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Original Research Article

**Parasitemia and its relation to blood indices and liver function among malarial patients**

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Department of Biochemistry,  
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Dewas (M.P.), India**\*Article History:****Received:** 09/05/2017**Revised:** 02/06/2017**Accepted:** 02/06/2017**DOI:** <https://doi.org/10.7439/ijbar.v8i5.4151>**Abstract****Objectives:** Objective of the study is to observe effects of Parasitemia on blood indices and liver function among malarial patients.**Material & Method:** A total of 67 malarial patients attending the OPD were enrolled as test subjects. Venous blood was collected. Malarial parasite density was determined in various blood smears and analyzed for blood indices- Hb, PCV, total WBC, lymphocytes, neutrophils, platelets, AST, ALT, ALP, total and direct bilirubin.**Result & Discussion:** There were significant decreases in the mean values of the hemoglobin (Hb), packed cell volume (PCV), total leucocyte counts (t WBC), lymphocytes and platelets. Neutrophils were significantly higher in patients with falciparum malaria in comparison to healthy subjects. There were significant increases in the mean activity enzyme values of AST, ALT, ALP and serum total and direct bilirubin. The present study reports a significant increase in neutrophil level of individuals infected with *P. falciparum* as compared to healthy subjects.**Conclusion:** Haemoglobin, platelet count, lymphocyte level, total WBC and neutrophil level were seen associated with mild, moderate and severe parasitaemia infection. Acute falciparum malaria infection is associated with an increase in serum activity of aspartate and alanine aminotransferase and alkaline phosphatase thus indicating that the infection is associated with acute liver injury.**Keywords:** ALT= alanine amino transferase, AST= aspartate amino transferase, ALP= alkaline phosphatase.**1. Introduction**

Malaria continues to be a great health problem in some of the most populated areas of the world. Infection is caused by a parasite of genus *Plasmodium* which is transmitted to human beings by infected female anopheline mosquito. Studies have revealed that hematological and biochemical changes occur in malaria infected blood and there are common complications associated with this disease. Hematological changes that are associated with malaria infection include anemia, thrombocytopenia and disseminated intravascular coagulation [4-6]. Changes in physiochemical parameters of *Plasmodium falciparum* infested blood may vary with level of malarial endemicity, presence of haemoglobinopathies, nutritional status, demographic and level of malarial immunity [7,8].

The infection of liver cells by the sporozoite form of malarial parasite can cause organ congestion, sinusoidal blockage and cellular inflammation. These changes in hepatocytes can lead to the leakage of parenchymal (transaminases) and membranous (alkaline phosphatase) enzymes of the liver to the circulation. Hence increase in liver enzymes and ALP observed in malaria infected patients also demonstrated that the serum activities of these liver enzymes increased with increase in malarial parasite density. This change could confirm that the hepatic stage of the parasite's life cycle in human host is accompanied by significant perturbation in hepatocytes parenchyma and membrane leading to leakage of liver enzymes into the general circulation [9,10].

Liver involvement in malaria is common in patients of severe malaria and may manifest as jaundice, hepatomegaly and elevated liver enzymes like aspartate and alanine transaminases.[11] Hyperbilirubinemia mainly unconjugated is a common feature of falciparum malaria and is attributed to both parasitized and non-parasitized erythrocytes and partly due to liver damage[12]. Therefore well informed changes in blood parameters and hepatic function in malarial infection enable the clinician to establish reliable diagnosis and therapeutic interventions. Hence this research attempts to evaluate the changes in both hematological parameters and liver functions and in addition to evaluate the relationship between the levels of parasite density in blood and changes in hematological and hepatic function in falciparum malaria infected patients admitted in Amaltas Medical College Dewas (M.P.).

## 2. Material and method

### 2.1 Selection of subjects

A total of 67 patients with malaria parasitaemia attending the O.P.D of Amaltas Medical College Dewas were enrolled as the test subjects who were reported ill with fever(temp.>37.5 °C) headache, vomiting, chills, diarrhoea and other clinical signs. Patients of age 18-48 yrs were selected who confirmed to be infected with the falciparum malarial parasite by microscopic examination of giemsa stained thin blood smears. Patient selection was done by simple random sampling of males and females between October 2016 and April 2017. 30 normal subjects free from malaria were used as controls. Patients with hepatitis, smokers and those taking any of the antimalarial drugs were excluded from the study.

### 2.2 Malarial parasite density determination

*P. falciparum* parasitaemia was determined in various blood smears stained by giemsa stain. Parasitaemia was calculated based on WHO [13]: low (+) 1-10/100 fields, mild (++) 11-100/100 fields, moderate (+++) 1-10/one field and high parasitaemia (++++)>10/one field.

### 2.3 Sample collection and preparation

Venous blood was collected aseptically from the subjects using 5ml disposable syringes. The blood samples were collected and 4ml was transferred in to plain bottles for the biochemical assays whereas the remaining 1ml was transferred in to plain bottles for malaria parasite tests. The blood samples in the plain bottles were allowed to collect and retract after which they were centrifuged at 3000rpm for 10min and the serum was transferred in to sterilized plain bottles for the biochemical analysis.

### 2.4 Haematological analysis

Whole blood samples were collected in EDTA tube for determination of haematological parameters including haemoglobin(Hb) concentration, WBC count, neutrophils, lymphocytes, platelets and packed cell volume (PCV) using automated() hematology analyzer.

### 2.5 Assays of liver function parameters

Serum aspartate transaminase and alanine transaminase parameters were determined using ERBA EM 200 systems automatically. This assay follows the recommendations of the IFCC but was optimized for performance and stability by [14,15]. Alkaline phosphatase (ALP) parameter was determined using ERBA EM 200 automatically by colorimetric assay in accordance with a standardized method. In presence of magnesium and zinc ions, p-nitro phenyl phosphate is cleaved by phosphatases in to phosphate and p-nitro phenol [16].

Serum bilirubin concentration was determined using ERBA EM 200 systems automatically by colorimetric assay and total bilirubin was determined by Diazo method [17], while direct bilirubin was determined by Diazo method (special)[18].

### 2.6 Statistical analysis

The data were expressed as mean  $\pm$ SE. The results were analyzed statistically using column t test. Correlation among the investigated parameters was tested by curves and regression using linear regression to test departure from linearity with runs test. These analyses were carried out using computer statistics. The minimum level of statistical significance was set at  $p < 0.05$ , 0.01 or 0.001.

## 3. Result

Of the 67 patients infected with *P. falciparum* 11(16.4%) had low intensity of infection low 1+ (1-10/100fields), 14(20.9%) had mild intensity of infection 2+(11-100/100fields), 23(34.3%) had moderate intensity of infection 3+(1-10/one field) and 19(28.4%) had high intensity of infection 4+(>10/one field).(Table 1).

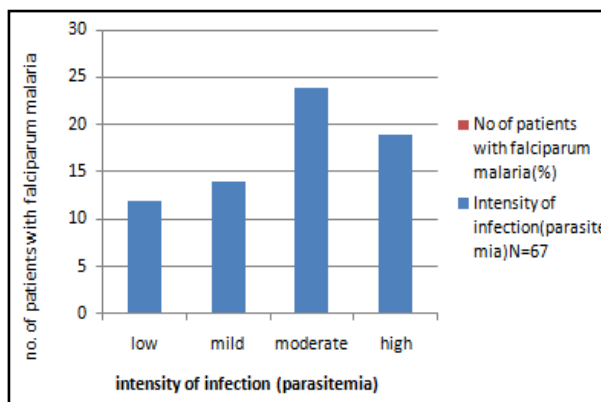
There were significant decreases in the mean values of the haemoglobin (Hb), packed cell volume (PCV), total leucocyte counts (t WBC), lymphocytes and platelets. Neutrophils were significantly higher in patients with falciparum malaria in comparison to healthy subjects (Table 2). Table 3 shows the relationship between parasite density haemoglobin and platelets. Table 4 shows the changes in liver function biomarkers of the patients *P falciparum* malaria and healthy subjects. There were significant increases in the mean activity enzyme values of AST, ALT, ALP and serum total and direct bilirubin.

Table 5 shows the relationship between parasite density and liver enzyme activities in serum. Level of parasitaemia correlates positively with mean liver enzyme activities specifically moderate and high parasitaemia showing higher values of liver enzyme activities when compared with patients having low and mild parasitaemia. Total and direct bilirubin showed increase with increase in severity of parasitaemia of malarial infections (Table 5).

All tabular details were correlated when plotted graphically.

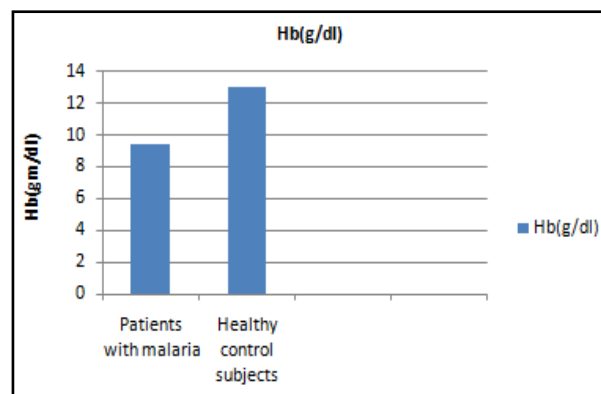
**Table 1: Occurrence of plasmodium falciparum malaria in parasitaemia patients by intensity of infections**

Intensity of infection (Parasitemia) N=67	No of patients with falciparum malaria (%)
Low(+)	12(16.6)
Mild(++)	14(20.9)
Moderate(+++)	24(34.5)
High(++++)	19(28.4)



**Table 2: Comparison of haematological parameters between the cases of P. falciparum malaria infection and healthy subjects**

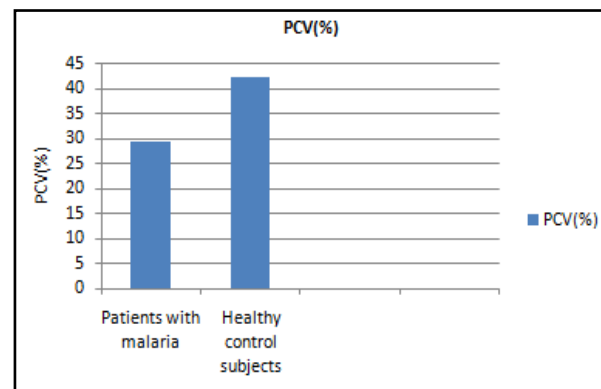
Parameters	Patients with malaria N=67	Healthy control subjects N=30
Hb (g/dl)	9.4±0.28***	13.08±0.36
PCV (%)	29.47±0.82***	42.52±0.86
T.WBC(10 <sup>3</sup> /μl)	5.91±0.35**	7.95±0.60
Lymphocytes (%)	24.64±1.63***	39.17±2.19
Neutrophils (%)	71.28±1.60***	48.46±2.30
Platelet (x10 <sup>3</sup> /μl)	116.6±7.03***	353.4±18.72



\*\*p<0.01; \*\*\*p<0.0001

**Table 3: The level of haemoglobin and platelets in patient P.falciparum malaria with parasitaemia**

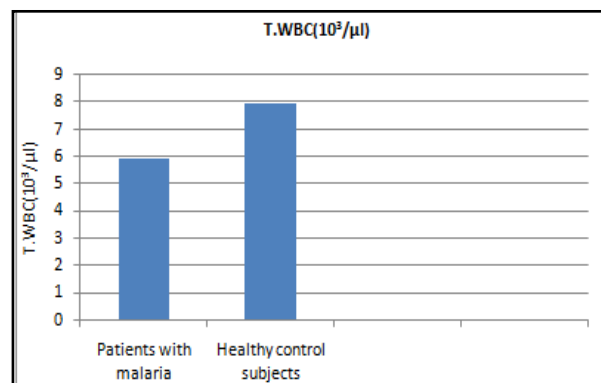
Parasitaemia/field	Mean hemoglobin	Mean platelets
Low(+)	12.75±0.2	177.5±5.1***
Mild(++)	10.95±0.1***	131.5±7.8***
Moderate(+++)	8.64±0.3***	83.8±2.6***
High(++++)	8.14±0.4***	53.1±6.9***
Healthy	13.08±0.3	353.4±18.7.

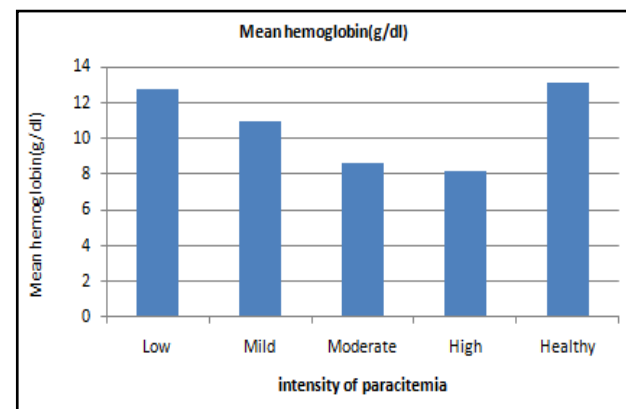
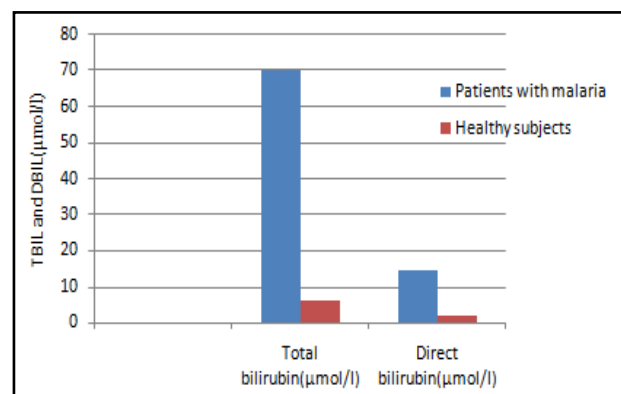
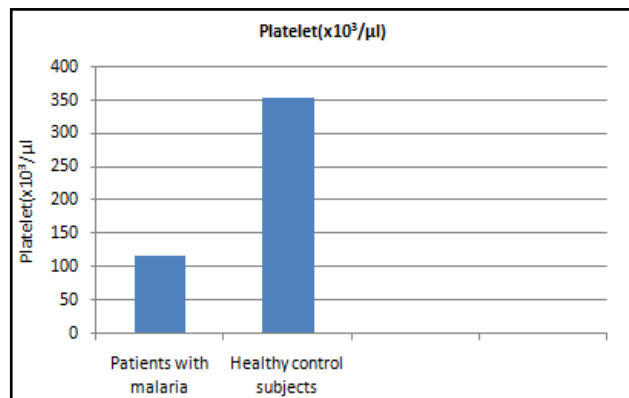
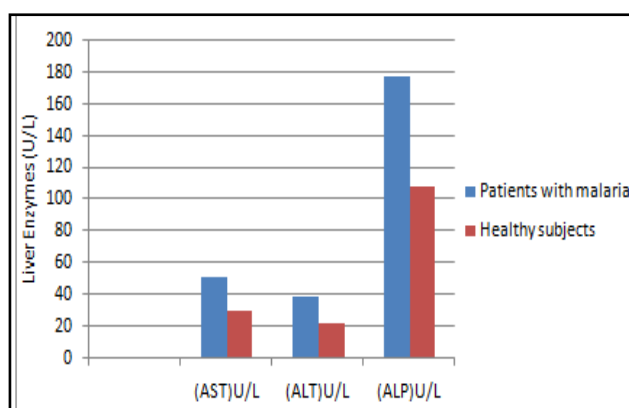
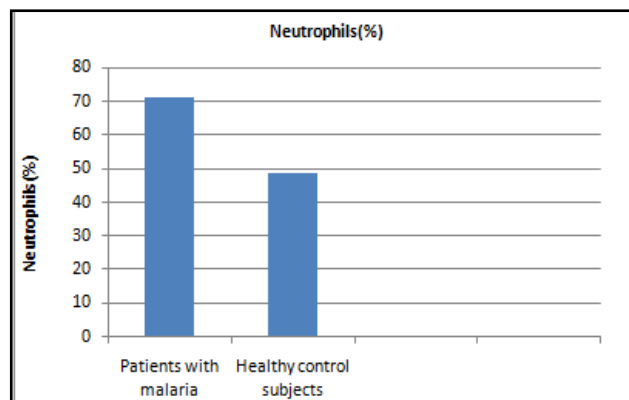
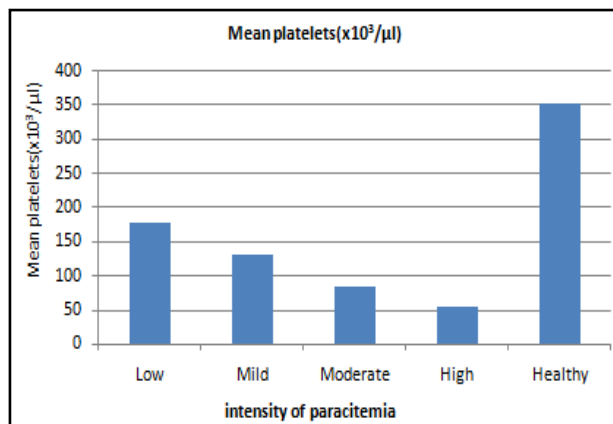
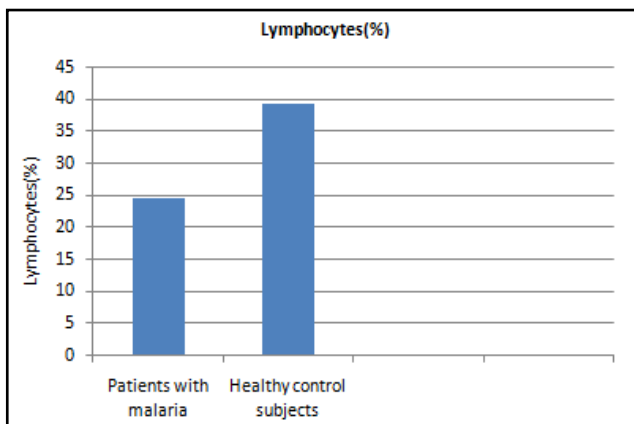


\*\*\*=p<0.0001.

**Table 4: Changes in liver function biomarkers of the patient's P. falciparum malaria and healthy subjects**

Parameters	Patients with malaria N=67	Healthy subjects N=30
Aspartate aminotransferase (AST)U/L	51.12±2.85***	29.20±1.50
Alanine aminotransferase (ALT)U/L	37.92±2.04***	20.97±1.42
Alkaline phosphatase (ALP)U/L	177.4±10.49**	108.2±6.81
Total bilirubin(TB)μmol/l	70.13±7.04***	6.15±0.39
Direct bilirubin(DB)μmol/l	14.61±1.78***	2.05±0.18





#### 4. Discussion

In this study haemoglobin (Hb) and packed cell volume (PCV) decreased in *P falciparum* affected patients compared to healthy subjects. This finding agrees with previous reports [5,19]. The earlier studies reported a significant reduction in haemoglobin concentration and packed cell volume in patients with malaria parasitaemia. In this study the mean haemoglobin concentration showed 9.3g/dl but in the study done by Nadeem *et al*[20] haemoglobin level in *P falciparum* affected patients was 13.7g/dl. This value was more than that observed in our study. Reduced haemoglobin in malaria may be attributed to increased breakdown of red blood cells by the parasites.[21] according to national guidelines for diagnosis treatment

and prevention of malaria Anemia is defined as (Hb)<5.5g/dl. Therefore the drop in haemoglobin concentrations in malarious subjects in our study ranged between 5 and 10.5g/dl, ranging approximately from mild to moderate anemia. In the study done by Bakhubaira [22] anemia found to be observed more common; amongst the patients infected by *P falciparum*.

Lower mean value for leucocyte count in the falciparum malaria patients compared to healthy subjects was observed in our study. This was in concordance with other studies [23]. Similarly decrease in WBC in malaria patients has been observed by S Chandra and F Chandra [24] who reported that low leucocyte count may be used as probable indicator for malaria in endemic countries. The present study reports a significant reduction in lymphocyte level in individuals infected with *P falciparum* as compared to healthy subjects. Reduction in counts of lymphocytes was observed in some studies [25]. The decrease in lymphocyte count associated with malaria may be due to reflecting redistribution of lymphocytes with sequestration in the spleen [26].

The present study reports a significant increase in neutrophil level of individuals infected with *P falciparum* as compared to healthy subjects. These findings are in agreement with previous reports [5,25]. Increase in neutrophil in these cases could be a representation of early release of neutrophil from the bone in response to infection. In our study thrombocytopenia emerged as a predictor of malaria which is an observation of many studies which confirm it [19,27,28]. Low platelet count is a finding of malarial infection and thrombocytopenia may be more common than anemia in malarial infection.

The results reported in this study show some significant increases in activities of enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) among patients with *P falciparum* malaria. The increased serum levels of hepatic enzymes transaminases (AST and ALT) and ALP are biomarkers of liver disorders. Our results are consistent with other studies which reported that majority of patients show elevation in serum activities (AST, ALT and ALP) indicating liver damage [29,30]. The increases in serum levels of hepatic enzymes, transaminases (SGOT, SGPT) and alkaline phosphatase are the markers of liver damage. Ignatius *et al* reported that the liver enzyme leakage and bilirubin increased with increase in malarial parasite density. Usually in uncomplicated malaria raised bilirubin is mainly due to hemolysis of parasitized and non-parasitized RBC and/or hepatocyte damage [32]. In this study raised bilirubin was mainly due to hepatic dysfunction. Molyneux *et al* [33] suggested that jaundice which may be deep is usually accompanied by only moderate elevation of hepatic

enzymes and results more from hemolysis than from hepatic damage. In the present study the cause of jaundice was attributed mainly to intravascular hemolysis and hepatic dysfunction (42.1%) as reported by other studies [34,35].

## 5. Conclusion

Haemoglobin, platelet count, lymphocyte level, total WBC and neutrophil level were seen associated with mild, moderate and severe parasitaemia infection. Acute falciparum malaria infection is associated with an increase in serum activity of aspartate and alanine aminotransferase and alkaline phosphatase thus indicating that the infection is associated with acute liver injury.

## Ethical approval

Ethical approval was given by the hospital management and center of malaria in the area.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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