Serum C-Reactive Protein and Cholesterol as Predictors of Severity in Childhood *Falciparum* Malaria Infestation Among Nigerians

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Abstract

Background: *C-Reactive-Protein (CRP) is regarded as one of the most sensitive indicators of acute inflammation and its concentration often increases or decreases by about 2%. Although CRP is a nonspecific marker of inflammation, very high CRP levels are observed during attacks of malaria. CRP is a good positive predictive indicator for the diagnosis of malaria and is also useful in epidemiological studies on malaria. Depletion of red cell cholesterol has no detectable effect on major red cell membrane function but can block malaria invasion. Malaria parasite count on peripheral blood film has been reported not to be indicative of an acute infestation, thus highlighting the need for an alternative indicator of acute malarial infestation.*

Materials and Methods: *A total of 120 paediatric subjects were randomly recruited for the study from among those diagnosed routinely in the Haematology Laboratory of University of Ilorin Teaching Hospital of acute malaria. They were grouped into those with mild, moderate and severe malaria with 40 persons in each group. Their samples were further subjected to tests for malaria density, CRP and cholesterol levels determination.*

Results: *The mean ages of our subjects with mild, moderate and severe malaria were 10.3±1.5 years, 7.1±2.2 years and 3.8±1.3 years respectively. Serum CRP levels significantly increases while that of cholesterol significantly reduces with increase in parasite scoring and absolute count (p values <0.005). Also the levels of serum CRP correlated positively while that of cholesterol correlated negatively with parasite scoring and count.*

Conclusion: *In conclusion, serum C-reactive protein can be said to be a good indicator of the severity of malaria in our environment although it is not routinely done. Cholesterol level which correlated negatively with parasite density, can also serve as an indicator of severity of falciparum malaria infestation.*

Keywords: *falciparum* malaria, Parasite scoring, Parasite density, CRP and cholesterol

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Introduction

Acute phase proteins levels fluctuate in response to tissue injury, such as trauma, myocardial infarction, acute infections, burns and chronic inflammation (as in Crohn's disease, rheumatoid arthritis and malignancy). The acute-phase response is general and nonspecific.

The stimulus for production is likely to be inflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor (TNF)[1]. These acute phase proteins include C-reactive protein (CRP), Alpha-1 acid glycoprotein, Alpha-1 antitrypsin, Haptoglobins, Ceruloplasmin, Serum amyloid A, Fibrinogen, Ferritin and Complement components C3, C4.

C-Reactive-Protein (CRP) is widely regarded as one of the most sensitive indicators of inflammation[2]. It is an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis and detoxification of substances released from damaged tissues. During acute inflammation, concentrations of CRP and other acute phase proteins increase or decrease by about 2%[3, 4]. CRP derives its name from its ability to react with the C polysaccharide of Streptococcus pneumoniae, but it may also bind to chromatin in nuclear DNA-histone complexes. Once bound, it is able to activate the classical complement pathway. Its concentration characteristically return to normal after 7 days of appropriate treatment for bacterial meningitis or malaria if no complications develop.

These proteins are called acute phase proteins because of the sharp alteration in their concentrations during acute inflammation. Of all, CRP is the most widely used because of its early rise and rapid kinetics[5]. CRP is a sensitive indicator of early phase of inflammation or tissue destruction process. It is synthesized and secreted mainly by the liver.

CRP levels have been used extensively in clinical practice. It is used as a simple measure of disease severity[6], efficacy of therapy[7] and severity of complications[8]. CRP is not normally present in human serum but if present, its normal concentration is 0-8mg/L and does not show alterations with difference in age and sex[9]. Although CRP is a nonspecific marker of inflammation, very high CRP levels are measured during attacks of malaria [10, 11].

Malaria is a very widespread disease in tropical countries, affecting up to 400 million people each year. The disease continues to claim the lives of more children worldwide than any other infectious diseases. It is caused by Plasmodium species and produces an inflammatory reaction in the body. Mononuclear cells, which are activated by the plasmodium during malaria attacks, produce inflammatory cytokines such as TNF, IL-1 or IL-6. These cytokines stimulate the hepatic synthesis of acute phase proteins including CRP, orosomucoid and haptoglobin which all rise in malaria[12]. Rise in CRP in malaria has been reported by several authors[13, 14]. CRP has been found to be a good positive predictive indicator for the diagnosis of malaria in febrile people returning from a tropical areas[15] and is a marker for malarial epidemiological studies[16].

During malaria attack, the invading merozoites of the parasite invaginates the red cell membrane to reside in an immunologically priviledged intracellular parasitophorous vacuolar membrane (PVM). Although the bulk of erythrocyte proteins is excluded from the PVM, proteins that reside in cholesterol –rich-detergent -resistant membrane (DRM) rafts in the host membrane are recruited into the membrane[21]. Mild depletion of red cell cholesterol has no detectable effect on major red cell membrane function but disrupts DRM rafts and blocks malaria infection[22].

Clinically, malaria infestation could be mild, moderate or severe depending on the parasite load or density. The mainstay of malaria diagnosis has been the microscopic examination of blood, using blood film[17]. Other samples less frequently used are saliva and urine which are less invasive alternatives[18]. Recently modern methods utilizing antigen-

antibody reaction or polymerase chain reaction have been discovered, though they are not widely implemented in malaria endemic regions[19, 20]. Areas that cannot afford these modern methods depend on the history of subjective fever and other symptoms together with blood film finding of malaria parasites. Presently parasite load is used to grade the severity of malaria in developing countries. The malaria parasite load has been reported to be high in apparently healthy individuals and may also be low in subjects with severe malaria. There is need to have another indicator of malaria infestation and also one that can predict the severity of infestation.

We therefore decided to study the relationship between the levels of parasitaemia, CRP and serum cholesterol in malaria infestation in Ilorin which is an area of intense perennial Plasmodium falciparum transmission. We also hope our findings will aid in the diagnosis, management and therapeutic monitoring of malaria.

Materials and Methods

This was a prospective study in which blood samples of 120 paediatric subjects that had acute malaria infestation were were analysed for the analytes in question. They included forty samples each of the subjects with mild, moderate and severe form of malaria. The samples were picked randomly based on their malaria parasite positivity results in the Haematology Department of the hospital. Their samples(in EDTA bottle) were retrieved and subjected to further analysis which included thick film for malaria parasite scoring, malaria parasite counting per microlitre of blood, the levels of C-reactive protein and their serum cholesterol. Inclusion criteria includes subjects with clinical and laboratory evidences of malaria infestation with onset not more than 72 hours while exclusion criteria onset of signs and symptoms more than 72 hours. Subjects with other forms of acute inflammatory reactions apart from malaria infestation, such as typhoid enteritis, septicaemia and acute infections like hepatitis as well as malignancies were also excluded from the patients.

Methodology

The diagnosis of malaria was confirmed by the demonstration of the different stages of malaria parasite on the subject's blood film. Subsequently parasite load was determined in both the thick and thin films. C-reactive protein was determined in the subject's plasma obtained from the EDTA samples.

Accurate measurement of the Plasmodium falciparum count in blood samples was done using the World Health Organization (WHO) expert panel on the measurement of malaria vaccine efficacy in phase III clinical trials which recommends a method based on a reading linked to a defined number of white blood cells and use of individually calculated white cell counts[21]. Scoring of the Falciparum parasitaemia was also categorized as mild (+), moderate (++) and severe (+++) malaria respectively using absolute count.

Measurement of CRP

The biochemical analysis of CRP was done using high sensitive CRP turbilatex agglutination method, manufactured by AGAPPE (Switzerland) and supplied by NUMS diagnostic Nigeria Ltd. Latex particles coated with specific human anti-CRP in agglutination causes absorbance changes depending upon the content of CRP in the patient's sample. This can be quantified by comparison with the absorbance of a calibrator of known concentration using the formula below:

CRP concentration=A2-A1 (sample)/A2-A1 (calibrator) X concentration of calibrator, where A2 and A1 are absorbance or optical density of the sample and calibrator at 1 and 0 minute respectively.

Measurement of Serum Cholesterol:

The biochemical analysis of Cholesterol was done using enzymatic method, manufactured

by AGAPPE (Switzerland) and supplied by NUMS Diagnostics Nigeria Ltd. Optical densities of the reacting species depends upon the concentration of the Cholesterol in the patient's sample. This can be quantified by comparison with the absorbance of a calibrator of known concentration using the formula below:

Cholesterol concentration=Absorbance of the sample/Absorbance of the calibrator X concentration of calibrator.

Results

The paediatric subjects with mean ages of 10.3±1.5 years, 7.1±2.2 years and 3.8±1.3 years had mild, moderate and severe *falciparum* malaria infestation respectively, Table 1.

Mean absolute MP counts of 2.92±0.76/ 200wbc, 51.3±6.9/200wbc and 665.3±54.7/ 200wbc, mean CRP levels of 29.0±9.7mg/l, 134.0±33.1mg/l and 176.3±16.6mg/l and mean total cholesterol of 3.3±0.6mmol/l, 3.1±0.4mmol/l and 2.8±0.5mmol/l were recorded for subjects with mild, moderate and severe malaria respectively, Table 1. There was a significant increment in absolute parasite counts and CRP levels and decrease in total cholesterol levels with increment in parasite scoring.(p values <0.005.)

Table 2 shows the levels of correlation and significance between the parasite scoring, absolute parasite count, CRP and serum total cholesterol. There was a positive correlation as well as a statistical significant difference between CRP and both parasite density and scoring (r=0.870 for parasite count, 0.917 for parasite scoring and p values <0.005 in both). We found a negative correlation between total cholesterol and parasite count and density (r=-0.176 for parasite count and -0.333 for parasite scoring, p=0.230, 0.021 respectively). A significant negative correlation was also observed between CRP and serum total cholesterol with r=-0.449 and p=0.001.

Table 2: Correlation between parasite count, parasite scoring, CRP and serum cholesterol

Parameters	Serum CRP		Serum Total Cholesterol	
	'r'	p-value	'r'	p-value
MP count	0.870	0.001	-0.176	0.230
MP scoring	0.917	0.001	-0.333	0.021
CRP			-0.449	0.001

Table 1: Relationship between parasite scoring, absolute parasite counts, serum total cholesterol and CRP

MP scoring	Mean absolute parasite count/ 200wbc	Total cholesterol (mmol/L)	CRP (mg/L)	Mean age in years
+	2.92±0.76	3.3±0.6	29.0±9.7	10.3±1.5
++	51.3±s6.9	3.1±0.4	134±33.1	7.1±2.2
+++	665±54.7	2.8±0.5	176±16.6	3.8±1.3
Level of significance	p=0.001	p=0.001	p=0.001	

Discussion

In this study the levels of CRP was found to increase significantly with parasite count and scoring. This is in keeping with the findings of previous researchers[13, 14]. As high as 85.2% of patients suffering from acute malaria was reported to have a positive result with C-reactive protein. More than 50% of these patients with positive results showed a strongly positive reaction[24]. The higher the number of parasites, the greater the possibility of obtaining a highly positive result. High positive results are usually noted after about 3-4 days; subsequently it falls but still remains on the high level[24]. A negative correlation between total cholesterol and parasite count and CRP was not in keeping with the findings of previous researchers[22, 23]. Although the mechanism of parasite entry into red cell is poorly understood, our observation could be explained because we measured serum cholesterol but not red cell membrane cholesterol which the previous researchers reported. It is believed that red cell membrane cholesterol is in equilibrium with serum cholesterol. Decrease in total cholesterol noticed in this study could be because of reduced production from the liver due to invasion of the liver by sporozoites of malaria parasite.

Conclusion

C-reactive protein can be said to be a good predictive factor to determine the severity of malaria in our environment although it is not routinely done.

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