

# Activity and Isoenzymes of Alkaline Phosphatase in Patients with Urinary Schistosomiasis in Balad Rouz Town, Diyala Province

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## Abstract

A total of 1550 urine samples were collected from pupils and households in Balad Rouz town, Diyala province and examined for urinary schistosomiasis during the period from October 2005 till December 2006. The total infection rate with *Schistosoma haematobium* was 2.13%. Serum and urine from all the 33 infected individuals and 25 controls were assayed for the estimation of alkaline phosphatase (ALP) activity. The study revealed a significant increase in ALP values in both serum and urine of infected individuals. No significant differences in ALP activity were recorded among males and females and among different age groups. Through polyacrylamide gel electrophoresis, ALP isoenzymes revealed three banding patterns which differ from the three zymodemes obtained from control group. This is a cheap, reproducible and rapid method for detection of progressive disease in *Schistosoma haematobium* infection especially in endemic areas.

## Introduction

Alkaline phosphatase, orthophosphoric monoester phosphohydrolase or phospho-monoesterase (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 [1]. The optimum pH for the reaction is around 10 [2]. Significant amounts of this enzyme are found in the liver, placenta, intestinal epithelium, kidney tubules, osteoblasts, leucocytes and platelets in a decreasing order [1].

ALP consists of a group of isoenzymes encoded by at least four different gene loci [3]. These are: tissue (non specific), placental, intestinal and germ cell phosphatase [4]. 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 ALP [5].

The normal ALP values in adult human plasma are 35-129 u/l [6]. Raised serum levels are noticed in different bone disorders [7], in some liver diseases [1], and in some dual infections such as malaria and dengue [8] or hepatitis virus related cirrhosis and *Leishmania* infection [9]. Elevated levels of ALP were found in the urine of patients with kidney and urinary tract disorders including bladder carcinoma [5].

Studies concerning the correlation between ALP and parasites indicated that parasitic infection with *S. mansoni* induced elevation of ALP level in serum as a marker of liver fibrosis and associated with hepatic complications [10]. Measurement of ALP is usually done by using colorimetric methods [2]. A continuous spectrophotometric method has been described under optimum conditions [3]. Electrophoresis methods can also be used to differentiate ALP isoenzymes [11].

## Materials and Methods

The present study was based on screening primary school children and households in Balad Rouz town by urine examination. The study covered 1550 individuals during the period from October 2005 to December 2006. Twenty-five individuals (15 males and 10 females) were selected as a control group for this study. Among those 25 controls, 15 individuals, living in this endemic area, were negative for *S. haematobium* eggs in their urine (positive controls), while the other 10 individuals were living in non-endemic areas, some from Baghdad city and others from Al-Khalis city (negative controls). Their ages ranged from 5-40 years. They were supplied with marked test tubes for urine sample which included the last urine drops. The samples were collected between 10:00-14:00 hours and were transferred in a cool-box to the laboratory of Medical Research Center, College of Medicine, University of Al-Nahrain where the direct examination was carried out.

During the next day of urine collection, each pupil infected with *S. haematobium* (as indicated with the presence of ova in the urine) was asked to provide ten ml of venous blood. The collected sample was immediately transferred into a plain plastic tube and the serum was obtained by centrifugation at 2000 rpm for 10 minutes at 4°C. The serum was dispensed into sterile tubes (0.5 ml in each tube) and stored at -20°C for the determination of ALP activity and processing of the electrophoresis of ALP enzyme. ALP activity of serum was assayed by using bioMerieux phosphatase alkaline kit. This kit was also used for ALP determination in urine for comparison with that of serum.

The gel solution was made by mixing 7.5 ml distilled water, 33.3 ml tris-glycine buffer stock, 22.2 ml acrylamide solution, 3.2 ml ammonium persulphate and 0.1 ml tetra methyl ethylene diamine (TEMED). The final volume of this prepared gel solution was 66.3 ml. This solution was immediately poured into the modulating set. The gel was left for 45 minutes, and then kept at 4°C for 24 hours [12].

The gel plate was placed on a cooling plate; 7-8 layers of electrode wicks (Whatman No. 1 filter paper) were soaked with the tris-glycine buffer. One edge was applied at the gel plate and the second edge was in the buffer tank. Pre-electrophoresis was performed with a pre set constant current (50 mA) for 30 minutes. Ten  $\mu$ l of the tested samples and one  $\mu$ l bromophenol blue were placed in each slot of the gel. Immediately after the application of the sample in the slot, the electrophoresis was started by switching on the power supply current of 20 mA for 10 minutes. Then, the current was adjusted to 50 mA and the electrophoresis was ended when the bromophenol blue was reached to the end of the gel. Then, position of the bands was detected by using the proper stain [13].

The gel plate was placed on the staining kit tray and was then easily transferred between solutions for developing [13]. The solution for developing ALP isoenzyme was made by mixing 0.01 gm nitroblue tetrazolium (NBT), 0.005 gm 5-bromo-4-chloro-3 indolyl phosphate, 0.07 gm MgCl<sub>2</sub>, 20 ml tris-glycine buffer, pH=10.2. This solution was kept at 40°C till the appearance of the bands.

## Results and Discussion

Thirty- three individuals were infected with *S. haematobium* in Balad Rouz town, which bring the overall percentage of infection to 2.13%. High significant increase (p value=

0.0001) in the level of activity of ALP in sera of schistosomiasis patients was noticed compared to that of the control group ( $178.88 \pm 2.745$  u/l versus  $87 \pm 6.714$  u/l). Also, high significant increase (p value= 0.0001) in level of activity of ALP in urine was noticed in comparison with the control group ( $21.907 \pm 2.757$  u/l versus  $7.817 \pm 1.557$  u/l). These findings come in agreement with some other studies which showed an increase in ALP activity in patients with acute schistosomiasis and bladder cancer [14], falciparum malaria [1], leishmaniasis [15], fascioliasis [6], hydatidosis [16] as well as some dual infections [8, 9]. Nubian goats (with no scientific name given) infected with *Trypanosoma evani* also showed an increase in their serum ALP [17].

No significant changes (p value= 0.0001) were detected in ALP levels in sera of patients in relation to sex ( $180.82 \pm 3.050$  u/l for males and  $175.66 \pm 2.193$  u/l for females) and in relation to age ( $183.53 \pm 5.63$  u/l for 6-10 years age group,  $178.52 \pm 4.29$  u/l for 11-15 years age group and  $171.71 \pm 2.176$  u/l for  $\geq 16$  years age group). Also, no significant changes (p value= 0.0001) were detected in ALP levels in urine in relation to sex ( $22.86 \pm 3.305$  u/l for males and  $18.67 \pm 2.121$  u/l for females) and in relation to age ( $22.4 \pm 5.151$  u/l for 6-10 years age group,  $21.16 \pm 3.466$  u/l for 11-15 years age group and  $18.137 \pm 1.6$  u/l for  $\geq 16$  years age group). The absence of significant changes of ALP level in both serum and urine in relation to age and sex of the present study may be attributed to the small number of patients involved (27 males against six females and 12, 20 and one for the above age groups, respectively).

Three banding patterns for ALP enzyme activity were discerned among the sera of 33 patients which differ from the three zymodemes obtained from the 25 healthy controls. These differences are due to isoenzymes activity of patients with urinary schistosomiasis (Figs. 1 and 2), as following: ALP enzyme of 25 healthy controls sera (100%) was separated into three major fractions (liver, bone and intestine). Liver ALP normally runs as the  $\alpha$ -2 band, while bone ALP runs in the  $\alpha$ -2/ $\beta$  region. Intestinal isoenzyme is usually found in the  $\beta/\gamma$  region (Fig. 1).

Thirty - three patients (100%) had different bands (with excess amount) between  $\alpha$ -2 and  $\beta$  regions towards the normal liver bands in addition to the presence of a thick band of skeletal isoenzyme. The intestinal isoenzyme had a thin band in comparison with that of the control. The present results indicated that, in addition to the normal ALP enzymes fraction, there exists some variations in the amount of these fractions which may be associated with genetically determined differences occurred due to the infection, represented in different electrophoresis mobility [18]. Some cytogenetic differences in schistosomiasis patients were confirmed [14]. So, alkaline phosphatase isoenzymes represent non-invasive, inexpensive, reproducible and rapid method to detect progressive disease in *S. haematobium* infection especially in endemic areas.

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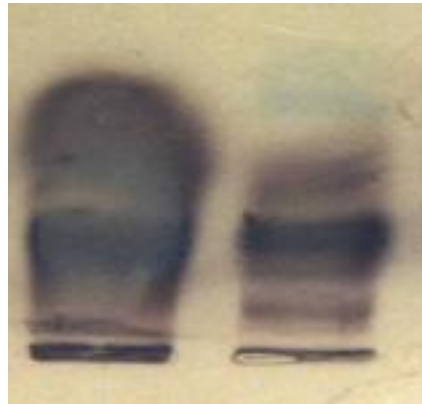


Fig. (1) Photograph of the electrophoresis patterns obtained with urinary schistosomiasis for ALP isoenzymes. Left: serum from patient's sample. Right: control serum.



Fig. (2) Zymograph of ALP iso enzymes obtained with urinary schistosomiasis. Left: serum from patient's sample. Right: control serum

## نشاط ومنتازرات إنزيم الفوسفاتيز القاعدي في المرضى المصابين بالبلهارزيا البولية في مدينة بلد روز، محافظة ديالى

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### الخلاصة

تم فحص 1550 عينة إدرار من تلامذة وأهالي مدينة بلد روز، محافظة ديالى المدة من شهر تشرين الأول 2005 الى كانون الأول 2006 بحثا عن الإصابة بالبلهارزيا البولية. سجلت نسبة إصابة كلية تعادل 2.13%. تم فحص عينات مصل وإدرار من جميع المصابين (من الموجبي الفحص) البالغ عددهم 33 شخصا وتلك لتحديد نشاط أنزيم الفوسفاتيز القاعدي. تم الكشف عن حصول زيادة معنوية في قيم هذا الأنزيم في كل من المصل والبول للأشخاص المصابين. لم تظهر فروق معنوية في هذا المجال بين الذكور والإناث ولا بين مختلف المجاميع العمرية. ومن خلال الترحيل الكهربائي باستخدام هلام البولي أكريلاميد، أظهرت منتازرات هذا الأنزيم ثلاثة أنماط للحزم والتي تختلف عن الحزم الثلاث التي ظهرت لدى مجموعة السيطرة. وتعد هذه الطريقة رخيصة الكلفة ومثمرة وسريعة لكشف تطور الإصابة بمرض البلهارزيا البولية ولاسيما في المناطق الموبوءة.