

Research Article

Prognostic value of Serum C-Reactive Protein in Malaria

Mitul Chhatrivala*, Bhavita Patel, Rita Shah, Nadeem Shaikh, Ruchi Gokani,
Anup Nilawar

Department of Biochemistry, S.B.K.S Medical Institute & Research Centre, Sumandeep Vidyapeeth, Piparia, Gujarat

***Correspondence Info:**

Dr. Mitul Chhatrivala,
Resident,
Department of Biochemistry,
S.B.K.S MI & RC, Sumandeep Vidyapeeth, Piparia, Vadodara, Gujarat, India
E-mail: drchhatri@gmail.com

Abstract

Introduction: C-Reactive protein is known as a marker of morbidity and mortality in malaria. It correlates closely with other complications in malaria and can be used to predict severity. Thus, measurement of CRP can be useful in understanding the pathogenesis of severe malaria. However, studies regarding CRP in malaria are very rare from India.

Aims & objectives: The present study aimed to study CRP levels in patients with malarial infection and to find out the usefulness of CRP in assessment of disease severity.

Material and Methods: The study population consisted of 50 patients with malarial infection, of them 29 were *Plasmodium falciparum*, 21 were *P. vivax*-infected and no patient found with dual infection in our study. Out of 50 patients, 17 patients required admission for various reasons and of them 5 patients died. Serum C-reactive protein concentrations were measured in all the patients. Percentage parasitemia, platelet count and Liver function tests were measured on the day of admission. Statistical tests included were Students t-test, chi-square test and Pearson correlation coefficient.

Results: Serum C-reactive protein concentration was significantly elevated in all the malaria patients and it was significantly higher ($P < 0.01$) in *Plasmodium falciparum* (28.81 ± 10.91 mg/L) as compared to *P. Vivax* malaria (15.91 ± 8.73 mg/L). Admitted patients had higher CRP levels (47.80 ± 12.73 mg/L) compared to patients treated on OPD basis (24.14 ± 12.17 mg/L) ($P < 0.0001$). Also CRP levels were higher in patients with multiple complications and in the patients who died compared to survivors. A significant positive correlation was found between serum CRP and percentage parasitemia, serum bilirubin, serum alanine aminotransferase and serum aspartate aminotransferase in *P. falciparum* infected patients but not in *P. Vivax*.

Conclusion: There is significant increase in C-reactive protein in malaria infected patients and can be considered a cost - effective and reliable tool in assessment of prognosis in malaria.

Keywords: C-Reactive Protein, Malaria, *Plasmodium falciparum*, *Plasmodium Vivax*, Prognostic marker

1. Introduction

Malaria is a major health problem in India, being one of the biggest burden in terms of morbidity and mortality among all infectious diseases.¹ It is transmitted by the bite of female *Anopheles* mosquito and caused by protozoan parasites of the genus *Plasmodium*. Four species of the *Plasmodium* parasite, namely *P. Falciparum*, *P. Vivax*, *P. ovale*, *P. malariae* can infect humans. Most serious forms of the disease are caused by *P. Falciparum*.²

Plasmodium causes biological disorders which are not totally elucidated. When malaria occurs there is destruction of infected erythrocyte, opsonisation of red cells and dyserythropoiesis.³ When schizonts rupture, host monocytes and macrophages secrete pro-inflammatory cytokines stimulating the production of acute phase protein. An acute-phase protein is defined as one of which plasma concentration increases (positive acute phase proteins) or decreases (negative acute-phase proteins) by at least 25 percent during inflammatory disorders.⁴

Recently some acute phase reactants have emerged as biomarkers in malaria infection in addition to chemokines and cytokines. In particular C-reactive protein (CRP) and nitric oxide (NO) have been identified as important inflammatory biomarkers.^{5,6} CRP is an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis and detoxification of substances released from the damaged tissue. Measurement of serum CRP is most frequently used for the evaluation of injury in the body tissue or for the detection of inflammatory event somewhere in the body. In malaria CRP secretion is induced by pro-inflammatory cytokines that are secreted by host mononuclear cells and strong correlations have been found between CRP levels and parasitemia.⁷

In malaria, CRP is said to have a pathogenic role. CRP is said to bind to infected erythrocytes and help in their clearance. This immune activation towards infected RBCs also results in various deleterious manifestations. Also, CRP activates complement pathway and platelet activation, and results in various untoward effects. Thus, measurement of CRP can be useful in understanding the pathogenesis of severe malaria.⁸

However, studies involving measurement of CRP in malaria patients are quite few in India, this study was designed to evaluate whether measurements of CRP in malaria patients can be used to assess disease severity and can be used as prognostic marker in Malaria.

2. Material and Method

The present study included 50 untreated malaria patients attending the Out-patient - Department at Dhiraj General Hospital, Sumandeep Vidyapeeth, Piparia, with symptoms of fever, rigor, headache and vomiting between June 2013 to December 2013. Patients who tested positive for malaria by slide microscopy were enrolled in the study. In all the patients' malaria infection was the only diagnosis.

A finger prick blood sample was taken to prepare thick and thin blood films and stained with Geimsa stain to determine the presence of malaria parasites. The following lab investigations were performed when the patient was admitted: - Percentage parasite count, platelet count,

serum bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Patients with complication of malaria like renal failure, Anaemia, Jaundice or Altered consciousness were admitted in the ward.

Estimation of C-reactive protein was done by nephelometry method with the commercially available Kit on MISPA- i instrument, with normal value ≤ 5 mg/L. The study was approved by Institutional Ethics Committee and written informed consent was obtained from all patients. All patients above 15 years of age of both sexes were included. Patients with other infection and diseases that can alter CRP levels and on drugs like steroids and immunosuppressants were excluded from the study group.

2.1 Statistical Analysis

The data were collected, recorded and analyzed statistically to determine the significance of different parameters by using Med-Calcv11.5.0. Statistical analysis software. All discrete variables were expressed number and percentages and continuous variables as mean \pm SD (standard deviation). To compare different biochemical parameters Students' t-test and when number was small Mann-Whitney U-test was used. To find out the correlation between two variables, Pearson's correlation coefficient was used. A value of $P < 0.05$ was considered as statistically significant.

3. Results

In this study total 50 patients were included, out of which male to female ratio was 34:16. Out of them, 29 patients (58 %) were *Plasmodium falciparum* (Pf) and the rest 21 patients (42 %) were *P. vivax* - infected and no patient with dual infection was included in this study. Fever, headache and tremors were the commonest presenting symptom in both the cases. Table-1 shows demographic and clinical profile of the patients.

Table-1: Demographic and Clinical profile of the patients

Parameters	<i>P. falciparum</i>	<i>P.vivax</i>	Total (n=50)
Male	20	14	34 (68 %)
Female	9	7	16 (32 %)
No. of patients Admitted	15	2	17 (34%)
No of patients died	5	0	5 (10 %)

Out of 50 patients, 17 patients (34 %) needed admission. Of the 17 patients admitted, the complications seen were Hepatic (n= 4 {Pf = 3, Pv =1}), Renal (n = 2 {Pf = 2,Pv =0}), Haematological (n =2 {Pf = 2,Pv = 0}), Metabolic (n= 1 { Pf =1,Pv =0}), Cerebral (n= 3 {Pf 3=,Pv =0}) and in 5 patients who died, more than one complication was seen and the causes of death were cerebral cause (n= 3), renal (n =1) and haematological (n = 1). Most of the admitted patients & the patients who died were of *Plasmodium falciparum* infected. (Table 1).

Mean CRP levels in patients with malaria infection was 34.85 ± 19.90 mg/L. Of these, mean CRP concentration was significantly high in both Pf (28.81 ± 10.91 mg/L) and Pv (15.91 ± 8.73 mg/L) patients ($P < 0.01$). The serum bilirubin concentration and platelet count were statistically different between the two groups. (Table 2)

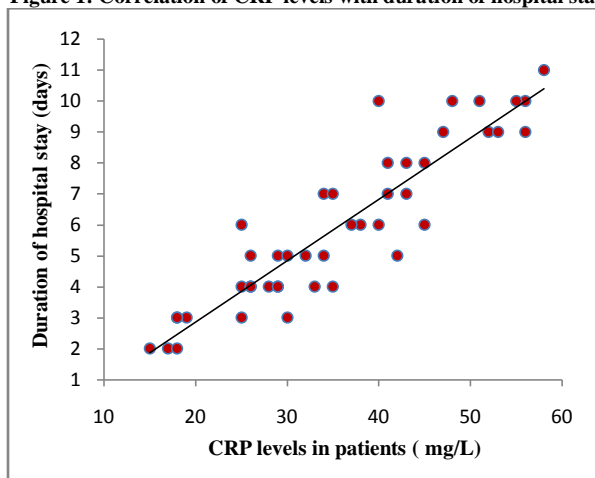
Table 2: shows CRP levels and other investigations in *Plasmodium falciparum* and *Plasmodium vivax* infected patients

Investigations	Mean \pm SD		P- value
	<i>P. falciparum</i> (n = 29)	<i>P. vivax</i> (n = 21)	
CRP level (mg/L)	28.81 ± 10.91	15.91 ± 8.73	0.001
Total Bilirubin (mg %)	4.9 ± 1.9	1.5 ± 0.6	0.001
Alanine Transferase (U/L)	89.61 ± 68.46	32.50 ± 12.80	0.05
Aspartate Tranaminase (U/L)	139.51 ± 121.41	38.93 ± 15.48	0.065
% Parasitemia	2.9 ± 1.9	1.5 (0.9)	0.038
Platelet count (lac/cumm)	0.8 ± 0.3	1.4 ± 0.5	0.001

A significant correlation was found between CRP levels and various haematological and biochemical investigations in *Plasmodium falciparum* infected patients. CRP correlated positively and significantly with Total Bilirubin ($r = 0.75$, $P < 0.01$), Alanine transferase ($r = 0.90$, $P < 0.001$), Aspartate transaminase ($r = 0.91$, $P < 0.001$), % Parasitemia ($r = 0.87$, $P < 0.001$) and correlated negatively but not significantly with Platelet count ($r = - 0.51$, $P < 0.081$) in *Plasmodium falciparum* infected patients. But in *Plasmodium vivax*, no significant correlation was found between any of the haematological and Biochemical Parameters (Total Bilirubin ($r = 0.17$, $P = 0.52$), Alanine transferase ($r = -0.04$, $P = 0.82$), Aspartate transaminase ($r = -0.20$, $P = 0.22$), % Parasitemia ($r = 0.29$, $P = 0.23$)).

A strong correlation was found between CRP levels and duration of hospital stay ($r = 0.62$). ($P < 0.001$) (Figure 1)

Figure 1: Correlation of CRP levels with duration of hospital stay



The patients who needed admission had higher CRP levels (47.80 ± 12.73 mg/L) compared to patients who were treated on OPD basis (24.14 ± 12.17 mg/L) ($P < 0.0001$). Among the 5 patients who died had significantly higher CRP levels (48.22 ± 13.56 mg/L) than patients who survived (27.47 ± 14.76 mg/L) ($P < 0.001$). In the patients who were having multiple complications ($n = 5$) CRP levels (52.08 ± 9.51 mg/L) were significantly higher than patients with single complication ($n = 14$) (40.21 ± 12.34 mg/L) ($P < 0.001$).

4. Discussion

Malaria causes high mortality and morbidity in various tropical regions. The indirect burden of malaria like malnutrition and anemia are also highly prevalent in these populations of endemic zones. Thus, measures to identify severity of this disease are needed so as to institute timely therapy and avoid the complications.⁸ CRP is an acute phase reactant and is one of the most widely used acute phase inflammatory proteins because of its early rise and rapid kinetics. It binds damaged host cells including erythrocytes infected by *P. falciparum* resulting in their clearance by both humoral and cellular immune mechanisms.⁷ Also, CRP activates complement pathway and platelet activation, and results in various untoward effects. Thus, measurement of CRP can be useful in understanding the pathogenesis of severe malaria.⁸

The results of the study showed that CRP levels are increased significantly in patients with malarial infection. Also CRP levels were significantly higher in patients with *Plasmodium falciparum* infection compared to patients with *Plasmodium vivax* infection. Paul *et al*⁸ in their study found that CRP Levels are increased significantly in Malaria infected patients but found no significant difference between *P. falciparum* and *P. vivax* cases. However our results are in accordance with Agrawal *et al*⁷ who found mean CRP to be higher in the plasma of *P. Falciparum* infected patients compared to *P. vivax* infected patients. Other studies have reported a higher serum CRP concentration in *P. vivax* patients.⁹

In our study we found that patients who died had significantly higher CRP levels than patients who survived. Also patients who were admitted had higher CRP levels than patients who were treated on OPD basis. Similar findings were observed by Paul *et al*⁸ & Bainik Adhikari¹¹ in their study. In our study, we measured CRP levels only at the time of admission or first contact, However serial measurement of CRP levels, can be even better at assessment of course of the disease. Study by Agarwal *et al*⁷ involved sequential measurements of serum CRP concentrations in Malaria patients over a period of seven days. Highest values of mean CRP were found on the second day of treatment; this finding was consistent with the fact that increase in parasitemia occurs upto 18 hrs after initiation of antimalarial chemotherapy. We also found that CRP levels were significantly higher in patients with multiple complications compared to patients with single complications. These findings were similar to studies by Paul *et al*⁸ & Dongho *et al*¹² who found that CRP levels rise in complicated malaria, compared to uncomplicated.

In our study, CRP levels correlated significantly with haematological & biochemical markers of severity of malaria like Total Bilirubin, SGPT, SGOT & % parasitemia in *P. falciparum* infected patients, but not in *P. vivax* patients which were similar to Studies by Lima-Junior Jda *et al*⁹ & Gillespie *et al*.¹⁰ We also found that in malaria children CRP concentrations depended on parasitaemia levels. According to Naik and Voller¹³, the more parasite density is increased, the more both biochemical parameters are elevated too. Some novel markers like TNF and interleukins have also been shown to correlate well with disease severity¹⁴ but in a developing country like India where cost effectiveness of the test is a major concern, these markers may not be feasible. Hence measurement of serum CRP concentrations can provide a simple measurement of disease severity and can be used as prognostic marker in Malaria.

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