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Case Report

Artemisinin-based combination therapy successfully treated two hyperparasitaemic *Plasmodium falciparum* cases

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Abstract

Hyperparasitaemia is an important event in the cascade of *Plasmodium falciparum* severe malaria (SM), and may also lead to SM associated complications and death, if left untreated. Here, we report two hyperparasitaemic patients with no life-threatening complications. Malaria diagnosis was performed using thick and thin blood smears and immunochromatographic-based rapid diagnostic tests (RDTs) purchased from three different manufacturers. Parasitaemia was calculated following the World Health Organization (WHO) guidelines. Haematological and biochemical investigations were also performed. Weekly follow-up of blood smear examination, blood pressure and temperature were recorded up to day 63. The first patient had 42% parasitaemia (100% asexual parasites). The second patient had 9.5% parasitaemia, comprising 46% asexual and 54% sexual stages, with a 1:1 male to female ratio. On the day of admission, both had presented abnormal haematological and biochemical parameters compared to the reference values. Remarkably, both the patients recovered successfully with oral artemisinin-based combination therapy (ACT) and a single dose of primaquine on day 1. Weekly follow-up did not show any parasite suggesting successful treatment with ACT without any side effects. The presence of hypergametocytaemia may hinder malaria elimination efforts, if not treated immediately.

Key words: *Plasmodium falciparum*; severe malaria; hyperparasitaemia; hypergametocytaemia; ACT; primaquine.

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Introduction

Plasmodium falciparum infection is characterized by >10% parasitized red blood cells (PRBCs), generally known as hyperparasitaemia, which is one of the features leading to severe malaria (SM) [1]. Cerebral malaria (CM), severe anemia, multiple organ dysfunction, and respiratory failure are the common complications associated with severe *P. falciparum* infection. This is suggestive of sequestered parasites in the vital host organs and a threat for recrudescent infections [2,3]. Hyperparasitaemia has also been associated with high gametocyte carriage [4] and *de novo* anti-malarial drug resistance [5], both of which have implications for malaria elimination efforts. Generally, hyperparasitaemia is found in non-immune [6] and semi-immune individuals [7]. Hyperparasitaemia among the Thai population was associated with poor prognosis in adults suffering from CM [8], whereas, it increased the risk of CM in Nigerian children [9]. Hyperparasitemia in Indian patients has been correlated with an amplified risk of mortality with multi-organ dysfunction [10], and death within a short period of time [11]. Here, we report the cases of two Indian adults who had *P. falciparum* hyperparasitaemia with no severe or adverse events. However, one of them presented hypergametocytaemia.

Methodology

Study design

The two prospective adult males infected with hyperparasitaemia of *P. falciparum* visited the malaria clinic at the Wenlock District Government Hospital, Mangalore, Karnataka State, India for diagnosis and treatment. Clinical, biochemical, hematological, and parasitological outcomes as well as treatment details of two patients have been presented in this brief report. The Indian reference value system for these parameters was considered [12].

Ethical statement

Prior written informed consent was obtained from both the patients. The institutional review board of the ICMR-National Institute of Malaria Research, New Delhi, India, reviewed and approved the study (Ref. No-ECR/NIMR/EC/2012/39). The research and ethics committee of the Kasturba Medical College (KMC) under the Manipal Academy of Higher Education, Mangalore, Karnataka, India, also approved the study (Ref. No. IEC KMC MLR 03-16/49). All essential regulatory procedures were followed strictly in compliance with the Helsinki Declaration.

Malaria diagnosis and quantification of parasites

Both thick and thin blood smears on glass micro slides were prepared from spirit-swabbed finger pricking [13]. These smears were stained with 10% Giemsa and examined under 100X immersion oil objective lens using Carl Zeiss Primo Star light microscope (Zeiss, Jena, Germany) [14]. Images were captured on Zeiss AxioPhot microscope (Zeiss, Jena, Germany). Two trained expert microscopists examined both thick and thin smears for diagnosis and determination of parasite count; the senior expert confirmed the results. The mean values of two counts were taken for estimating final parasitaemia. The percent parasite density in the thin films was determined by counting the number of infected red blood cells (iRBCs) examined against 5000 RBCs in 20 microscopic fields considering the actual number of RBCs/μL for each patient, i.e., 3.87 and 3.69 million/mm^3, respectively. Similarly, parasitaemia per μL of blood in the thick smears was calculated by enumerating the number of parasites counting 200 white blood cells (WBCs) in relation to the actual number of WBCs/μL, i.e. 6900 and 6000/mm^3, respectively. In addition, the National Center for Vector Borne Diseases Control (NCVBDC) approved rapid diagnostic test (RDT) kits were used as per the manufacturer's instructions to confirm *Plasmodium* infections. These kits were FalciVax™ Rapid Test for Malaria Pv/Pf (Ref. No.: 50301002), (Tulip Diagnostics, Bangalore, India), Onsite Malaria Pf/Pv Ag Rapid Test (Ref. No.: R0112C), (CTK Biotech, Poway, California, USA) and SD Bioline Malaria Ag P.f/P.v (Ref. No.: 05FK80), (Standard Diagnostics Inc. Yongin-si, South Korea) targeting both *P. vivax*-specific *Plasmodium* lactate dehydrogenase (pLDH) and *P. falciparum*-specific histidine rich protein-2 (HRP-2) antigens.

Laboratory procedures

Upon admission, 4mL venous blood (pre-treated) from the two patients was collected in Ethylenediamine tetraacetic acid (EDTA) vacutainers (BD Vacutainer®; Cat. No. 367861, Becton Dickinson, New Jersey, USA) for haematological tests. Another 4 mL of blood was collected in Clot Activator Vacutainers (BD Vacutainer® 367812, Becton Dickinson, New Jersey, USA) for biochemical analysis for liver and kidney

Table 1. Physical and clinical characteristics of two patients (from day 0 to day 7).

Characteristics	Patient 1			Patient 2		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
Weight (kg)	66	65	64.5	60	60.8	59
Height (cm)	165	165	165	162	162	162
Blood Pressure (mmHg)	130/70	150/100	124/84	140/90	130/88	126/82
Axillary temperature	39 °C	37.5 °C	36.8 °C	38.5 °C	37.2 °C	36.4 °C
Heart rate (bpm)	100	84	72	88	84	76
Eyes	Burning sensation	Normal	Normal	Burning sensation	Normal	Normal
Throat	Sore	Normal	Normal	Sore	Normal	Normal
Chest	Burning sensation	Normal	Normal	Burning sensation	Normal	Normal
Neurological system	Normal	Normal	Normal	Normal	Normal	Normal
Lymphatic system	Normal	Normal	Normal	Normal	Normal	Normal
Chills	Yes	Yes	No	Yes	Yes	No
Headache	Yes	Yes	No	Yes	Yes	No
Nausea	Yes	No	No	Yes	No	No
Vomiting	Yes (3 times)	No	No	Yes (> 3 times)	Yes	No
Cough	Yes	Yes	No	Yes	No	No
Shivering	Yes	No	No	Yes	No	No
Loss of appetite/anorexia	Yes	Yes	No	Yes	No	No
Fatigue	Yes	Yes	Yes	Yes	No	No
Myalgia (back and limbs)	No	No	No	No	No	No
Jaundice	Yes	Yes	No	Yes	No	No
Hepatomegaly	No	No	No	No	No	No
Splenomegaly	Palpable	Palpable	No	Palpable	Palpable	No

function tests. The DxH 800 Hematology (Beckman Coulter, Brea, California, USA) and Cobas® 6000 (Roche, Basel, Switzerland) analyzers were used for haematological and biochemical tests, respectively. All the tests were performed at the National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited laboratory services at Kasturba Medical College, Mangalore, India.

Treatment and follow-up

Both the inpatients were treated orally with artemisinin-based combination therapy (ACT) – artesunate 200 mg and 1500 mg sulfadoxine plus 75 mg pyrimethamine on day 0; artesunate 200 mg on day 1 and day 2, respectively, followed by a single dose of 45 mg primaquine on day 1 as per the guidelines of the national programme [15]. All required safety measures and prognosis of oral ACT therapy were monitored in the hospital as per the protocol.

Both the patients were followed weekly up to day 63. Blood smear examination and temperature were recorded for each visit.

Results

Clinical manifestations

The two adult patients aged 35 and 36 years, respectively, presented almost similar clinical manifestations with high-graded fever of 39 °C and 38.5 °C, respectively. Upon admission, both the patients had higher heart rates and abnormal breathing issues. Clinical assessment revealed no neurological and lymphatic abnormalities, but the patients had palpable splenomegaly at the time of admission. Detailed information on physical and clinical manifestations are presented in Table 1.

Haematological and biochemical analyses

On the day of admission i.e., day 0, both the patients presented abnormal haematological and biochemical parameters as shown in Table 2. However, total leukocyte count, total protein, albumin, globulin and their ratios were within the normal limits. Patient 2 had severe anaemia and acute kidney injury. Both patients presented hypoglycemia and clinical jaundice. Further details on haematological and biochemical test results are shown in Table 2.

Patient 1

The microscopic examination of peripheral blood smears confirmed only asexual stages of *P. falciparum* parasites based on typical morphological characteristics. Parasitaemia was 1668824/µL of blood based on thick smear examination and 42% on a thin smear. Figures 1A and 1B show thick smear images,

Table 2. Serological, haematological and biochemical test results of two patients.

	Patient 1	Patient 2	Reference values
HIV	Negative	Negative	
HBsAg	Negative	Negative	
Hb (gm/dL)	11.1	6	Male (14-18); Female (12-16)
TLC (million/mm^3)	6900	6000	4500-11000
PCV %	34.9	30	35-45
Total RBC (million/cu.mm)	3.87	3.69	4.0-6.0
Total Platelets (lakh/cu. mm)	24000	50000	150000-400000
Blood Sugar (mg/dL)	45	49	70-140
Blood urea (mg/dL)	58.4	49	10-45
Serum Creatinine (mg/dL)	0.66	11	0.4-1.4
Total Bilirubin (mg/dL)	8.37	3.89	0.3-1.2
Dir. Bilirubin (mg/dL)	3.5	2	0.0-0.3
Ind. Bilirubin (mg/dL)	4.87	1.95	0.2-0.7
SGOT/AST (IU/L)	96.5	96	17-59
SGPT/ALT (IU/L)	70	56.7	< 50.0
Alkaline Phosphate (IU/L)	563.3	389.9	98-279
Total Protein (g/dL)	6.54	7.91	6.0-8.3
Albumin (g/dL)	3.85	3.98	3.5-5.2
Globulin (g/dL)	2.69	2.5	2.5-3.5
A/G Ratio	1.43	2	1.0-2.3
Parasitaemia/µL	16,68,824	304000	
Asexual %	100%	136800 (46%)	
Sexual %	0	167200 (54%)	
Male gametocyte %	0	51%	
Female gametocyte %	0	49%	

HIV: Human immunodeficieny virus; HBsAg: Hepatitis B surface antigen; Hb: Haemoglobin; PCV: Packed cell volume; TLC: Total leucocyte count; RBC: Red blood cell; SGOT: Serum glutamic-oxaloacetic transaminase; AST: Aspartate aminotransferase; SGPT: Serum glutamate pyruvate transaminase; ALT: alanine transaminase; A/G Ratio: Albumin/globulin ratio.

Table 3. Clinical follow-up from day 0 to day 63 for fever (°C) and parasitaemia (per µL) of two adult patients.

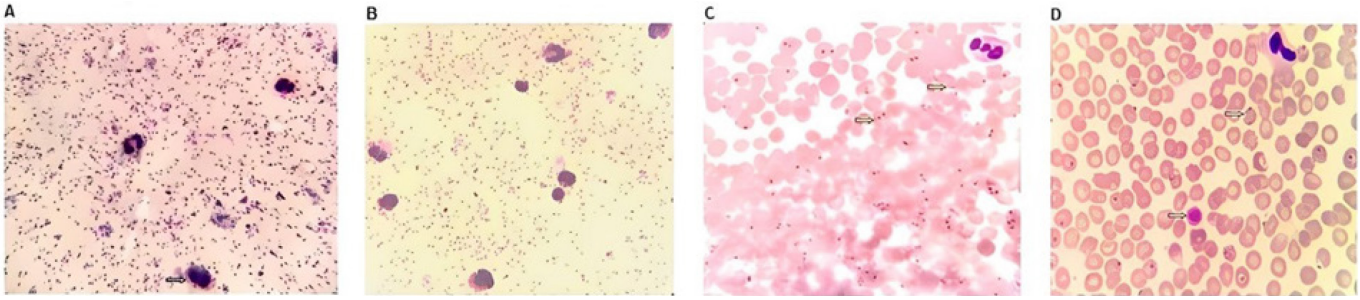
Patients	Day	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63
Parasitaemia and Temperature	**(00.00 hr)**	**(22-24 hr)**	**(44-48 hr)**	**(66-72 hr)**	**(154-168 hr)**	**(308-336 hr)**	**(462-504 hr)**	**(616-672 hr)**	**(770-840 hr)**	**(924-1008 hr)**	**(1078-1176 hr)**	**(1232-1344 hr)**	**(1386-1512 hr)**
Patient 1													
Parasitaemia	1668824/µL	300673/µL	9887 /µL	00 /µL	00/µL	00/µL	00/µL	00/µl	00/µL	00/µL	00/µL	00/µL	00/µL
Temperature	39.1 °C	37.6 °C	37.9 °C	36.8 °C	37.0 °C	36.9 °C	36.3 °C	36.9 °C	36.7 °C	37.0 °C	36.8 °C	36.6 °C	36.7 °C
Patient 2													
Parasitaemia	304000 /µL	100075/µL	1989/µL	00 /µL	00µL	00/µL	00/µL	00/µL	00/µL	00/µL	00/µL	00/µL	00/µL
Temperature	38.8 °C	37.7 °C	37.5 °C	37.0 °C	36.8 °C	36.9 °C	37.0 °C	36.7 °C	36.6 °C	37.0 °C	36.5 °C	36.8 °C	36.9 °C

and Figures 1C and 1D panels represent thin smear images. A large number of typical chromatin dots of ring form were seen on thick smears (Figure 1A and 1B). The parasitological assessment of thin smear for species identification was the cytoplasm, which makes the complete ring formation in young trophozoites followed by thickening and invariably contains several vacuoles to develop the trophozoites. The chromatin (parasite nucleus) was characteristically contained as a single bead and double bead forms on thin smear examination (Figure 1C). The most important feature of the rings was found on the margin or edge of the red blood cells, called 'accolé/appliqué' forms (Figure 1C and 1D). Accolé forms were seen in early stages of *P. falciparum* parasites and there were three distinct types – common form, rim form, and displaced form (16%). The large mass of golden-brown pigment (haemozoin) was seen in the pre-schizont and schizont stages (20%) (Figure 1D). No sexual forms, i.e., gametocytes, were detected in this patient.

Patient 2

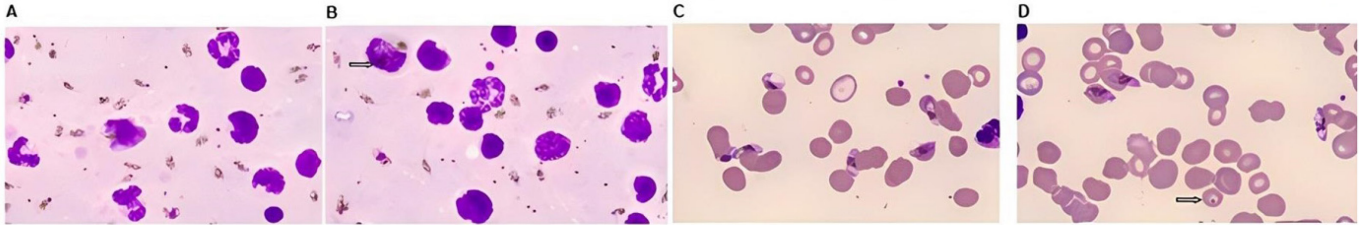
Patient 2 had mixed stages of parasites. Total parasitaemia on the thick smear was 304000/µL of blood, and 9.5% on the thin smear (Figures 2A to 2D). Infected RBCs in the thin smear were normal in size and contained young rings, and some mature stages, showing occasionally thin accolé/appliqué forms (Figure 2C). Asexual stages constituted about 46% of the 9.5% parasitaemia. Occasionally, pigments were evident in mature trophozoites and schizonts. Phagocytosed monocytes, macrophages and polymorphonuclear neutrophils were also detected. Different developmental stages of gametocytes - the sexual stage of the parasite, were seen in both thick and thin peripheral blood smears (Figures 2A to 2D), and constituted about 54% of 9.5% parasitaemia. The

Figure 1. Images of asexual stage of *P. falciparum* parasites (Patient 1).



Panels A and B showing thick smear images with huge ring form of *P. falciparum* parasites. Panels C and D representing thin smears. Accolé form was seen in both C and D panels. A double-chromatin ring was seen in panel C, indicated by the arrows.

Figure 2. Images of sexual and asexual stages of *P. falciparum* parasites (Patient 2).



Panels A and B showing thick smear images; arrow indicating pigments of *P. falciparum* gametocyte. Plenty of gametocytes along with rings can be seen on thick smear panels. C and D panels showing gametocytes on thin smears. One ring was seen at the bottom in the panel D, indicated by the arrow.

female gametocyte or macrogametocyte was more slender and longer than the male, cytoplasm was deep blue in colour and nucleus was small, compact, staining dark red in colour. However, the male gametocyte or microgametocyte was broader than the female and sausage-shaped. The cytoplasm was either pale blue or tinted pink; the nucleus was stained dark pink in colour and was seen in peripheral thin blood smear. In this patient, 54% of 9.5% parasitaemia were gametocytes comprising 51% of males and 49% of females (1:1).

Both the patients were discharged from the hospital on day 7 when blood smears were negative and other parameters were normal. Weekly blood examination of both the patients did not show any parasite, and temperature was recorded below 37.5 ºC during each visit from day 3 onwards (Table 3). This indicated that both the patients successfully responded to ACT treatment.

Discussion

Asexual hyperparasitaemia in *P. falciparum* has been observed both in children and adults by many investigators. But high gametocytaemia in *P. falciparum* is not commonly encountered. In the present study, we found 54% gametocytaemia and to our knowledge, this is the first report. Occasionally, a disastrous mistake takes place when stains and microscopes are of poor quality and microscopists misread hyperparasitaemic slides as negative [1]. Both the patients presented uncomplicated hyperparasitaemia and were treated as per national malaria drug policy and hospital protocol. Axillary temperature became normal on day 1 for both the patients. Based on their clinical assessment and prognosis, oral therapy of ACT was administered; the patients responded successfully and hyperparasitaemia started to decline. The basic definition of hyperparasitaemia needs to be updated based on transmission intensity [16]. The World Health Organization (WHO) has defined hyperparasitaemia as > 5% or 250,000/μL in high transmission stable malaria areas or > 2% or 100,000/μL in low transmission areas [17]. However, in low transmission areas parasitaemia of 0.5% was considered a cut-off point for discrimination between severity levels of *P. falciparum* malaria patients [18]. The WHO has re-defined hyperparasitaemia criteria to >10% parasitaemia irrespective of transmission settings and patient conditions [19]. Here we calculated parasitaemia taking the actual WBC and RBC counts, instead of WHO recommendations considering 8000 WBC/μL and 5 million/mm^3 for counting and estimating parasitaemia [14]. Taking actual numbers gives accurate estimation especially in clinical drug trials or therapeutic efficacy studies.

The accolé/appliqué forms were seen only in hyperparasitaemia of severe *P. falciparum* malaria cases and indicated the presence of CM complication [20]. In the present cases, 16% and 5% such forms were recorded, but no sign of CM was found, suggesting that both the patients were semi-immune. The presence of > 20% mature parasite stages in peripheral blood has been associated with a poor prognosis in severe *P. falciparum* malaria [3, 20]. However, the first patient in our study who had ≥ 20% mature parasite stages was found to have normal prognosis, suggesting early diagnosis and prompt treatment were critical for a positive outcome in SM. This observation aligns with prior results where a 4-year girl child survived 70% *P. falciparum* parasitaemia in Odisha, India [21]. The first patient in this study also presented no gametocytes despite having 42% parasitaemia, which contradicts with the previous finding, where hyperparasitaemia was associated with enhanced gametocyte carriage [4] suggesting this patient may have gametocytes neutralizing antibodies.

Mangalore city in the southwestern coast of India is endemic for malaria and contributes 70 to 80% of total malaria cases in Karnataka [22] and *Anopheles stephensi* is the main urban malaria vector [23]. Recent changes in the malaria control operations in Mangalore [24] and national malaria control programme [23] have led to a significant reduction in malaria transmission in India. Hypergametocytaemia (54%) with 1:1 of male and female gametocytes ratio in the second patient further confirms the hypothesis that in low-transmission areas, parasites invest more in transmission to new hosts via reproduction and less in within-host replication than parasites found in high-transmission areas [25].

Conclusions

In summary, we show that two male adults with *P. falciparum* hyperparasitaemia were successfully recovered with the ACT and a single dose of primaquine. However, the presence of hypergametocytaemia may hinder the elimination efforts with increased infectivity, if not treated immediately.

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Authors' contributions

BM participated in fieldwork, collected clinical and epidemiological data, performed laboratory analysis and wrote the first draft of the manuscript. HG, AKRA, BS participated in data interpretation and critical reviewing of this article. KP participated in follow-up patients' record collection and microscopy. SKG carried out the final microscopy examination, generated the resources and wrote the final manuscript.

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