Clinical Correlation of Coagulopathy in Vivax Malaria

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ABSTRACT

Background: Malaria is a disease with a great global burden. Understanding the pathogenesis of the disease with particular emphasis on the complications is necessary. The coagulation system plays a key role in the pathogenesis of complicated as well as uncomplicated malaria. Pathogenesis of vivax malaria is less focused upon as compared to that of falciparum malaria and studies on the role of coagulation have yielded conflicting results.

Aims: To study the prothrombin time (PT) and activated partial thromboplastin time (aPTT) in malaria with focus on vivax cases to determine the presence or absence of coagulation abnormality and to correlate these with the clinical features.

Settings and Design: Hospital based prospective cross-sectional study.

Methods: A single citrated blood sample of patients diagnosed with falciparum and vivax malaria was analysed in semi-automated coagulation analyzer at the time of presentation. The values were compared with healthy controls. Correlations with clinical features and effect of treatment on coagulation profile have been studied. Data was analyzed by mean standard deviation and by ‘t’ test using SPSS software version 16 for windows. ‘p’ value is obtained by Mann Whitney U test

Results: Prolonged PT and aPTT was noted in vivax malaria as compared to the controls. The difference in the coagulation profile of vivax and falciparum cases was not significant. PT and aPTT were prolonged in 38% and 56% of the malaria patients. The sample obtained at the time of presentation had no significant correlation with the clinical symptoms, antimalarial treatment and complications.

Conclusions: Coagulation is involved in the early stages pathogenesis of vivax malaria to the same extent as falciparum malaria.

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Introduction

Malaria is one of the most widespread diseases causing nearly 120000 deaths annually. Haematological alterations are the commonest complications encountered in malaria irrespective of the species. Etiology is almost equally attributable to the two major species namely Plasmodium vivax and Plasmodium falciparum except in regions of Africa and the western pacific Falciparum malaria is the cause of majority of deaths and complications and its pathogenesis has been studied extensively.[1,2]

Factors contributing to complication in malaria include sequestration of parasitized RBCs in microcirculation, activation of inflammation with production of cytokine storm, widespread activation of coagulation system accompanied by formation of thrombi in microvascular bed producing multorgan dysfunction and disseminated intravascular coagulation (DIC).[3]

By virtue of PfEMP expressed on the membrane the parasitized RBCs (pRBCs) sequester in different organs adhering to the endothelial cells by using the host receptor adhesion molecules. Sequestration helps the parasite avoid killing by the splenic macrophages and is the root of all major complications. [3,4]

Clinical severity of the disease depends also upon the response of the host. The two factors that play a key role in development of complications are a) the release of the inflammatory cytokines especially TNF and interleukins and b) the activation of coagulation system. [9]

Malaria infection is a procoagulant state. Multiple pathways are involved in activating the coagulation system-

- The parasitized RBCs become procoagulant by altering the the phospholipid orientation of their cell membrane.[6,7]
- The sequestrated and procoagulant RBCs induce release of tissue factor from activated blood monocytes, endothelial cells and microparticles.TF activates the extrinsic pathway of coagulation after complexing with FVII. The thrombin generated can activate platelets, produce microthrombi and activate the intrinsic pathway of coagulation [9-12]
- In addition to coagulation, TF can also potentiate the inflammatory response. TF thrombin and activated FX together can trigger the inflammatory system by enhancing the production of cytokines, especially interleukins and Tumor Necrosis Factor (TNF).[6,10]
- TNF is responsible for systemic manifestations of malaria such as fever and headache. Additionally TNF also induces expression of adhesion molecules on the surface of endothelial cells which leads to recruitment of inflammatory cells and release of their contents such as elastase. Elastase damages the endothelium which in turn potentiates coagulation.[13,14]
- Platelets when activated not only aggregate at the site of endothelial damage but also release several procoagulant and proinflammatory cytokines contributing significantly to the pathogenesis of complications.[3,7]
- Activation /damage to endothelium by pRBCs causes release of ultra large multimers of Von-Willebrand factor which together with deficiency of ADAMTS13 in affected individuals causes platelet adhesion and aggregation and relase of procoagulant substances. [15]

Understanding the role of coagulation system in pathogenesis of malaria and its complications was initiated when prolongation of prothrombin time and aPTT was first recognised in uncomplicated cases. Research into its etiology led to the identification of the interdependence of the coagulation and inflammation and the fundamental role of TF in activation of the two. [16-18]

Functional activation of coagulation system in falciparum malaria is undisputed but its activation is still contested in vivax malaria. The aim of this study was to explore this incongruity by doing baseline investigations of PT and aPTT in patients with vivax and falciparum malaria and comparing them with controls and with each other. Correlation with clinical features and complications was done. Research was centred in a region endemic for malaria and hence the cases as well as the controls may be considered as partially immune to malaria. [11,19]

Materials and Methods

It was a prospective cross-sectional study done over two years. Patients were included in this study after obtaining the necessary clearance from institutional ethics committee and obtaining consent from participating patients and controls.

Inclusion criteria: Patients diagnosed with P vivax, P falciparum and co infection with these two species were included in this study.

Exclusion Criteria:
- Patients on anticoagulant therapy
- Known disease states having abnormal coagulation profiles like hemophilia.
- Pregnant women
- Other associated diseases like Diabetes mellitus and renal failure
A detailed history with emphasis on presenting complaints was obtained. Findings of clinical examination were recorded. The patients were followed up for the duration of stay in hospital.

A single citrated sample was obtained at the time of admission. PT and aPTT were performed on semiautomated Sysmex CA-50 using Thromborel S and aPTT kit from Dade Behring. An EDTA sample was also collected simultaneously. Platelet count was estimated in Coulter LH 500.

A few of the patients already on antimalarial therapy at the time of admission were also included in the study. PT ratio and aPTT ratio of >1.5 were considered to be prolonged. MNPT and MNaPTT were determined from the samples obtained from the healthy controls.

Data was analyzed by mean standard deviation and by ‘t’ test using SPSS software version 16 for windows. ‘p’ value is obtained by Mann Whitney U test.

**Results**

A total of total 300 slide positive malaria patients were included in the study.

Two hundred and twenty two were males and 78 were females. The age of the patients ranged from 15 years to 65 years. The distribution of cases by the species was as in Fig.1.

The most common symptom was fever. It was seen in almost all patients with malaria. Other common symptoms were headache, bodyache and vomiting. Pain abdomen associated with fever was seen in six patients. One patient was diagnosed with *P. vivax* malaria incidentally when she presented with loss of consciousness after an accidental fall.

One of the patients had associated acute suppurative otitis media and one patient had breast engorgement and secretion. Clinical examination revealed hepatomegaly and splenomegaly in eighteen patients each and both in three patients. One patient presented with mild right pleural effusion. Six of the patients had icterus associated with fever.

Complicated malaria was diagnosed in twenty patients and surprisingly twelve patients had infection with *P. vivax* and the remaining eight with *P. falciparum*. Complications included septicaemia, acute respiratory distress syndrome (ARDS), multi-organ dysfunction and septic shock. One patient with complicated vivax malaria subsequently died due to septicaemia and ARDS.

Haematological profile revealed thrombocytopenia (<1,50,000/cmm) in 96% of the patients. Severe thrombocytopenia with platelet count of 20,000/cmm or less was seen in 42 patients. However none of the patient had any bleeding manifestations. (Fig 2)

**Comparison of malaria cases vs controls**

PT and aPTT values were prolonged in 38% and 56% of the malaria patients respectively. The differences in the PT and aPTT between patients with malaria and healthy control was highly significant (*p* <0.001). (Fig.3 & Fig.4, Table 1)

There was no significant differences in aPTT and PT values between genders.

**Comparison of *P vivax* vs control**

The PT and aPTT values of *P. vivax*, were significantly different from those of the control groups. (*p* <0.001).

**Comparison of *P falciparum* vs control**

aPTT was significant. (*p* = 0.025).

**Comparison of mixed infection vs control**

The PT and aPTT values of mixed infection groups were significantly different compared to the control groups. (*p*<0.001).
Comparison of P falciparum vs P vivax vs mixed infection
There was no significant difference in PT and aPTT values in between patients with \textit{P.falciparum}, \textit{P.vivax} and mixed malaria (p>0.05)

Comparison of patients on treatment vs untreated cases
Antimalarial treatment did not alter the aPTT and PT values in the patients with malaria. Also among patients who had antimalarial treatment, there was no difference in the values between patients who had started on antimalarials less than 10 hours and more than 10 hours prior to the collection of blood sample.

Comparisons depending on clinical and laboratory parameters
The PT and aPTT values of patients with clinical history and findings of headache, bodyache, cough, pain abdomen, spleenomegaly and hepatomegaly were not significantly different from patients with negative history for these symptoms and signs. However the PT value was significant prolonged in patients who presented with vomiting (‘p’ value:0.033), as compared to those who had no history of vomiting.

The correlation between PT and the platelet count was not significant (‘p’value:0.086). However there was a significant correlation between the aPTT values and the platelet count in patients with counts above 50,000/cmm (‘p’value:0.035).

Interestingly, PT and aPTT values did not differ significantly between the patients with raised and normal liver parameters (Bilirubin, Aspartate amino transferase(AST) and Alanine amino transferase (ALT))

Comparison between complicated and uncomplicated cases.
There was no difference in PT and PTT values between patients with complicated and uncomplicated malaria. There was also no correlation between the values and duration of hospital stay.

Discussion
In our study the difference in the PT and aPTT values between the vivax group the controls was highly significant. This definitely suggests activation of coagulation in vivax malaria.

Coagulation factors once activated are used up. Depleted levels of various factors either in the common pathway or both in intrinsic and extrinsic pathway leads to prolonged PT and PTT.

There was no significant difference when the values of vivax, falciparum and the mixed infection groups were compared with each other which suggests rather uniform degree of activation of coagulation in all the species.

PT and aPTT was prolonged in 38% and 56% of the malaria patients respectively The derangement in values is comparable with those of Prasad et al. and Netha et al who noted prolongation of PT in 47.5%, and 22% of cases and deranged aPTT in 35% and 11% of cases, respectively [20,21] (Fig.5)
The study conducted by Rojanasthein et al. also revealed significantly prolonged PT and aPTT values in patients with *P. falciparum* malaria but no difference in values in patients with vivax malaria. These coagulation abnormalities were attributed by him to liver dysfunction. However in our study there was no significant difference in the PT and aPTT values between patients who had elevated liver enzymes and those who did not indicating that the changes were unrelated to liver involvement[11].

In our study we found no significant differences in the coagulation parameters between patients with complicated and uncomplicated malaria of either species. However derangements of PT and PTT were seen in 80% and 100% of all the complicated cases respectively.

These were comparable to the study conducted by Srinivas et al who found no correlation between the coagulation parameters and severity of the disease. Netah et al also did not have correlation of PT, aPTT and thrombocytopenia with mortality[19, 21].

PT and aPTT alone are probably inefficient in predicting course of the disease. Additional information regarding the status of the fibrinolytic and the anticoagulant mechanisms are probably required to recognize the degree of impairment in the homeostasis.

DIC is an uncommon state of decompensation in malaria as evidence by the fact that none of the 300 patients included in the study had any bleeding manifestations including those with liver enzyme abnormalities.

Most of the cases of malaria remain in a state of compensated DIC wherein the activated factors are kept in check by the anticoagulant mechanisms. Decompensated state occurs when the continued presence of the offending agent leads to consumption of the anticoagulants which are then no longer available to manage the activated factors leading to large scale thrombosis. This hypothesis bears investigation by research which has shown a greater depletion of anticoagulant proteins and presence of fibrin degradation products in severe disease[5,11,16].

Haematological profile revealed thrombocytopenia (<1,50,000/cmm) in 96% of the patients. However none of the patient had any bleeding manifestations supporting the idea that platelets in malaria are hyperresponsive and hence prevent any bleeding.

It has been shown that platelet activation and degranulation occurs early in the disease. As the disease progresses the platelets become exhausted and are cleared from circulation which may be one of the mechanisms of thrombocytopenia in malaria. Immune destruction, sequestration and invasion of platelets by parasite with subsequent platelet destructions are other theories proposed for thrombocytopenia[12,21].

There was no significant differences in PT and aPTT values between genders. Antimalarials do not have effect on the mechanisms that trigger the coagulation system as evidenced by the fact that there was no difference in the PT and PTT values in patients who had received treatment and who had not. Among patients who had antimalarial treatment, there was no difference in the values between patients who had started on antimalarials less than 10 hours and more than 10 hours prior to the collection of blood sample.

It has been demonstrated that administering heparin in patients with severe malaria did not affect the outcome of the disease. However it is interesting to know that anti TF antibodies were in fact able to bring down the severity of the disease[6].

Correlating the PT and aPTT values with the symptoms the patients came with, showed no significant value except that the PT value was significant for patients who presented with vomiting (‘p’ value=0.033). Further studies into this parameter may throw light upon this matter.

**Conclusion**

This study has compared the PT and aPTT values in patients with malaria and normal healthy controls derived from the same population. We conclude that

There is a significant difference between the PT and aPTT values of the two groups. This indicates that in patient with malaria there is an activation of intrinsic and extrinsic pathways of coagulation.

activation of coagulation cascade is not confined to *P. falciparum* alone but is also seen in significant percentages of patients with *P. vivax* infection most of the cases derangements with or without accompanied thrombocytopenia did not lead to hemorrhagic

![Coagulation parameters in different studies](http://www.pacificejournals.com/aabs)
manifestations probably because of compensatory anticoagulant/ fibrinolytic activity.

Coagulation system is activated even in patients without complications.

Since the PT and aPTT values are unaffected by treatment we could hypothesize that antimalarials cannot prevent activation of coagulation however they may contribute in reducing the severity of the disease by clearing off the antigen

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Conflicts of interest
Nil

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