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PREDICTORS OF C-REACTIVE PROTEIN RESPONSE IN CHILDREN INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA

E. E. UTUK, E. E. IKPEME, J. J. UDO and M. U. AKPAN

ABSTRACT

***Objective*: To assess the predictors of C-reactive protein response in *plasmodium falciparum* malaria as seen in children in a malaria endemic region of Nigeria.**
***Design*: A prospective cross-sectional study.**
***Setting* : The Children Out-patient (CHOP) Clinic, Children Emergency Unit (CHEU), Child Welfare/Growth Monitoring Clinic, Immunisation Centre and Paediatric Ward of the University of Uyo Teaching Hospital (UUTH), Uyo in Akwa-Ibom State.**
***Subjects* : Three hundred and sixty children aged six to sixty months with microscopically confirmed *P. falciparum* malaria compared with 360 healthy children without malaria parasitaemia matched for age and gender.**
***Results*: The predictors of the C-reactive protein response in malaria (CRP ≥ 10mg/l) were fever (t = 6.867; p = 0.001), malaria parasite count (t = 5.387; p = 0.001), severe anaemia (t = -11.23; p = 0.001) and age. Younger children had a greater CRP response. The logistic regression curve showed a 66.9% sensitivity, 92.1% specificity, positive predictive value, 83.2% and negative predictive value of 82.2% of predicting C-reactive protein response in malaria.**
***Conclusion*: *P. falciparum* malaria induces significant CRP responses. Younger children who present with fever, hyperparasitaemia and severe anaemia are more likely to have C-reactive protein response with malaria.**

INTRODUCTION

The global burden of malarial disease and its complications in childhood still constitutes a major public health problem and calls for sustained efforts towards its control. The pathophysiological response of individuals to malaria is highly variable. It depends on several factors including previously acquired immunity and the production of cytokines (1,2). Host immune markers of inflammation like C-reactive protein (CRP) which is a non-specific acute phase reactant, has been found to play an important role in the immune responses to malaria (3). Its measurement therefore has physiological relevance as an indirect measure of inflammatory events that occur during malaria infection (4). A prospective characterisation of CRP levels during malaria infection could provide additional insight to its pattern of response in the disease.

Various host and parasite factors are responsible for the pathological events seen in malaria. At the completion of schizogony within red blood cells, newly developed merozoites are released, and alongside numerous waste substances and toxic factors such as glycosylphosphatidylinositol (GPI) into the blood (5,6). The vigorous cytokine responses to parasite proteins released during schizogony contributes to adverse clinical outcomes. Severe malaria has been associated with high levels of inflammatory cytokines such as Tumour necrotic

factor-α, Interleukin-I and Interleukin-6, and these cytokines induce the production of C-reactive protein from hepatocytes (5,6).

In hyperendemic or holoendemic malarial areas in sub-Saharan Africa, including Nigeria, these immunological markers may serve as useful indicators for active malarial episodes (7,8). There is an association of elevated levels of C-reactive protein in uncomplicated and severe forms of malaria. Therefore, knowledge of C-reactive protein response may be useful as a tool in malaria immuno-epidemiology, serving as a malaria episode marker for use in government and non-governmental malaria control programmes. Recent studies on new therapeutic agents that lower the circulating CRP levels might also open up new adjuncts to treatment options against malaria to reduce its incidence and promote better health among children in Nigeria and the sub-Saharan Africa.

MATERIALS AND METHODS

The study was carried out in the Children Out-patient (CHOP) Clinic, Children Emergency Unit (CHEU), Child Welfare/Growth Monitoring Clinic, Immunisation Centre and Paediatric Ward of the University of Uyo Teaching Hospital (UUTH), Uyo in Akwa-Ibom State. It was conducted over a six month period, from November 2010, to April 2011. Uyo, the capital city of Akwa Ibom State, is located in the south-south region of Nigeria. The Teaching Hospital which is the only tertiary healthcare facility is located on the outskirts of Uyo six kilometres from the centre of the city. The hospital is a three hundred and fifty-five bed health facility. It serves as a referral centre and also accepts self reported cases. Ethical approval was obtained from the University of Uyo hospital's ethics committee while the informed consent was obtained from parents/guardian of children included in the study after initial assessment.

Included were children with fever or a history of fever in the previous 48 hours prior to presentation in the hospital with a parasitological proof of *P. falciparum* malaria, and those with one or more features of severe malaria such as prostration, repeated convulsions, hyperpyrexia, impaired consciousness, severe anaemia, respiratory distress, jaundice and haemoglobinuria were included. Excluded were children with any obvious *foci* of infection and/or a positive blood culture examination and those with evidence of chronic illnesses, chronic liver disease, inflammatory diseases, viral exanthema and those receiving anti-inflammatory drugs.

During the six month study period, every consecutive patient, aged six to sixty months, who presented with fever, defined by an axillary temperature ≥ 37.50 Celsius (9) or a history of fever had a detailed history, general and systemic physical examination, including examination of the ears and throat. The controls were afebrile, apparently healthy children aged six to sixty months, matched for age and gender with no clinically identifiable *foci* of infection after a thorough and detailed clinical examination. They were selected from children attending child welfare clinic for growth monitoring and those presenting for immunisation.

For every child recruited, thick and thin blood films for malaria parasite were prepared for confirmation of malaria parasitaemia and determination of malaria parasite count. A clinical diagnosis of malaria was confirmed on the basis of the presence of the asexual forms (trophozoites / ring forms) of malaria parasites. A blood culture was done on every subject to exclude concurrent bacterial infection and a lumbar puncture to exclude meningitis in children with altered sensorium, or presenting with an episode of seizure.

The serum C-reactive protein assay was done by quantitative determination of C-reactive protein using the Enzyme Linked Immunosorbent Assay method, (High Sensitivity C-reactive protein Enzyme Immunoassay test kit. Catalog number BC-1119, USA) (10). The obtained values of the patient samples and control sera were multiplied by the dilution factor of 100 to obtain CRP results in mg/l.

The data were analysed using the Statistical Package for Social Sciences (SPSS) version 17.0. P-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

A total of seven hundred and forty-five children aged six to sixty months were recruited. Twenty-three children were excluded from this study for different reasons which included eight controls with asymptomatic malaria parasitaemia, six patients with no malaria parasitaemia, six children with viral exanthem and three children who had a positive blood culture. Thus, a total of three hundred and sixty (360) patients, and three hundred and sixty (360) controls were subsequently studied. Among the patients and controls studied, 200 (55.6%) were males and 160 (44.4%) were females giving a male to female ratio of 1.25 : 1, Table 1.

Table 1
Age and gender distribution of study population

Age group (months)	Control n (%)	Subject n (%)	Total n (%)
<12	121 (33.60)	121 (33.60)	242 (33.60)
13 – 24	104 (28.90)	104 (28.90)	208 (28.90)
25 – 36	46 (12.80)	46 (12.80)	93 (12.80)
37 – 48	43 (11.90)	43 (11.90)	84 (11.90)
49 – 60	46 (12.80)	46 (12.80)	93 (12.80)
Gender			
Male	200 (55.60%)	200 (55.60%)	360 (100%)
Female	160 (44.40%)	160 (44.40%)	360 (100%)
TOTAL	360 (100.00)	360 (100.00)	720 (100.00)

The mean ages and weights of the male and female patients and that of the control group were similar. The duration of fever in the patients was one to four days (mean 1.9 ± 0.8) and mean temperature 37.83 ± 0.890Celsius (range 36.5 – 40.10Celsius). No child in the control group had fever. The overall mean value of serum CRP for the subjects was 11.97 ± 13.97 (range 0.2 – 70.0mg/l), greater than the 2.21 ± 2.30 (range 0.2 -10.0mg/l) in the control group. This difference was statistically significant ($t = 13.09$; $p < 0.001$). There was no association between the age ($p = 0.71$), weight ($p = 0.9$) and gender ($p = 0.4$) of the subjects in the prediction of a CRP response.

The relative frequency of the manifestations of severe malaria in the fifty-five patients depicts hyperpyrexia as the most common, in 54 (98.2%), prostration in 31 (53.4%), and jaundice the least, in three (5.5%) of the 55 patients. Of the seven patients presenting with altered sensorium (Blantyre coma score < 4), three of these were deeply comatose, (Blantyre score of 0 to 2) at presentation, with a diagnosis of cerebral malaria. Of the variables, only temperature, severe anaemia and hyperparasitaemia were independent predictors of a CRP response in the patients presenting with severe malaria.

Table 2
Logistic regression model for predicting CRP response in malaria.

Variable	Unstandardised B	Co-efficients Standard Error	Standardised Beta	Co-efficients T	P-value
Constant	-8.616	1.551		-5.554	0.000
Age (months)	0.000	0.001	0.0004	0.104	0.917
Temperature	0.252	0.037	0.468	6.867	0.001*
Prostration	0.073	0.101	0.043	0.726	0.468
Impaired consciousness	0.073	0.160	0.021	0.455	0.649
Hyperpyrexia	0.014	0.098	0.011	0.148	0.883
Multiple convulsions	-0.051	0.132	-0.017	-0.388	0.698
Severe anaemia	-36.38	2.237	0.521	-11.23	0.001*
Hypoglycaemia	3.11	5.52	0.026	0.56	0.57
Malaria parasite count	0.148	0.028	0.283	5.387	0.001*
Duration of fever	0.015	0.026	0.024	0.581	0.562

*Statistically significant

Table 3
Distribution of the malaria parasite count and serum CRP levels in the patients.

Malaria parasite count/μl (range)	n	C-reactive protein (mg/l) Mean ± S.D	Mean difference	F (p value)
500 – 5000	195	5.96 ± 7.01		87.13 (0.001)
>5000 – 100,000	86	11.43 ± 10.97	-5.47 (0.001)*	
>100,000 – 250,000	72	25.20 ± 16.73	-16.59 (0.001)*	
> 250,000	7	49.71 ± 10.79	-36.62 (0.001)*	
Total	360	11.97 ± 13.96		

ANOVA F = 87.13; p = 0.001

*Statistically significant

Table 4
Mean Serum C-reactive protein levels and temperature pattern in patients

Temperature	N	CRP (Mean ± S.D)	Mean difference	F (p-value)
Afebrile	144	4.13 ± 5.07		156.82 (0.001)
Pyrexia	162	11.67 ± 10.35	-7.67 (0.001)*	
Hyperpyrexia	54	32.84 ± 17.40	-28.71 (0.001)*	

*Statistically significant

Table 5
Mean serum C-reactive protein and duration of fever in patients

Duration of fever (days)	N	CRP (Mean ± SD)	Mean difference	F (p-value)
1	120	10.34 ± 11.77		0.81 (0.48)
2	164	12.83 ± 14.83	1.67 (0.53)	
3	68	12.69 ± 15.74	2.12 (0.74)	
4	8	12.95 ± 9.02	5.10 (0.98)	

The logistic regression model in Table 2 showed that the significant predictors of a C-reactive protein response in malaria were the malaria parasite count (t = 5.387; p = 0.001), temperature (t = 6.867; p = 0.001) and severe anaemia (t = -11.23, p = 0.001). The other clinical variables were not significant predictors of a CRP response. The logistic model had a 66.92% sensitivity, 92.07% specificity, negative predictive value of 82.16% and positive predictive value of 83.18% of predicting elevation of C-reactive protein in children with malaria.

The distribution of the malaria parasite count and serum C-reactive protein levels in the subjects is shown in Table 3. The subjects with hyperparasitaemia (>250,000 parasites/μl) had the highest mean serum C-reactive protein level. The parasite count was a significant predictor of a CRP response in patients and this was significantly higher in children with severe than uncomplicated malaria (t = -7.24; p<0.001).

Table 4 shows temperature as a significant predictor of a CRP response. A significant difference in the mean serum C-reactive protein level (mg/l) occurred in the subjects as temperature increased using the analysis of one way variance (ANOVA). Subjects who presented with hyperpyrexia had greater mean serum CRP level of 32.84 ± 17.40 than the 11.67 ± 10.35mg/l in subjects with fever and that in afebrile subjects (4.13 ± 5.07), (F = 156.82; p = 0.001). There was however, no significant difference between the mean serum CRP levels and the duration of fever at presentation (t = 0.81; p = 0.48) Table 5.

Figure 1
Pattern of C-reactive protein response in study population.

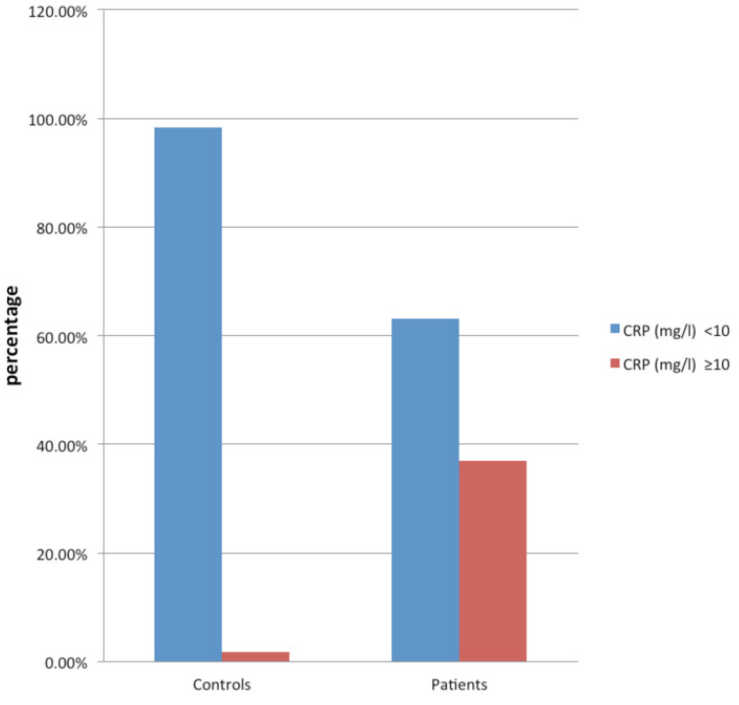

Figure 2 shows that one hundred and thirty-three (36.9%) of the 360 patients with malaria had a CRP response (CRP ≥ 10mg/l), while only six (4.32%) of the 360 controls had a CRP response ($P < 0.001$). This pattern of CRP response indicated that children with a C-reactive protein level ≥10mg/l were twenty-two times more likely to be infected with malaria [chi-square (p-value); 143.79 (0.001) OR = 1.29; RR = 22.16)].

DISCUSSION

This study demonstrated the presence of a greater C-reactive response in the malaria infected children than in the controls. This was consistent with findings by other authors (11,12) who acknowledged a greater concentration of C-reactive protein in the sera of children infected with *plasmodium falciparum* malaria. The malaria-attributable CRP response of 36.9% obtained in this study was lower than the 51.7% documented in Tanzanian children (13), but higher than the 8.2% documented in children residing in Papua New Guinea (14). The difference in reports may be because the study in Tanzanian children (13) was community based, and in a hyperendemic malaria region. Moreover, children with bacterial infections were not excluded. This could have provoked a greater induction of CRP response. Imrie *et al* (14) in Papua new guinea studied only healthy children with asymptomatic low-grade parasitaemia. This probably explains the lower prevalence obtained from the Papuan children.

It has been established that CRP responses are greater in children presenting with the acute phase of uncomplicated or severe malaria than in asymptomatic children. This is as a result of the greater release of bioactive molecules, and host inflammatory response including cytokine production (15-17). Also, there is a genetic predisposition to the secretion of CRP in malaria and this is demonstrated in varying degrees in different population groups (18,19).

The comparatively low level of CRP response in control children in this study was similar to reports by Riberio (11) in Brazil and Kindmark (12) in Sweden in their study populations of healthy children. The trauma prone tendencies common in young children, with accompanying injuries and also sub-clinical infections not usually obvious on clinical examination could all elicit these varying degrees of C-reactive protein resp.

The higher levels of mean serum CRP concentration between the subjects and controls in the various age groups in present study reinforces the effect of malaria antigen in the induction of CRP production (20,21). Younger children mostly under two years of age constituted a greater percentage (62.5%) of the patients in this study. These children, who though live in a holoendemic region are yet to develop full malaria immunity which comes from prolonged and repeated exposure to malaria parasitaemia. They therefore elicited a more marked immune and inflammatory response to invading parasitaemia reflecting in their greater CRP levels.

Acquired malarial immunity is known to play an important role in the modulation of the immune responses to invading parasites (22,23).

The significantly higher response observed in the febrile patients compared with the afebrile ones was similar to studies in other African children (13,14). In Tanzania, Hurt *et al* (13) documented this trend of significantly increasing serum CRP levels in children with temperatures greater than 37.4ºC. The reason for this observation may be explained by the greater parasite densities and corresponding cytokine release from the macrophage response. These cytokines including interleukins 1 and 6, tumour necrosis factor are also responsible for the stimulation of CRP in malaria as well as fever (24,25).

The lack of a significant difference between the mean serum CRP levels and the duration of fever in the patients presenting in the present study indicates that once the stimulus for induction of CRP is triggered, the secretion of CRP is sustained, irrespective of the temperature periodicity, as occurs in malaria. Serum CRP levels gradually and slowly rises with the level of parasitaemia and cytokine release. The present study suggests that increased levels of CRP may also occur in clinical episodes of malaria which symptoms do not include a measurable fever. This indicates that CRP levels reflect recent histories of fever as well as the current episodes (13). This is a useful finding for malaria epidemiological surveys, and may accurately present malaria morbidities in a given population.

The study indicates a moderate CRP response in children with malaria, especially younger children, those with hyperparasitaemia, severe anaemia and hyperpyrexia. This is a useful test in identifying children with a risk to life-threatening manifestations such that prompt and appropriate treatment can be instituted. Larger scale studies which include more severe cases of malaria may indicate more independent predictors of the CRP response in malaria.

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