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Elevated total and isoenzyme forms of acid phosphatase in *falciparum* malaria

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Abstract

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 109 adult male and female patients presenting acute *falciparum* malaria infection, and a normal, healthy control group comprised of 82 subjects. All the three forms of acid phosphatase were found to be significantly ($p < 0.05$) higher during infection as compared to their activity in the control group. This result suggests that the measurement of acid phosphatase, particularly the erythrocyte isoenzyme, in serum could be potentially used as a biomarker of acute *falciparum* malaria infection. ***To cite this article: I.H. Garba et al., C. R. Biologies 329 (2006).***

Résumé

Teneurs totales élevées en formes isoenzymes de la phosphatase acide dans le paludisme aigu à *Plasmodium falciparum*. Les activités de la phosphatase acide sérique totale (E.C. 3.1.3.2) et deux de ses isoenzymes, la phosphatase acide tartrate-resistante et la phosphatase acide érythrocyte-spécifique, ont été mesurées chez 109 patients adultes, hommes et femmes, souffrant d'une infection palustre aiguë à *Plasmodium falciparum*, et un groupe témoin constitué de 82 sujets sains. Toutes les trois formes de la phosphatase acide ont été significativement ($p < 0{,}05$) élevées pendant l'infection, comparées à leurs activités chez les témoins. Ce résultat suggère que l'activité sérique de la phosphatase acide, particulièrement l'isoenzyme érythrocytaire, pourrait être potentiellement utilisée comme biomarqueur du paludisme aigu à *Plasmodium falciparum*. ***Pour citer cet article : I.H. Garba et al., C. R. Biologies 329 (2006).***

Keywords: Acid phosphatase; *Falciparum* malaria; Isoenzyme; Biomarker

Mots-clés : Phosphatase acide ; Paludisme, *Falciparum* ; Isoenzymes ; Biomarqueur

1. Introduction

Disease states usually lead to moderate or extensive tissue damage depending on the time of onset and severity of the disease. Such conditions are usually associated

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with the release of enzymes specific to the diseased organ or tissue into circulation. The consequence is an increase in activity of such enzymes in body fluids [1–4]. However, every organ or tissue damage does not lead necessarily to a rise in the activity of the organ or tissue-specific enzyme concerned. Lum et al. [5] have reported some disease associated with a decrease in organ or tissue-specific enzyme activity in body fluids. Therefore minimal changes in the concentration of an enzyme can be reflected in easily measured alterations in enzymatic activities. Thus, measurement of enzymatic activity in serum/plasma and other body fluids has been employed in the diagnosis of diseases. Enzymatic methods have proven to be crucial to the diagnosis of acute myocardial infarction [6], liver diseases [7] and acute pancreatitis [8]. Apart from their value as general diagnostic indicators, enzyme activity measurements in body fluids are providing scientists and clinicians with new insights into the pathological basis of some diseases. In some instances, they are used as predictors of the progress of diseases, particularly certain types of cancers [1,8,9]. *Falciparum* malaria is a disease caused by an obligate intracellular parasite of the *Plasmodium* complex known as *P. falciparum*. It is the commonest malaria infection in Africa, particularly south of the Sahara [10]; *falciparum* malaria infection is at the root of hyper endemic malaria with great regional epidemics [11] and fatalities in the region of 1.5–2 million persons annually [12,13].

In this work, we assayed for serum activity of total acid phosphatase and two of its isoforms, tartrate-resistant acid phosphatase (TRAP) and erythrocyte-specific acid phosphatase (ESAP), otherwise known as Band Three Acid phosphatase in patients with acute *falciparum* malaria infection. This was with a view to assessing the effect of the disease on the levels of these enzyme and isoenzymes that might be of importance in the diagnosis of this pathological condition.

2. Materials and methods

2.1. Study locale

The southern and northern limits of Bauchi State where the study was conducted are demarcated by latitudes 9°30′ north and 10°30′ north, respectively. Its western and eastern limits are bounded by longitudes 8°45′ east and 11°0′ east, respectively. Two thirds of the land area is in the south of latitude 11°15′. Mean daily temperature in August, the month in which the study was conducted is 29.2 °C and a humidity range of 68%. August is the month when the incidence of *falciparum* malaria endemicity is at its highest peak because of the highest average rainfall, which occurs during this month.

2.2. Study design

Patient selection and pre-qualification was done by simple random sampling of individuals at the Bauchi Specialist Hospital Outpatient Department presenting with a history of fever and malaise within a period of 1–8 days, and who were confirmed to be infected with the *falciparum* malaria parasite by microscopic examination of Giemsa stained thin blood slides. Based on the following selection criteria, only 109 patients were found to be qualified for participation in the study. Among the qualified patients, 67 were males, while the females were 42. Both groups of patients fell within the age range of 18–45 years.

2.3. Patient selection criteria

Patients whose case history showed a concomitant presentation with the following conditions: anaemia, cancer, metabolic bone disease, multiple myeloma and Gaucher's disease and Paget's were excluded from this study. This is because these conditions are known to be associated with raised serum acid phosphatase activity [14]. Similarly, patients on self-medication with any antimalarial drug prior to presentation were also excluded from the study.

For comparative purposes, a control group of 82 healthy adults (age range, 18–45 years) were also enrolled in the study.

2.4. Serum preparation

Venous blood (5 ml) was obtained from each of the patients by venepuncture of the antecubital vein using a sterile needle and syringe between the hours of 9–11.00 am local time. The blood samples were then transferred into clean, sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged at 3000 *g* for 10 min to obtain the sera. Enzyme assay was carried out within 24 h of collection.

2.5. Enzyme assays

Total serum acid phosphatase activity was assayed according to the method described in Bergemeyer [15]. Tartrate-resistant acid phosphatase (TRAP) and erythrocyte-specific acid phosphatase (ESAP) activities were also assayed according to the method described in Bergemeyer [15] in the presence of 0.4 M tartrate (for TRAP activity) and 0.4 M fluoride (for ESAP activity).

Enzyme activities are reported in International Units (IU).

2.6. Statistical analysis

Data analyses were carried out using the MINITAB-10 Statistical Software. Comparison of mean total serum acid phosphatase, tartrate-resistant acid phosphatase (TRAP) and erythrocyte-specific acid phosphatase (ESAP) activities between the control group and patients were done using one-way analysis of variance (ANOVA). Where p values are < 0.05, the Duncan's Multiple Range Test was used to test the difference between pair of means; p values < 0.05 were considered significant.

2.7. Ethics

This work was conducted in accordance with the following ethical declarations:

- World Medical Association's Declaration of Helsinki [16];
- APA Ethical Principles in the Conduct of Research With Human Participants [17];
- World Medical Association's Declaration of Lisbon on the Rights of the Patient [18];
- CIOMS/WHO International Guidelines for the Conduct of Research Involving Human Subjects [19].

3. Results

Table 1 shows the serum activity of total acid phosphatase and its isoforms in normal and infected males and females. The infected females had the highest serum activity of acid phosphatase in the range of 2.35 ± 0.13 IU. This value is roughly two-fold higher than the activity in normal males (1.40 ± 0.07 IU) and females (1.30 ± 0.04 IU). Total serum acid phosphatase activity in infected males was found to be 2.25 ± 0.06 IU. Acid phosphatase is significantly elevated among both infected males and females in relation to its activity in normal males and females ($p < 0.05$). Tartrate-resistant acid phosphatase (TRAP) activity in serum of infected males and females was also highly elevated: almost double the activity in normal males and females ($p < 0.05$). TRAP activities were 1.22 ± 0.13 IU (infected females), 1.08 ± 0.06 IU (infected males), 0.79 ± 0.04 IU (normal females) and 0.83 ± 0.07 IU (normal males), respectively. Here also, the infected females showed the highest activity for tartrate-resistant acid phosphatase. The infected females also had the highest elevation in the activity of erythrocyte-specific acid phosphatase (ESAP) with a value of 1.07 ± 0.05 IU. This activity is significantly higher than the normal female erythrocyte-specific acid phosphatase activity of 0.45 ± 0.06 IU. ESAP activity is also significantly higher in infected males (0.94 ± 0.02 IU) when compared to the normal male activity of 0.48 ± 0.09 IU ($p < 0.05$). These elevations in erythrocyte-specific acid phosphatase activity during infection are about two-fold the normal serum values.

4. Discussion

The only clinical situation in which serum acid phosphatase activity assays have proven to be of importance for more than 60 years is in its application as a marker in the diagnosis of prostatic carcinoma [2,20,21]. This is because cancer-induced damage to the prostate gland, a rich source of this enzyme causes its leakage in significant amounts into the victim's serum [22]. However, Chambers et al. [23] have reported a 5–50-fold elevation in serum acid phosphatase activity in patients with the adult, non-neuropathic form of Gaucher's disease, suggesting its potential in monitoring this inborn error in lipid metabolism. Also, Gatsing et al. [1] reported a significant increase in serum acid phosphatase activity in patients with laryngeal carcinoma. In this work, highly significant elevations in both total and tissue-specific isoenzymes of acid phosphatase in adult *falciparum* malaria patients have been found. One of the important pathophysiological processes that contribute to the debilitation associated with this infection is the

Table 1
Serum activity (IU) of acid phosphatase and its isoforms in adult male and female controls and adult male and female *falciparum* malaria patients

Enzyme	Male control	Male patients	Female control	Female patients
Total serum acid phosphatase	1.40 ± 0.07 ($n = 38$)	2.25 ± 0.06[a] ($n = 67$)	1.30 ± 0.04 ($n = 44$)	2.35 ± 0.13[a] ($n = 42$)
Tartrate-resistant acid phosphatase	0.83 ± 0.07 ($n = 38$)	1.08 ± 0.06[a] ($n = 67$)	0.79 ± 0.04 ($n = 44$)	1.22 ± 0.13[a] ($n = 43$)
Erythrocyte-specific acid phosphatase	0.48 ± 0.09 ($n = 38$)	0.94 ± 0.02[a] ($n = 67$)	0.45 ± 0.06 ($n = 44$)	1.07 ± 0.05[a] ($n = 41$)

[a] $p < 0.05$ vs. control.

destruction of both parasitized and unparasitized erythrocytes [24] with its consequent release of parasite-derived toxic waste products during erythrocytic merogony. The haemolytic process invariably leads to the release of significant amounts of this enzyme into the patient's serum, since red blood cells are also among some of the rich sources of acid phosphatase. This is evident in the fact that the erythrocyte-specific acid phosphatase activity accounts for about 42% and 46% of the total acid phosphatase activity in infected males and females, respectively. In addition, tartrate-resistant acid phosphatase activity represents up to 48% and 52% of the total acid phosphatase activity in infected males and females, respectively. The proportion of the activity of both TRAP and ESAP during infection is about 90% and 97% of total serum acid phosphatase activities in infected males and females, respectively. However, the ratio of total serum acid phosphatase to that of erythrocyte-specific acid phosphatase activity is higher in infected males (2.4) relative to their female counterparts (2.2). This is a finding of potential importance because measurement of serum levels of acid phosphatase, particularly the erythrocyte isoenzyme can be used to diagnose this infection. Furthermore, the elevation in tartrate-resistant acid phosphatase activity during this infection indicates some degree of damage to other tissues or organs, which are rich sources of this enzyme particularly the liver and possibly macrophages. In addition, it may be indicative of tissue lysosomal function in malaria infection, since the lysosomes also contain high amounts of this isoenzyme [25].

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