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Original Research Article

Study of Serum Acid Phosphatase Levels in Children with Malaria

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ABSTRACT

Objective and Study Design: This was a cross-sectional study aimed to evaluate the significance of the serum acid phosphatase (ACP) activity along with Haemoglobin and other biochemical and haematological activity in patients with malaria and non malarial normal healthy control children.
Material and Methods: This study was conducted at Smt. Kashibai Navale Medical College and General Hospital, Pune (Maharashtra), India. A total of 60 children in the age group of six months to twelve years were included in the study. They were divided into two groups. The study was carried out during the period between September 2012 and February 2013. Venous blood samples were collected from all participants. The whole blood and sera were analyzed for various haematological and biochemical parameters respectively. The statistical analysis was done by EPI Information statistical package.
Results: ACP was significantly increased (P=0.037) and Hb level (P= 0.027) was significantly decreased in malarial group as compared with the controls. There is a negative correlation (r=-.035) between ACP and Hb in malarial patients, which is statistically significant. The difference in the levels of haematological parameters namely TLC, Platelet count, MCV, MCH, MCHC. The levels of biochemical parameters namely Total Bilirubin, Unconjugated Bilirubin and Conjugated Bilirubin were statistically not significant. Similarly the mean age and gender was statistically not significant.
Conclusion: These findings suggest that the measurement of ACP can be used as a marker for malaria.

Keywords: Malaria, Haemoglobin, Acid Phosphatase.

INTRODUCTION

Malaria is a protozoal disease caused by infection with parasites of genus Plasmodium and transmitted to man by certain species of infected female anopheline mosquitoes, which inoculates plasmodium sporozoites from the salivary gland into the human host during a blood meal.[1] It is the most important of the parasitic diseases of humans, with

transmission in 103 countries affecting greater than one billion people and causing between 1-3 million deaths each year .[2]

Malaria continues to pose a major public health threat in India (World Malaria Report 2008).[3] Four species of genus plasmodium cause nearly all malarial infections in humans. These are *P .falciparum, P. vivax, P. ovale, P. malariae*. Almost all deaths are caused by P falciparum malaria.

The clinical features of the disease include the primarily fever; it is marked by paraoxyms which corresponds to the development of the parasites in the red blood cells. The complications of P falciparum malaria are cerebral malaria, Acute renal failure, liver damage, GI symptoms, dehydration, collapse, anaemia, blackwater fever etc. The diagnosis of malaria depends on the demonstration of the parasite in the blood. The clinical picture of malaria in children in endemic zones may often be atypical and a high degree of suspicion for malaria is required. In tropical country like India we need to have a simple reliable and objective marker for malaria, hence this study on ACP was planned.

Acid phosphatase (EC 3.1.3.2), is a hydrolytic lysosomal phosphatase,enzyme.[4] The measurement of acid phosphatase, in serum could be potentially used as a biomarker of malaria infection.[5]

The malarial parasite uses host erythrocyte hemoglobin as a major nutrient source. During the intraerythrocytic phase of its life cycle, the malarial parasite matures within a cell in which hemoglobin is the single major cytosolic protein. While in the trophozoite stage, the parasite avidly ingests and degrades host erythrocyte Hemoglobin.

Hemoglobin degradation is a massive catabolic process that is essential for the intraerythrocytic development of malaria parasites which is unique of the organelle and this is responsible for degradation and breakdown of cell membrane of RBC resulting in anaemia.[6] It has been reported that red blood cells contain an excess quantity of ACP, when the cell membrane of the exhausted corpuscle bursts, merozoites, toxic products and the enzymes like ACP are released into the blood plasma.

The aim of this study was to find any association between the ACP and Hb levels, and can ACP be used as a marker for estimation of Malaria in children and also to study various biochemical and haematological parameters that could provide credential clues in understanding malaria pathogenesis, diagnosis and management.

MATERIALS AND METHODS

This study was conducted at Smt. Kashibai Navale Medical College and General Hospital, Pune (Maharashtra), India sixty children were included in the study. They were divided into two groups from the age 6 months to 12 years. Group I (cases) consisted of patients who were positive for malarial parasites, diagnosed by positive test on blood smear due to any of the four species of the genus plasmodium. Group II(controls) consisted of healthy age and sex matched subjects.

The entire experimental protocol was approved by institutional ethics committee and informed consent of the parent was taken. Utmost care was taken during the experimental procedure according to the Helsinki Declaration of 1964.

A total of four ml venous blood was collected by venipuncture and two ml was used in dried EDTA containers for hematological tests(Hb,TLC, Platelet count, MCV, MCH,MCHC) and another two ml was used for biochemical investigations (ACP, Total, Direct and Unconjugated bilirubin) All the estimations were done by standard procedures.

All biochemical parameters were measured on fully automatic analyzer EM-360 (Transasia) on the same day of sample collection. Serum Bilirubin was estimated by Walter and Gerade method using diazotized sulfanilic acid.[7] Serum ACP was estimated by the kinetic method using disodium phenyl phosphate.[8] All the hematological parameters were measured by using fully automated Hematology analyzer Sysmax KX-21.

RESULTS

The mean age of patients was 5.69 years as compared with the controls which was 6.94 years and the difference was statistically not significant Gender difference was also statistically not significant which included 58 percent males and 42 percent females.

The difference in the levels of Haematological parameters namely Total Leucocyte Count (TLC), Platelet count, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC).The levels of biochemical parameters namely Total Bilirubin, Unconjugated Bilirubin and Conjugated Bilirubin were statistically not significant as shown in Table no 1 and 2 respectively.

Table no 1 - Haematological Parameters in Cases and Controls.

Variables	Cases (mean±SD)	Controls (mean±SD)	p-value	t-value
Hb	9.9 ± 2.04	11.0 ± 1.5	0.027	2.283
TLC	9600 ± 3278.3	9006 ± 3322.2	0.5143	0.6567
PLT	3.53 ± 1.15	3.62 ± 0.97	0.7563	0.312
MCV	76 ± 7.19	74.64 ± 8.09	0.5221	0.645
MCH	23.79 ± 3.75	22.64 ± 3.55	0.254	1.154
MCHC	30.83 ± 2.37	29.87 ± 2.06	0.1175	1.591

Table 2- Biochemical Parameters in Cases and Controls:

Variables	Cases (mean±SD)	Controls (mean±SD)	p-value	t-value
ACP	5.45 ± 2.04	4.2 ± 2.2	0.037	2.142
T BIL	1.48 ± 0.88	1.53 ± 0.809	0.829	0.217
D BIL	0.86 ± 0.29	0.93 ± 0.31	0.40	0.8483
I BIL	0.49 ± 0.23	0.5 ± 0.23	0.875	0.159

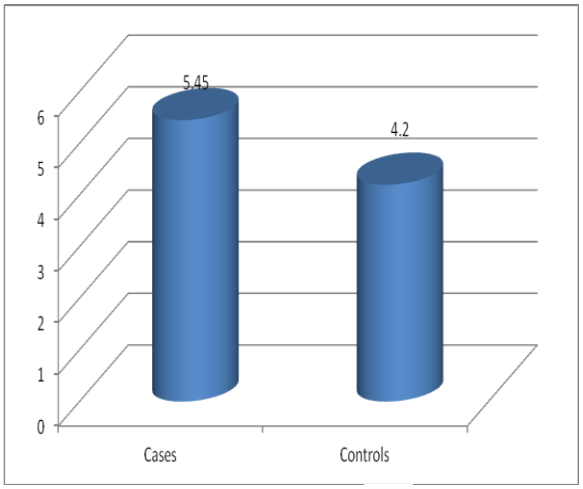
The Hb content and the serum levels of ACP in malaria patients and normal controls are given in Table no. 1 and 2 respectively. The serum ACP levels are significantly increased in malaria patients when it's compared with the non malaria group (P=0.037) There is also statistical difference in the levels of mean Hb levels in cases compared with control group. There are no significant differences in case of other haematological and biochemical parameters. There is a negative correlation between ACP and Hb in malaria patients. (r = - 0.35)

DISCUSSION

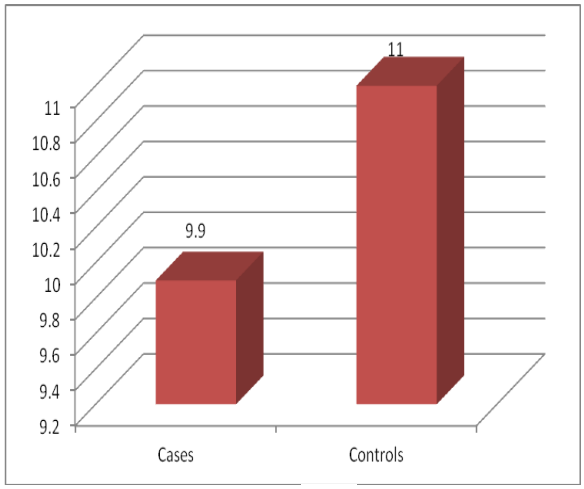
The present study shows that there is inverse relation between ACP levels and Hb levels this is due to the fact that during the intraerythrocytic phase of its life cycle, the malaria parasite matures within a cell in which hemoglobin is the single major cytosolic protein. While in the trophozoite stage, the parasite avidly ingests and degrades host erythrocyte haemoglobin. Parasite possess an efficient and probably highly specific pathway for hemoglobin proteolysis. Parasites obtain many of the nutrients they need directly from their host cell. The absence of typical lysosomal phosphatase and glycosidases indicates that the digestive vacuoles of P. falciparum are specialized organelles that have evolved with the primary purpose of degrading hemoglobin.

There are many studies which indicate that precise haematological changes may vary with category of malaria with the background of haemoglobinopathy, nutritional status, demographic factors and malaria immunity. Many studies have shown that anaemia is associated with malaria infection and mostly of normocytic normochromic type. The pathogenesis of anaemia in malaria is particularly complex and incompletely understood. It is thought to

result from a combination of hemolysis of parasitized red blood cells; accelerated removal of both parasitized and innocently un-parasitized red blood cell, depressed as well as ineffective erythropoiesis with dyserythropoietic changes and anemia of chronic disease. Other factors causative to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and/or pooling, so they have an increased rate of clearance from the circulation. Tumour necrosis factor alpha (TNF-α) has also been implicated and may cause ineffective erythropoiesis. Nonrmocytic normochromic pattern was observed as the predominant type of anaemia and it correlate with the degree of parasitemia.[9]



Graph 1:- Mean Levels of Serum ACP(U/L) in Cases and Controls.



Graph 2 :- Mean Levels of Hb(g/dL) in Cases and Controls.

The present study shows that increased ACP activity observed in malaria patients was statistically significant as compared with the controls. The malaria parasite itself generates large quantities of Reactive oxygen species (ROS) and also through its interaction with phagocytic cell system. Some of these radicals attack the plasma membranes and haemoglobin. The ROS generated in the host parasite interactions can cause several biochemical changes like lysis of erythrocytes.[10]The alterations in the major antioxidants of the erythrocytes and the peroxide lysis of the erythrocytes may result in release of enzyme like ACP. Our results indicate that there is an increased level of ACP in malaria patients where as the levels of Hb are decreased, this is due to lysis of RBC by the host parasitic interactions and increased oxidative stress.

Since the parasite has a limited capacity to synthesize amino acids de novo or to take them up exogenously, the Hb is thought to be broken down to provide amino acids for its growth and maturation. Thus increase in serum ACP levels in malaria patients could serve as a marker for hemolysis indicating the active stage of the disease, which may be used as an additional investigation in the diagnosis of malaria. Levels of ACP are also indicative of the severity of the disease. The negative correlation between ACP and Hb in malaria patients also confirms this finding.[11]

CONCLUSION

Thus our results of increased levels of serum ACP in malarial patients could be

used as a marker for haemolysis & anemia. Since very few studies are done on Paediatric age group, there is a further need to study to use this enzyme along with its isoenzyme as a marker in malaria in addition to the routine test involved.

***Limitation of the study**:* This study was taken in malarial patients. We did not divide malaria into its different subgroups. We plan to undertake further study considering sub groups of malaria and finding any association between ACP and Hb.

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