Acid Phosphatase as a Marker in Malaria

Benedicta D'Souza, Rajeevalochana Parthasarathy, Sreekantha & Vivian D'Souza

Indian Journal of Clinical Biochemistry

ISSN 0970-1915
Volume 26
Number 4

Ind J Clin Biochem (2011) 26:396-399
DOI 10.1007/s12291-011-0137-z

Springer

Ind J Clin Biochem (Oct-Dec 2011) 26(4):396–399
DOI 10.1007/s12291-011-0137-z

ORIGINAL ARTICLE

Acid Phosphatase as a Marker in Malaria

Benedicta D'Souza · Rajeevalochana Parthasarathy · Sreekantha · Vivian D'Souza

Received: 16 July 2010 / Accepted: 17 April 2011 / Published online: 24 June 2011

Abstract The serum acid phosphatase (ACP) activity and Hemoglobin (Hb) levels were measured in malaria patients and nonmalarial fever patients. The results were compared with normal healthy control subjects. ACP was significantly increased ($P < 0.001$) in all the malaria patients. ACP was significantly higher in *Plasmodium falcifarum* malaria and mixed malaria when compared to *Plasmodium vivax* malaria. Hb levels were significantly decreased in all the malaria patients which indicates that malaria parasite uses host erythrocyte Hb as a major nutrient source. There is negative correlation($r = -0.478$) between ACP and Hb in malaria patients, which is highly significant. These results suggest that the measurement of ACP could be used as a marker for malaria.

Keywords Acid phosphatase · Malaria · Hemoglobin

Introduction

Acid phosphatases are a family of enzymes that are widespread in nature and can be found in many animal and plant species [1, 2]. Mystery surrounds the precise functional role of these molecular facilitators, despite much research. Yet paradoxically human acid phosphatase (ACP) have a considerable impact as tools of clinical investigation and intervention. Its levels are increased in various diseases like prostate cancer that has spread to the prostate gland and to the bone, Paget's disease, hemolytic anemia, prostatitis, thrombophlebitis, Gaucher's disease, hyperparathyroidism etc. which helps in their diagnosis.

This enzyme is a phosphatase of low specificity. It hydrolyses phosphoric acid esters. The optimum pH is between 4 and 5.5. This enzyme occurs in the prostate and a variety of tissues like the liver, spleen, erythrocyte etc. isoenzymes of ACP are described.

Erythrocytic ACP gene is located on chromosome 2, osteoclast ACP is on chromosome 19 and prostatic ACP gene is on chromosome 13. The prostatic isoenzyme is inactivated by tartaric acid and cupric ions inhibit the erythrocytic ACP [3, 4]. The total value of ACP is increased in prostatic carcinoma and bone metastasis.

Little is known about the levels of ACP in infectious diseases like malaria. Malaria is caused by the protozoan plasmodium species. Four species of the genus *Plasmodium* infects humans. They are *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these *P. Vivax* and *P. falciparum* account for more than 95% of the cases of malaria.

Human infection begins when a female anopheline mosquito inoculates the sporozoites from its salivary glands during a blood meal. Hence the parasite completes its life cycle in two hosts- man and female anapheline mosquito [5, 6]. Malaria is a major cause of mortality in developing countries. It is endemic in Mangalore. The aim of this study is to evaluate the levels of acid phosphatase in malaria and to check its possible use as a marker enzyme in its detection and prognosis.

Electronic supplementary material The online version of this article (doi:10.1007/s12291-011-0137-z) contains supplementary material, which is available to authorized users.

B. D'Souza (✉) · Sreekantha · V. D'Souza
Department of Biochemistry, Manipal University,
Centre for Basic Sciences, Kasturba Medical College, Bejai,
Mangalore 575004, India
e-mail: benedicta_7@yahoo.com

R. Parthasarathy
Department of Medicine, Manipal University,
Kasturba Medical College, Manipal, India

Materials and Methods

The study group consisted of 62 subjects of the age group 20 to 50 years. These subjects were divided into 3 groups. Group 1 included 25 malaria patients. Of these 7 patients had *P. falciparum* malaria, 11 patients had *P. vivax* malaria and 7 patients had mixed malaria, that is having both *P. falciparum* and *P. vivax* malaria. These patients attended the OPD at the Wenlock District hospital, Mangalore and Padmavathi Hospital, Mangalore. The groups selected had no prostate problems, previous anemia or any kind of bone disorders. The patients presented symptoms of intermittent high fever, chills, rigors, vomiting and headache. They also presented with hepatomegaly and spleenomegaly. Group 2 included 12 nonmalarial fever patients and group 3 included 25 healthy individuals of both the sexes of the same age groups. A finger prick sample was taken to prepare thick and thin blood films to determine the presence or absence of the malarial parasite.

5 ml of venous blood was collected randomly in EDTA bottles from malaria patients and normal healthy subjects. It was centrifuged for 10 min. The plasma was collected taking care to avoid hemolysis and was used for the estimation of the ACP level. Estimation of ACP was done by kit method using Teco Diagnostics kit-Acid phosphatase reagent set [7, 8]. The α-naphthol released from the substrate α-naphthyl phosphate by acid phosphatase is coupled with fast red TR to produce a colored complex which absorbs light at 405 nm. The reaction can be quantified photometrically because the coupling reaction is instantaneous. L-tartarate inhibits prostatic acid phosphatase but does not interfere with the reaction mechanism.

The hemoglobin (Hb) content of erythrocytes was determined by the Cyanmethaemoglobin method [8].

The statistical analysis was done using the Mann–Whitney's *U* test. *P*-value < 0.05 is considered to be significant. Correlations between the parameters were estimated by spearman's rank correlations.

Results

The serum levels of ACP and Hb content in malaria patients, nonmalarial fever patients and normal control subjects is given in Table 1. The serum ACP levels are highly increased in malaria patients when compared to nonmalarial fever patients and the controls. The increase in ACP is greatest in malaria falciparum and mixed malaria when compared to malaria vivax. The Hb content is significantly decreased in malaria patients when compared to the control subjects.

There is no significant difference in the rise in the ACP levels in the males and females suffering from malaria. There is negative correlation between ACP and Hb in malaria patients($r = -0.478$) which is statistically highly significant ($P = 0.016$) (Table 2).

Discussion

The present study shows that increased ACP activity observed in malaria patients was statistically highly significant. The ACP levels were significantly higher in malaria falciparum and mixed malaria when compared to malaria vivax.

It has been reported that the red blood cells contain an excess quantity of ACP. The cell membrane plays a central role in the growth and propagation of the malarial parasite in the blood. On one hand, it carries the parasite specific receptor sites on its surface, while on the other, it allows the parasite to derive from the host blood plasma the nutrients essential for the intracellular parasite development and growth [8, 9].

The invasion of the human erythrocytes by the malarial parasite is during the phase of erythrocytic scizogony. The RBC's are attacked by the pre erythrocytic cryptomerozoites or the later exo erythrocytic micro-meta crypto merozoites. Each merozoite buries itself in the RBC's and gradually increase in size to produce at least six signet ring

Table 1 Serum levels of ACP and Hb in malaria patients, nonmalarial fever patients and control subjects

	Control (25)	*P.falci* [7]	*P.vivax* [11]	Mixed [7]	Nonmalarial [12]
Age	30.06 ± 0.074	36.86 ± 9.173	39.18 ± 12.015	40.00 ± 6.928	33.82 ± 14.176
ACP	2.29 ± 0.658	6.65 ± 0.393*,#	5.96 ± 0.933*,#	7.77 ± 0.602*,#	3.36 ± 0.381
Hb	12.59 ± 1.43	10.17 ± 2.18*	10.59 ± 1.855*	10.06 ± 1.165*	11.26 ± 0.73[a]

Statistical comparison among the groups was carried out by Mann–Whitney "*U*" test

* $P < 0.001$ vhs (Compared with control very highly significant)

\# $P < 0.001$ vhs (Compared with nonmalarial fever group very highly significant)

[a] *NS* Non significant compared with malaria

Table 2 Correlations

GROUP	TYPE		Hb
	Vivax ACP	*r*	−0.824
		P	0.002 hs
		N	11
	Falciparum ACP	*r*	−0.692
		P	0.085
		N	7
	Mixed ACP	*r*	0.302
		P	0.574
		N	7
	Total ACP	*r*	−0.478
		P	0.016 Sig
		N	25
	Fever ACP	*r*	−0.516
		P	0.008
		N	12
	Control ACP	*r*	0.152
		P	0.574
		N	25

Correlations between the values were estimated by spearmann's correlation coefficient

stages. It is during this stage that hemozoin pigments are seen. After sometime the cell membrane of the totally exhausted corpuscle bursts and the merozoites, toxic products and the enzymes like ACP are released into the blood plasma [10].

Malaria even today is a major health problem in many tropical and sub tropical countries. The genus *Plasmodium* is considered to be one of the main killers of man in malaria endemic foci. Invasion of the human erythrocytes by the malarial parasite is accompanied by a variety of biological responses in the human host and in order to survive, the plasmodium parasite brings about considerable metabolic changes in the host cell. The host cells may then become more vulnerable to damage due to toxic metabolites derived from both the host and the parasite through the basic pathophysiology involved in the RBC destruction [11–13].

A number of biological factors are involved during the reactive interaction. These include the reactive oxygen species generated in the major host parasite interactions. Increase in ROS and decrease in antioxidants has been reported in malaria patients [14–16]. The alterations in the major antioxidants of the erythrocytes and the peroxide lysis of the erythrocytes may result in release of enzyme like ACP [17]. Our results indicate that ACP levels are very highly increased in malaria patients where as Hb levels are decreased. This may be due to hemolysis caused by host parasite interactions and increased oxidative stress. The malaria parasite uses host erythrocyte Hb as a major nutrient source. Since the parasite has a limited capacity to synthesize amino acids denovo or to take them up exogenously, the Hb is thought to be broken down to provide amino acids for its growth and maturation [10, 14].

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.

The higher levels seen in the case of malaria falciparum and mixed malaria indicates that the extent of hemolysis is the greatest in these cases and therefore there is a corresponding increase in the ACP levels. Therefore it is also indicative of the severity of the disease and *P. falciparum.* The negative correlation between ACP and Hb in malaria patients also confirms this finding.

There is no significant difference in the rise in ACP levels in males and females.

Finally, to conclude, there seems to be a significant increase in the serum ACP levels in malaria patients as shown in the present work. There is a need for further study to use this enzyme as a marker in malaria in addition to the routine tests involved.

References

1. George YS, Lois MJ, Hary LR. Th estimation of serum inorganic phosphate in acid and alkaline phosphatase activity. J Biol Chem. 1942;142:921–33.
2. Dissing J. Immunochemical characterization of human red cell acid phosphatase isoenzymes. Biochem Genet. 1987;25:11–2.
3. Vasudevan DM, Sreekumari S. Text book of biochemistry. 3rd ed ed. New Delhi: Jaypee Brothers Medical Publishers (P) Limited; 2001. p. 50–2.
4. Palmarino R, Agostino R, Zgloria F, Lucarelli P, Businco L, Antognoni G, Maggioni G, Workman PL, Bottini E. Red cell acid phosphatase: another polymorphism correlated with malaria. Am J Phys Anthrpol. 2005;43(2):177–85.
5. White NJ. The pathophysiology of malaria. Adv Parasitol. 1992; 31:34–173.
6. Bruce-Chwatt LJ. History of malaria from prehistory to eradication. Malaria, principles and practices of malariology. Edinburgh: Churchill Livingstone; 1988. p. 1–59.
7. Bergmeyer HV. Methods of enzymatic analysis. Weinheiam: Verlag Chemie; 1984. p. 92.
8. Varley H. Practical Clinical Biochemistry. 5th ed. In: Determination of serum acid phosphatase. London: William Heinemann Medical Books Ltd; 1980. p 913–915.
9. Anderson HR, Nielsen JB, Nielsen F, Philippe G. Antioxidative enzyme activities in human erythrocytes. Clin Chem. 1997;43(4): 562–8.
10. Gupta CM. Red cell membrane alterations in malaria. Ind J Biochem Biophys. 1988;25:20–4.
11. Prassannachandra, D'Souza V, D'Souza B. Comparative study on lipid peroxidation and antioxidants vitamins E & C in falciparum and vivax malaria. Ind J Clin Biochem. 2006;21(2):103–6.
12. Goldberg DE, Slater AF, Cerani A, Henderson GB. Hemoglobin degradation in malaria parasite *Plasmodium falciparum*: an

ordered process in a unique organelle. Proc Natl Acad Sci. 1990; 87(8):2931–5.
13. Kremsner PG, Greve B, Lell B, Luckner D, Schmid D. Malarial anemia in African children associated with high oxygen radical production. Lancet. 2000;355:40–1.
14. Bhabhani SD, Nilini KN. Evidence for erythrocyte lipid peroxidation in acute falciparum malaria. Trans R Soc Trop Med Hyg. 1999;93:58–62.
15. Clark IA, Hunt NH. Evidence for reactive oxygen intermediates causing the hemolysis and parasite death in malaria. Infect Immun. 1983;39:1–6.
16. D'Souza B, D'Souza V, Swagatha H, Vijayalaxmi K, Namratha AS. Erythrocyte superoxide dismutase and catalase and their correlation with malondialdehyde in falciparum and vivax malaria. Biomed Res. 2009;20(1):25–7.
17. Garba IH, Gatsing D, Uborn G. Elevated total and isoenzyme forms of acid phosphatase in falciparum malaria. Comput Rendus Biol. 2006;329(2):75–8.