

Full Length Research Paper

Study about relationship between C-reactive protein (CRP) and other indicators in children with malaria

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Measurement of C reactive protein rate in children suffering from falciparum malaria was done in order to determine its relationships according to parasite density, white blood cells, age and hemoglobin. This study was a prospective cross-sectional with descriptive and analytical purpose. It focused on 50 children aged from 0 to 15 years admitted in the Pediatric departments of university hospitals in Cocody and Treichville for malaria (Abidjan). Venous blood samples were collected on ethylenediaminetetraacetic acid (EDTA) for blood cells count, parasite density and identification of *Plasmodium falciparum*. The samples, collected without EDTA were used to measure C Reactive Protein. With the blood collected, the serum was processed on the same day and preserved at -20°C. Giemsa-stained thin and thick blood films were analyzed by microscope for plasmodium species and parasite densities. Hematological parameters were determined using hematology cell counter. Turbidimetric test was used for quantitative detection of C Reactive Protein. Statistical analysis was carried out using SPSS (Statistical Package for Social Science) Version 18.0 and Excel 2007. For all test p-value <0.05 below was considered significant. High levels of C reactive protein were observed in all of patients. Younger children had higher C-reactive protein (CRP) level. Positive strong correlation was noted between CRP and both parasite density and leukocytes. There was negative correlation between C reactive protein rate and age. In children suffering from severe anemia, the negative correlation observed between CRP and hemoglobin level was stronger than those suffering from moderate anemia. The main finding of this study was the involvement of CRP in malaria anemia. The levels of CRP according to age in children with falciparum malaria could be used as a biomarker for assessing anemia.

Key words: Falciparum malaria, C reactive protein (CRP), children.

INTRODUCTION

The C-reactive protein (CRP) is a protein belonging to the family of pentraxines. In the phylogenetic evolution, this

protein appears well before immunoglobulins. The main secretion site, but not exclusive of CRP is the hepatocyte,

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responsible for the basal rate of 1 mg/l. However an extra hepatic secretion was demonstrated in neurons, in some lymphocytes, and finally within atherosclerotic plaques (Yasojima et al., 2001). The C-reactive protein (CRP) is well known to be an inflammatory protein. It is a pivotal molecule between innate and adaptive immunity. It is more than a marker of acute inflammation. The development of more sensitive detection methods of hs-CRP, has sparked renewed interest in this protein of the acute phase. It is now recognized that a moderate and chronic increase in CRP is a risk factor for cardiovascular disease (Omair Yousuf et al., 2013). Many studies have shown an increased CRP level in Alzheimer's disease (In-Uk Song et al., 2015) and strong association with cancer (Mieke Van Hemelrijck, 2011; Xu, 2015).

About malaria, clinical manifestations of *Plasmodium falciparum* are associated with an inflammatory syndrome characterized by hematological changes (George and Ewelike-Ezeani, 2011; Momodu et al., 2013; Latif and Jamal, 2015). The main findings in the works of Walisa (Walisa et al., 2006, 2009) about the implication of CRP in malaria anemia, led us to assess the level of this acute phase protein during falciparum malaria in children aged 0 to 15 years, and its relationship according to parasite density, white blood cells (WBC), age and hemoglobin. This study had to confirm a previous one we have done, using slide agglutination test (CRP-latex) which revealed the involvement of CRP in falciparum malaria anemia in children aged 0 to 15 years (Hien et al., 2013). Indeed, malaria anemia every year kills from 190000 to 974000 sub-Saharan children less than 5 years. The objective of this study was to evaluate whether the measurement of CRP in children with falciparum malaria can be used as a biomarker to assess anemia particularly in rural areas where majority of laboratories had no hematological counter to determine hemoglobin level.

METHODOLOGY

Research design

This study was a prospective cross-sectional with descriptive and analytical purpose. It focused on children aged from 0 to 15 years admitted in the Pediatric departments of university hospitals in Cocody and Treichville for malaria. The study ran from September 2013 to January 2014. In total 50 patients with *Plasmodium falciparum* malaria were included. These patients had no infection associated on clinical examination and were not on antimalaria treatment.

Sample

The blood collected by venipuncture was collected in tubes containing EDTA to perform thick film, smear and complete blood count (CBC), and in dry tubes for the determination of C-reactive protein. Blood samples were transported to the emergency laboratory of Treichville University Hospital for various laboratory tests.

Laboratory procedure

The determination of parasite density was made by counting the number of parasites in 200 leukocytes and assuming a mean WBC at 8000/ μ l. The smear helped identify the parasite species and take into account only *P. falciparum* infections. The blood count was performed using the hematological counter CELTAC α NIHON Kohden MEK-6500K with Nihon Kohden reagents. Parasitological examinations (thick and thin smears) were performed and read by means of a binocular microscope Leitz Laborlux K. The CRP assay was performed by immuno-turbidimetry using a multiparametric analyzer of Biochemistry HITACHI 704. The reagent used was CRP FS * from Diasys laboratory.

Data collection and preparation

All the study parameters and their values were entered in Microsoft Excel. Then according to the objectives, parameters were ranged in order to compare their mean levels in two groups of age. They were also ranged to determine their relationship with CRP by using regression lines.

Data analysis

Statistical analysis of the data was performed using SPSS software version 18.0 and Excel 2007 at the 5% threshold. Averages, standard deviations, regression lines and correlation coefficients, and mean comparisons (Student test) were obtained with the same software.

Ethical consideration

Official letters were sent to the Heads of Pediatrics Departments and Emergency Laboratories for each University Hospital Center (Cocody and Treichville). Written permissions for this study were received from them. For each patient, admitted to these departments and who meets the inclusion criteria, informed consent was obtained from a member of their families. Their families were informed on the objectives of this study.

RESULTS

Characteristics of study population

The study population was composed of 58% of males and 42% of females with a sex ratio of 1.38. The mean age was 3.68 ± 3.46 years with extremes of 1 month and 13 years. But for the sake of respect for pediatric division, we maintained the age group of 0 to 15 years. The majority age group was that of 0 to 5 years with 74%. We noted 40% of severe cases and 60% of simple forms.

Values of parameters

According to Table 1, the mean parasite density was 4602.7 ± 7214.63 with extremes of 200 and 28000 Tpz/ μ l (Tpz: Trophozoite); Anemia was present with hemoglobin level varying from 4 to 11.20 g/dl and an average of 7.53 ± 2.07 g/dl; the mean leukocyte count

Table 1. Values of parameters in the study population.

Parameter	Number	Minimum	Maximum	Average	Ecartype
CRP (mg/l)	50	6	486	145.32	155.03
Parasite density (Tpz/ μ l)	50	200	28000	4602.7	7214.63
Leucocytes ($\times 10^3$ / μ l)	50	2.20	46.70	12.51	9.67
Hb (g/dl)	50	4	11.2	7.53	2.07

Tpz, Trophozoite.

Table 2. Distribution of patients according to parasite density and CRP rate.

Parasite density (Tpz/ μ l)	Number	CRP rate (mg/l)
200-1000	16	30.93
1000-5000	24	98.75
>5000	10	437.90
Total	50	145.32

Table 3. Values of parameters according to age.

Parameter	Age < 5 years	Age \geq 5 years	p-value
	N = 36	N = 14	
CRP (mg/l)	169.47	83.21	0.038
Parasite density (Tpz/ μ l)	5524.86	2231.43	0.074
Leucocytes ($\times 10^3$ / μ l)	14.53	7.31	0.008
Hb (g/dl)	7.39	7.89	0.224

was $12.51 \pm 9.67 \times 10^3 / \mu$ l; all patients had high levels of CRP (≥ 6 mg/l) (Table 1), with an average rate of 145.32 ± 155.03 mg/l. In Table 2, the patients were distributed according to the average rate of CRP and parasite density. Table 3 allowed the comparison of the values of different parameters according to the age group. The subjects under 5 years had significantly higher levels of CRP (165.38 vs 88.23 mg/l; $p = 0.038$) and leucocytes (14.53×10^3 vs $7.31 \times 10^3 / \mu$ l). Any difference was observed in parasite density and level of hemoglobin.

Relationship between parameters

A strong positive correlation between CRP level and parasite density ($r = 0.9$; $p < 10^{-3}$) was demonstrated in Figure 1. On the other hand the secretion of this protein of inflammation appeared negatively correlated with age (Figure 2; $r = -0.26$ $p = 0.012$). Similarly, a negative correlation exists between the rate of leucocytes and the age of patients (Figure 3; $r = -0.34$ $p = 0.009$). Considering CRP, hemoglobin and leucocytes levels on the one hand in patients with hemoglobin levels ≤ 5 g/dl and on the other hand in those with a rate > 5 g/dl;

Figures 4 and 5 showed the existence of a positive correlation between CRP and leucocytes ($r = 0.91$ $p < 10^{-3}$; $r = 0.38$ $p < 0.05$). As for Figures 6 and 7, they revealed a negative correlation between the levels of CRP and those of hemoglobin ($r = -0.52$ $p < 0.05$; $r = -0.29$ $p < 0.05$) depending on the severity of anemia.

DISCUSSION

Patients who had a disease associated with malaria were excluded in order not to overestimate the rate of CRP. The majority of the patients are children under 5 years (Table 3) because children of this age are more susceptible to malaria. It was noted in these children a normal average WBC (Table 1), which corroborated the results of Imoru et al. (2013). In fact the standard rate of leucocytes of the age group studied varies between 6×10^3 and $13.5 \times 10^3 / \mu$ l. The rate of CRP was elevated in all patients (Table 1). These high values are due to the inflammatory condition in these patients because inflammation is one of the characteristic reactions of *P. falciparum* malaria (Sarah et al., 2008). In this study, we observed a strong positive correlation between the levels

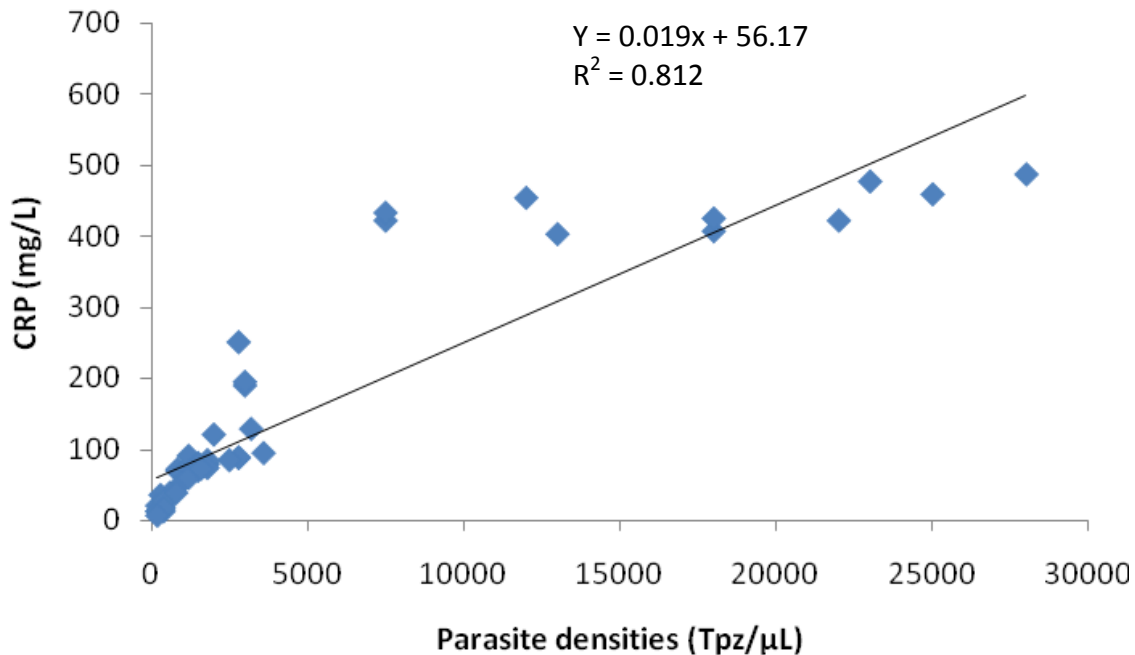


Figure 1. Relationship between C reactive protein rate and parasite densities in study population. $R = 0.90$; $p < 10^{-3}$.

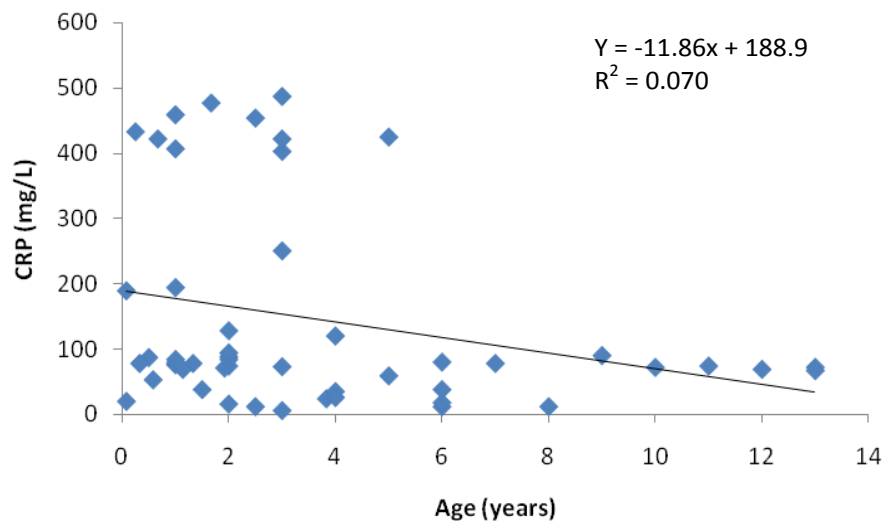


Figure 2. Relationship between C Reactive Protein rate and age in study population ($R = -0.26$; $p = 0.012$).

of CRP and parasite density in Figure 1 as other studies had also mentioned (Vandana Agrawal et al., 2013; Utuk et al., 2014; Pelkonen et al., 2015). Indeed *P. falciparum*, once in the body is recognized by the actors of innate immunity, phagocytic cells through PRRs receptors that bind to glycosil-phosphatidyl-inositol anchored in MPS-1 and MPS-2 parasite proteins of *P. falciparum*. This recognition activates phagocytes which then produce pro-inflammatory cytokines (TNF α , IL-1, IL-6). These cytokines stimulate hepatic synthesis of proteins of the

inflammatory reaction which include CRP. Table 2 demonstrated that CRP rate was proportional to parasite density. There is therefore a relationship between parasite density, levels of cytokines and CRP. Figure 2 showed a negative correlation between CRP level and age. The younger children had higher CRP level (Table 3), which corroborated the results of Utuk et al. (2014). The similarity of correlations between levels of CRP and leukocytes and age of patients (Figures 2 and 3) lead us infer that there would be a link between CRP and

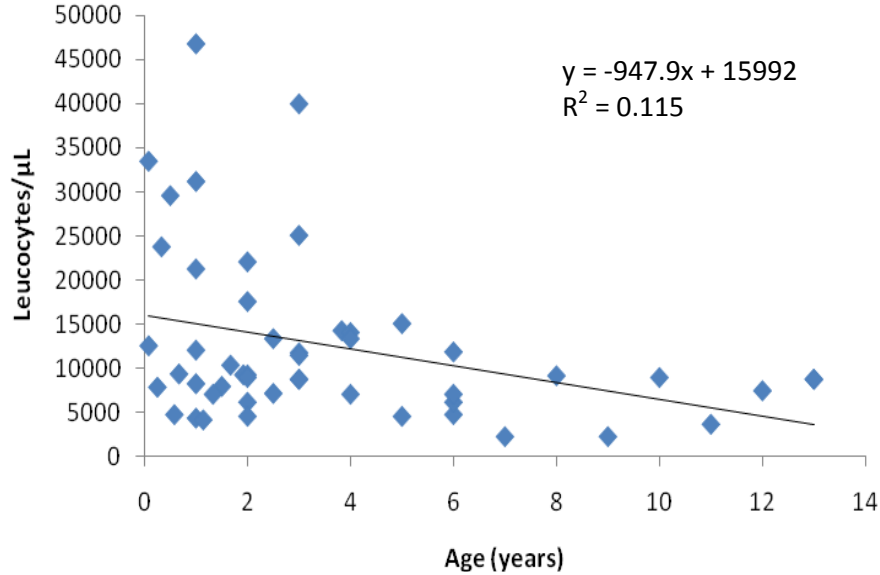


Figure 3. Relationship between leucocytes rate and age in study population ($R = -0.34$; $p = 0.009$).

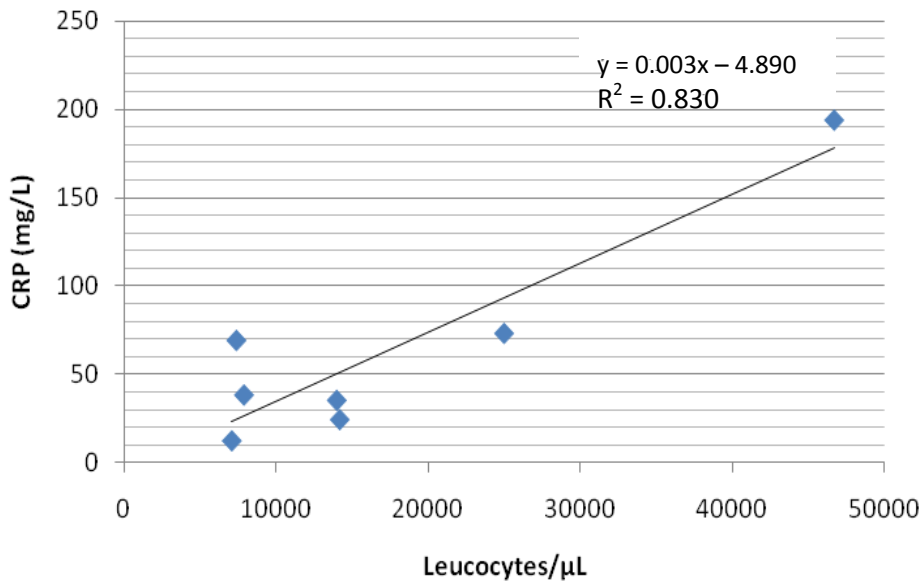


Figure 4. Relationship between C reactive protein and leucocytes rates in patients suffering from severe malaria anemia ($Hb \leq 5$ g/dL) ($R = 0.91$; $p < 10^{-3}$).

leukocytes. CRP in fact has receptors on phagocytes, which activates monocytes, macrophages and neutrophils, enhances phagocytosis and production of pro-inflammatory cytokines via receptors FcγRI (CD64), FcγRIIA (CD32A) (Stein et al., 2000; Lorraine et al., 2005). The main determinant of plasma levels of CRP is synthesis rate which depends on the number of hepatocytes recruited and therefore the circulating levels of cytokines related to the number of activated

phagocytes. For the similarity of the correlations observed in Figures 2 and 3, the following explanation may be given. Indeed the rate of CRP does not vary with age in normal physiological conditions. But during an infection as described above, the pathogen agent causes secretion of cytokines among which Il-6 is the major component of the secretion of all proteins of the acute phase, including CRP. This negative correlation between leucocytes levels and age follows the decrease in their

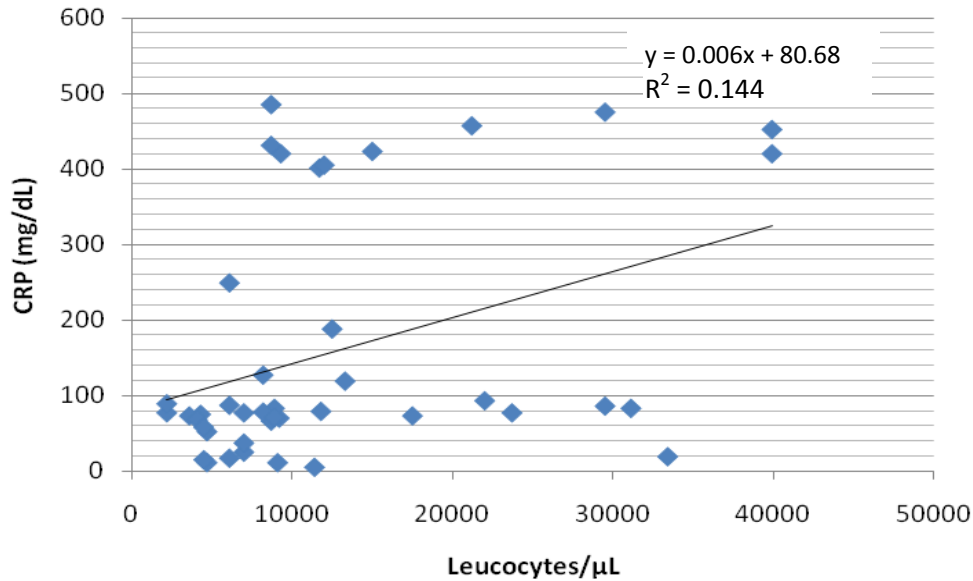


Figure 5. Relationship between C Reactive Protein and leucocytes rates in patients suffering from moderate malaria anemia (Hb > 5 g/dL) (R = 0.38; p < 0.05).

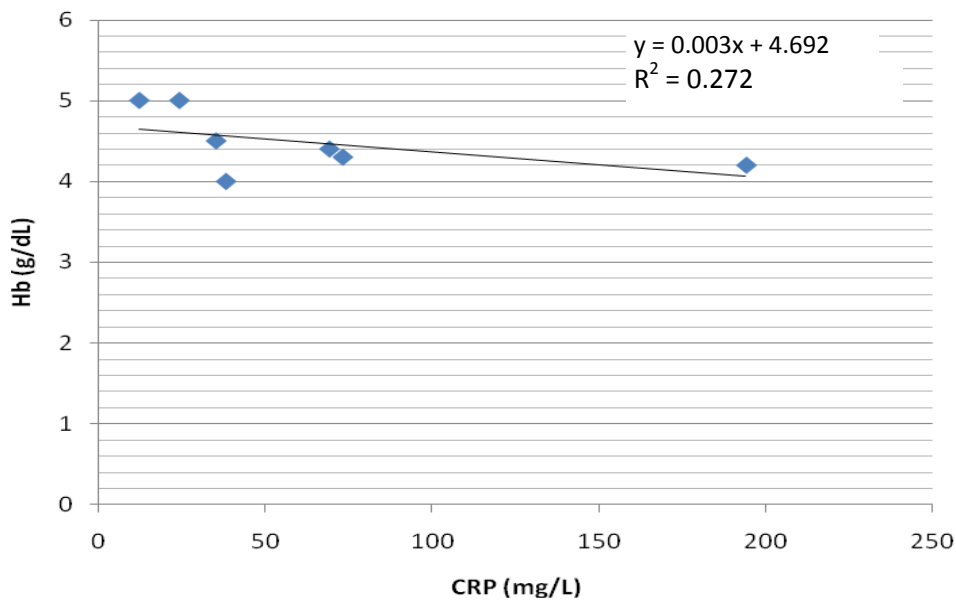


Figure 6. Relationship between C reactive protein and hemoglobin rates in patients suffering from severe malaria anemia (Hb ≤ 5 g/dL) (R = -0.52; p < 0.05).

amount in normal blood counts in children in the age group of our study (Kanakia Health Express, 2006). The rate of CRP produced would be proportional to the number of leukocytes activated so to the amount of cytokines secreted by each age group, but inversely proportional to age overall. Another explanation could be the decrease of parasite density according to progresiv acquired immunity against Plasmodium antigenic

polymorphism, observed in children living in endemic areas (Ndungu et al., 2012; Hviid and Jensen, 2015). We have above demonstrated in Figure 1, the link between parasite density and CRP level. Considering the severity of anemia, we showed interest in the relationship between leukocytes-CRP and CRP-Hemoglobin. Figures 4 and 5 allowed us to demonstrate a positive correlation between CRP and leukocytes. This positive correlation,

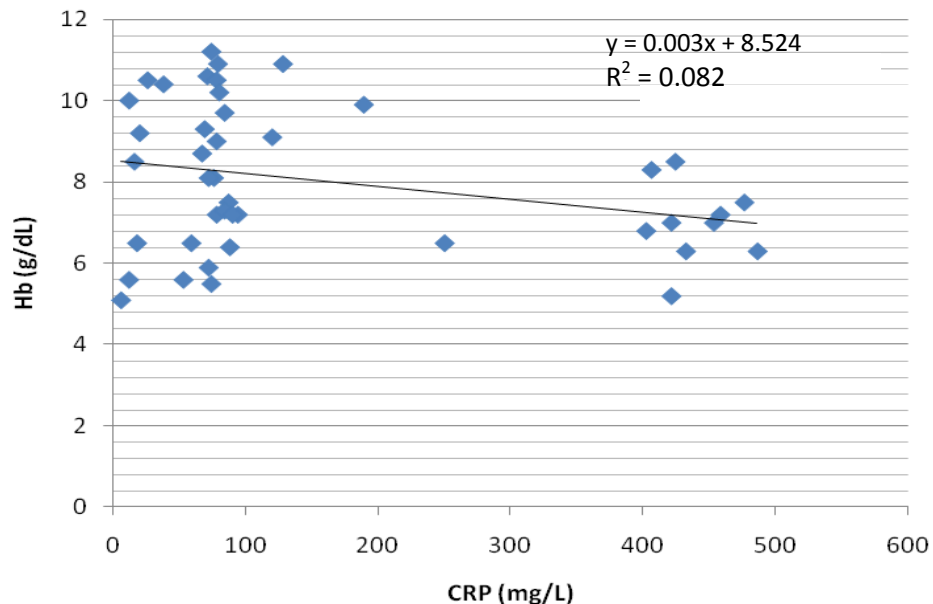


Figure 7. Relationship between C Reactive Protein and hemoglobin rates in patients suffering from moderate malaria anemia (Hb > 5 g/dL). (R = - 0.29; p < 0.05).

higher in cases of severe anemia (Hb \leq 5 g/dl) may be explained by the fact that leukocytes, particularly phagocytes produce cytokines among which Il-6 is the key to the synthesis of CRP. Figures 6 and 7 revealed a negative correlation between the levels of CRP and hemoglobin.

This negative correlation is stronger in cases of severe anemia (Hb \leq 5 g / dl), suspecting so the involvement of CRP in malaria anemia as suggested in the work of Waliza et al. (2006, 2009) and Pelkonen et al. (2015). Indeed, during malaria infection, the CRP produced is able to bind to the infected red blood cells and activate the classical pathway of the complement system, leading to complement-dependent hemolysis. Red blood cells opsonized by CRP may also be phagocytosed because this protein of inflammation as mentioned previously has Fc γ RI (CD64) and Fc γ RIIa (CD32A) receptors on phagocytes. Both erythrocyte lysis mechanisms would participate in malaria anemia. Another study revealed that in children with malaria, severity of anemia was associated to high expression of Fc γ RIIIA (CD16A) which could enhance erythrophagocytosis and TNF α production (Lilian et al., 2010).

This study had some limits. In fact, we have not taken into account that the study population could have other parasites infections or nutritional deficiencies, which could affect the levels of CRP and hemoglobin. Younger children have higher probability of presenting higher CRP values, exactly because they are more prone to have acute, apparent or subclinical, infections (Marcos Borato Viana, 2011). Concerning anemia, many mechanisms can contribute to its onset, such as nutritional

deficiencies (Leonard, 2015). In the Democratic Republic of Congo, a study found that malaria and *Shistosoma mansoni* infection were strongly associated with high prevalence of anemia in schoolchildren (Matangila et al., 2014).

Conclusion

The assay of C-reactive protein during *P. falciparum* malaria in children aged from 0 to 15 years has allowed us to demonstrate that subjects under 5 years had a CRP levels significantly higher. A negative correlation was noted between the levels of CRP and the age of children. It was observed a positive correlation between the levels of this acute phase protein and parasite density. It also allowed us to note a negative correlation between the levels of CRP and hemoglobin, suspecting so the involvement of this protein in acute malaria anemia.

Conflict of Interests

The authors have not declared any conflict of interests.

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