JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Pretreatment elevated erythrocyte sedimentation rate and C-reactive protein as a predictor of malarial complications

Sachin Vemula[1], Vidyalakshmi Katara[2], Unnikrishnan Bhaskaran[3], Sushma Adappa[1], Mahabala Chakrapani[1]

[1] *Department of Medicine, Kasturba Medical College, Manipal University, Mangalore, India*
[2] *Department of Microbiology, Kasturba Medical College, Manipal University, Mangalore, India*
[3] *Department of Community Medicine, Kasturba Medical College, Manipal University, Mangalore, India*

Abstract

Introduction: Complications of malaria can develop suddenly and unexpectedly. Although various parameters have been associated with severity of malaria, they have not been studied as predictors of these events. Many of the malarial complications are inflammatory in nature, and C-reactive protein (CRP) and elevated erythrocyte sedimentation rate (ESR) could be early markers of these complications and might precede and predict the development of complications.

Methodology: A total of 122 inpatients with uncomplicated newly diagnosed malaria were studied. CRP, ESR, hemoglobin, and platelets were measured before initiating treatment. Patients were monitored closely for the subsequent development of complications based on the World Health Organization's definition of severe malaria.

Results: Seven patients (5.7%) had worsening of symptoms compared to the day of admission and had higher pretreatment CRP and increased ESR compared to those patients who did not develop complications. Area under receiver operator characteristic curve was 0.761(p=0.02) for CRP and 0.739 (p = 0.035) for ESR. CRP>124 mg/L and increased ESR (>34.5 mm in the first hour) had a sensitivity of 71.4% and specificity of 79.1%, respectively, for predicting complications of malaria. Other parameters did not reach statistical significance for predicting complications. Elevated CRP and elevated ESR had a negative predictive value of 97.8%.

Conclusions: Elevated CRP>124mg/L and increased ESR>34.5 mm in the first hour at the time of diagnosis in patients with uncomplicated malaria identifies patients who might subsequently develop complications of malaria.

Key words: Severe malaria; C-reactive protein; acute-phase reactant; inflammatory marker; erythrocyte sedimentation rate.

J Infect Dev Ctries 2016; 10(12):1332-1337. doi:10.3855/jidc.8053

(Received 28 December 2015 – Accepted 02 May 2016)

Introduction

Development of complications during the treatment of malaria leads to severe malaria, which has higher mortality and morbidity [1]. Complications of malaria can develop suddenly and unexpectedly during treatment [2-4]. Malaria is an exception to the rule that patients with infectious disease who apparently look stable and well at presentation rarely develop complications [2]. Complications such as renal failure and respiratory failure can develop even when patients seem to be clinically improving or even when parasite load decreases [2].

Many parameters have been studied as possible predictors of malaria, since it is extremely important to differentiate stable patients who will remain stable from those who could rapidly progress to develop complications. Some of the parameters include anemia, spleen enlargement, glucose, lactate, cytokines, and strain multiplicity. Most of these parameters have been studied in the setting of mixed populations consisting of uncomplicated and complicated cases [5]. Although various parameters, such as C- reactive protein (CRP), have been associated with severity of malaria [6], they have not been studied as predictors of these events. Severity scores for malaria have been developed and validated [7-8]; these are useful in predicting outcomes once the complications have developed. They are not used to predict the development of these complications in a pretreatment setting of stable, uncomplicated patients. Inflammatory markers such as tumor necrosis factor, interferon-gamma, and CRP have been shown to be closely associated with disease severity in *vivax* malaria [6]. Flow cytometry was found to be a good tool to identify multiple-infected red blood cells (RBCs), which could be a marker of severe disease; however, correlation to clinical endpoints of severe disease has

not been established for flow cytometry [9]. Parasite load has not been found to be useful in predicting the development of severe malaria due to the cyclical nature of the peripheral parasitemia and to the phenomenon of sequestration of parasites in vital organs [2,10]. Even studies involving severe malaria showed that parasite load had poor prognostic ability in terms of mortality prediction [3]. Parasite load had poor area under receiver operating characteristic (ROC) curve for prognostication in severe malaria, whereas elevated lactate level and acidosis at admission in severe malaria were found to be very good predictors of mortality [3]. Abnormal host inflammatory response is one of the possible pathogenetic mechanisms in severe malaria. Parasite load was inferior in predicting subsequent mortality [11]. Early diagnosis and risk stratification are crucial for preventing complications in malaria. Parasite load does not predict complications adequately [12].

Prediction of complications has been shown with malaria severity prognostic score by Tangpukdee *et al.* [4]; however, the study involved cases with falciparum malaria only, and dehydration was one of the parameters in the formula. However, signs of dehydration may themselves be a marker for the unstable patient. There is a need to predict the complications in the stable pretreatment stage. A retrospective study of patients admitted for malaria showed that hemoglobin and parasitemia at admission predicted subsequent multiple organ dysfunction syndrome (MODS); however, the study did not exclude complicated cases at admission [13]. Intra-neutrophilic pigments correlate well with parasitemia and severity of complications, but the utility of this finding has not been studied in an uncomplicated pretreatment setting. ROC and predictive ability of granulocyte pigment inclusions was poor for predicting mortality [14].

It is necessary to identify those malarial patients who are likely to develop complications at the beginning of treatment itself, which will help in focusing resources to these patients. Measurement of acute-phase reactants such as CRP or serum amyloid A have been used to monitor the response to antibacterial treatment [15]. Many of the malarial complications are inflammatory in nature [6]. Abnormal inflammatory host response is the possible pathogenetic mechanism in severe malaria [11]. At the cellular level, various pro-inflammatory cytokines and several adhesion molecules play important roles in the pathogenesis of complicated malaria [7], and CRP and increased erythrocyte sedimentation rate (ESR) might represent this state of inflammation. Higher levels of high mobility group box (HMGB1) were associated with higher mortality in patients with severe malaria [11]. Although these studies predicted higher mortality using biomarkers, the study population included patients who already had severe malaria at baseline. This study was undertaken to determine if CRP and ESR levels at the beginning of treatment of stable, uncomplicated malaria could be used as markers to predict the subsequent development of complications.

Methodology

Clinicians recruited patients who had been diagnosed with malaria into the study. Non-probability sampling was done. Diagnosis of the patient was done by thick smear examination, and species identification was done by thin smear. Consecutive patients diagnosed with *falciparum*, *vivax*, and mixed malarial infections, fulfilling inclusion and exclusion criteria, and willing to be part of the study were included in this study. Patients were examined by clinicians every day to for signs of hypotension, tachypnea, acidotic breathing, symptoms of uremia, oliguria, and altered mental status. Based on clinical signs, liver function test (LFT), creatinine levels, hemoglobin, blood gas analysis, chest X-ray, and electrocardiogram were ordered. Patients with any parameter of severe malaria [16], with previous malarial infections within two months, concurrent bacterial infections, human immunodeficiency virus (HIV), diabetes mellitus, chronic kidney disease, pre-existing rheumatologic conditions, active tuberculosis, active malignancies, and patients on steroids were excluded. Based on World Health Organization(WHO) guidelines, the parameters considered for severe malaria were cerebral malaria (unarousable coma with Glasgow coma scale <11/15), severe anemia (Hb<5gm/dL, hematocrit <15%), respiratory distress (rapid labored acidotic breathing), renal failure (urine output <400mL in 24 hours and serum creatinine >3gmL/dL), hypoglycemia (<40mg/dL), and circulatory collapse (systolic blood pressure <70 mm Hg or core skin temperature difference >10°C) [16]. Patients with concurrent bacterial infections (those who had had fever for 7 days, who had been diagnosed with respiratory infection, urinary tract infection, and sepsis) were excluded; also, those who had had fever from dengue in the preceding 7 days were excluded from the study. Chronic kidney disease was defined by based on National Kidney Foundation guidelines as either kidney damage or Glomerular filtration rate (GFR) <60mL/min/1.73m^2 for ≥ 3 months [17]. Diabetes was defined based on the American Diabetes Association (ADA) guidelines as

persons on treatment or fasting plasma glucose ≥126 mg/dL (7.0 mmol/L), 2-hour postprandial glucose (PG) ≥200 mg/dL (11.1 mmol/L) during oral glucose tolerance test (OGTT) (75g), glycosylated hemoglobin (A1C) ≥6.5% (48 mmol/mol), and random PG ≥200 mg/dL (11.1 mmol/L) [18]. Blood from each patient was used for detection and quantification of CRP by nephelometry, ESR, hemoglobin, and platelet count. Laboratory examination was done in a tertiary care hospital in Mangalore, in the National Accreditation Board for Testing and Calibration Laboratories (NABL). The study protocol was approved by the institutional ethics committee, and informed consent was obtained from each participant prior to the study. This cohort of uncomplicated and stable newly diagnosed patients was treated with standard WHO guidelines and was monitored closely for the subsequent development of any of the complications listed in the WHO definition of severe malaria. Treatment was given based on 2010 WHO guidelines: artesunate and mefloquine for 3 days for uncomplicated falciparum malaria (4 mg/kg/day artesunate once a day for 3 days and 25 mg/kg of mefloquine 8.3 mg/kg/day once a day for 3 days); chloroquine (25 mg/kg over 3 days and primaquine 15 mg/kg/day for 14 days) for uncomplicated vivax malaria, and mixed malaria was treated with artemisinin-based combination therapy (ACT) and primaquine [19].

Statistics

Sample size was calculated using a diagnostic test calculator with expected sensitivity of 80%, specificity of 60%, and prevalence of 5%. Continuous variables (ESR, hemoglobin, platelets, and CRP) were compared with Mann-Whitney Test, with $p<0.05$ considered statistically significant. ROC curve analysis was performed to evaluate the performance of CRP, ESR, hemoglobin, and platelets in predicting complications. Area under the ROC curve was calculated with corresponding 95% confidence intervals for ESR, CRP, hemoglobin, and platelets. Co-ordinates of ROC curve were analyzed to find the optimal cut-off value for the relevant parameters. Statistical analysis was done using SPSS 15.0 statistical software package (IBM, Armonk, USA).

Results

In total, 122 patients with uncomplicated malaria were studied. The mean age of the patients was 33.98±13.85 years. There were 88 (72.13%) males and 34 (27.87%) females. In total, 86 (70.5%) patients had *vivax* malaria, 15 (12.3%) had *falciparum* malaria, and 21 (17.2%) had mixed malaria.

In total 7 patients (5.7%) developed complications during follow-up. Acute respiratory distress syndrome (ARDS) developed in 4 (3.2%), severe anemia developed in 6 (4.9%), and significant hypotension developed in 6 (4.9%) patients during treatment. The median day of onset of complications was 2 days (range, 2–3 days). There was statistically significant elevated CRP and ESR in those patients who subsequently developed complications compared to those who remained free of complications (Table1). Differences in hemoglobin and platelet count were not statistically significant. ROC curve analysis showed a statistically significant area under the curve for pretreatment CRP and pretreatment ESR for predicting complications (Table2). Analysis of the coordinates of the ROC curve showed that optimal sensitivity and specificity was obtained at CRP of 124 mg/L and ESR

Table 1. Comparison of pretreatment parameters in patients who developed complications and those who remained free of complications.

Parameter	Developed complications (n = 7) Median (IQ)	No complications (n = 115) Median (IQ)	P value
CRP (mg/L)	133 (72–192)	66.5 (38–97.7)	0.016
ESR (mg/L)	43 (28–45)	24 (18–32)	0.038
Platelets (per mm^3)	0.69 (0.15–1.04)	0.82 (0.53–1.24)	0.173
Hemoglobin (gm/L)	11.6 (11.0–13.8)	12.75 (11.3–13.8)	0.358

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Table 2. Area under receiver operator characteristic (AUROC) curve for pretreatment CRP and ESR, with 95% confidence interval, for predicting malarial complications.

Variable	Area under the curve	P	95% confidence interval	
			Lower bound	Upper bound
ESR	0.739	0.035	0.526	0.951
CRP	0.761	0.021	0.635	0.887

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

of 34.5 mm at the first hour. Both parameters had a sensitivity of 71.4% and specificity of 79.1% for predicting complications of malaria (Table 3). There was a very high negative predictive value (NPV) (98%), suggesting that if these parameters were not elevated, the possibility of subsequent complications was around 2%. In *vivax* malaria, complications developed in 5.8% of cases (Table 4).

Table 3. Level of CRP and number of patients developing complications.

CRP level (mg/L)	Developed complications (n)	Did not develop complications (n)
CRP > 124	5	24
CRP < 124	2	91

Sensitivity: 71.4%; Specificity: 79.1%; Positive predictive value: 17.2%; Negative predictive value; 97.8%; CRP: C-reactive protein.

Table 4. Type of malaria, number of patients in each type, and percentage of patients developing complications.

Type of malaria	Number of patients	Complications
Vivax malaria	86	5 (5.8%)
Falciparum malaria	15	1 (6.7%)
Mixed malaria	21	1 (4.7%)

Discussion

Although various markers and scoring systems have been developed to predict outcome after the development of severe malaria [6], simple early markers for the prediction of complications are lacking [2]. Complications such as renal failure and respiratory failure can develop unexpectedly during treatment of malaria [2]. In this study, we evaluated the role of acute inflammatory response markers in predicting subsequent development of malarial complications in a cohort of patients with clinically stable uncomplicated malaria. Pretreatment CRP and ESR, done at the time of diagnosis, were significantly elevated in those patients who subsequently developed complications compared to those who remained free of complications. Our observations showed that ESR or CRP alone had good predictive value for complications, similar to the malaria severity prognostic score of Tangpukdee *et al.* [4]. Low albumin, which is also an inflammatory marker, was shown to be an important parameter in that study [4]. Since rising ESR and CRP precede a fall in albumin [21], checking ESR in the primary care setting might be a useful parameter to risk-stratify patients with malaria before clinically recognizable complications have set in.

Seven patients (5.7%) developed complications during follow-up, which was similar to Tangpukdee *et al.*'s findings [4]. Predicting development of complications has been done successfully in bacterial infections and sepsis using acute-phase reactants [7]. Pro-inflammatory cytokines, which are secreted in severe sepsis, have been the stimuli for these reactants. Since unregulated and aggressive host inflammatory response is the basis for poor outcome an extensive organ damage [12], it seems logical that acute-phase reactants are markers for severe malaria. Complications of malaria such as acute lung injury and renal failure are inflammatory in nature [10]. It is also known that CRP elevation occurs within 24 hours of the onset of the inflammatory process [21]. Hence, there appears to be a strong biological basis for the observation of raised CRP levels in those who subsequently develop complications.

Unregulated and aggressive host inflammatory response that is out of proportion to the infection is the basis for poor outcome and extensive organ damage. Parasite load was inferior in predicting malarial mortality [3]. Even studies involving severe malaria showed that parasite load had prognostic capability in terms of mortality prediction. An acute-phase protein has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25% during inflammatory disorders [20]. Cytokines, which are inflammatory mediators, stimulate the production of acute-phase proteins, including CRP [16]. CRP and amyloid A are very early markers of inflammatory responses [20]. In addition to elevated CRP in bacterial infections, higher levels have also been seen in parasitic disease [22]. Previous studies observed higher levels of pro-inflammatory mediators in severe malaria and improvement in level markers following recovery from severe malaria, but the role of pro-inflammatory mediators in predicting these complications has not been studied [6].

Among all the acute-phase reactants, only CRP and amyloid A have optimal kinetics with rapid elevation to very high levels and subsequent rapid fall during recovery [20]. Measurement of amyloid A is not done in routine practice. The availability of CRP and ESR measurement at point of care [22] makes them ideal markers for identifying complications at an early stage of malaria and offers an additional advantage for prognostication at the primary care level.

Interestingly, ESR also was found to be a good predictor of subsequent complications, though the area

under the curve was slightly less than that for CRP. Since ESR is a simple and cost-effective investigation and is available in primary care settings, it could be a substitute for CRP in predicting complications at a pretreatment stage in resource-limited settings.

CRP is found in trace amounts in normal human serum and is markedly increased in inflammatory conditions. CRP activates the complement cascade and could be an important contributor to the accelerated destruction of RBCs in malaria [23]. Thus, CRP, in addition to being an acute-phase marker for complication, could be an active participant in the pathogenesis of this event, which explains the observation of this study that a rise in CRP precedes the development of complications of malaria. It was also observed in another study that CRP and serum amyloid A were valuable in assessing the response to antimalarial treatment [16], which reinforces the role of elevated CRP in the pathogenesis of malarial complications.

Another interesting observation of this study was the occurrence of complications in vivax malaria. Traditionally, vivax has been considered a benign disease. But recently, more cases of vivax-related complications and mortality are being reported [10]. Our study confirmed the recent observations of complications developing in vivax malaria in about 5.8% of cases (Table 4). Most of the previous studies for predicting complications were done exclusively in falciparum malaria [4]. Our study has demonstrated that ESR and CRP have very good prognostic capability in falciparum and vivax malaria.

The results of this study have important clinical implications, especially in third-world countries where malaria is endemic. Very high NPV (91/93; 97.8%) suggests that only 2% of those with CRP <124mg/L developed complications, whereas 17% with CRP >124 mg/L developed complications. Those with pretreatment CRP <124 mg/L or ESR <34.5mm in the first hour can safely be assumed to remain free of complications with a reasonable amount of confidence, which could prevent aggressive and expensive monitoring of these patients. Patients with higher levels of inflammatory markers can be monitored closely and managed aggressively because the outcome of treatment for severe malaria is better if it is identified and managed early [2]. All the 7 patients experienced these complications between the second and third day. Physicians managing these patients should be watchful during this period when maximum complications are anticipated.

One important limitation of this study was the fact that we did not observe any case of cerebral malaria, an extremely dangerous complication, in this cohort. Moreover, unlike other complications, cerebral malaria does not involve any significant inflammation [10]. The observations of this study cannot be expected to be true for cerebral malaria. Another limitation is the relatively small number of patients who developed complications. Though statistical and clinical significance was achieved in this cohort, it needs to be validated in larger samples and various clinical and geographic settings. A final limitation was that this was a hospital-based study, so extrapolation of the results to the general population would be difficult.

Conclusions

CRP and ESR may help to identify uncomplicated malarial patients with a higher risk of developing complications during the course of treatment. These patients must be monitored closely, especially between the second and third day of diagnosis.

Acknowledgements

The authors are grateful for the financial support provided by Manipal University PG research grant to Sachin Kumar Vemula to carry out this study

References

1. World Health Organization (2000) Severe falciparum malaria: World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg 94 Suppl 1:1-90.
2. Moore DAJ, Jennings RM, Doherty TF, Lockwood DN, Chiodini PL, Wright SG, Whitty CJM (2003) Assessing the severity of malaria. BMJ 326:808-809.
3. Stauga S, Hahn A, Brattig NW, Fischer-Herr J, Baldus S, Burchard GD, Cramer JP (2013) Clinical relevance of different biomarkers in imported *plasmodium falciparum* malaria in adults: a case control study. Malar J 12:246.
4. Tangpukdee N, Krudsood S, Thanachartwet V, Duangdee C, Paksala S, Chonsawat P, Srivilairit S, Looareesuwan S, Wilairatana P (2007) Predictive score of uncomplicated falciparum malaria patients turning to severe malaria. Korean J Parasitol 45:273-282.
5. Shankar AH, Fawzil WW (2010) Moving toward hematological predictors of disease severity in malaria: going with the flow. Am J Hematol 85:225-226.
6. Andrade BB, Reis-Filho A, Souza-Neto SM, Clarencio J, Camargo LMA, Barral A, Barral-Netto M (2010) Severe *Plasmodium vivax* malaria exhibits marked inflammatory imbalance. Malar J 9:13.
7. Mohapatra MK, Das SP (2009) The malaria severity score: a method for severity assessment and risk prediction of hospital mortality for falciparum malaria in adults. J Assoc Physicians India 57:119-126.
8. Mishra SK, Panigrahi P, Mishra R, Mohanty S (2007) Prediction of outcome in adults with severe falciparum malaria: a new scoring system. Malar J 6:24.

9. Bei AK, DeSimone TM, Badiane AS, Ahouidi AD, Dieye T, Ndiaye D, Sarr O, Ndir O, Mboup S, Duraisingh TM (2011) A flow cytometry –based assay for measuring invasion of red blood cells by *plasmodium falciparum*. Am J Hematol 85:234-237.
10. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM (2014) Malaria. Lancet 383:723-735.
11. Day NPJ, Phu NH, Mai NTH, Chau TTH, Loc PP, Chuong LV, Sinh DX, Holloway P, Hien TT, White NJ (2000) The pathophysiologic and prognostic significance of acidosis in severe adult malaria. Crit Care Med 28:1833-1840.
12. Higgins SJ, Xing K, Kim H, Kian DC, Wang F, Dhabangi A, Musoke C, Cserti-Gazdewich CM, Tracey KJ, Kain KC, Liles WC (2013) Systemic release of high mobility group box 1 (HMGB1) protein is associated with severe and fatal *Plasmodium falciparum* malaria. Malar J 12:105.
13. Walton E, Oliveros H, Villamor E (2014) Hemoglobin concentration and parasitemia on hospital admission predict risk of multiple organ dysfunction syndrome among adults with malaria. Am J Trop Med 91: 50-53.
14. Kremsner PG, Valim C, Missinou MA, Olala C, Krishna S, Issifuo S, Kombila M, Bwanaisa L, Mithwani S, Newton CR, Agbenyega T, Pinder M, Bojang K, Wypij D, Taylor T (2009) Prognostic value of circulating pigmented cells in African children with malaria. J Infect Dis 199:142-150.
15. Gillespie SH, Dow C, Raynes JG, Behrens RH, Chiodini PL, McAdam KP (1991) Measurement of acute phase proteins for assessing severity of *Plasmodium falciparum* malaria. J Clin Pathol 44:228-231.
16. World Health Organization (2009) Severe malaria. Trop Med Int Health 19:7-131.
17. National Kidney Foundation (2002) K/DOQL Clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kidney Dis 39Suppl 1:1-266.
18. American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33Suppl 1:62-69.
19. World Health Organization (2010) Guidelines for the treatment of malaria. Available:http://www.ncbi.nlm.nih.gov/pubmed/25473692 Accessed 15April 2016.
20. Gabay C, Kushner I (1999) Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448-454.
21. Stuart J, Lewis SM (1988) Monitoring the acute phase response. BMJ 297:1143-1144.
22. Watson J, Round A, Hamilton W (2012) Raised inflammatory markers. BMJ 344:e454.
23. Ansar W, Habib SKH, Roy S, Mandal C, Mandal C(2009) Unraveling the C-reactive protein complement-cascade in destruction of red blood cells: potential pathological implications in *Plasmodium falciparum* malaria. Cell Physiol Biochem 23:175-181.

Corresponding author
Mahabala Chakrapani, Professor
Department of Medicine, Kasturba Medical College
Manipal University, Mangalore, India
Light House Hill Road, Hampankatta, Mangalore
Phone: + 919448812207
Fax: 0824-2428183
Email: chakrapani.m@manipal.edu

Conflict of interests: No conflict of interests is declared.