

Plasma Activity of Tartrate Resistant Acid Phosphatase in Acute Malaria Infection

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Abstract

Enzymes are biological molecules that modulate rate of chemical reactions within an optimum temprature and Hydrogen potential without themselves been used up. The activity of tartrate-resistant acid phosphatase E.C .3.1.3.2. Orthophosphoric monoester phosphohydrolase was assayed using the plasma of patient presenting with acute malaria. Objective of this study is to explore an alternative enzymatic approaches for diagnosis of severe malarial parasite infection. Total plasma tartrate-resistant acid phosphatase was assayed according to procedure of (Henry and Steer, 1999). The results obtained showed mean activity of 69.09 ± 1.56 IU, 110.02 ± 0.74 IU and 125.46 ± 0.79 IU, for infected male adults, female adults, and children respectively. These were found to differ significantly ($p > 0.05$) from that of normal adult Males, Females and Children having mean activities of 182.68 ± 0.93 IU, 162.01 ± 0.79 IU and 178.37 ± 1.03 IU respectively. The research work revealed plasma activities of tartrate-resistant acid phosphatase for infected males, females and children, this means that tartrate resistance acid Phosphatase may possibly serves as an index in diagnosis of malaria parasite infection and its severity.

Keywords

Tartarate, Phosphatase, Malaria, Damaturu, Plasmodium, Marker Enzyme

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1. Introduction

Critical health issues and development (HIV/AIDS, Malaria, etc.) is item (v) of what Millennium Development Goals (MDGs) seek to address. Malaria being a parasitic disease; it is by far the world's most important tropical disease and kills more people than any other communicable diseases such as HIV/AIDS and tuberculosis. It has continued to be the major source of morbidity and mortality in many developing countries especially in Africa.

Since 2002, rich countries have poured more than \$10 billion into malaria control. The money has help pay for planeloads of bed nets treated with insecticides, hundred of million of dosea of a powerful combination therapy, widespread indoor spraying of homes, and prophylactic treatment of pregnant women, an especially vulnerable group. The generous, large-

scale programs have saved the lives of hundreds of thousands of people, most of them African children (Jon, 2014).

The most important human parasite among the sporozoa is Plasmodium, the causative agent of malaria, which is of four different species: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Among these, *Plasmodium falciparum* accounts for the majority of infections and is the most deadly. It has estimated that more than one hundred and fifty million (150, 000,000) peoples are infected, and about one million (1, 000,000) peoples dies annually of malarial in Africa alone. About one thousand cases are reported each year in the United States, divided between returning U.S. Citizen and non-U.S. citizen (Prescott, Harley, and Klein, 2002). *Plasmodium falciparum* is the most common in the tropical

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region, and *Plasmodium vivax* is the most common in the temperate zone. (WHO, 1998). The objective of this study is to explore an alternative enzymatic approaches for diagnosis of severe malarial parasite infection.

2. Enzyme Changes in Disease Conditions

Enzymes are biological catalysts that function by speeding up or slow down the rate of chemical reactions taking place within the cells and do not suffer any loss of the changes. The reactants termed substrates. Each enzyme is quite of specific in the activity. Enzymatic activity measurement in plasma and other body fluids had employed for more than thirty years. Changes in enzyme activity have been proven to be the most sensitive in diagnosis, a reflection of the practical implication inherent in the catalytic property of enzymes.

Disease state usually leads to extensive or moderate tissue damage depending on the time of onset of the disease. Such disease conditions usually lead to the release of enzyme specific to the disease organ or tissue into the circulation with a resultant increase in activity of such enzymes in body fluids. However not all organ or tissues-specific damage leads to rising in the activity of the organs or tissue specific enzymes concerned. Some disease conditions have been reported to involve a decrease in organ or tissue specific enzyme activity in the body fluids. (www.freepatentsonline.com/search).

Normal plasma level reflects the balance between the synthesis and release of enzymes during ordinary cell turnover and their catabolism and excretion. The proliferation of cells, an increase in the rate of cell turnover, cell damage or enzyme induction usually results in raised plasma level. These are readily demonstrable because very low concentrations produce easily measured activity *in vitro*. Hence, enzymes can be used as "markers" to detect and localized cell damage or proliferations.

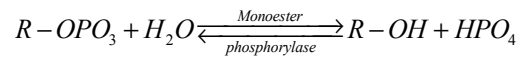
- 1 The rate of release from the cell in which in turn depend on the rate of cell damage
- 2 Or the degree of proliferation or induction of enzyme synthesis.

These two factors balanced against the rate of enzyme excretion and catabolism. (Nelson and Cox, 2005).

3. Phosphatases

Phosphates are a group of enzymes that are distributed throughout most cells and body fluids they belong to the class of hydrolases. That catalyse the hydrolysis of compounds containing Acyl or phosphoryl (phosphatases)

ester bonds and also compounds containing peptides amide, hemiacetal bond. As these bonds split, a simultaneous splitting of an equal molecule of water takes places.



They are characterized by their ability to hydrolyzes a wide variety of monophosphate ester to alcohol and inorganic phosphate. They catalyze the ester of phosphoric acid and are very important in the absorption and metabolism of carbohydrates, nucleotide and phospholipids and the calcification of bone.

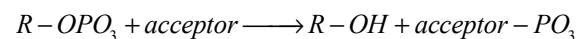
Different groups of phosphomonoesterase of diagnostic importance have been identified which includes:

1. Acid phosphatase
2. Alkaline phosphatase.

The splitting of inorganic phosphate from a phosphorylated substrate is due to the presence of these enzymes (phosphatase) which is located in the cytoplasm of cell i.e. alkaline and acid phosphatases.

4. Acid Phosphatase

Orthophosphoric monoester phosphohydrolase is acid optimum EC. 3.1.3.2. This enzyme promotes the hydrolysis of number of orthophosphate esters, given by this generic reaction.



The R may be one of a number of substrates. Presently there is no clearly define specific physiological substrate for the enzyme (Calibreath, 1992). The enzyme contains tightly bound manganese ion in the ± 2 or ± 3 oxidation state, in the ratio of one manganese ion per enzyme molecule.

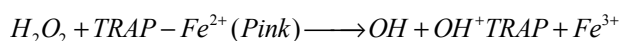
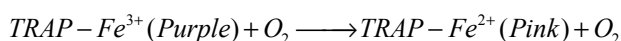
Acid phosphatase is found mostly in the prostate followed by the liver, red blood cells, platelets and bone. The acid phosphatase is family of an enzyme that are widespread in nature. Mystery surrounds the precise functional role of these molecular facilitators despite much research, yet paradoxically human acid phosphatase have a considerable impact as a tool for clinical investigation and intervention. Slight raised acidic phosphatase occasionally found in these osteoblastic bone disease (including Paget's disease) or cholestatic liver disease. In Gaucher and when there is the destruction of platelets and red blood cells in the case of malaria infection (Zilva, Pannal, and Mayne, 1988).

5. Tartrate Resistant Acid Phosphatase (TRAP)

Tartrate resistant acid phosphatase is glycosylated monomeric metalloenzyme found in mammals with a molecular weight of approximately 35KDa, a basic isoelectric point of 7.6 – 9.5, characteristic purple colour, and optimal activated in acid conditions. It is synthesized by as latent proenzyme and activated by proteolytic cleavage and reduction. (Ljusberg, Rylander, and Anderson, 2005). *It differs from other mammalian acid phosphatases by its resistance to inhibition by tartrate.*

The mechanism of phosphate ester hydrolysis by tartrate resistance acid phosphates is through a nucleophilic attack mechanism (Klabunde, 1996). Whereby catalysis occurs with binding of phosphate substrate to Fe^{2+} in the active site of tartrate-resistant acid phosphatase. The reaction is followed by a nucleophilic attack by a hydroxide ligand bond phosphorus atom resulting in cleavage of the phosphate ester bond and production of alcohol. The exact identity and mechanism of the hydroxide is unclear. But is thought to be either a hydroxide that bridges the metal ions with the active site or a terminal hydroxide bound to Fe^{3+} , with conflicting both mechanisms.

Normally (TRAP) is highly expressed by osteoclast, activated macrophages, neurons and the porcine during pregnancy (Burstone, 1959), (Minkin, 1982). There are also pathological conditions whereby (TRAP) is expressed, they include patients with leukaemia (Hairy cell like) gaucher disease, prostatic carcinoma and metabolic bone disease. Reactive oxygen species (ROS) are generated in macrophages and osteoclasts from superoxide form. The action of NADPH-oxide oxygen (O_2). (Darden, 1996) They play an essential role in the functioning of phagocytic cells. Tartrate resistant acid phosphatase is containing a redox active ion, catalyzes the generation of ROS through Fenton's chemistry.



Producing hydroxyl radicals, hydrogen peroxide and singlet oxygen. In osteoclast, reactive oxygen species are generated in ruffled border and seen to require for resorption and degradation to occur.

The exact physiological role(s) of (TRAP) is unknown. But many functions have been attributed to this in knockout mice. Studies were those with the phenotype of tartrate-resistant acid phosphatase - / - showed mild osteopetrosis, while those with great osteoclast activities resulted in thickening and

shortening of the cortices (Hayman, 1996). It has been shown that osteopontin and bone sialopontin, bone matrix phosphoproteins, are high in the *in vitro* tartrate-resistant acid phosphatase substrate which bonds to osteoclast when phosphorylated. Upon partial dephosphorylation, osteopontin and bone sialopontin are incapable of binding to osteoclast. From this effect, it has been hypothesised that tartrate-resistant acid phosphatase secreted from the ruffled border dephosphorylates osteopontins and allow migration and further resorption to occur (Rylander, 1994).

6. Acid Phosphatase Changes in Disease

The major clinical situation for which measurement of acid phosphatase is valuable is prostatic carcinoma, particularly where the turnovers have extended beyond the gland or exist with bone metastases. This condition associated with increased serum acid phosphatase. A significant number of women with malignant neoplasm of the breast show an increase in serum acid phosphatase as do patients with a variety of other types of cancer.

P – nitrophenyl phosphate is the most commonly employed substrate for the assay of acid phosphatase. P – nitrophenol, the product of the reaction of acid phosphates with p-nitrophenyl phosphatase is bright yellow in colour. At the end of the incubation period, the addition of concentrated base stop the reaction and shifts the pH to the alkaline range where the P – nitro phenolate ion has a strong absorbance at 405nm. The amount of p- nitrophenol measured at the end of the assay is directly proportional to enzyme activity.

7. Methodology

7.1. Assay and Calculation of Total Plasma Tartrate Resistant Acid Phosphatase

Total plasma tartrate-resistant acid phosphatase was assayed according to the procedure of (Henry and Steer, 1999). The total plasma tartrate resistant acid phosphatase activity was calculated for each sample using the following relationship below (Henry and Steer, 1999):

$$\text{Volume activity} = \frac{\Delta E \times 3.1 \times 100}{30 \times 18.5 \times 0.1} = \Delta E \times 55.9$$

Where, ΔE = Absorbance of Sample, 18.5 = Extinction Coefficient for 4-nitrophenyl Phosphate, 0.1 = Volume of plasma, 30 = Incubation period, 1000 = Scaling factor per litre of plasma, and 3.1 = Final volume of assay mixture.

7.2. Statistical Analysis

All samples result were calculated from triplicate assay and expressed as the means ± standard error of mean (SEM) at $\alpha=0.05(\alpha=5\%)$.

8. Results

Table 1. Showing the mean activity of tartrate-resistant acids phosphatase in normal individuals.

SUBJECT	ACTIVITY (IU)			MEAN±SD
Males	183.14	181.60	183.29	182.68±0.93
Female	162.57	161.45	—	162.01± 0.79
Children	179.10	177.64	—	178.37±1.03

Table 2. Showing the mean activity of tartrate-resistant acid phosphatase of infected individuals.

SUBJECT	ACTIVITY (IU)			MEAN± SD
Males	66.20	68.90	66.17	67.09±1.56
Female	110.55	109.49	—	110.02±0.74
Children	126.02	124.90	—	125.46±0.79

Table 3. Showing comparison between normal and infected male adults.

SUBJECT	MEAN± SD(IU)
Normal Males	182.67±0.93
Infected Males	67.09±1.56

Table 4. Showing comparison between normal and infected female adults.

SUBJECT	MEAN±SD (IU)
Normal Females	162.01± 0.79
Infected Females	110.02± 0.74

Table 5. Showing comparison between normal adults and infected children.

SUBJECT	MEAN±SD (IU)
Normal Adults	174.41±11.34
Infected Children	125.46± 0.79

Table 6. Showing comparison between infected male adults and infected children.

SUBJECT	MEAN± SD (IU)
Infected males	67.09±1.56
Infected Children	125.46±79

From the results above, Table 1 shows the plasma Tartrate, resistant acid Phosphatase (TRAP) activity of normal individuals. The males had the activity of 182.68±0.93 IU, whereas the females had 162.01±0.79IU and the children had 178.37±1.03 IU. Table 2 shows the plasma tartrate-resistant aid Phosphatase activity of infected individuals where the males had an activity of 67.09±1.56IU. While the infected females had 110.02±0.74 IU, and infected children had 125.46±0.79 IU.

The comparison between the plasma tartrate-resistant acid Phosphatase activity of normal and infected male adults is shown in Table 3, where normal male adults had an activity

of 182.68±0.93 IU. It differs significantly to that of infected male adults whose activity was 67.09±1.56±1.56 IU.

Table 4 shows the comparison between the plasma activities of tartrate-resistant acid Phosphatase of normal female adults and infected ones, where the normal female adults were found to have the activity of 162.01±0.79 IU as against 110.62±0.74 IU for that of infected female adults, showing a significant difference.

Table 5 shows the comparison between the plasma activity of tartrate-resistant acid Phosphatase of normal adults and that of infected children with activity of 174.41±11.34 IU for normal adult was found to differ significantly from that of infected children with activity of 125.46 ±0.79 IU.

Table 6 compares the plasma activity of tartrate-resistant acid phosphatase of infected male adults to that of infected children. Infected male adults had the activity of 67.09±1.56 IU that significantly differs from that of infected children with the activity of 125.46±0.79 IU.

9. Discussion

The results obtained show a general increase in the activity of Tartrate resistant acid Phosphatase (TRAP) in normal individuals. There is a general decrease in the activity of Tartrate resistant acid Phosphatase in infected individuals when compared to that of normal individuals. Briggs, Rice, Daly, Urguhart, and Gornall, (2011) in their finding revealed a previously unrecognized link between Tartrate resistant acid Phosphatase activity and interferon alpha levels using a biological assay of antiviral, five cases were assayed serially, with greater than one month intervals between.

It has been reported in the literature that the level of acid Phosphatase rises in many disease conditions. The rises in the level of the enzyme are due to its release from the liver; prostate, platelets, spleen, kidney e.t.c. The release of this enzyme from the liver especially during malaria infection has also been reported. Role of Tartrate resistant acid Phosphatase in bone and immune cells had been reported and it was suggested that TRAP may be complicated in autoimmune disorders and cancers (Hayman, 2008).

The activities of total serum acid phosphatase (E.C.3.1.3.2.) and of two of its isoenzymes, tartrate-resistant acid phosphatase and erythrocyte-specific acid phosphatase were measured in patients presenting acute falciparum malaria infection, and a normal, healthy control. All the three forms of acid phosphatases were found to be significantly higher during infection as compared to their activity in the control group. This finding suggests that the measurement of acid phosphatase, particularly the erythrocyte isoenzymes, in serum could be potentially used as a biomarker of acute

falciparum malaria infection (Garba, Gasting, and Ubom, 2006). This decrease in TRAP activities may be caused by many factors as reported by Calibreath (1992) which is due to the inhibition of the prostatic fraction of the activity of the enzyme in the plasma of malaria-infected patients. Also, the result of this work shows a decrease in the activity of tartrate-resistant acid phosphatase, especially in infected male adults.

10. Conclusion

Today, malaria is one of the world's largest killer diseases, especially in the developing world like Africa where it was about 1 million people annually, and an estimate of 150 million people get infected. The malaria parasite gets into the blood stream through the bite of an infected female anopheles mosquito. These results in the destruction of erythrocytes and eventually leads to the release of tartrate-resistant acid phosphatase into the bloodstream, this TRAP plasma activity is lowered during infection. The research work revealed plasma activities of tartrate-resistant acid phosphatase for infected males, females and children; 67.09 ± 1.56 IU, 110.02 ± 0.74 IU and 125.46 ± 0.79 IU respectively. These result when compared with normal value of sampled individual, Table 1-6, is on the lower side, this means that tartrate resistance acid Phosphatase may serve as an index to be used in diagnosing the level of malaria infection and its severity. If the MDGs goals are to achieve massive campaign should be launched in order to partially eradicate malaria disease.

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