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# IN MALARIA, ACUTE INFLAMMATION LEVEL DEPENDS ON PARASITAEMIA

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## ABSTRACT

*Malaria is a parasitic disease of the tropics. It is accompanied by an inflammatory reaction, consequence of the immune cells activation.* ***The objective*** *of this work was to evaluate the intensity of inflammation in relation to parasitaemia.* ***Method:*** *On the blood samples of 300 patients detected malaria positive by thick smear were quantified C-reactive protein (CRP), an acute inflammatory marker and made the leukocyte formula.* ***Results:*** *The study of the general characteristics of the patients showed a very high proportion of young people with a slight predominance of the male sex (53% of the patients). The cases of moderate parasitaemia were more frequent and represent 46% of the total. Those of low and high parasitaemia accounted for 28% and 26% respectively. The serum level of CRP was increased significantly in cases of moderate parasitaemia and very significantly in cases of high parasitaemia compared to cases of low parasitaemia. The numbers of inflammatory cells, such as neutrophils and monocytes, increased in significantly in cases of moderate parasitaemia and significantly in cases of high parasitaemia compared those of low parasitaemia. By cons, the number of lymphocytes, chronic inflammatory cells decreased very significantly in cases of high parasitaemia, compared to those of low parasitaemia.* ***Conclusion:*** *In malaria, blood levels of CRP, neutrophils and monocytes were proportional to parasite density. These inflammatory markers may serve as an indicator for the therapeutic management of the disease.*

## KEY WORDS

*CRP, inflammation, leucocytes, Malaria.*

## INTRODUCTION

Malaria is a disease that infects 5–10% of human in word and causes around two million deaths each year, mostly in children[i,ii]. It occurs mostly in sub-tropical regions. In 2015, an estimated 438.000 deaths were attributed to malaria, mostly due to *P. falciparum* infection among children under 5 years old in sub-Saharan Africa[iii]. The infection causes human suffering, and severely handicaps economic growth in the affected areas[iv].

A major characteristic of malaria is anemia due to haemolysis of parasitized red blood cells, especially in children and pregnant women[v,vi,vii].

The disease in most cases is not severe in adults and complications only occur in 1 to 2% of cases[viii]. This lesser severity of disease in adults is due to partially acquired immunity against the parasite. Both types of immunity, namely humoral immunity and cellular immunity, are likely to contribute to protection against the parasite[ix,x].

However, the immune system may be sometimes defeated by the parasite, which could become more virulent through antigenic variation strategies and its system of massive invasion of red blood cells[xi,xii].

Another major manifestation of malaria is fever whose episodes are correlated with the release of pro-inflammatory cytokines such as TNF, IFN-c and IL-1b. These cytokines are released by immune cells following the schizont breakdown and contribute to reactions of the acute phase of the disease[xiii,xiv,1,xv].

This work aimed to evaluate acute inflammation according to the malaria parasite density in blood.

## MATERIAL AND METHODS

### Characteristics of the study participants

The study involved 300 patients admitted from June to August 2017 to the hospital of Army Instruction (HAI) in Parakou in Benin republic. These selected patients all suffered from malaria, detected positive for malaria parasite at thick blood smears. All other infection tests were negative in these patients. They all consented to participate in the study and the data processing was made anonymously.

### Sample collection

In all patients, venous blood was collected in a dry tube for the C-reactive protein (CRP) detection, and in EDTA tube for blood count.

### Semi-quantitative determination of Protein C Reactive (CRP)

C-reactive protein is a serum protein of hepatic origin. It is a marker of the acute phase of inflammation[xvi,xvii,xviii].

This was performed with an immunological test, carried out with a SPINREACT Brand CRP kit which was a suspension of IgG (goat anti-human CRP) covered with latex particles. A positive reaction resulted in agglutination of the latex particles and indicated a presence of CRP in the sample. The detection limit was 6 mg/L. When the reaction was positive, the concentration of CRP was semi-quantified. For this purpose, the title of the CRP was determined by serial dilutions of the sample. The concentration was obtained by multiplying the title by the threshold concentration (6 mg/l).

### Thick blood smears for malaria parasite

It detects the presence of plasmodium in the infected blood. For this purpose, a drop of blood is deposited on a glass plate and then spread in a thick layer by circular movements using a glass rod. The spreading was then dried for 30 minutes in the open air. A few drops of water were added to hemolysate the erythrocytes, and then the slide was stained with Giemsa solution diluted one-tenth for 15 minutes. The preparation was then rinsed with water and dried in the air. It was read under the microscope at the objective 100 with the immersion oil. Trophozoites appeared as dots or commas. The trophozoites were count for 500 leukocytes on the slide. To obtain the parasite density, the number of the parasites counted was divided by 500 and the result was multiply by the number of leucocytes per µL obtained at blood count. The plasmodial species was determined by observing the shape of the trophozoites in the parasitized erythrocytes on the blood thin smears made and stained with May Grunwald Giemsa for the leukocyte formula.

### Thin blood smear preparation

It consisted of a single layer of red and white blood cells spread on the surface of the glass slide. For this purpose, a drop of blood was deposited at one end of the blade. Against the drop was placed a lamella inclined at about 45 °. The drop stretched by capillary action along the lamella. The lamella was then slid towards the other end of the slide by a regular, continuous and uniform movement until the drop of blood was exhausted. The smear was then dried in air for 20 minutes.

### May Grunwald Giemsa staining

After drying the smear, it was covered with May Grunwald dye for two minutes, then a few drops of water were added to the May Grunwald and acted for 10 minutes. The colorant was then rinsed and replaced with Giemsa diluted tenth for 10 munites. It was then rinsed and then dried a few minutes in the air for reading.

### Leukocyte count

It was carried out by automatic cell counting and confirmed by manual method. The coulter counter used was Mindray. It counts the different types of blood cells, the different parameters of the red blood cells and those of the white blood cells including the leukocyte formula.

For the manual method, blood was diluted to one-twentieth in Lazarus liquid which hemolysed the red blood cells. The diluted blood was then mounted in a counting chamber of the Mallassez hematometer.

After three minutes, the leukocytes were counted in five strips of the hematometer. The number of leukocytes per µL of blood was obtained by multiplying the number of leukocytes counted by 40.

**Liquid of Lazarus composition**

Methylene blue 0.25 g; Acetic acid 30 mL; Distilled water qs 1000 mL

**Leukocyte formula by manual method**

It consisted in counting the different types of leukocytes in a volume of blood. On the stained smear with May Grunwald Giemsa and observed under a microscope with oil immersion objective, were counted on cent leukocytes, the number of neutrophils, eosinophils, basophils, monocytes and lymphocytes. The absolute leucocyte formula was obtained by multiplying the frequencies found by the number of leukocytes counted per µL of blood.

**Statistical analysis**

The graphs were made in the Graphpad software. The means obtained for the measured parameters of moderate (PL ++) and high (PL +++) parasitemia cases were compared with those of low parasitemia (PL +) using the Dunnett multiple comparison test. The significance level was set at 5%.

## RESULTS

**1- The majority of patients with malaria were young**

Of the 300 studied cases, 36% were under 10 years old, 24% were between 10 and 20 years old, 21% were between 21 and 30 years old, and the remaining 19% were over 30 years old (Table 1). This result showed that the majority of affected patients were young.

Both sexes were represented with little disparity at 53% for males and 47% for females.

**Table 1: General characteristics of patients**

| **Age (years)** | < 10 | 11 to 20 | 21 to 30 | > 30 | Total | **Sex** | Male | Female | Total |
|---|---|---|---|---|---|---|---|---|---|
| **Number** | 108 | 72 | 63 | 57 | 300 | **Number** | 159 | 141 | 300 |
| **Frequency (%)** | 36 | 24 | 21 | 19 | 100 | **Frequency (%)** | 53 | 47 | 100 |

**2- Malaria severity was commonly moderate**

Of the 300 samples analyzed, parasitaemia was low (<500 trophoïtes / µl of blood) in 84 cases, moderate (500 to 10000 trophozoites / µl of blood) in 138 cases and high (> 10000 trophozoites / µl of blood) in the remaining 78 cases. Thus, cases of modarate parasite density dominate, followed by cases of low parasite density and finally cases of high parasite density with frequencies of 46%, 28% and 26% respectively (Table 2).

**Table 2: Distribution of cases according to parasitaemia level**

| **Parasitemia level (PL)** | | **Number** | **Frequency (%)** |
|---|---|---|---|
| Low | (+) | 84 | 28 |
| Moderate | (++) | 138 | 46 |
| Hight | (+++) | 78 | 26 |
| Total | | 300 | 100 |

**3- Expression of the C Reactive Protein (CRP) increased with the parasitemia**

The Table 3 and Figure 1 showed the evolution of CRP according to the parasite density.

The frequency of positive CRP increased with parasitemia. It increased from 70.3% of cases for low parasitaemia (PL+) to 100% of cases for high parasitaemia (PL+++).

The mean CRP value was 24.5 ± 13 mg / l for low parasite densities. This mean significantly increased to 35.3 ± 14.6 mg / l (P value <0.05) for the moderate parasite densities (PL +) and very significantly at 44 ± 12 mg / l (P value <0.01) for High parasite densities (PL ++).

**Table 3: CRP frequency according to parasitaemia**

| CRP | | Parasitemia level (PL) | | |
|---|---|---|---|---|
| | | + | ++ | +++ |
| Negative | (n) | 25 | 5 | 0 |
| | (%) | 29.7 | 3.2 | 0 |
| Positive | (n) | 59 | 134 | 77 |
| | (%) | 70.3 | 96.8 | 100 |
| Total | (N) | 84 | 139 | 77 |

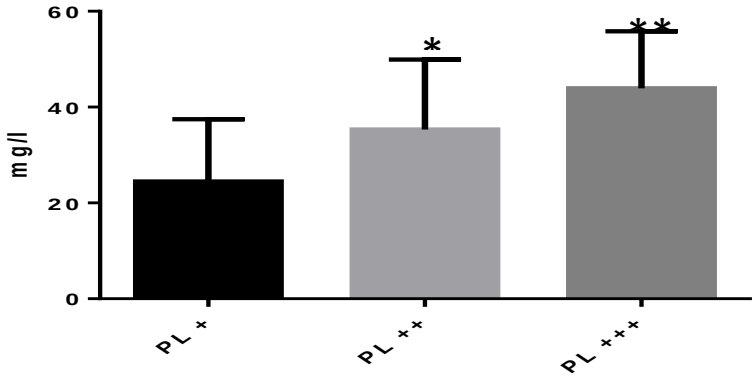


**Figure 1: Mean value of the CRP based on parasitaemia**

** : P value <0.05; ** : P value <0.01; PL : Parasitemia level*

**4- The total number of blood leukocytes did not vary with parasitaemia**

The mean number of blood leukocytes was 7120 ± 2359 / µl of blood for low parasite densities (PL +). It did not change significantly when the parasite density was moderate (PL ++) or high (PL +++). The respective values were 6952 ± 2391 / µl for PL ++ and 7021 ± 2341 / µl for PL +++ (Figure 2).

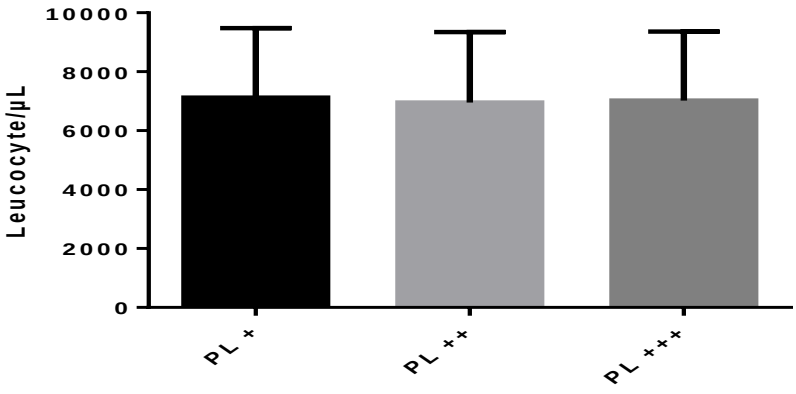


**Figure 2: Mean number of leukocytes according to parasite density**

**PL: Parasitemia Level**

**5- The number of neutrophils increased with parasitaemia**

The Mean number of neutrophils was (3650 ± 1189) /µL of blood for low parasitemia (PL +). It increased insignificantly for moderate parasitaemia (PL ++) and significantly for high parasitaemia (PL +++). Means were respectively (3898 ± 1491) / µl for PL ++ and 4557 ± 1334 for PL +++ (Figure 3).

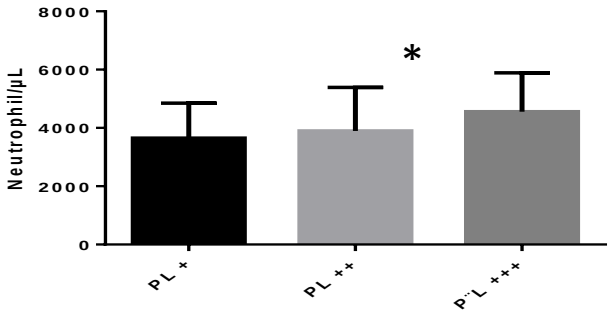


**Figure 3: Mean number of neutrophils according to parasitaemia**

**** : P value< 0.05; PL : Parasitemia Level***

**6- The number of eosinophils cells did not change with parasitaemia**

The mean number of eosinophilic polynuclear cells did not significantly vary in the cases of moderate (PL ++) or high (PL +++) parasitaemia compared to those of low parasitaemia (PL +). The means were 143 ± 75 / µl for PL +, 142 ± 78 for PL ++ and 175 ± 87 for PL +++, respectively (Figure 4).

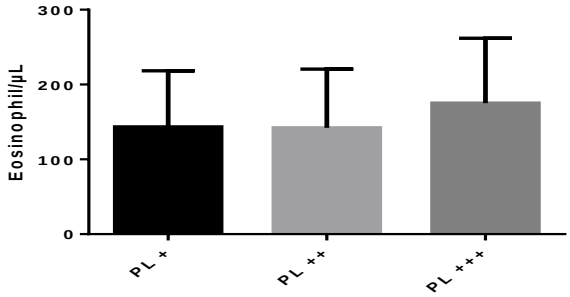


**Figure 4: Mean number of eosinophilic polynuclear cells according to parasitaemia**

***PL : Parasitaemia Level***

**7- The number of monocytes increased with parasitaemia**

The mean number of blood monocytes was 423 ± 180 / µl of blood in cases of low parasitaemia (PL +). This number increased insignificantly in cases of modrate parasitaemia (PL ++) and significantly in cases of high parasitaemia (PL +++). The values were 501 ± 192 / µl for PL ++ and 573 ± 190 / µl for PL +++, respectively (Figure 5).

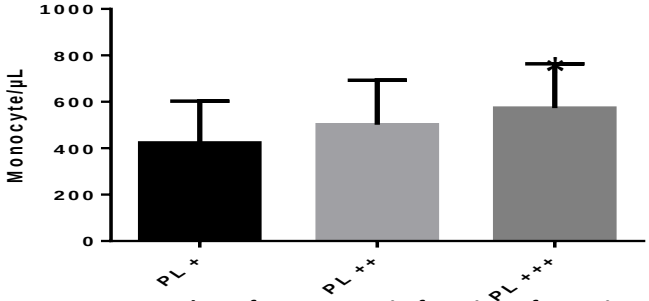


**Figure 5: Mean number of monocytes in function of parasitaemia**

****: P value <0.05;PL: Parasitaemia Level***

**8- The number of lymphocytes decreased with parasitaemia**

The mean number of lymphocytes was 3019 ± 1098 / µl for cases of low parasitaemia (PL +). It decreased insignificantly in cases of moderate parasitaemia (PL ++) and very significantly in cases of high parasitaemia (PL +++). The values were 2793 ± 1103 / µl for PL ++ and 1978 ± 854 / µl for PL +++, respectively (Figure 6).

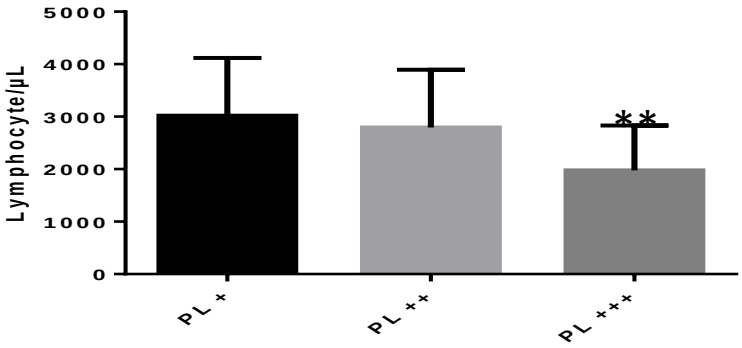


**Figure 6: Mean number of lymphocytes in function of parasitaemia**

*****: P value < 0.05; PL: Parasitemia Level***

## DISCUSSION

Malaria is a widespread disease in tropical countries, particularly in Africa4,[xix]. It is caused by a parasite Plasmodium genus and is accompanied by inflammation[xx]. This study evaluated the importance of acute inflammation depending on parasitaemia. Thus, in 300 patients detected positive for malaria in thick blood smear, were evaluated for both the expression of C-reactive protein and leukocyte formula, two inflammatory parameters.

The study of the general characteristics of malaria showed that its frequency decreased with the evolution of the age of the patients, indicating that it affected mainly the children, in concordance with the observations of the WHO[xxi] (2016). This may be partly explained by the immaturity of the immune system of children, which protects them less from the parasite[10]. It affected patients of both sexes with a slight predominance of male sex. This observation was also made by other authors [xxii , xxiii , xxiv]. The classification according to the parasite load showed that malaria of moderate severity was more frequent and accounted for almost half of the cases (46%). The less severe and severe types accounted for only 28% and 26 of the cases respectively. The correlation of malaria severity and mortality with parasite density was established by previous studies[xxv,xxvi,xxvii]. Our frequencies differ from those obtained in Thailand by Manas[23], who placed first the cases of low parasitaemia (65.1%), followed by that of moderate parasitaemia (26.8%) and that of high parasitaemia (8, 1%).

We investigated the expression of C-reactive protein (CRP), a protein of the acute phase of the inflammation produced by the liver[16] in different cases of parasitaemia. CRP was positive in 100% of cases of high parasitaemia, in 96.8% of cases of moderate parasitaemia and in 70.3% of cases of low parasitaemia. Comparison of the mean serum CRP level between the different groups showed that it increased significantly in the cases of moderate parasitaemia ($P < 0.05$) and very significantly in cases of high parasitaemia ($P < 0.01$), compared with cases of low parasitaemia. This observation confirmed that of other authors who showed a positive correlation between serum CRP and parasitaemia [xxviii ,22, xxix ,24]. Indeed, the production of CRP by the liver is initiated by the pro-inflammatory cytokines of blood phagocytes[28]. For this purpose, we examined the blood levels of the different types of leukocytes.

The total number of white blood cells did not vary depending on the parasitaemia. This result was discordant with that of McKenzie [xxx] who found a significant increase in the total number of white blood cells with the parasite density. The mean number of neutrophils increased insignificantly in moderate parasitaemia and significantly in cases of high parasitaemia compared to those of low parasitaemia, consistent with the findings of Kakom[xxxi] and Manas[23]. This result was not surprising because neutrophils are acute inflammatory cells whose blood number increases initially during the early stage of inflammation before gradually decreasing as the chronic phase progresses[xxxii,xxxiii].

For cons, the number of eosinophils did not vary significantly with parasitaemia, which was in agreement with their role as chronic inflammatory cells. It was even emphasized that Plasmodium falciparum suppresses preexisting eosinophils before infection, causing their blood levels to drop[xxxiv].

Like neutrophils, the number of monocytes also increased insignificantly in cases of moderate parasitaemia and significantly in cases of high parasitaemia compared those of low parasitaemia. Indeed, monocytes are macrophages precursors involved in innate immunity by their phagocytic potency and production of pro-inflammatory cytokines such as TNF-α and IL-6 whose blood levels increased in malaria[20, xxxv] . This may explain the increase in their rates in malaria. This result contrasts with that of Manas[23] who observed a significant decrease in their number depending on the parasite load.

On the other hand, the number of blood lymphocytes collapsed in cases of high parasitaemia compared to those of low parasitaemia. This observation was also made by different authors[xxxvi, xxxvii, 23], and confirmed their characteristic of cells of the chronic phase of inflammation.

## CONCLUSION

This work showed an increase in serum C-reactive protein level with plasmodial parasitaemia. It was the same for neutrophils which also were markers of acute inflammation. The unexpected increase in the number of monocytes would be related to their intervention in innate immunity. The value of these different parameters could serve as indicators of the severity of malaria, and orient the therapists in the disease management.

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