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Overt bleeding in complicated *P. falciparum* malaria: An experience from east coast of India

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Abstract. Delay in diagnosis of falciparum may result in complicated, life-threatening conditions. Though haematological abnormalities and coagulopathy are common complications that occur in malaria but complications with rare manifestations like overt bleeding do pose challenges for the clinicians worldwide. This study reports the incidence and prognosis of overt bleeding from the east coast of India and makes an attempt to relate it with the pathogenesis of the disease in severe *Plasmodium falciparum* malaria patients. This study was conducted in Sriram Chandra Bhanj Medical College Hospital, Cuttack, Odisha, India. A total of 120 complicated (multi organ dysfunction) malaria cases were included in this study. Amongst them 54 (45.0%) showed signs of overt bleeding and clinically the bleeding was either from one or multiple sites. Out of the total overt bleeding cases, 79.6% had elongated prothrombin time (PT), activated partial prothrombin time (aPTT) and elevated D-dimer with thrombocytopenia indicating disseminated intravascular coagulopathy (DIC). It was observed that case fatality rate was very high in coagulopathy group than the non coagulopathy group. Our observations highlight that awareness of overt bleeding in *P. falcipatum* infection is necessary for general practitioners in endemic areas for malaria like in Odisha for effective and timely management of complicated patients.Timely diagnosis and treatment of DIC with appropriate prescribed drugs can prevent and cure the complications of severe falciparum malaria with anti-malarial treatment.

INTRODUCTION

Falciparum malaria remains a major cause of morbidity and mortality worldwide. As per World malaria report the annual clinical case load may well be over 212 million, leading to 429 000 deaths globally (WHO 2016). Currently malaria is endemic in around 91 countries or territories located in tropical countries including India, where an estimated 3.3 billion people are at risk of infection. In Odisha, malaria is still an important public health problem. With only about 4.8% land area and 3.4% population of the country, accounted for 26.9% of malaria cases, and about 17.6% of all reported deaths in 2013 nationwide (NVBDCP 2014). Whilst the majority of cases of falciparum malaria worldwide are mild and can be treated with oral drugs, a minority, mainly because of delays in diagnosis or treatment, may develop complicated, life-threatening disease requiring parenteral therapy (Pasvol 2005). The leading causes of death are severe anaemia, cerebral malaria, acute renal failure, jaundice and hepatic failure, pulmonary oedema (Harinasuta & Bunnang 1988). But sometimes complications with rare manifestations do pose challenges for the clinicians worldwide. Though haematological abnormalities and coagulopathy are common complications that occur in malaria, but very rarely malaria patients present with overt bleeding. In the present study we are reporting the incidence and prognosis of overt bleeding in severe

Plasmodium falciparum malaria patients presenting multi organ dysfunction (MOD) in Odisha, an eastern Indian state, with an aim to link it to the pathogenesis of the disease.

MATERIALS AND METHODS

Study participants

The cases positive for *P. falciparum* infection either by blood smear examination and/or by ICT was recruited from in-patients admitted to the SCB Medical College Hospital, Cuttack for treatment during 2002-2005. As a tertiary care hospital majority of the cases were referred from peripheral hospitals (n=120) with complications. The clinical signs/ symptoms of these patients with reference to anaemia, jaundice, purpuric spots, pulse, blood pressure, respiration, temperature, level of consciousness (Galsgow Coma Score), signs of meningial irritation, local neurologic deficits, fundoscopy, hepatosplenomegaly, respiratory system and cardio vascular system were recorded to classify the patients as complicated malaria as per WHO criteria (WHO 2000). The exclusion criteria were (i) known case of bleeding disorder / family history of bleeding disorder (ii) patient with known cause of leucocytosis / leucopenia (iii) patients on chronic anticoagulant therapy (iv) cases of chronic liver disease (v) cases of chronic renal failure. The patients without overt bleeding (n=66) were considered as control for comparison of haematological investigations. The patients have been given appropriate and adequate treatment by the hospital as per the guideline. Informed and free consent was obtained from all the participants at the time of enrollment. The human ethical committee of RMRC, Bhubaneswar has approved the study.

Laboratory investigations

At least 5ml of venous blood was drawn aseptically and preserved using EDTA as anticoagulant (1.5 mg/ml of blood) so as to perform ICT test, prepare peripheral smear, complete blood cell count (CBC), biochemical investigations and isolation of plasma sample after centrifugation of the blood at 3000RPM for 5 minutes for PT, aPTT and D-dimer assay.

Peripheral blood smear examination

At least 25-30 µl of blood was used to prepare thick and thin blood smear to diagnose malaria by microscopy after staining with Giemsa following the standard procedure (Gills 1993). The thin smear was used to identify the species and thick smear to count the parasite [Parasites/µl of blood= (parasites/WBCs) × 8,000].

Malaria antigen detection test

The OptiMAL-IT malaria test kit (Bio-Rad, USA) was used to diagnose malaria by detection of circulating antigen following the instruction of the manufacturer.

Other blood infections

The blood culture was done using BacT/ Alert 3DTM (B3D) automated blood culture system to detect the blood stream bacterial infections. The serum sample was used to diagnose the enteric fever (*Salmonella typhi* and *Salmonella paratyphi*) by using the commercially available Widal test (slide) kit (Recombigen Lab Pvt Ltd, New Delhi) and leptospira/ dengue fever by the commercially available immunochromatographic test (ICT) kit (Standard Diagnostics, Inc, South Korea).

Haematological investigations

The complete blood count (CBC) has been done using automatic haematological analyser (Melet Schloesing Laboratories, USA). The plasma sample was used to test the PT, aPTT and D-dimer level (semi quantitative) using reagent of Tulip Diagnostics, India. The average bleeding time (BT) and clotting time (CT) was done from direct blood by capillary tube method.

Biochemical Investigations

The liver function test, renal function test and blood sugar was analysed by the automatic biochemistry analyser and the reagents supplied by the company (Logo Tech, Italy).

Statistical Analysis

The geometric mean intensity of malaria parasites was calculated as (log 10) antilog [Σlog (x+1)/n], where x is the number of parasites per µl of blood. Quantitative data were expressed a±SD and were compared by using Student's unpaired "t" test the mean. Qualitative data were compared by use of Yates corrected χ^2 test. Statistical analysis was performed using the SPSS 7.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

RESULTS

A total of 120 complicated (multi organ dysfunction) malaria cases were included in the study. Majority of the patients (n=66, 55%) studied were with cerebral malaria (Glasgow coma score < 9) followed by jaundice (n=64, 53.3%), mostly due to liver damage as evidenced by the increased levels of serum bilirubin and liver enzymes. Acute renal failure was found in 41.7% (n=50), convulsions in 38.3% (n=46), severe anaemia (Hb < 5g/dl) in 21.7%, (n=26) acute respiratory syndrome (ARDS) in 6.6% (n=8), hypotension in 3.3% (n=4) and hypoglycaemia (fasting blood sugar <40mg/dl) in 3.3% (n=4) of patients. Concerning the bleeding presentation 54 cases (45%) showed signs of overt bleeding. But none of them had past history of upper gastrointestinal symptoms, bleeding tendencies or overt bleeding episodes. Clinically the bleeding was either from one site or multiple sites in different patients. However majority of them showed subconjuctival haemorrhage (30 out of 54) followed by gastrointestinal bleeding (18 out of 54), epistaxis (14 out of 54), haematuria (4 out of 54), retinal haemorrhage (4 out of 54), intracranial bleed (2 out of 54) and purpuric spots (2 out 54) (Table 1).

The frequency of clinical signs and symptoms of two study groups (MOD with overt bleeding and MOD without overt bleeding) have been depicted in Table 2. All cases in both groups shared a common manifestation with high fever (>38°celsius) with chills. In the overt bleeding group the haemoglobin level and platelet count was significantly low, while bilirubin level, serum urea and serum creatinine levels were significantly high compared to the patients without bleeding. One of the patients among the overt bleeding group with GI bleed had premature cells (metamyelocyte and myelocyte) and neutrophil with malarial pigment and 4 patients with atypical lymphocytes in peripheral blood smear. Blood culture, Widal test, IgM for dengue fever and leptospirosis were negative in both the groups.

The average BT, CT, PT, aPTT and D-dimer level were found to be higher in patients with overt bleeding compared to non bleeding group of patients (Table 3). However among the overt bleeding group at least 79.6% (43 out of 54) had elongated PT (>1 sec), aPTT (>40 sec) and elevated D-dimer (>200 ng /ml) with thrombocytopenia indicating that overt bleeding has been precipitated in majority of the cases due to DIC and rest of the patients (11 out of 54) were having only thrombocytopenia indicating general bleeding.

While analysing the prognostic value of the complicated patients without coagulopathy / bleeding compared to patients with overt bleeding/coagulopathy it was observed that fatality rate was very high in coagulopathy group (27 out of 43, 62.8%) than the non coagulopathy group (9 out of 66, 13.3%) even though carefully monitored for haemodynamic instability and treated with Artemesinin Combination Therapy (ACT) and supportive drugs.

Table 1. Sites of overt bleeding in malaria patients with MOD

Manifestations	No of Cases	Percentage (%)
Subconjuctival haemorrhage	30	55.6
GI bleed	18	33.3
Epistaxis	14	25.9
Haematuria	4	7.4
Retinal haemorrhage	4	7.4
Intracranial bleed	2	3.7
Purpuric spot	2	3.7

Table 2. Laboratory and clinical characteristics of MOD patients with and without overt bleeding

	MOD with Overt Bleeding (n=54)	MOD without Overt Bleeding (n=66)	P-value
Median Age (Years)	29.5	33.0	NS
Geometric mean of *P. falciparum* density (95% CI)	32687 (21985 – 33226)	39879 (25651 – 39879)	NS
Temperature (°C)	38.4 ± 0.5	38.7 ± 0.6	NS
Hepatomegaly (n)	0	17	P < 0.0001
Splenomegaly (n)	3	14	P < 0.0001
Pallor (n)	41	39	NS
Haemoglobin (g/dl)[a]	6.60 ± 1.64	8.90 ± 2.82	P < 0.0001
Total leukocyte count (m/mm^3)[a]	8.4 ± 2.3	9.9 ± 5.8	NS
Total platelet count (m/mm^3)[a]	46.2 ± 4.7	151.7 ± 7.7	P < 0.0001
Biluribin (mg/dl)[a]	14.1 ± 3.8	8.8 ± 5.52	P < 0.0001
Aspartate transferase (IU/L)[a]	157.9 ± 110.5	162.9 ± 111.4	NS
Alanine transferase (IU/L)[a]	127 ± 38.4	111 ± 49.6	NS
Alkaline phosphatase (IU/L)[a]	166.0 ± 20.9	167.6 ± 86.1	NS
Serum urea (mg/dl)[a]	234.2 ± 45.2	106.8 ± 66.3	P < 0.0001
Serum creatinine (mg/dl)[a]	5.7 ± 2.6	3.2 ± 2.9	P < 0.0001
Fasting Sugar (mg/dl)[a]	59.2 ± 11.3	61.3 ± 19.6	NS

Table 3. Coagulation Parameters in complicated malaria with and without overt bleeding

Coagulation parameter	Control (Without Overt bleeding) (n=66)	Cases (Overt bleeding) (n=54)	P-value
Prothrombin Time in sec	1.12±0.003	1.82±0.71	<0.0001
Activated partial prothrombin time in sec	30.04±1.75	42.25±10.29	<0.0001
D-dimer (ng/ml)	167±12.7	494±121.1	<0.0001
Bleeding time in min	2.55±0.93	5.29±1.48	<0.0001
Clotting time in min	6.24±0.89	8.25±1.42	<0.0001

DISCUSSION

Our study revealed a high rate of overt bleeding in complicated *P. faciparum* infection (54 out of 120 severe cases) presenting with multi organ failure in population of Odisha, situated along the Bay of Bengal and known to be highly endemic for malaria (API ≈10 in 2016, NVBDCP-Odisha). This is in contrast to the reports from other parts of the world where it has been described that even though the complicated *P. falciparum* infections develop bleeding manifestations, very rarely do they present with overt bleeding (Kueh & Yeo 1982; Gall *et al.*, 1999; Corne *et al.*, 2001). Further mortality rate was also observed to be very high in this group of patients (27 out of 54), who were with low platelet count, prolonged PT, elevated aPTT and elevated fibrin-degradation products (FDP) ord-DIMER (an indication of *in vivo* fibrin deposition and degradation) suggesting the manifestation was due to disseminated intravascular coagulation (DIC).

DIC is characterized by systemic activation of blood coagulation, which results in generation and deposition of fibrin, leading to microvascular thrombi in various organs and contributing to multiple organ dysfunction syndrome (MODS) (Vincent & De Backer 2005; Thachil 2016).Derangement of the fibrinolytic system further contributes to intravascular clot formation, but in some cases, accelerated fibrinolysis may cause severe bleeding. Hence, a patient with disseminated intravascular coagulation (DIC) can present with thrombotic and bleeding problem simultaneously (Levi & Ten 1999), which corresponds to our observation. DIC in malaria is believed to be due to immunological mechanism. An immune reaction, associated with complement activation in malarial infection, is believed to contribute to injury of red blood cells and platelets, and to promote the development of DIC and other serious clinical complications (Srichaikul *et al.*, 1975). Rojanasthien and others (1992), however, recently reported that coagulation factors V, VII, and IX were the most sensitive parameters in the expression of coagulation defects, and that most coagulation abnormalities were due to liver involvement. We however based from our earlier observations in severe *falciparum* malaria patients belonging to this region predict that the activation of coagulation could be partially responsible for thrombocytopenia in this study population and release of high levels of microparticles as reported in our earlier study (Sahu *et al.*, 2013) due to endothelial injury might be triggering the 'consumptive coagulopathy' leading to DIC. Because microparticles contains tissue factor (TF) and phosphatidylserine (PS), which respectively generate FXa and thrombin, they presumably contribute to formation of a fibrin meshwork, platelet accumulation, and inflammation *in vivo* at sites of endothelium activation (Mallat *et al.*, 2000; Engelmann *et al.*, 2003; Muller *et al.*, 2003; Gomez and McVey 2006). In this context, endothelial cell (EC) microparticle levels are increased in severe malaria but not in uncomplicated cases (Combes *et al.*, 2004). Microparticles are also increased under conditions associated with increased thrombotic risk (Mackman 2004; Steffel *et al.*, 2006). This needs an extensive study with respect to DIC in complicated malaria presenting overt bleeding because understanding this will in the development of new therapies and prognostic tools.

CONCLUSION

Since mortality from severe malaria continues to be high, even when many effective drugs are available, because most deaths occur within few hours of admission to hospital (Maitland *et al.*, 2003). Therefore awareness of overt bleeding in *P. falcipatum* infection is necessary for general practitioners in the malaria endemic areas like Odisha for effective and timely management of complicated patients. Because timely diagnosis and treatment of DIC with appropriate prescribed drugs can prevent and cure the complications of severe falciparum malaria with anti-malarial treatment (Sailo *et al.*, 2014).

Conflict of interest

None of the authors of this paper have any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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