Serum Alkaline Phosphatase Activity As A Potential Biomarker For The Intergrity Of The Hepatic Drainage System In Acute Falciparum Malaria Infection

I Garba, U Gregory

Citation

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Abstract

Introduction: The serum activity of the tissue-specific, membrane-bound enzyme alkaline phosphatase (Orthophosphoric monoester phosphohydrolase, alkaline optimum (E.C. 3.1.3.1))was assayed in the sera of 110 adult patients comprising of 50 males and 60 females (age range, 18 – 40 years) presenting with acute falciparum malaria infection and a control group of 48 healthy adults.
Methods: Serum samples were collected from a randomly selected group of individuals confirmed to be infected with the falciparum malaria parasite by microscopic examination of Giemsa stained thin blood slides.
Results:Serum alkaline phosphatase activity was significantly increased (6.35 ± 0.39 IU) in female patients relative to the normal females (4.33 ± 0.23 IU), p = 0.003. Serum alkaline phosphatase activity was also higher in male patients (5.85 ± 0.36 IU) when compared to the control activity of (4.83 ± 0.35 IU) in males, p = 0.001.
Conclusion: The increased serum alkaline phosphatase activity among the patients indicates that the liver stage of falciparum malaria infection is accompanied by a perturbation of the host hepatocytes drainage pathways where alkaline phosphatase is localized on the membrane. This makes alkaline phosphatase a potentially important biomarker for the assessment of the integrity of the hepatic drainage system in acute falciparum malaria infection.

INTRODUCTION

Alkaline Phosphatase (Orthophosphoric monoester phosphohydrolase, alkaline optimum (E.C. 3.1.3.1)) is an enzyme which catalyses the hydrolysis of a number of phosphate esters, transferring the phosphate group to an acceptor molecule. The pH optimum for the reaction is in the alkaline range, around 10. Alkaline phosphatase is a membrane-bound metalloenzyme comprising a group of isoenzymes encoded by at least four different gene loci [1]. They are: tissue-specific, placental, intestinal and germ cell alkaline phosphatase. Each isoform of this enzyme has a slightly different pH optimum as well as different substrate preferences and concentration for maximum activity[2]. The two major and clinically most relevant isoenzymes in human serum are bone and liver alkaline phosphatase formed through post-translational modifications of the tissue non-specific gene product [3]. They mainly circulate in soluble dimeric forms. Several theories have been postulated with respect to the biochemical role of alkaline phosphatase isoenzymes[4]. The liver fraction is believed to be involved in the transport processes. The bone isoenzyme is thought to enhance bone formation via enzymic hydrolysis of orthophosphate, an inhibitor of calcium deposition [5]. The intestinal fraction is thought to be involved in some manner with metabolite transport across cell membranes [6] and calcium absorption [3]. Phosphate absorption is believed to be facilitated by both intestinal and kidney isoenzymes. All fractions show some ability to regulate the synthesis of DNA. No specific function has yet been suggested for the placental fraction, although some studies suggest it is part of the process of the nutrient transport to the fetus [7]. Alkaline phosphatase is located in a wide variety of tissues. Significant amounts of the enzyme are found in the liver, placenta, intestine, kidney, bone and platelets in decreasing order [8]. The primary clinical utility of alkaline phosphatase is in cases of suspected bone disorders and obstructive liver diseases. Raised serum levels are seen in different bone disorders including Paget's disease, osteomalacia, rickets,

hyperparathyroidism, osteogenic sarcoma, fractures and osteoblastic metastases. Increased serum levels are also seen in liver disease associated with extra or intra-hepatic obstruction, obstructive jaundice, diabetes [9] infectious mononucleosis [10] biliary cirrhosis and cholestasis. Low serum levels are associated with protein-energy malnutrition, cardiac surgery, low dietary magnesium, hypothyroidism, pernicious anemia [11], hypervitaminosis D, scurvy, achondroplasia in children and estrogen replacement therapy in post-menopausal women with osteoporosis [12]. In this work, we assayed for serum activity of alkaline phosphatase in adult patients with acute falciparum malaria infection. The location of this enzyme on the membrane of the hepatic drainage system [13] makes it a potentially important biomarker for the assessment of the integrity of this system during malaria infection. 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 [14] and is at the root of hyper endemic malaria with great regional epidemics [15] and fatalities in the region of 1.5 - 2 million persons annually[16, 17].

PATIENTS AND METHODS

Patients and Study Design: Patient selection and pre-qualification was done by simple random sampling of individuals presenting at the Bauchi Specialist Hospital Outpatient Department 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 110 patients were found to be qualified for participation in the study. Among the qualified patients 50 were males while 60 were females. Both group of patients fall within the age interval of 18 – 40 years.

Patient Selection Criteria: Patients whose case history showed a concomitant presentation with the following conditions: Pregnancy, anemia, liver diseases including cirrhosis and hepatitis, alcoholism, metabolic bone disease, cancer, gastrointestinal tract infections, kidney disorders, protein energy malnutrition, diabetes, infectious mononucleosis and magnesium/vitamin D deficiency were excluded from this study. This is because all these conditions are associated significant changes in the serum activity of alkaline phosphatase [6, 7, 9, 10, 11, 12, 18]. Similarly, patients on self-medication with any antimalarial drug prior to presentation were also excluded from the study.

Controls: For comparative purposes, a control group of 48 made up of 24 each of healthy male and female adults (age interval, 18 – 40 years) were also enrolled in the study.

Serum sample collection and preparation:Blood samples were collected between the hours of 9.00 a.m. and 11.00 a.m. by venepuncture of the antecubital vein into clean, sterile, plastic centrifuge tubes. The samples were centrifuged at 3000g for ten minutes after clotting. Sera was collected by aspiration using a Pasteur pipette and assayed within 24 hours.

(ii) Enzyme assay: Total serum alkaline phosphatase activity was assayed according to the method described in Bergemeyer [19]. Enzyme activities are reported in International Units (IU/L).

Statistical analysis: The results are given as mean ± standard error of the mean. Data analyses were effected using MINITAB-10 Statistical Software. Comparison of mean total serum alkaline phosphatase activity 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.

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

A. World Medical Association's Declaration of Helsinki [20]. B. APA Ethical Principles in the Conduct of Research With Human Participants [21]. C. World Medical Association's Declaration of Lisbon on the Rights of the Patient [22]. D. CIOMS / WHO International Guidelines for the Conduct of Research Involving Human Subjects [23].

RESULTS

The results are shown in tables 1 and 2 below.

Figure 1

Table 1: Total serum alkaline phosphatase activity in healthy and adult falciparum malaria patients (IU/L).

Subjects	Mean serum alkaline phosphatase activity(IU/L)
Male patients	5.85 ± 0.36[a] (n = 50)
Control males	4.83 ± 0.35[a] (n = 24)
Infected females	6.35 ± 0.39[b] (n = 60)
Control females	4.33 ± 0.25[b] (n = 24)

Values with the same subscript differ significantly at $p < 0.05$

Figure 2

Table 2: Total serum alkaline phosphatase activity in male and female adult falciparum malaria patients (IU/L).

Subjects	Mean serum alkaline phosphatase activity (IU/L)
Infected males	5.85 ± 0.36 * (n = 50)
Infected females	6.35 ± 0.39 * (n = 60)

* No significant difference at $p = 0.05$

Alkaline phosphatase activity in infected males was 5.85 ± 0.36 IU/L. This activity was found to differ significantly from the control alkaline phosphatase activity of 4.83 ± 0.35 IU/L in normal, healthy males, as shown in table 1, $p = 0.001$. The picture is similar in females. A significant increase was found in the serum activity of alkaline phosphatase in infected females. The mean serum alkaline phosphatase activity was found to be 6.35 ± 0.39 IU/L in infected females and 4.33 ± 0.23 IU/L in normal, healthy females, $p = 0.003$, table 1. Serum alkaline phosphatase level was also found to be lower in infected males (5.85 ± 0.36 IU/L) relative to their female counterparts (6.35 ± 0.39 IU/L) $p > 0.05$, table 2. Among the controls, alkaline phosphatase activity was not found to differ significantly between the males and the females $p > 0.05$.

DISCUSSION

In terms of pathogenesis, the host liver is among the organs affected in the early stage of falciparum malaria [24] leading to significant alterations in host hepatocyte physiology and morphology [25]. The observed elevation in serum alkaline phosphatase activity is an indication that the hepatic stage of the parasite's life cycle in its human host and is accompanied by significant perturbation in the hepatocytes membrane leading to leakage of this enzyme out of the liver cells. This finding correlates positively with earlier reports by Maegraith [26] that centrilobular liver damage is one of the factors involved in hepatic dysfunction in acute falciparum malaria infection, leading to hyperbilirubinaemia [14]which is a direct consequence of the impaired drainage capacity of the liver. However, considering the magnitude of the elevation in serum alkaline phosphatase reported in this study, which is less than 50 % of the control activity even among the female patients, the observed increase is not as high as that found to be associated with cholestasis [7] where serum level is over twice the normal limit. In addition other diseases like liver cirrhosis and diabetes were found to be associated with a 92% and 48 % increases above the normal limits of serum alkaline phosphatase respectively, [6, 9]. The variation in the relative percentage increases in serum alkaline phosphatase in malaria relative to these diseases can be due to the differences in the etiology and pathogenic outcome of acute falciparum malaria infection relative to these diseases. The significantly higher serum alkaline phosphatase activity among the patients is evidence of the fact that changes in serum alkaline phosphatase activity can be used as a potential biomarker in assessing the integrity of the hepatic drainage system during acute falciparum malaria infection. The higher serum alkaline phosphatase activity in female patients relative to their male counterparts is not an unexpected finding. This is because sex-related changes in serum alkaline phosphatase activity are not uncommon [12]although patient sex is not a significant factor with regards to the pattern of results obtained in this study.

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Author Information

Ibrahim Hassan Garba, M.Sc.
Chemistry Programme, School of Science, Abubakar Tafawa Balewa University

Ubom Gregory, Ph.D.
Department of Biochemistry, Faculty of Medical Sciences, University of Jos