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Fibrinolytic Activities in Malaria Patients at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria

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Abstract: Malaria is one of the prevalent infections with high mortality in >90 countries in the world and it is associated with the coagulation cascade and fibrinolytic activities. The study investigated fibrinolytic activities in individuals with malaria infection at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, South-Western Nigeria. Seventy samples were collected, fifty from malaria patients and twenty from healthy individuals which were used as control. Plasma samples were obtained from the subjects and these were used to investigate the fibrinolytic activities using the euglobulin clot lysis time and fibrinogen concentration methods. It was discovered that all the fifty malaria positive samples had a prolonged euglobulin clot lysis time with 395.54±50.20 indicating hypofibrinolysis and these eventually had high fibrinogen concentration in comparison with the control samples which had normal euglobulin lysis time with 138.85±18.37. The values were significantly different when both test and control samples were compared ($p<0.05$). There was also high fibrinogen concentration of 297.94±24.23 when compared with the control that had 159.30±7.34 which is also significantly different ($p<0.05$). Malaria infection had been seen as contributing to thromboembolic process in any infected individual. The results necessitate the need for scrupulous prevention and prompt treatment of malaria in infected patients.

Key words: Malaria, fibrinolytic activities, euglobulin clot lysis time, hypofibrinolysis, blood, tissue

INTRODUCTION

Fibrinolysis is the process of removing unwanted, insoluble deposit formed as a result of coagulation. It is the process wherein a fibrin clot, the product of coagulation is broken down (Cesarman-Maus and Hajjar, 2005). Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium*. It remains a major cause of morbidity and mortality in tropical and subtropical countries including parts of the Americas, Asia and Africa. Each year, there are approximately 350-500 million cases of malaria, killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa. About 90% of malaria-related deaths occur in Sub-Saharan Africa (Snow *et al*., 2005). The parasite attacks and destroys red blood cells and it may affect vital body organs including the brain. Most deaths due to falciparum malaria are the result of cerebral malaria.

Activation of the intrinsic pathway which only uses factors contained within the blood is often associated with activation of the fibrinolytic system. Fibrinolytic activity is primarily determined by the balance between tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1 levels. Endothelial cells are responsible for the production and release of t-PA and contribute to the release of PAI-1. Multiple factors including lipoproteins, cytokines and inflammatory markers, modulate Endothelial Cell production of t-PA and PAI-1(Gross, 2000).

Fibrinolytic activity has been postulated as one of the risk factors associated with severity of malaria infection (Omoigberale *et al*., 2005). Marked changes in blood coagulograms and high levels of serum fibrin degradation products appeared only in cases with very severe cerebral involvement and also in cases with very high parasiteamia alone. In India, different parameters of fibrinolytic systems like t-PA, PAI, D-dimer and inhibitors of blood coagulation and Antithrombin III (AT-III) have

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been studied in cases of acute malaria due to *Plasmodium falciparum* and *Plasmodium vivax* infection and these patients were followed up. It was observed that the plasma PAI-1 was very high in cases of *Plasmodium falciparum* malaria infection as compared to uninfected controls and *P. vivax* infection. The changes in complicated cases of *Plasmodium falciparum* were remarkable as compared to uncomplicated ones. The Factor VIII levels were invariably high in acute malaria. On follow-up of some of these cases the values came back to normal after the antiparasite treatment. The monocyte procoagulant activity was found to be significantly higher in *Plasmodium falciparum* infection as compared to that of *P. vivax* infection. All these findings therefore contribute towards the production of a hypercoagulable state in *Plasmodium falciparum* infection and partly explain the complications of *Plasmodium falciparum* infection like cerebral malaria (Sucharit *et al*., 1975).

MATERIALS AND METHODS

All the chemicals used were of analytical grade and the purest quality available. They include acetic acid, sodium borate, calcium chloride, thrombin, khan tubes and water bath set at 37°C.

Study centre: The study was carried out at the Parasitology Department of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife between July and August 2010. The Teaching Hospital Complex is the major referring centre in this area.

Study population: The population comprises mainly of adults within the age range of 25-60 years who were diagnosed of having malaria caused by *Plasmodium falciparum* and were confirmed positive of the infection through laboratory investigation in The Parasitology Department of the Laboratory. A total of 50 samples were collected for investigation. About 20 samples were collected from uninfected individuals as control samples. The subjects were grouped based on age, sex and severity of the malaria infection.

Collection of samples: Samples were collected over a period of 2 months from July to August 2010. The samples were collected through vein puncture and the prominent vein was chosen from the median cubital vein. The site was clean with 70% alcohol to remove dirts and microorganisms from the site.

About 5 mL needle and syringe was inserted into the vein and the plunger was gently drawn back and the blood moved slowly to the syringe. Approximately 4.5 mL of venous blood was collected from each subject into 0.5 mL of sodium citrate as the anticoagulant. The blood was then mixed with the anticoagulant so that the blood would not clot. The blood was centrifuged at room temperature for 2-3 min and the plasma is then removed into the plain bottle by Pasteur pipette.

Euglobulin clot lysis time: The plasma samples were precipitated with 1% acetic acid and resuspended with borate buffer. The euglobin fractions in the sample are clotted by thrombin and the clot incubated and the period of lysis was noted. About 1 mL of plasma was added to 9 mL of 0.1% acetic acid to acidify it. The mixture was incubated at 4°C for 30 min to precipitate the euglobulin. After incubation the mixture was centrifuged at 2000 rpm. for 5 min. The supernatant was discarded and the tubes were inverted to drain the excess liquid. The precipitate was resuspended and dissolved in 0.5 mL of 1% borate solution. The 0.5 mL of thrombin was added and incubated at 37°C for 10 min and the record for the time for the clot to be formed. This was inspected at time interval for complete clot lysis. Average ranges 70-300 min.

Dry Clot Weight Method for fibrinogen: Fibrinogen in plasma was converted to fibrin by clotting with thrombin and calcium. The resulting clot was weighed. The reagents and plasma were incubated at 37°C for 15 min. About 1 mL of pre-warmed plasma was put into labeled test tubes. Wooden applicator stick was put into each tube and 0.1 mL of pre-warmed calcium chloride and 0.9 mL of thrombin were added into each tube and then mixed. The tubes were incubated at 37°C for 15 min for clot formation. The fibrin clots were blotted with filter paper and the removed clots were dried in air oven. These were allowed to cool and then weighed.

Statistical analysis: Sigma Stat® 3.5 (statistical package for social sciences) was used. The data are presented as median value and range (25-75%). Correlations between variables were analyzed using Spearman Correlation test. A multiple logistic regression model was used for survival analysis. Probability level of $p<0.05$ was considered as statistically significant.

RESULTS

Table 1 shows the grouping based on age and sex of the subject while Table 2 shows that of the control using the same criteria. Table 3 shows the grouping based on age and the severity of the malaria infection.

It was discovered after the assay that all the fifty samples had prolonged euglobulin clot lysis time

compared with the control samples irrespective of the age, sex and the severity of the condition. The mean and standard deviation of euglobulin clot lysis time for the test samples and control were shown in Table 4. Table 5 shows the mean and standard deviation of fibrinogen concentration for the test samples and control statistically prepared by SPSS. Their results were expressed as mean and standard deviation.

From the Table 1, thirty one of the subjects are male while nineteen are female and twenty nine of the subjects are within the age range of 25-40 while twenty one are within the age range of 41-60.

From the Table 2, thirteen of the control samples are male and seven are female; ten falls within the age range of 25-40 and ten are also within the age range of 41-60.

From Table 3, thirty four of the subjects had 11-100 malaria parasites per 100 High Power Field (HPF) and sixteen had 1-10 malaria parasites HPF.

The Table 4 shows that the test group has a mean value and Standard deviation of 395.54 and ±50.20, respectively when compared with the control group that has 138.85 and ±18.37. There was a statistical significant difference between the mean ECLT of the test sample and the control ($p<0.05$).

The statistical data showed that there was a significant difference between the fibrinogen level of the control and the test samples, that is 159.30±7.34 and 297.94± 24.23, respectively $p<0.05$.

Table 1: Subjects grouping based on age and sex

	Sex		
Age	Male	Female	Total
25-40	20	9	29
41-60	11	10	21
Total	31	19	50

Table 2: Showing grouping of the control samples based on age and sex

	Sex		
Age	Male	Female	Total
25-40	5	5	10
41-60	8	2	10
Total	13	7	20

Table 3: Severity of malaria infection

	Severity		
Age	11-100 parasites/100 HPF (++)	1-10 parasites/HPF (+++)	Total
25-40	20	9	29
41-60	15	6	21
Total	34	16	50

Table 4: Mean and standard deviation of euglobulin lysis time for test samples and control

ECLT	N	Mean±SD	p-value	Decision	Reason
Control	20	138.85±18.37	0.00	Significant	p<0.05
Test	50	395.54±50.20	0.00	Significant	p<0.05

SD = Standard Deviation

Table 5: Mean and standard deviation of fibrinogen concentration of test samples and control

Fibrinogen level	N	Mean±SD	p-value	Decision	Reason
Control	20	159.30±7.340	0.00	Significant	p<0.05
Test	50	297.94±24.23	0.00	Significant	p<0.05

SD = Standard Deviation

DISCUSSION

Malaria remains a highly prevalent disease in >90 countries in the world and accounts for at least 1 million deaths every year. It has become a growing problem all over the world with drug resistant strain of the malaria parasite. Malaria infection is often associated with the activation of the coagulation cascade and fibrinolytic activities (Francischetti *et al*., 2010). The present study was undertaken to demonstrate a significant alteration in fibrinolytic activities in malaria infection vis a vis euglobulin clot lysis time and fibrinogen concentration.

In this study, 50 individuals with malaria infection were investigated for fibrinolytic activities and all of them had prolonged euglobulin clot lysis time which signified hypofibrinolysis compared with 20 individuals without malaria infection that were used as control. The patients also had high fibrinogen level which may contribute to the possible thromboembolic process in these patients and hence higher risk of mortality from malaria infection. In agreement with this result; previous studies by Mohanty showed that in severe malaria there have been consistent elevated concentrations of fibrin degradation products, usually with increased levels of fibrinogen and suggesting increased fibrinogen turnover.

Studies carried out by Francischetti *et al*. (2010) on blood coagulation, inflammation and malaria indicated that there is a compensated state of blood coagulation activation in malaria which must have been as a result of malaria pathogenesis. This agrees with the result obtained in this present study when hypofibrinolysis was observed and also high fibrinogen concentration.

Sucharit *et al*. (1975) investigated coagulation and fibrinolysis in cases of falciparum malaria. The study was conducted using 18 cases of severe falciparum malaria including cases with parasiteamia. The investigation indicated that intravascular coagulation occurred only in patient who developed cerebral manifestation and in cases with high parasiteamia whereas in this study all the patient developed hypofibrinolysis regardless of the severity of the condition. Hypofibrinolysis was observed in a study carried out by Mohanty where plasminogen activator inhibitor was high with decrease in tissue plasminogen activator in cases of malaria infection as compared to uninfected control. The present study also agrees with this observation.

Kanjaksha and Shrimati (2007) reported that when malaria parasites infect the red cells, it leads to changes in the red cell phospholipids composition supporting blood coagulation. Red cells infected with *Plasmodium falciparum* also adhere to deeper tissue capillary endothelium leading to profound damage to endothelial cells leading to further activation of blood coagulation. He also observed heavy parasiteamia leading to occlusion of hepatic microcirculation and hence abnormalities in synthesis and secretion of coagulation factors and their inhibitors. This could explain the rationale for the results obtained in this study.

Again, in a study carried out at the University of Benin, Nigeria by Omoigberale *et al.* (2005), the fibrinolytic activity was measured in children with malaria infection. It was discovered that Euglobin Lysis Time (ELT) was found to be higher in children with *Plasmodium falciparum* malaria infection (430±149) than in the controls (158±21.7). It was then concluded that children who have malaria infection have decreased fibrinolytic activity and proportionately high fibrinogen level which may contribute to the possible thromboembolic process in these children and hence higher risk of mortality from *Plasmodium falciparum* malaria infection (Omoigberate *et al*., 2005). This report was in total agreement with the result of this study where the euglobulin lysis time was 395.54±50.20 as compared to the control which is 138.85±18.37.

Other hematological and immunological changes have been reported to occur in malaria infection such as increase tumor necrosis factor alpha, high frequency of thrombocytopenia, anemia lymphopenia and monocytosis (Abdus, 1997).

CONCLUSION

The results of this study established that malaria infections showed hypofibrinolysis and increase in the severity of the malaria infection increased hypofibrinolytic condition of the subjects. It was equally ascertained that malaria infection contributes to thromboembolic process in any infected individual. This necessitates the need for scrupulous prevention and prompt treatment of malaria in infected patients.

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