falciparum* and *P. vivax* are the two predominant species, with an incidence of almost 1:1.

The study was an observational study to determine of time duration required for restoration of normal haemoglobin level and PCV among microscopically positive mono-infected *P. falciparum* and *P. vivax* cases after completion of anti-malarial treatment without any

supplementary treatment. The study was approved by the Institutional Ethics Committee of the Calcutta School of Tropical Medicine.

Screening and enrolment of patients:
The febrile patients from the surrounding areas attending the Malaria Clinic of the Calcutta School of Tropical Medicine were screened for malarial parasite by examining Giemsa stained thick and thin peripheral blood smears (PBS) and dual RDTs (SD Bioline One step Malaria HRP2 (Pf) and pLDH (Pv) antigen rapid test kit).Confirmed *P. falciparum* and *P. vivax* mono-infection patients were explained about the study protocol and requested to participate in the study. The patients who fulfilled the following inclusion criteria like microscopically confirmed *P. falciparum* and *P. vivax* mono infection, with fever (axillary temperature $\geq 37.5^{0}$C) or history of fever in preceding 24 hours, and no history of malaria or anti-malaria treatment within last one months, no signs and symptoms of complicated malaria were enrolled after obtaining written, informed consent.

Treatment and follow-up: The study enrolled patients were treated as per the Guidelines for Diagnosis and Treatment of malaria in India, 2011. Confirmed *P. falciparum* mono-infected patients were treated with AS+SP combination – artesunate at 4 mg/kg body weight once daily for 3 days and a single dose of SP at 25/1.25 mg base/kg body weight on day 0 and a single dose of primaquine at a dose of 0.75 mg/kg body weight on day 1. Whereas, Confirmed *P. vivax* mono-infected cases were treated with chloroquine in full therapeutic dose of 25 mg/kg body weight divided over a period of three days and primaquine at a dose of 0.25 mg/kg body weight daily for 14 days. The day, the patient was enrolled and received the first dose of medicine was designated day 0. After enrolment all patients were given a follow-up schedule for attending the clinic on days 3, 14 and 28. During this follow-up period, study participants were screened for haemoglobin level and packed cell volume (PCV).

Laboratory Methods:
Microscopic blood examination and parasite count: Collected thick and thin blood smear slides PBS were stained with Giemsa stain and examined by expert microscopist for presence malaria parasites. The number of parasites per 200 WBC were counted in Giemsa-stained thick blood films collected on day 0. Assuming a WBC count to be 8000/µL, parasitaemia was calculated and expressed as per µL of blood. A thick smear was considered as negative on initial review if no parasites were seen in 100 oil immersion fields. All positive and negative slides were cross checked by a second microscopist.

Rapid diagnostic test:
The study participants were also tested by dual RDT kit (SD Bioline One step Malaria HRP2 (Pf) and pLDH (Pv) antigen rapid test kit) for detection of malarial antigen on day 0. The RDTs are rapid, qualitative immunochromatographic test for the differential detection of HRP2 antigen specific to *P. falciparum* and pLDH antigen specific to *P. vivax* in human whole blood. All RDTs were performed as per manufacturer instruction.

Estimation of Haemoglobin: The haemoglobin level of the study patients was estimated on day 0, 3, 14, and 28. The cyanmethemoglobin method was used for this. In brief, blood was mixed with Drabkin's solution and all forms of haemoglobin present in the blood were converted into cyanmethemoglobin. After completion of the reaction absorbance of the solution was measured in a spectrophotometer at 540 nm. The amount of haemoglobin present in the unknown sample was estimated by comparing its absorbance with that of the standard cyanmethemoglobin solution by using the following formula.

$$\text{Hemoglobin in gm/dl} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \times \frac{\text{Dilution factor}}{100}$$

Determination of packed cell volume (PCV): The PCV value of the study patients was estimated on day 0, 3, 14, and 28. The Wintrobe method was used for this. In brief, anticoagulant was mixed thoroughly with blood sample and draw it in the Wintrobe tube which is filled exactly up to the100 marks. The sample was centrifuged at 2300 g for 30 min and the length of the column of red cells was taken as reading.

Data analysis: The recorded observations of all study variables were double entered in MS Excel and validated. The data analysis and calculation of basic statistics was carried out by using Minitab version 17. The association of different factors and level of haemoglobin and PCV in different follow up days were analysed by chi square test by using Minitab version 17.

RESULTS:
Base line characteristics of the study participants:
During the study period, a total of 1,321 patients with fever attended the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata and screened for malaria parasites by PBS and RDTs examination. Among the tested individuals, 253 were positive for *P. falciparum* and 361were positive for *P. vivax*, while 17 had mixed infections. Among the confirmed mono-infected *P. falciparum*-positive cases, 98 subjects were consented and enrolled in the study, while 103 mono-infected *P. vivax* patients were participated in the study. The base line characteristics of the study patients were summarized in **Table 1**. The mean age, temperature and parasite count of the *P. falciparum* positive patients at D0 was 36.58 years (95% CI: 33.91 – 39.26), 37.86 °C (95%CI: 37.68 – 37.98) and 5257.20 (95%CI: 3892.2 – 6622.2). While, at D0 the mean age, temperature and parasite count of the *P. vivax* positive patients was 33.44 years (95% CI: 30.78 – 36.09), 37.92°C (95%CI: 37.86 – 38.05) and. 4171.94 (95%CI: 3458.3 – 4985.5). The day 0 mean haemoglobin level was 13.17 g/dl and 13.26 g/dl whereas mean day 0 PCV value was 41.01% and 41.28% among the *P. falciparum* and *P. vivax* patients, respectively. At day 0, 3.06%, 16.3% and 79.6% *P. falciparum* patients were moderate anaemic, mild anaemic and normal respectively. While 1.9%, 17.5% and 80.6% patients with *P. vivax* infections were moderate anaemic, mild anaemic and normal respectively.

Table 1: Base line characteristics of study patients

Characteristics	Pf (n = 98)	Pv (n = 103)
Age (yr)		
Mean	36.58	33.44
Range	12 – 65	16 – 69
SD	± 13.33	± 13.54
95% CI	33.91 – 39.26	30.78 – 36.09
Temperature (°C)		
Mean	37.86	37.92
Range	37.5 – 38.3	37.6 – 39.1
SD	± 0.17	± 0.41
95% CI	37.68 – 37.98	37.86 – 38.05
Parasite count (no/µl)		
Mean	5257.20	4171.94
Range	400 – 36840	320 – 21800
SD	± 6975.5	± 4052.1
95% CI	3892.2 – 6622.2	3458.3 – 4985.5
Haemoglobin (g%)		
Mean	13.17	13.26
Range	9.0 – 14.8	8.0 – 14.80
SD	± 1.25	± 1.28
95% CI	12.93 – 13.43	13.02 – 14.8
Anaemia gradation [n, (%)]		
Moderate (Hb% = 8-10)	3 (3.06)	2 (1.9)
Mild (Hb% = 10-12	16 (16.3)	18 (17.5)
Normal (Hb%>12)	78 (79.6)	83 (80.6)
PCV		
Mean	41.01	41.28
Range	29.0 – 55.0	25.0 – 50.0
SD	± 4.59	± 4.15
95% CI	40.08 – 41.93	40.47 – 42.09

Treatment and follow-up: All *P. falciparum* mono-infected study patients were treated with AS+SP combination whereas, all *P. vivax* mono-infected cases were treated with chloroquine and primaquine as described earlier. After enrolment, study patients were screened clinically and their haemoglobin and PCV level were estimated on day 3, 14 and 28 days. During the follow-up it was observed that clinical sign and symptoms were subsidised within 3-7 day of post treatment whereas the haemoglobin and PCV level of study participants initially lowered on day 3 in respect to the day 0 level and then haemoglobin and PCV level started increasing in subsequent days.

Pattern of changes in haemoglobin during follow-up:
Among the *P. falciparum* positive patients mean haemoglobin level on Day 0 and day 28 was 13.17 g/dl and 13.31 g/dl whereas among *P. vivax* cases mean haemoglobin ievel was 13.28 g/dl and 13.29g/dl, respectively. On day 3, it was recorded that both *P. falciparum* and *P.*

vivax cases mean haemoglobin level was lower than the day 0 values i.e., 11.67 g/dl and 11.68g/dl, respectively which again increased during follow up period (Table 2). No significant difference was found between the mean haemoglobin level of *P. falciparum* and *P. vivax* patients group containing parasite count <5000/µl and >5000/µl blood (Table 3, 4).

Table 2: Pattern of changes in Hb% among *P. falciparum and P. vivax* malaria cases

Factors		Mean Hb level			
		D 0	D 3	D 14	D 28
Parasite species	Pf (n = 98)	13.17	11.67	12.35	13.31
	Pv (n = 103)	13.28	11.68	12.4	13.29

Table 3: Pattern of changes in Hb level among *P. falciparum* cases with different parasite count

Factors		Mean Hb level			
		D 0	D 3	D 14	D 28
Parasite count	PC<5000 (n = 67)	13.05	11.55	12.18	13.17
	PC>5000 (n = 31)	13.44	11.94	12.71	13.61

Table 4: Pattern of changes in Hb% among *P. vivax* cases with different parasite count

Factors		Mean Hb level			
		D 0	D 3	D 14	D 28
Parasite count	PC<5000 (n = 73)	13.45	11.89	12.67	13.22
	PC>5000 (n = 30)	13.44	11.94	12.67	13.47

Among the *P. falciparum* cases (n = 98), 4.08%, 16.33% and 79.59% had moderate anaemia, mild anaemia and normal, respectively. Mean haemoglobin level on day 3 was reduced by 4.68%, 7.67% and 12.66% with respect to day 0 haemoglobin level among moderate anaemic, mild anaemic and normal cases, respectively (Table 5).

Table 5: Pattern of changes in Hb level among *P. falciparum* malaria cases with different grade of anaemia at day 0

Factors		Mean Hb level			
		D 0	D 3	D 14	D 28
Day 0 anaemia level	Moderate (Hb% = 8-10) [n = 4]	9.60	9.15	10.8	11.35
	Mild (Hb% = 10-12) [n = 16]	11.73	10.83	11.64	12.41
	Normal (Hb%>12) [n = 78]	13.66	11.93	12.58	13.59

Among the P. vivax cases (n = 103), 1.94%, 17.47% and 80.58% had moderate anaemia, mild anaemia and normal, respectively. Mean haemoglobin level on day 3 was reduced by 4.54%, 10.44% and 12.65% with respect to day 0 haemoglobin level among moderate anaemic, mild anaemic and normal cases, respectively (Table 6).

Table 6: Pattern of changes in Hb% among P. vivax malaria cases with different grade of anaemia at day 0

Factors		Mean Hb level			
		D 0	D 3	D 14	D 28
Day 0 anaemia level	Moderate (Hb% = 8-10) [n = 2]	8.8	8.4	9.4	10
	Mild (Hb% = 10-12) [n = 18]	11.59	10.38	11.1	11.99
	Normal (Hb%>12) [n = 83]	13.75	12.01	12.75	13.66

Pattern of changes in PCV during follow-up:

Among the *P. falciparum* positive patients mean PCV value on Day 0 and day 28 was 41.01% and 41.23% whereas among *P. vivax* cases mean PCV value was 41.28% and 41.35, respectively. It was recorded that both *P. falciparum* and *P. vivax* cases mean PCV value was lower than the day 0 values i.e., 36.35% and 36.38%, respectively which again increased during follow up period (Table 7). No significant difference was found between the mean PCV value of *P. falciparum* and *P. vivax* patients group containing parasite count <5000/µl and >5000/µl blood (Table 8, 9).

Table 7: Pattern of changes in PCV among *P. falciparum* and *P. vivax* malaria cases

Factors		Mean PCV			
		D 0	D 3	D 14	D 28
Parasite species	Pf (n = 98)	41.01	36.35	38.26	41.23
	Pv (n = 103)	41.28	36.38	38.17	41.35

Table 8: Pattern of changes in PCV among *P. falciparum* cases with different parasite count

Factors		PCV			
		D 0	D 3	D 14	D 28
Parasite count	PC<5000 (n = 67)	40.79	36.04	37.97	40.88
	PC>5000 (n = 31)	41.48	37.03	38.90	42.0

Table 9: Pattern of changes in Hb% among *P. vivax* cases with different parasite count

Factors		PCV			
		D 0	D 3	D 14	D 28
Parasite count	PC<5000 (n = 73)	42.13	37.13	37.69	41.14
	PC>5000 (n = 30)	41.48	37.03	39.3	41.87

Among the *P. falciparum* cases (n = 98), 4.08%, 16.33% and 79.59% had moderate anaemia, mild anaemia and normal, respectively. Mean PCV value on day 3 was reduced by 4.13%, 7.2% and 12.57% with respect to day 0 PCV value among moderate anaemic, mild anaemic and normal cases, respectively (Table 10).

Table 10: Pattern of changes in PCV value among *P. falciparum* malaria cases with different grade of anaemia at day 0

Factors		Avg. PCV			
		D 0	D 3	D 14	D 28
Day 0 anaemia level	Moderate (Hb% = 8-10) [n = 4]	30.25	29.0	33.0	35.0
	Mild (Hb% = 10-12) [n = 16]	36.25	33.63	36.19	38.56
	Normal (Hb%>12) [n = 78]	42.54	37.19	38.96	42.10

Among the *P. vivax* cases (n = 103), 1.94%, 17.47% and 80.58% had moderate anaemia, mild anaemia and normal, respectively. Mean PCV value on day 3 was reduced by 4.35%, 10.61% and 12.48% with respect to day 0 PCV value among moderate anaemic, mild anaemic and normal cases, respectively (Table 11).

Table 11: Pattern of changes in PCV value among *P. vivax* malaria cases with different grade of anaemia at day 0

Factors		Avg. PCV			
		D 0	D 3	D 14	D 28
Day 0 anaemia level	Moderate (Hb% = 8-10) [n = 2]	28.0	26.5	29.5	31.5
	Mild (Hb% = 10-12) [n = 18]	36.67	32.78	34.56	37.5
	Normal (Hb%>12) [n = 83]	42.6	37.28	39.16	42.42

DISCUSSION:

The comparison of malaria associated anaemia between pre-infection and following treatment is very difficult, as no records on haemoglobin level are available for any patients before infection. The role of antimalarial treatment in correction of malaria associated anaemia may be estimated by determining the level of haemoglobin just before initiation of treatment and weekly follow up for one to two months post treatment period. In the present study, microscopically positive 103 P vivax and 98 P falciparum patients were followed up for one month to determine the changing pattern of haemoglobin level following treatment.

The initial mean haemoglobin level was almost similar among *P. falciparum* and *P. vivax* patients (Pf: mean 13.17, range 9.0-14.8; Pv: mean 13.26, range 8.0-14.8). The parasitaemia was higher for *P. falciparum* than that *P. vivax* positive patients (Pf: 5257.2, Pv: 4171.94). The initial declined level of haemoglobin was almost similar in both the cases. It might be due to removal of absolute no. of RBCs were same for both the malarial parasite [17, 18, 19]. Evidences showed that about 34 normal RBCs are cleared against every infected cell by *P. vivax* [20]. This ratio is lower in case of *P. falciparum* [14, 21].

In the present study, it was observed that most of the patients had normal haemoglobin level (>12 g/dl) with few mild (10-12 g/dl) and very little moderate (8-10 g/dl) anaemic cases. In all these cases haemoglobin level dropped initially up to 3 days post treatment period which gradually regained to their initial level (day 0) by 28 days of follow up period. This event was found to be similar among *P. falciparum* and *P. vivax* positive patients. Similar haemoglobin changing pattern following anti-malarial treatment was also reported from different studies [14, 22]. The pattern of haemolysis over the follow up period and its recovery following anti-malarial treatment has been classified as delayed and persistent haemolysis [7]. In the presence study no case of delayed and persistent haemoglobin was

recorded. Normal recovery of haemoglobin level was observed in all cases irrespective of parasite species and drug used. In contrast late haemolysis following treatment with normal recovery has been reported by other studies [23-27]. Observed initial decreased haemoglobin level may be due to drug induced haemolysis of both parasitized and normal erythrocytes as reported by other studies [14].

Present study showed that most of the malaria patients infected either *P. falciparum* or *P. vivax* showed normal haemoglobin level on day 0. In both *P. falciparum* and *P. vivax* cases, following anti-malarial treatment haemoglobin level declined steadily up to day 3 which gradually regained to their day 0 level by day 28 without any supplementary treatment.

Acknowledgements:
We sincerely acknowledge to the Director of Calcutta school of tropical medicine for his permission to publish the data. We also acknowledge to the laboratory members of the Department of Microbiology, Calcutta School of Tropical Medicine.

Conflict of interest: We have no conflict of interest

REFERENCES:

1. WHO, 2021. World Health Organization, Malaria fact sheet. https:// www. who.int/ news-room/fact-sheets/detail/malaria.
2. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, Price RN, 2012. The anaemia of Plasmodium vivax malaria. Malaria Journal 2012 :135.
3. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, et al., 2011. Severe malarial anemia: innate immunity and pathogenesis. Int J Biol Sc. 7: 1427–42.
4. Krystal JE, Hansen DS, van Rooijen N, Buckingham LA, Schofield L, 2006. Severe malarial anemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes. Blood.107:1192–9.
5. WHO, 2015. World Health Organisation: Guidelines for the treatment of malaria. Third edition (2015) http://apps.who.int/iris/bitstream/10665/162441/1/ 9789241549127 eng.pdf.
6. Finaurini S, Ronzoni L, Colancecco A, Cattaneo A, Cappellini MD, et al., 2010. Selective toxicity of dihydroartemisinin on human CD34+ erythroid cell differentiation. Toxicology 276:128–34.
7. Rehman K, Lötsch F, Kremsner PG, Ramharter M, 2014. Haemolysis associated with the treatment of malaria with artemisinin derivatives: a systematic review of current evidence. Int J Infect Dis. 29:268–73.
8. Adjuik M, Agnamey P, Babiker A, Borrmann S, Brasseur P, et al., 2002. Amodiaquine artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. Lancet.359: 1365–72.
9. Olliaro P, Nevill C, LeBras J, Ringwald P, Mussano P, et al., 1996. Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet. 348: 1196–201.
10. Sirima SB, Tiono AB, Gansane A, Diarra A, Ouedraogo A, et al., 2009. The efficacy and safety of a new fixed-dose combination of amodiaquine and artesunate in young African children with acute uncomplicated Plasmodium falciparum. Malar J. 8:48.
11. Gasasira AF, Kamya MR, Achan J, Mebrahtu T, Kalyango JN, et al., 2008. High risk of neutropenia in HIV-infected children following treatment with artesunate plus amodiaquine for uncomplicated malaria in Uganda. Clin Infect Dis. 46:985–91.
12. Zwang J, Ndiaye JL, Djimdé A, Dorsey G, Mårtensson A, et al., 2012. Comparing changes in haematologic parameters occurring in patients included in randomized controlled trials of artesunate-amodiaquine vs single and combination treatments of uncomplicated falciparum in sub-Saharan Africa. Malar J. 11:25.
13. Legssyer R, Ward RJ, Crichton RR, et al. 1999. Effect of chronic chloroquine administration on iron loading in the liver and reticuloendothelial system and on oxidative responses by the alveolar macrophages. Biochem Pharmacol 57:907–11.
14. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirijaroen L, ter Kuile F, Chongsuphajaisiddhi T, White NJ, 2001. Factors contributing to anemia after uncomplicated falciparum malaria. Am J Trop Med Hyg. 65:614–622.
15. Kongpatanakul S, Chatsiricharoenkul S, Khuhapinant A, Atipas S, Kaewkungwal J, 2009. Comparative study of dihydroartemisinin and artesunate safety in healthy Thai volunteers. Int J Clin Pharmacol Ther 47:579–586.
16. Poravuth Y, Socheat D, Rueangweerayut R, Uthaisin C, Phyo AP, Valecha N, Rao BHK, Tjitra E, Purnama A, Borghini-Fuhrer I, Duparc S, Shin C, Fleckenstein L, 2011. Pyronaridine-artesunate versus chloroquine in patients with acute Plasmodium vivax malaria: a randomized, double-blind, noninferiority trial. PLoS One 6:e14501.
17. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, Lampah DA, Price RN, 2008. Multidrug-resistant Plasmodium vivax associated with severe and fatal malaria: a prospective study in Papua, Indonesia. PLoS Med. 5:e128.
18. Rodriguez-Morales AJ, Sanchez E, Vargas M, Piccolo C, Colina R, Arria M, Franco-Paredes C, 2006. Is anemia in Plasmodium vivax malaria more frequent and severe than in Plasmodium falciparum? Am J Med. 119:e9–e10.
19. Fernandes AA, Carvalho LJ, Zanini GM, Ventura AM, Souza JM, Cotias PM, Silva-Filho IL, Daniel-Ribeiro CT, 2008. Similar cytokine responses and degrees of anemia in patients with Plasmodium falciparum and Plasmodium vivax infections in the Brazilian Amazon region. Clin Vaccine Immunol. 15:650–658.
20. Collins WE, Jeffery GM, Roberts JM, 2003. A retrospective examination of anemia during infection of humans with Plasmodium vivax. Am J Trop Med Hyg. 68:410–412.
21. Jakeman GN, Saul A, Hogarth WL, Collins WE, 1999. Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. Parasitology. 119:127–133.
22. Leslie T, Mayan MI, Hasan MA, Safi MH, Klinkenberg E, Whitty CJ, Rowland M, 2007. Sulfadoxine-pyrimethamine, chlorproguanil-dapsone, or chloroquine for the treatment of Plasmodium vivax malaria in Afghanistan and Pakistan: a randomized controlled trial. JAMA. 297:2201–2209.
23. Rolling T, Wichmann D, Schmiedel S, Burchard GD, Kluge S, Cramer JP, 2013. Artesunate versus quinine in the treatment of severe imported malaria: comparative analysis of adverse events focussing on delayed haemolysis. Malar J. 12:241.
24. Eder M, Farne H, Cargill T, Abbara A, Davidson RN, 2012. Intravenous artesunate versus intravenous quinine in the treatment of severe falciparum malaria: a retrospective evaluation from a UK centre. Pathog Glob Health. 106:181–7.
25. Zoller T, Junghanss T, Kapaun A, Gjorup I, Richter J, Hugo-Persson M, et al., 2011. Intravenous artesunate for severe malaria in travelers, Europe. Emerg Infect Dis, 17:771–7.
26. Kreeftmeijer-Vegter AR, van Genderen PJ, Visser LG, Bierman W, Clerinx J, van Veldhuizen C, et al., 2012. Treatment outcome of intravenous artesunate in patients with severe malaria in the Netherlands and Belgium. Malar J. 11:102.
27. Rolling T, Agbenyega T, Issifou S, Adegnika AA, Sylverken J, Spahlinger D, et al., 2014. Delayed hemolysis after treatment with parenteral artesunate in African children with severe malaria—a double-center prospective study. J Infect Dis 209(Jun (12)):1921–8.