J Parasit Dis (Oct-Dec 2019) 43(4):633–637
https://doi.org/10.1007/s12639-019-01142-2

ORIGINAL ARTICLE

A comparative study assessing the effect of haematological and biochemical parameters on the pathogenesis of malaria

Sindhusuta Das[1] · Nonika Rajkumari[1] · Palanivel Chinnakali[2]

Received: 14 January 2019 / Accepted: 5 July 2019 / Published online: 12 July 2019
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Abstract Malaria is one of the most common parasitic disease affecting mankind since millennia. The most pronounced changes related to malaria involve the blood and the blood forming system, the spleen and the liver. The abnormal haematological and biochemical parameters observed in malaria cases adversely affect the prognosis of the disease. The aim of this study was to assess the severity of malaria by observing the significant abnormalities in haematological and biochemical parameters in the malaria infected cases as compared to the healthy controls. The study population comprised of 138 individuals, of which 69 were malaria cases and 69 were apparently healthy controls. All the 138 individuals were subjected to haematological and biochemical workup, following which statistical analysis was done to observe any association of altered haematological and biochemical parameters with severity of malaria, as compared to the healthy controls. Among the 138 study population, 69 patients were malaria cases whereas the other 69 were healthy controls. Haematological investigations revealed, that the haemoglobin levels, total RBC counts and haematocrit were significantly altered in the malaria cases as compared to the healthy controls. Also the leucogram profile showed significant leucopenia and neutropenia in the malaria patients as compared to the controls. Thrombocytopenia was also seen to be more pronounced in the malaria infected. The liver enzymes and serum bilirubin levels were raised in the malaria cases more than the controls. Altered haematological and biochemical parameters are indicators of disease progression to severity. Early detection and management of these parameters, will prevent the development of complications in malaria.

Keywords Malaria · Anaemia · Haematological abnormalities · Biochemical abnormalities · Plasmodium

✉ Nonika Rajkumari
nonika.raj@gmail.com

Sindhusuta Das
sindhusuta87@gmail.com

Palanivel Chinnakali
palaniccm@gmail.com

[1] Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Educations and Research, 2nd Floor, Institute Block, Dhanvantri Nagar, Puducherry 605006, India

[2] Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Educations and Research, Puducherry 605006, India

Introduction

Malaria remains one of the major parasitic disease in the tropics with increased morbidity and mortality. In 2016, an estimated 216 million cases of malaria occurred worldwide with 7% of cases in WHO South-East Asia Region (World Malaria Report 2018). *Plasmodium falciparum* is the most prevalent malarial parasite in sub-Saharan Africa, while outside of Africa, *Plasmodium vivax* is the predominant parasite and is above 30% in the WHO South-East Asia (World Malaria Report 2018). Mortality rate is usually high in severe malaria and cases with altered haematological and biochemical parameters, that usually lead towards common complications associated with malaria (Khuraiya et al. 2016).

Haematological changes in malaria are diverse and they vary according to the *Plasmodium species*, age, gender and various other factors. The presence of anaemia is a serious

pathology in malaria patients and, its concurrence with other associated haematological alterations further complicates the existing disease condition in the infected hosts (Abdulkareem et al. 2017). The haematological changes that have been reported to accompany malaria include anaemia, thrombocytopenia and leucocytosis, leukopenia, mild atypical lymphocytosis, monocytosis, eosinophilia and neutrophilia (D'souza et al. 2017; Das et al. 2017). There was a positive association between levels of inflammatory biomarkers and eosinophilia in the malaria patients, indicating that inflammatory reactions or T cell activation may play a role in eosinophil induction during acute illness (Kurtzhals et al. 1998). Thrombocytopenia has been seen as a common finding in both *vivax* and *falciparum* malaria. It is also observed that leukopenia and leukocytosis, lymphopenia, monocytosis, and neutrophilia are directly linked to severity of disease (Tobón-Castaño et al. 2015). The degree of thrombocytopenia is considered relevant to prognosis of the disease, since platelet survival is reduced in severe falciparum malaria (D'souza et al. 2017). Biochemical abnormalities observed in malaria infected patients include a high bilirubin, increased liver enzymes, high creatinine all of which contribute to complicating the existing disease. Acute malaria infection is associated with an increase in serum activity of liver enzymes and bilirubin levels thus indicating that the infection is associated with acute liver injury (Al-Salahy et al. 2016). Renal abnormalities like raised blood urea, decreased creatinine clearance are directly associated with heavy parasitaemia levels in malaria cases (Sharma et al. 2012). Such abnormal blood and biochemical profiles play an important role and in causing fatal complications, contributing to the high morbidity and mortality.

So, an understanding of haematological and biochemical parameters will help in diagnosis and treatment and also prevent various complications. This study was conducted to observe the frequency of malaria in Pondicherry and to find out whether haematological and biochemical abnormalities were more severe in malaria infected cases, also whether the effect on the pathogenesis of the disease are directly attributable in causing complications of malaria.

Aims of the study

This study was aimed to compare the haematological and biochemical parameters of the malaria infected patients and that of the controls.

Also to observe if any significant correlation existed between the abnormal haematological and biochemical parameters and occurrence of malaria related complications in human host.

Materials and methods

Selection of subjects

A total of 138 individuals were included in the study group, among which 69 samples were taken from the patients who were diagnosed positive for presence of *Plasmodium species*, by the routine laboratory methods, that is, giemsa stained blood film examination, as per WHO protocol (WHO editor, Basic Malaria Microscopy 2010) and Immunochromatography, and the other 69 samples were from the apparently healthy individuals, who were negative for malarial parasites.

Sample collection and processing

With sterile aseptic precaution, about 5 mL of venous blood was collected from each individual, out of which 2 mL was kept in EDTA vacutainer for the haematological assays and 2 mL was kept in plain vial for the biochemical analysis. Rest 1 mL was used for the smear preparation, ICT and QBC for microbiological lab diagnosis of malaria.

All haematological parameters were performed in the haematology auto-analyzer (Sysmex XTI 2000). The haematological parameters which were tested included the haemoglobin (Hb), red blood cell (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell (WBC) count, differential count, total platelet count and the absolute eosinophil count (AEC).

The biochemical parameters which were tested included aspartate transaminase (SGOT or AST), alanine transaminase (SGPT or ALT), blood urea, creatinine, total protein, total bilirubin and direct bilirubin. These parameters were estimated by using bio chemistry analyser (Roche).

Statistical analysis

The data are expressed as mean (SD) and p value was calculated for each parameter. Based on normality of data, independent t test or Wilcoxon rank-sum test were used to compare continuous variables between malaria infected patients and controls. The minimum level of statistical significance was set at p value < 0.05.

Results

A total of 138 individuals were taken as the study group, among which, 69 were malaria-infected patients and the other 69 were engaged as controls. Out of the 69 infected

cases of malaria, 23 had mono-infection with *Plasmodium falciparum*, 43 cases were infected with *Plasmodium vivax* and 4 were due to infection with other *Plasmodium* spp.

The mean haemoglobin level in the malaria infected cases was observed to be 9.9 g/dL whereas in the value was 10.2 g/dL in the control group. Again the average total RBC count was found to 3.1×10^6/μL whereas in the controls, the count was 4.3×10^6/μL. The mean haematocrit was 27.8% in the malaria cases, whereas in the controls it was 30.8%. Mean values for haemoglobin (Hb), total RBC counts and haematocrit values were significantly lower for the malaria group compared with the controls (p value < 0.05). The mean MCV of the malaria cases was seen to be 83.3 fL, whereas those of the controls was 82.9 fL. The average MCH of the malaria patients and the controls were 28.4 pg/cell and 26.9 pg/cell respectively. The mean MCHC was observed to be 33.4 g/dL in the malaria cases and 32.8 g/dL in the controls respectively. Although, the MCV values were not significantly different between the malaria-infected and the healthy controls (p value > 0.05), but the MCH and the MCHC were very much reduced in the malaria cases as compared to the controls (p value of 0.05 and < 0.05 respectively) (Table 1).

The mean total WBC count was found to be 6.7×10^3/μL in the cases suffering from malaria and 14.7×10^3/μL in the controls. Also the mean neutrophil count was 59.3% in the malaria cases as compared to a value of 67.7% in the controls. Therefore, leucopenia and neutropenia were significant in the malaria infected group (p value < 0.001 and < 0.005 respectively). The malaria infected cases showed a very significantly raised mean eosinophil count of 5.1%, as compared to 2.2% in the controls (p value < 0.001). But the basophil count was 0.7%, found to be lower in the malaria cases than the controls (p value 0.01). Also a significantly higher lymphocyte count is seen in the malaria infected cases (p value < 0.05). There was no significant difference in means of the monocyte counts between the cases and the controls. The total platelet count was observed to be very significantly low in the malaria-infected patients than the controls (p value < 0.001) (Table 1).

Since malaria affects liver functions, therefore among the biochemical parameters observed, the liver enzyme aspartate transaminase level, as well as total and direct bilirubin levels showed a significant reduction in the malaria infected cases as compared to the controls (p value < 0.05, < 0.0001 and < 0.05 respectively) (Table 2).

Discussion

The parasitic disease malaria is caused by the protozoan of genus *Plasmodium*. Alterations in haematological and biochemical parameters are also thought to worsen the prognosis of the malaria infected patients (Syamsundar 2013). It is essential to perform haematological and biochemical investigations in the cases of malaria infection so as to detect the early complications associated with acute malaria infection (Godse 2013).

Anaemia was observed to be the most common complication associated with the malaria patients. In this study, most of the malaria cases were found to have anaemia with

Table 1 Various effects of Plasmodium infection on the haematological parameters of the host

Parameters	Malaria patients Mean (SD)	Controls Mean (SD)
Haemoglobin (g/dL)	9.9 (1.8)*	10.2 (3.0)
Total RBC count ($\times 10^6$/μL)	3.1 (0.5)*	4.3 (4.1)
Haematocrit (%)	27.8 (6.3)*	30.8 (8.1)
MCV (fL)	83.3 (10.7)	82.9 (10.3)
MCH (pg/cell)	28.4 (3.2)*	26.9 (4.9)
MCHC (g/dL)	33.4 (1.4)*	32.8 (2.0)
Total WBC count ($\times 10^3$/μL)	6.7 (4.6)***	14.7 (27.8)
Differential count (%)		
Neutrophil	59.3 (11.9)**	67.7 (19.7)
Eosinophil	5.1 (7.4)***	2.2 (3.9)
Basophil	0.7 (2.3)**	1.7 (11.5)
Lymphocyte	29.6 (12.8)*	24.4 (15.9)
Monocyte	5.4 (7.5)	4.1 (3.4)
Total platelet count ($\times 10^6$/μL)	131.9 (137.7)***	199.7 (167.9)

*p value < 0.05; **p value < 0.01; ***p value < 0.001

Table 2 Various effects of Plasmodium infection on the serum biochemical parameters of the host

Parameter	Malaria patients Mean (SD)	Controls Mean (SD)
AST/SGOT (IU/L)	49.1 (30.6)*	75.3 (66.4)
ALT/SGPT (IU/L)	48.6 (30.8)	85.3 (140.1)
Blood Urea (mg/dL)	25.5 (10.4)	43.5 (48.7)
Creatinine (mg/dL)	0.9 (0.3)	1.3 (1.59)
Total protein (g/dL)	6.1 (0.8)	5.8 (1.0)
Total bilirubin (mg/dL)	1.3 (1.5)****	1.7 (3.9)
Direct bilirubin (mg/dL)	0.5 (0.5)*	0.6 (0.9)

*p value < 0.05; ****p value < 0.0001

significantly low levels of haemoglobin, low total RBC count and low haematocrit. Though the MCV was not affected much, but the MCH and MCHC of the malaria infected cases were lower than the controls, indicating the severity of the pathological effects in the malaria cases. Similar findings have also been reported by other studies (Khatib et al. 2015; Agrawal et al. 2015). This abnormality may be due to the obligatory destruction of red cells at merogony, accelerated destruction of non-parasitised red cells (major contributor in anaemia of severe malaria), bone marrow dysfunction, shortened red cell survival and increased splenic clearance.

Leucopenia was defined as total WBCs count $< 4000/\mu L$. There was significant difference in the total WBC count between the two groups of patients (p value < 0.001). Leukocyte components were also significantly affected. The neutrophil and basophil counts were significantly decreased in the patients whereas the eosinophil and lymphocyte counts were increased in the patients with malaria as compared to those in the control group, in the present study. The results are similar to the results obtained by Tobón-Castaño et al. (2015) and Jairajpuri et al. (2014). The leucopenia observed in the present study may be due to the localization of leucocytes away from the peripheral circulation, splenic sequestration and other marginal pools rather than actual depletion or stasis.

In our study, thrombocytopenia (platelet count $< 100{,}000/mm^3$) was significantly observed among malaria cases, as is the observation of many other studies (Kotepui et al. 2014; Martínez-Salazar and Tobón-Castaño 2014; Francis et al. 2014). This observation in malaria cases may be due to the peripheral destruction and excessive removal of platelets by spleen pooling as well as platelet consumption by the process of disseminated intravascular coagulopathy.

Since malaria affects liver and its biochemical parameters adversely, raised level of liver enzymes (transaminases) and high bilirubin levels are usually seen in patients of malaria. In this study, a significant statistical association was observed in aspartate transaminase as well as total and direct bilirubin levels in the malaria cases as compared to non-malarial controls was observed ($p < 0.05$, $p < 0.0001$ and $p < 0.05$ respectively). Similar observation was also made by other studies (Al-Salahy et al. 2016; Abro et al. 2009; Kotresh and Suresh 2016). Renal biochemical parameters and total protein levels were not significantly altered in the malaria cases in the present study.

Summary and conclusion

In the present study, a significant alteration was observed in the blood and biochemical parameters of the malaria cases. This indicated the existence of a strong statistical association between the abnormal haematological and biochemical parameters and occurrence of complications in a malaria case. Therefore, a detailed analysis and meticulous periodic monitoring of the haematological and biochemical parameters is vital in the cases of malaria, in order to foresee the advent of serious complications, and thus manage the patients effectively to reduce morbidity and mortality associated with it.

Author's contribution Dr. SD was responsible for sample and data collection, its processing and microscopy. She was responsible for writing the manuscript and its submission. Dr. NR was responsible for the conceptualization of the study, counterchecking the results and monitoring of the work carried out and analysis of the results. She also checked and refined the manuscript and also gave critical inputs to the paper. Dr. PC helped in the statistical modelling of the study, selection of the study parameters as well as gave critical inputs to the study and helped in refining the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was given by the JIPMER ethical committee.

Informed consent Informed consent has been taken from all the enrolled participants during the study.

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