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Original Research Article

Platelet count and superoxide dismutase as a marker for severity of plasmodium infection

Archana Shetty[1], Teerthanath Srinivas[2], Hariprasad S.[3]*

[1]K. S. Hegde Medical Academy, Deralakatte, Mangalore, Karnataka, India
[2]Department of Pathology, K. S. Hegde Medical Academy, Deralakatte, Mangalore, Karnataka, India
[3]Department of Surgery, Navodaya Medical College, Raichur, Karnataka

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***Correspondence:**
Dr. Hariprasad S.,
E-mail: raohariprasads@yahoo.co.in

ABSTRACT

Background: Malaria continues to be an important public health problem in developing and underdeveloped countries with high morbidity and mortality. It continues to be one of the major public health problems in India. Plasmodium vivax is the major parasite type causing malaria and Plasmodium falciparum is the major cause of serious and complicated disease. Haematological abnormality which is most commonly seen in malaria is thrombocytopenia followed by anaemia. Identification of predictors of disease severity is critical to improve patient care. This study was undertaken to evaluate the severity of thrombocytopenia and erythrocyte SOD level in infections by plasmodium vivax, falciparum and mixed infection.

Methods: A hospital based cross sectional study done on confirmed cases of malaria in a tertiary care hospital in costal Karnataka. All patients tested positive for malaria (either by rapid diagnostic test or peripheral smear) were included and patients presenting with fever who were but treated empirically for malaria were excluded in the study. The type of malarial infection was diagnosed with QBC and thin peripheral smear method; platelet count was done by automated cell counter and estimation of superoxide dismutase (SOD) by nitro blue tetrazolium chloride reduction method.

Results: A total of forty infected malaria patients were evaluated. The age range of the infective patients were between 15 to 70 years. Males were more commonly affected than females in the ratio of 5.6:1. The most common type of malaria infection was from P. vivax and mixed infection (40% each) followed by P. falciparum (20%). Majority (50%) of the patient had severe infection followed by mild infection (35%) and moderate infection. The mean platelet count was 1,14,250/cu mm in P.falciparum, 85,000/cu mm in P.vivax infection and 60,625/cu mm in mixed infection. The mean platelet count was least (56,181/cu mm) in severe infective patients than in moderate (91,666/cu mm) and mild (1,21,500/cu mm) infections. The SOD levels was reduced more in P. vivax (26.43U/mg Hb) and mixed infection (20.96U/mg Hb) than P.falciparum (32.74U/mg Hb). SOD levels were proportionally low in severe infection (14.94U/mg Hb), when compared to moderate (20.35 U/mg Hb) and mild infection (32.19 U/mg Hb).

Conclusions: Anaemia and thrombocytopenia are the most frequent haematological complications associated malaria. Thrombocytopenia is associated with increase in parasite density and severity of the infection, its more common in P.vivax and mixed infection than P.falciparum infection. The SOD level substantially reduces depending on the severity of malaria. Thrombocytopenia and reduced SOD level is a powerful predictor of disease severity. These parameters could be useful in the clinical approach of patients with malaria for prompt timely initiation of anti-malarial therapy and reduce the mortality.

Keywords: Mixed infection, *P. Vivax*, *P. Falciparum*, Platelet count, Superoxide dismutase

INTRODUCTION

Malaria continues to be one of the important public health problems in India, due to lack of proper health infrastructure, inability to control the disease in endemic areas, and movement of the population are some of the factors responsible for failure to curb malaria. It's estimated to be 70-100 million cases each year. The transmission of the disease varies with geographic areas, as the diversity and distribution of Anopheline and Plasmodium species vary. As per World Health Organization report 2015, Malaria caused 214 million infections and 438000 deaths worldwide, most of them occurred in the Africa region (90%) followed by South-east Asia region (7%).[1] Among South-east Asia region, India shares two-thirds of the burden (66%) followed by Myanmar (18%) and Indonesia (10%). The incidence of infection by *Plasmodium vivax* (*P. vivax*) is more common than plasmodium falciparum (*P. falciparum*) and *P. falciparum* causes the more serious form of the disease.[2] Haematological abnormality which is most commonly seen in malaria is thrombocytopenia followed by anaemia. Malarial Infection may become severe and fatal without early diagnosis and prompt appropriate case management. Rising documentation of drug resistance worldwide and complications of Plasmodium infection represents a global health threat. Prompt action is especially important for high risk groups such as young children and pregnant women. Therefore, identifying markers of disease severity is essential to improve the clinical management.

METHODS

This is a hospital based cross sectional study, during the period from January 2014 to August 2014, done on confirmed cases of malaria at K S Hegde Medical charitable hospital, Mangalore. Approval of institutional ethical committee was taken. All the patients tested positive for malaria (either by peripheral smear or rapid diagnostic test) were included in the study and paediatric patients and patients presenting with fever, who were treated empirically for malaria and were excluded in the study. All patients were informed about the study and informed consent was obtained. Malaria was diagnosed based on clinical features and positive QBC system for malarial parasites (QBC MP/ Microtube Agglutination stained by acridine orange) or thin blood smear examination using Giemsa stain. The patients were categorized based on the types of infective species as vivax, falciparum and mixed infections. Parasitaemia was commented as percentage of infected erythrocytes and the severity of infection was categorized based on based on the World Health Organization Definition of Severe Malaria of 2000. Platelet count was analysed on 2ml of anticoagulated (EDTA) blood using automated equipment (Mind ray-cell counter) on the day of admission along with routine investigations. Platelet count of <50x109/cu mm was considered severe thrombocytopenia and 100–150x109/cu mm was considered as mild thrombocytopenia. Estimation of superoxide dismutase (SOD) was done by Nitro Blue tetrazolium chloride reduction (NBT) method. The data were analysed using the statistical tools by SPSS software version 23.0.

RESULTS

In the study group, a total of forty malaria infected patients were evaluated and were compared with 40 healthy individuals. The age ranged of the infective patients were between 15 to 70 years, with a mean age of 36.6 years. Males were more commonly affected than females in the ratio of 5.6:1.

Plasmodium vivax infection was encountered in 40% of cases (16 nos), *P. falciparum* in 20% of cases (8 nos), and mixed infections in 40% of cases (16 nos). Severity of infection was graded into mild, moderate and severe parasitemia. Among the forty infected individuals, 35% of had mild infection, 15% had moderate infection and 50% suffered from severe infection.

The platelet count was analysed in all infected patients and their values were compared with the control group. It was observed that, all the infected individuals showed decrease in the platelet count with the mean platelet count of 81,100/cu mm. The decrease in platelet count was more seen in *P. vivax* and mixed infection than P. falciparum. The mean value of the platelet count observed in *P. falciparum* was 1,14,250/cu mm, mixed infection - 60,625/cu mm and *P. vivax* - 85,000/cu mm. The mean platelet count in severe infection was 56,181/cu mm, whereas in mild and moderate infection the value were 1,21,500/cu mm and 91,666/cu mm respectively. More the severity of infection, greater was the reduction in the platelet count.

The SOD was estimated in all the forty malaria infected patients and the control individuals. The mean SOD levels in controls was 42.31 U/mg Hb, while the mean SOD level in infection by P.vivax was 26.43 reduced in P. vivax and mixed infection than P.falciparum. The SOD levels in mild infection was 32.19 U/mg Hb, moderate and severe infection were 20.35 U/mg Hb, and 14.94 U/mg Hb respectively. These suggest that more the severity of the malaria infection, more will be the reduction in SOD levels (Table 1).

Table 1: Mean platelet count and SOD Level in severity of malaria infection.

Severity of infection	Mean platelet count	Mean SOD level
Mild	1,21,500/cu mm	32.19 U/mg Hb
Moderate	91,666/cu mm	20.35 U/mg Hb
Severe	56,181/cu mm	14.94U/mg Hb

In this study, it is observed that the reduction in the platelet count and SOD level is indirectly dependent on

the severity of malaria infection and degree of parasitemia. Therefore, both platelet count and SOD levels can be used as a marker for severity of malaria.

DISCUSSION

Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development.[3] Malaria transmission varies with geographic areas, as the diversity and distribution of Anopheline and Plasmodium species vary. The common clinical manifestation is fever with chills and rigors, headache, vomiting, jaundice and clinical signs being splenomegaly, pallor, and icterus. Malaria is a true haematological infectious disease affecting almost all blood components. Haematological changes are specific and may vary with demographic factors, nutritional status, hemoglobinopathy, background, malaria endemicity levels, and malaria immunity.[4] Anaemia and thrombocytopenia are the most frequent malaria associated haematological complications. Several clinical complications have been described in malaria infection including severe anaemia, cerebral malaria, acute pulmonary oedema and multi-organ failure. Anaemia may be linked to haemolysis, decreased cell deformity of parasitized cells, increased splenic uptake, decreased survival of platelets and decreased production of platelets.[5,6]

Platelet abnormalities in malaria are both qualitative and quantitative. Malaria induced thrombocytopenia (platelet count <150×109/L) is very common and has been reported in most of malaria studies.[7,8] The frequency of thrombocytopenia in malarial infection ranges from 24-94% in the literature.[9-11] Thrombocytopenia alone, rarely causes bleeding unless it is accompanied by coagulopathy, which is observed only in severe complicated falciparum infection. The mechanisms for platelet destruction are sequestration in spleen, coagulation disturbances, antibody mediated platelet destruction, oxidative stress, reduction of erythrocytic anti-oxidative enzyme activities, and Immune complexes mediated destruction.[12] Platelets in patients with acute malaria are highly sensitive to adenosine diphosphate (ADP) addition in vitro.[13] It is believed that ADP release following haemolysis could contribute to higher platelet aggregation. Platelets adhere to the endothelium when it is previously stimulated with tumor necrosis factor (TNF). Bruno et al, in their study noted that severe infected patients presented more severe thrombocytopenia and higher TNF levels.[14] There is also an inverse correlation between platelet count and TNF in patients with malaria infection. Immune-mediated destruction of circulating platelets has been postulated as a cause of thrombocytopenia seen in malaria. Malaria infected patients have elevated levels of specific IgG in their blood which binds to platelet-bound malaria antigens possibly leading to accelerated destruction. Platelet clumping is the most important platelet functional abnormality. Many small platelets are seen mixed or clumped with a few giant platelets that are falsely counted as single platelet by the analysers thus causing pseudo-thrombocytopenia. Factors associated with malaria induced thrombocytopenia include splenomegaly, splenic sequestration and platelet removal by macrophages.[15,16] Studies by Richards et al and Erhart et al suggests that a lower platelet count may be an indicator of higher biomass falciparum and vivax infections.[17,18] In this study, platelet counts were significantly reduced in malaria infected patients, the mean platelet count in P.falciparum was 1,14,250/cu mm, mixed infection was 60,625/cu mm and P.vivax was 85,000/cu mm; these platelet count values are comparable to study done by Ohtaka et al.[19] The platelet count was significantly reduced in severe infection (mean=56,181/cu mm) when compared to moderate infection (mean=91,666/cu mm) and mild infection (mean=1,21,500/cu mm). There is an inverse relation between the platelet count and degree of parasitemia and severity of infection. Present findings regarding severity of thrombocytopenia was in correlation with the studies done by Saravu et al, Fabio et al and among population in Thailand and South Korean.[7,8,18] Gerardin et al have studied the utility of thrombocytopenia as a prognostic marker in falciparum malaria alone.[20] Robert et al considered low platelet count and haemoglobin concentration as most important predictors of *P. falciparum* malaria infection.[9]

SOD represents an important defence against oxidative stress within a cell and is an important participant in the oxidative stress responses has been implicated in several other diseases and infections.[21] During malaria infection, reactive oxygen species are produced at high levels, inducing parasite killing and tissue damage.[22] Patients with acute non-complicated *P. falciparum* or *P. vivax* malaria have less catalase activity. Reduced catalase activity together may result in the accumulation of H2O2 and release of free oxygen radicals leads to a decrease in antioxidant enzymes in parasitized erythrocytes. Thus, the significant reduction of plasma antioxidant enzymes in high parasitaemia patients might be the predisposing factor to higher oxidative stress causing damage to erythrocyte membranes. These bring about consequential increase in severe anaemia, occlusion of peripheral microvasculature, cerebral pathology (hypoxia), and cardiac injury observed in severe malaria.[23] Erel et al found that thrombocyte count, platelet superoxide dismutase and glutathione peroxidase activities of patients with vivax malaria were lower and platelet lipid peroxidation levels were higher in patients than those of healthy subjects.[12] Bilgin et al in their study found the activities superoxide dismutase and glutathione peroxidase were found to be significantly lower in vivax malaria patients.[24] Narsaria et al also found that there is significant elevation of malondialdehyde and protein carbonyl levels reflect the increased oxidative stress, whereas decreased levels of glutathione and superoxide dismutase point toward utilization of the antioxidants in severe malaria.[25] Thus, these changes in oxidants and

antioxidants suggests the production of reactive oxygen species and their possible role in pathogenesis of severe malaria. In present study, the SOD level was substantially reduced depending on the density of the parasites in blood and severity of malaria. This was more common in *P. vivax* and mixed infection than *P. falciparum* infection. Our findings regarding the reduction in SOD level with that of severity of malarial infection is in correlation with the studies done by Erel et al, Bilgin et al and Narsaria et al.[12,24,25] The SOD levels though not specific for malaria, along with positive parasite identification could be a sensitive indicator for severity of malaria infection.

Platelet count and SOD levels could be used as an important marker for assessing the severity of infection and can be used in assessing the severity of the disease, before the development of fatal outcomes and monitoring the success of therapy and clinical recovery.

The present study is a short period cross-sectional study in one hospital and has no information on posttreatment follow up. Further study is needed to be concentrated on the association of platelet count and SOD in the large population of malaria infected patients may be needed.

CONCLUSION

Anaemia and thrombocytopenia are the most frequent haematological complications associated malaria. Thrombocytopenia is associated with increase in parasite density and severity of the infection, its more common in *P. vivax* and mixed infection than *P. falciparum* infection. The SOD level substantially reduces depending on the severity of malaria. Thrombocytopenia and reduced SOD level is a powerful predictor of disease severity. These parameters could be useful in the clinical approach of patients with malaria for prompt timely initiation of anti-malarial therapy and reduce the mortality.

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