Two hyperparasitaemic *Plasmodium falciparum* cases successfully treated with Artemisinin-based Combination Therapy

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Case Report

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Title

Two hyperparasitaemic *Plasmodium falciparum* cases successfully treated with Artemisinin-based Combination Therapy

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Abstract

Background

Hyperparasitaemia is an important event in a cascade of *Plasmodium falciparum* severe malaria (SM) but requires host responses to cause cerebral malaria (CM) leading to death, if left untreated. Here, we report two hyperparasitaemic patients with no CM.

Methods

Malaria diagnosis was performed based on thick and thin smears examination, and immunochromatographic-based rapid diagnostic test assay. Parasitaemia was calculated following World Health Organization (WHO) protocol. Haematological and biochemical investigations were also performed.

Results

The first patient had 42% parasitaemia (100% asexual parasites). The second one had 9.5% parasitaemia comprising 46% asexual, and 54% sexual stages with 1:1 male to female ratio. On the day of admission, both had presented abnormal haematological and biochemical parameters compared to the reference ranges. Remarkably, both the patients recovered successfully with oral artemisinin-based combination therapy (ACT) and a single dose of primaquine.

Conclusion

The presence of hypergametocytaemia may hinder the elimination efforts, if not treated immediately. This report also signifies the need of accurately estimating the parasitaemia in malaria patients and their timely management to prevent complications and mortality.

Keywords: *Plasmodium falciparum*, severe malaria, hyperparasitaemia, hypergametocytaemia, artemisinin-based combination therapy, primaquine

Background

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

Methods

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, India for diagnosis and treatment. Clinical, biochemical, hematological, parasitological outcomes as well as treatment details of the two patients are presented in this brief report. Indian reference value system for these parameters was considered [12].

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 (Germany) [14]. Two trained expert microscopists examined both thick and thin smears for diagnosis and determination of parasite counts – the senior expert (SKG) reconfirmed the final 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 RBCs examined against 5000 RBCs in 20 microscopic fields considering the actual number of RBCs/ μ L for each patient i.e. 3.87 and 3.69 million/ mm^3 , respectively. Similarly, parasitaemia per μ l of blood in the thick smears was calculated enumerating the number of parasites counting 200 white blood cells (WBCs) in relation to the actual number of WBCs/ μ l i.e. 6900 and 6000/ mm^3 , respectively.

Laboratory procedures

On admission, 4ml venous blood (pre-treatment) from the two patients was collected in EDTA vacutainers (BD Vacutainer®; Cat. No. 367861) for haematological tests. Another 4ml blood was collected in Clot Activator Vacutainer (BD Vacutainer® 367812) for biochemical analyses for liver and kidney function tests. DxH 800 Hematology (Beckman Coulter) and Cobas® 6000 (Roche) analyzers were used for hematological and biochemical tests, respectively. All the tests were performed at the National Accreditation Board for Testing and Calibration Laboratory (NABL)-accredited laboratory services at Kasturba Medical College, Mangalore, India.

Treatment

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 2 as per the guideline of 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 discharged from the hospital on day 7 when blood smears were found negative or other parameters showed normal.

Results

Clinical manifestations

The two adult patients aged 35 and 36 years, respectively presented almost similar clinical manifestations having high-graded fever of 39⁰C and 38.5⁰C, respectively. On admission both the patients had higher heart rates and abnormal breathing issues. Clinical assessment revealed no neurological and lymphatic abnormalities, but had palpable splenomegaly on admission. Detailed information on physical and clinical manifestations is presented in Table 1.

Table 1. Physical and clinical characteristics of two adult patients

Parameter	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

Haematological and biochemical analyses

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

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

Parameter	Patient 1	Patient 2
HIV	Negative	Negative
HBsAg	Negative	Negative
Hb (g/dl)	11.1	6
TLC (per mm ³)	6900	6000
PCV %	34.9	30
Total RBC (million/mm ³)	3.87	3.69
Platelet counts (per mm ³)	24000	50000
Blood sugar (mg/dl)	45	49
Serum urea (mg/dl)	58.4	49
Serum creatinine (mg/dl)	0.66	11
Total bilirubin (mg/dl)	8.37	3.89
Direct bilirubin (mg/dl)	3.5	2
Indirect Bilirubin (mg/dl)	4.87	1.95
AST levels (IU/L)	96.5	96
ALT levels (IU/L)	70	56.7
Alkaline Phosphate (IU/L)	563.3	389.9
Total Protein (g/dl)	6.54	7.91
Albumin (g/dl)	3.85	3.98
Globulin (g/dl)	2.69	2.5
A:G Ratio	1.43	2
Parasitaemia/ μ l	16,68,824	3,04,000
Asexual %	100%	13,6,800 (46%)
Sexual %	0	16,7,200 (54%)
Male gametocyte %	0	51%
Female gametocyte %	0	49%

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/ μ l of blood based on thick smear examination and 42% on thin smear. Fig. 1 A and B show thick smear images, and Fig. 1 C and D are thin smear images. A large number of typical chromatin dots of ring form are seen on thick smears (Fig. 1 A and B). 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, double bead forms on thin smear examination (Fig. 1 C). The most important feature of the rings was found on the margin or edge of the red blood cells, called 'accolé/appliqué' forms (Fig 1. C and D). Accolé forms were seen in early stage of *P. falciparum* parasites and these are 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 stage (20%). A low number of pigmented monocytes (0.19%), neutrophils (0.29%), and eosinophils (0.09%) was detected (Fig. 1 A). The unusual of marked multi parasitism (74.02%, 1 parasite/RBC; 14.95%, 2 parasites/RBC; 7.7%, 3 parasites/RBC; 3.14%, 4 or more parasites/RBC; 0.19%) was observed (Fig. 1 C and D). No sexual forms i.e. gametocytes were detected in this patient.

Fig. 1: Images of asexual form of *P. falciparum* parasites X 1000 (Patient 1)

Panels A and B show thick smear images with huge ring form of *P. falciparum* parasites. Panels C and D on thin smears. Accol form is seen in both C and D. A double-chromatin ring is seen in panel C (arrow in the middle). X 1000

Patient 2

Patient 2 had mixed stages of parasites. Total parasitaemia on thick smear was 304000/ μ l of blood, and 9.5% on thin smears (Fig. 2 A to D). Infected RBCs in thin smear were normal in size and contained young rings, and in some mature stages, showing occasionally thin accol/appliqu forms (Fig. 2 C). Asexual stages constituted about 46% of 9.5% parasitaemia. Occasional 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 (Fig. 2 A to D) constituted about

54% of 9.5% parasitaemia. The female gametocyte or macrogametocyte is more slender and longer than the male, cytoplasm is deep blue in colour and nucleus is small, compact, staining dark red in colour, but the male gametocyte or microgametocyte is border than female, sausage shaped, cytoplasm is either pale blue or tinted pink, nucleus is staining dark pink in colour was seen in peripheral thin blood smear. Pigments and nucleus were dispersed (Fig. 2 A to D). Some fragmented gametocytes were also detected (Fig. 2 C). In this patient, 54% of 9.5% parasitaemia were gametocytes comprising 51% males and 49% females (1:1). Pigmented neutrophils (1.2) and monocytes (0.4%) were present (Fig. 2 A and B). The unusual of marked multi parasitism asexual (14.5%), sexual (4.5%) comprising male gametocyte (2.3%) and female gametocyte (2.2%), 1 parasite/RBC (4.6%), 2 parasites/RBC (0.4%), 3 or more parasites/RBC (0.2%), schizonts (0.25%) and accolé/appliqué forms 1.5% were found.

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

Panels A and B thick smear images; arrow shows pigments of *P. falciparum* gametocyte. Plenty of gametocytes along with rings are seen on the thick smears. In C and D gametocytes on thin smears. One ring is seen at the bottom in D (arrow). X 1200.

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 – to our knowledge this is the first report. Occasionally, a disastrous mistake takes place when stains and microscopes are of poor quality, 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, and patients responded successfully and hyperparasitaemia started to decline. The basic definition of hyperparasitaemia needs to be updated based on transmission intensity [16]. 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 cutoff point for discrimination between severity levels of falciparum malaria patients [18]. Now WHO has clearly indicated that >10% parasitaemia is considered to be severe malaria irrespective of transmission settings and patient conditions [19]. Here we calculated the parasitaemia taking the actual WBC and RBC counts, instead of WHO recommendations considering 8000 WBC/μl and 5 million/mm³ for counting and estimating parasitaemia [14]. Taking actual number 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 indicate the presence of CM symptoms [20]. In the present cases, 16% and 5% such forms were recorded, but no sign of CM was found, suggesting 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 falciparum malaria [3,20]. However, first patient in our study who had ≥20% mature parasite stages still the prognosis was found normal, suggesting early diagnosis and prompt treatment are critical for a positive outcome in SM. This observation aligns with prior results where a 4-year girl child survived 70% falciparum parasitaemia in Odisha, India [21]. This patient also presented no gametocytes despite of 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 change 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 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].

Conclusion

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. This report also signifies the need of accurately estimating the parasitaemia in malaria patients and their timely management to prevent complications and mortality.

Availability of data and material

The data used in this study are archived with Dr SK Ghosh and available from them upon reasonable request.

Abbreviations:

ACT – Artemisinin-based combination therapy

SM – Severe malaria

CM – Cerebral malaria

WBCs – White blood cells

RBCs – Red blood cells

MOD – Multi-organ dysfunction

NABL – National Accreditation Board for Testing and Calibration Laboratory

WHO – World Health Organization

HIV – Human immunodeficiency virus

HBsAg – Hepatitis B surface antigen

Hb – Haemoglobin

AST - Aspartate aminotransferase

ALT - Alanine transaminase

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Authors' contributions: BM participated in fieldwork, collected clinical and epidemiological data, and laboratory analyses. AKRA and BS participated in data interpretation and critically reviewing this article. SKG carried out the microscopic examination, generated the resources, and together with HG wrote the first draft of this manuscript. All authors read, reviewed and approved the final manuscript

Ethical statement: Written prior informed consent was obtained from both the patients. The institutional review board of 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 Manipal Academy of Higher Education, Mangalore, Karnataka, India, approved the study (Ref. No- IEC KMC MLR 03-16/49). All essential regulatory procedures were followed strictly.

Consent for publication: Not applicable

Competing interests: The authors declare that they have no competing interests.

Figures

Figure 1

Images of asexual form of *P. falciparum* parasites X 1000 (Patient 1) Panels A and B show thick smear images with huge ring form of *P. falciparum* parasites. Panels C and D on thin smears. Accolé form is seen in both C and D. A double-chromatin ring is seen in panel C (arrow in the middle). X 1000

Figure 2

Images of sexual and asexual stages of *P. falciparum* parasites (Patient 2) Panels A and B thick smear images; arrow shows pigments of *P. falciparum* gametocyte. Plenty of gametocytes along with rings are seen on the thick smears. In C and D gametocytes on thin smears. One ring is seen at the bottom in D (arrow). X 1200.