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Original Research Paper

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ELEVATED LEVELS OF ACID PHOSPHATASE IN PATIENTS OF MALARIA WITH RESPECT TO TYPES OF MALARIA AND PARASITIC INDEX

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ABSTRACT

Malaria is one of the most prevalent parasitic disease. Acid phosphatase enzyme releases during erythrocytic phase of life cycle of malarial parasite. Acid phosphatase can act as a marker for Malaria as well as marker to assess severity of hemolysis in cases of Malaria.

Objectives: To estimate serum Acid phosphatase levels and haemoglobin levels in patients of Malaria and control subjects.

Material and methods: Collected 5 ml Blood from healthy individuals and patients of Malaria was divided in plane vacutainer and EDTA vacutainer for serum ACP and haemoglobin respectively.Blood Haemoglobin levels were analysed by Drapkin's Method. Serum Acid phosphatase levels were analyzed by Teco diagnostic kit.

Result: Acid phosphatase levels were significantly higher in study group (5.9 ± 1.4 U/L) compared to control group($p<0.001$). Negative correlation obtained between ACP and blood Hb, $r = -0.692$.

Conclusion: Enzyme ACP can be used as an Additional diagnostic marker in Malaria to the routine tests.

KEYWORDS : Acid phosphatase (ACP),Haemoglobin,Malaria,hemolysis

INTRODUCTION-

Malaria is major health problem in many tropical and sub-tropical countries. [1] Genus *Plasmodium* considered to be one of the main killers of man in malaria endemic foci. [2] It imposes great socio-economic burden on humanity and accounts for 85% of Global infectious disease burden. [3] According to survey of National Institute of Malaria research about 36% of the world population, i.e. 2020 million is exposed to the risk of contracting malaria in approximately 90 countries. [3] World Health Organization estimates are around 300–500 million malaria cases annually. [4]

There are five species of genus *Plasmodium* namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. [4] Amongst all these species, infection from *P.vivax*, *P. falciparum* and mixed malaria(co-infection of *P.vivax* and *P.falciparum*) is common. [4] Malaria is characterized by fever, chills and rigors. [5]

The parasite *Plasmodium* requires two host, mosquito and man. [6] The infected mosquito during its blood meal introduces sporozoites from its saliva into the person's circulatory system through proboscis which pierces the skin. In blood these sporozoites travel to the liver where it undergoes asexual division (Exoerythrocytic Schizogony) and releases merozoites in the blood which invade RBCs. In RBCs it gets further divided in Tropozoites, Schizonts and Merozoites. Finally RBC ruptures and releases merozoites which further invade fresh RBC. Some merozoites within red cell form gametocytes. The host carrying gametocytes is known as carrier. These gametocytes are taken up by mosquito during its blood meal and the cycle continues. [6] Thus the red cell plays a central role in growth and propagation of malarial parasite. [1] After formation of the six signet ring stage in RBCs, cell membrane of totally exhausted corpuscle bursts and merozoites, toxic products and enzymes like Acid Phosphatase are released in the blood plasma. [7,8]

Acid phosphatases(ACP) (EC 3.1.3.2) enzyme is present in the prostate and a variety of tissues like the liver, spleen, erythrocyte etc. Erythrocytic acid phosphatase belongs to a class of cytosolic low molecular weight (cLMW') acid phosphatase, with molecular weight of 35kDa. Its gene is located on chromosome 2. It has optimum pH between 4 to 5.5.This isoenzyme is tartrate and fluoride resistant, and highly sensitive to thiol reagents. [9] ACP is used as diagnostic marker in various diseases like prostate cancer, Paget's disease, haemolytic anemia, prostatitis, thrombophlebitis, Gaucher's disease, hyperparathyroidism etc [14] Human acid phosphatase (ACP) has a considerable impact as a tool of clinical investigation and interventions,But very little is known about the levels of ACP in infectious diseases like malaria. [7]

Anaemia is a common manifestation in all types of malaria. [6] During intraerythrocytic phase of lifecycle, Plasmodium uses haemoglobin as a major source of nutrition. Therefore haemoglobin levels significantly decrease in patients of malaria leading to anaemia. [7]

Peripheral blood smear has been and will remain the gold standard method for diagnosis of Malaria but the method is subjective and error prone. [10] In many remote and poor parts of India and places where malaria is endemic, facilities for malaria microscopy are either non-existent or inaccessible. Microscopes, stains, power sources or technicians are unavailable either in toto or in parts. So diagnosing malaria in such areas is indeed a challenge. [6] Other techniques like RDTs cannot be used for diagnosis of malaria as it lack reliability. [10]

Purpose of the study is to evaluate acid phosphatase levels in patients of malaria and to check whether it is possible to use it as an additional diagnostic marker for malaria and to assess haemoglobin levels in patients of Malaria and control subjects to find their co-relation.

MATERIAL AND METHOD

This is prospective, case control study conducted in Department of Biochemistry, MGM Medical College, Navi Mumbai.The aim of study is to estimate Acid phosphatase levels and Haemoglobin levels in Patients of Malaria and healthy individuals.Ethical clearance was taken from scientific and ethical committee of the institution.The

study was conducted as per ICH-GCP guidelines for human research.Written and informed consent was obtained from the patients before enrollment of subjects in this study.

Total 50 patients of Malaria between the age group of 25 -30 years and 30 healthy individuals after age and gender match were enrolled.Diagnosis of Malaria is confirmed by peripheral blood smear.Patients are categorized according to type of parasites and Parasitemia. Patients beyond study group age,who are on anti-malarial treatment,Hyperparathyroidism Osteoporosis,Benign Prostate Hyperplasia, Prostatic carcinoma, Hemolytic Jaundice and coagulation disorder, Diabetes Mellitus, known / suspected pregnancy were excluded.

5 ml of blood was collected from each subject by venipuncture with standard blood collection technique.Collected blood was divided in 2 vials,3 ml in plane vacutainer and 2 ml in EDTA vacutainer.Plane vacutainer was centrifuged for 15 mins at speed of 2500.Serum was separated for estimation of Acid phosphatase and blood in EDTA vacutainer was used for estimation of haemoglobin.Concentration of acid phosphatase was estimated spectrophotometrically by kinetic method using Teco diagnostic kit and haemoglobin levels were analysed by Drapkin's solution.Concentration of both parameters were compared and co-related statistically.

RESULT

Data reported were statistically analyzed by R-software which is freely available online.

Table I : Mean and SD of Acid Phosphatase levels in control and study group.

Groups	Acid phosphatase (U/L)	P value
Control group	2.01± 0.5	< 0.001**
Study group	5.9 ± 1.4	
**: Significant at 1% level of significance		

Figure I : Mean and SD of Acid Phosphatase levels in control and study Group

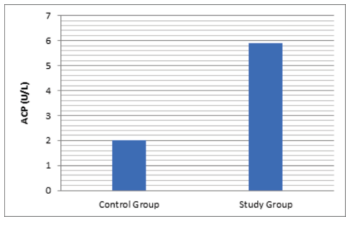


Table II :Mean and SD of ACP levels in different type of Malaria

Type of malaria	ACP levels
P.vivax	4.9 ± 0.66
P.falciparum	5.7 ± 0.64
mixed malaria	7.5 ± 1.18

Figure II: Mean and SD of ACP levels in different type of Malaria

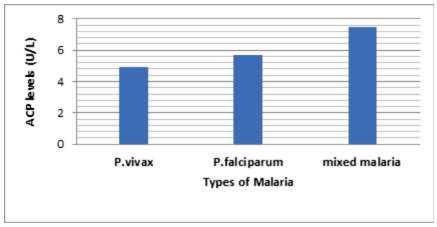


Table III : Mean and SD of ACP levels according to Parasitic Index in Malaria

Parasitic Index	ACP
1+	3.7 ± 0.15
2+	5.2 ± 0.53
3+	5.8 ± 0.73
4+	7.75 ± 1.11

Figure III: Mean and SD of ACP levels according to Parasitic Index in Malaria

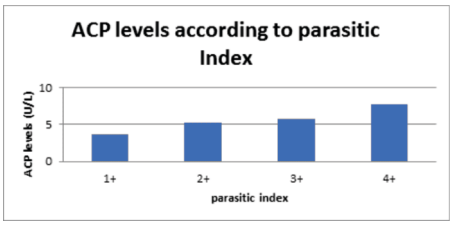


Table IV : Mean and SD of whole blood Haemoglobin levels in control and study group.

Groups	Haemoglobin (gm/dl)	P value
Control group	12.5 ± 0.9	<0.001**
Study group	9.8 ± 1.4	

**: Significant at 1% level of significance

Figure IV: Mean and SD of whole blood Haemoglobin levels in control and study Group

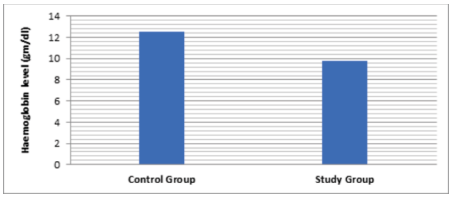
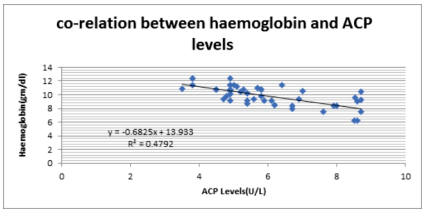


Table V: co-relation of ACP and whole blood Haemoglobin in study group

	ACP
ACP	1
Haemoglobin	**-0.692****

** Significant at 1% Level of Significance

Figure V : co-relation of ACP and whole blood Haemoglobin in study group



DISCUSSION

Malaria remains one of the most prevalent parasitic disease affecting human races in tropical and subtropical regions of the world.[8] According to the World Health Organization (WHO) report 2014, of all malaria cases in the world, 90% were occurring in Africa with 75% of global P. *falciparum* malaria cases, from which 80% mortality was documented. [11] According to World Malaria report 2018, 9.5 million cases of Malaria were registered in India.[12]Drug and insecticide resistance have aggravated the complexity of Malaria problem. [1]

In this study, utility of Acid phosphatase as additional diagnostic marker for malaria was assessed.Also levels of haemoglobin was estimated to assess degree of hemolysis.

During the study,cases of *P.vivax* , *P.falciparum* and Mixed Malaria was observed. Out of 50 patients, 24 patients were having infection of P. *vivax*, 10 patients of *P.falciparum* and 16 of Mixed Malaria. Patients were also assessed for parasitic index, indicated as 1+,2+,3+ and 4+ depending on parasitemia visible on blood smear.

We estimated levels of Acid phosphatase in diagnosed cases of malaria for assessment of its clinical utility as a diagnostic marker.

Table I shows Mean and SD of ACP levels in control and study group. Significant increase in ACP levels was observed in study group with mean of 5.9 ± 1.4 U/L compared to control group, 2.01± 0.5U/L with p ≤ 0.001.(Figure I)

Acid phosphatase levels were compared in different types of Malaria and according to parasitic index. Table II shows highest levels of ACP in patients of Mixed Malaria with mean 7.5 ± 1.18 U/L followed by *P.falciparum* (5.7 ± 0.64 U/L).*P.vivax* had mean 4.9 ± 0.66 U/L, lowest as compared to other types (figure II). It was suggested from table 3,levels of ACP were compared according to parasitic index, In Parasitic index 4+ and 3+ levels were of mean 7.75 ± 1.11 U/L and 5.8 ± 0.73 U/L respectively. Levels of ACP in patients with parasitic index 1+ and 2+ were 3.7 ± 0.15 U/L and 5.2 ± 0.53 U/L respectively. (Figure III)

Acid phosphatase (EC 3.1.3.2), is a hydrolytic lysosomal phosphatase enzyme of low specificity which hydrolyses phosphoric acid esters. The enzyme occurs in the prostate and variety of tissues like the liver, spleen, erythrocyte etc It has been reported that red blood cells contain an excess quantity of ACP. [7] In Malaria, the red cell membrane plays a central role in the growth and propagation of the malarial parasite. [1,8] During intra-erythrocytic stage of parasite, the cell membrane of the exhausted corpuscle bursts and releases merozoites, toxic products and the enzymes like ACP into the blood plasma. [7]

It was found that ACP levels were increasing depending upon degree of parasitemia. Higher levels were observed in cases of Mixed and *P.falciparum* due to greater extent of hemolysis associated with *P.falciparum*. [13]

Our findings of ACP in study group were consistent with the clinical studies done by D'Souza et al [2011], Pratinidhi et al [2013] which showed increased levels of Acid Phosphatase in patients of malaria.This study of D'Souza et al proposed that 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.[7]

Pratinidhi et al also found increased levels of ACP in patients of Malaria in children between age group of 6 months to 12 years. They proposed that the malaria parasite itself generates large quantities of Reactive oxygen species (ROS) 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. [14,15] The alterations in the major antioxidants of the erythrocytes and the peroxide lysis of the erythrocytes may result in release of enzyme like ACP. [14]

Our result was also in accordance with study of Garba et al which also showed increased levels of Acid Phosphatase in patients with *P.falciparum* Malaria. [16]

Table IV shows levels of whole blood Haemoglobin in control and study group. Control group had normal Haemoglobin levels with mean 12.5 ± 0.9 g/dl. On the other hand haemoglobin levels were found decreased with mean of 9.8 ± 1.4 g/dl in patients of Malaria (Figure IV).When the whole blood haemoglobin levels between study and control were compared, there was statistically significant decrease in study group (p value <0.001) is observed. Similar results were found by D'Souza et al [2011], Pratinidhi et al [2013] and Haldar et al [2009].

During the intraerythrocytic phase of life cycle, the malaria parasite avidly ingests and degrades the host erythrocyte's haemoglobin which is single major cytosolic protein. Parasite possesses an efficient and probably highly specific pathway for hemoglobin proteolysis. Parasites obtain many of the nutrients they need directly from their host cell. [7] Haldar and Mohandas explained role of IL6 induced hepcidin expression, which is a master regulator of iron trafficking, leading to decreased iron availability for erythropoiesis. TGF inhibits erythroblast proliferation. TNFα induces cleavage of major erythroid transcription factor, GATA-1. Interferon (IFN)-γ induces macrophage production of TNF-related apoptosis-inducing ligand (TRAIL). This TRAIL inhibits erythroblast differentiation, contributing to development of Anaemia. [17]

The levels of acid phosphatase were co-related with haemoglobin. It shows negative co-relation with co-relation coefficient r = -0.692 (figure V).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. [7,14]

CONCLUSION

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.

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