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# ENZYME LEVELS IN THE SERA AND ERYTHROCYTES OF PATIENTS WITH VISCERAL LEISHMANIASIS

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**ABSTRACT**

Twenty five blood samples from 6-month to 5-year old children with visceral leishmaniasis were analysed for various enzymes. Aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LD) and its various isoenzymes were estimated in the serum, while glucose 6-phosphate dehydrogenase (G6PD) was measured in red blood cells. For comparison, blood samples from healthy children of the same age group (controls) were analysed in similar manner. Significantly higher values were obtained for the activities and the specific activities of AST, ALT and total LD in the patients. The activity and the percentage of LD-5 isoenzyme of the kala-azar patients were also significantly higher than the controls, while the percentage of LD-1 isoenzyme was significantly decreased. Hematocrit levels and G6PD activities of the visceral leishmaniasis patients were also significantly diminished.

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## INTRODUCTION

Visceral leishmaniasis is an endemic disease in southern Iran. The disease is characterized by prolonged fever, hepatosplenomegaly, pancytopenia, hypergammaglobulinemia, reversed A/G ratio and proteinuria.[1] An increase of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LD), hydroxybutyrate dehydrogenase (HBDH) and a decrease in creatine phosphokinase (CPK) in serum of children suffering from infantile kala-azar has been reported in Iraq.[2] Also the activities of several enzymes including glucose-6-phosphate dehydrogenase (G6PD) were found to diminish significantly in the erythrocytes of 5 to 36-year old patients suffering from Indian kala-azar.[3]

The present comparative report centers on the activities of AST, ALT, total LD and LD-isoenzymes from the sera of children with infantile visceral leishmaniasis from the rural areas of Fars province in southern Iran and compares them with the respective values from the sera of healthy children. The hematocrit level and the erythrocyte G6PD activities of the kala-azar patients and the healthy children are also compared.

## MATERIALS AND METHODS

**Patients:** Sera and oxalated blood samples were obtained from 25 children, six months to five years old, of the rural areas of the Fars province in southern Iran, admitted to different university hospitals in Shiraz for the treatment of infantile visceral leismaniasis. Blood samples were collected either immediately after hospitalization or after a maximum of two to three days of treatment with meglumine antimonate (Glucantime). Twenty one out of the 25 patients were from six months to two years old, while the rest were in the two to five year age bracket. Clinical diagnosis of the disease was supported by demonstration of antibodies in the serum by indirect immunofluorescent antibody (IFA) titers of 1:256 or more.[4] Sera and oxalated blood samples of six month to five year old healthy children with no history

Table I- Protein, activities and specific activities of AST and ALT in sera of patients with visceral leishmaniasis and in controls.

| | | AST | | | ALT | | |
|---|---|---|---|---|---|---|---|
| No. of samples | Mean protein ± SEM(mg/ml) | Range (U/L) | Mean activity ± SEM(U/L) | Mean specific activitiy ±SEM (U/g protein) | Range (U/L) | Mean activity ± SEM (U/L) | Mean specific activity ±SEM (U/g protein) |
| Patients:25 | 64±4[a] | 21-336 | 110±18.2[a] | 1.8±30[a] | 8-106 | 26±5.0[a] | 0.42±0.08[a] |
| Controls:14 | 78±4[b] | 10-24 | 18±1.1[b] | 0.24±0.02[b] | 4-25 | 7±0.7[b] | 0.09±0.01[b] |

* Means denoted by different superscripts are significantly different ($p < 0.005$)

of leishmaniasis (controls) were also used for comparative studies.

**Analytical procedures:** Serum AST and ALT were measured by a modified colorimetric procedure.[5] A colorimetric method was used for the assay of serum lactate dehydrogenase (LD).[6] Serum LD-isoenzymes were estimated by a heat fractionation technique.[7] The method of Lowry, et.al.[8] was employed for the measurement of serum total proteins. Glucose 6-phosphate dehydrogenase (G6PD) in hemolysed red blood cells was measured by a spectrophotometric procedure while hematocrit was estimated by a micromethod.[9,10]

AST, ALT, LD and G6PD activities in terms of their respective spectrophotometric units per mL ($\Delta A_{340}$ per min per mL × 1000) were each multiplied by a factor of 0.428 to obtain the respective international units of the enzyme activity per liter of serum of erythrocyte hemolysate at 28°C.[11] Statistical differences were determined by Student's t test.

## RESULTS

The activities and the specific activities of AST and ALT together with the protein content of the sera of patients with visceral leismaniasis and those of the controls are shown in Table I. Although the sera of kala-azar patients had a significantly lower total protein than the controls, the activities and the specific activities of both ALT and AST were significantly higher than the controls. The mean specific activities of ALT and AST in patients increased by 4.7 and 7.5 fold, respectively.

Table II summarizes the data on total LD and LD-isoenzyme activities in the sera of kala-azar patients and in those of the controls. The mean activity of total LD in kala-azar patients is 3.5 times higher than that of the controls and the mean activities of LD-isoenzymes are 2.1 (LD-1), 6.4(LD-5) and 5.5($LD_2$ + $LD_3$ + $LD_4$) times higher than the corresponding values in the controls. As shown (Table II), the percentage of LD-5 and that of the sum of $LD_2+LD_3+LD_4$ in the patients are significantly higher than the controls, while LD-1 (the heart specific isoenzyme) has a significantly lower percentage in the patients.

The hematocrit levels and the G6PD activities of the erythrocyte hemolysates of the patients and the controls are given in Table III. As noted, the mean hematocrit level has decreased by a factor of 1.4, while the activity of G6PD is diminished by a factor of 2.1 in the patients with visceral leishmaniasis.

## DISCUSSION

Our data on the decreased total protein content of kala-azar patients (Table I) agree well with the studies of other authors who consider such a decrease to be due to liver degeneration and a decreased synthesis of albumin by the liver parenchymal cells.[12] Similarly the increased AST and ALT activities (Table I) and significant increase in the percentage of LD-5 (liver specific) and possibly, in that of LD-4 (as demonstrated by an increase in percentage of $LD_2+LD_3+LD_4$) in the sera of the patients (Table II) is considered to be a reflection of the involvement of the liver in these patients.[2] Wide

Table II- Total LD and LD-isoenzyme activities in sera of patients with visceral leishmaniasis and in controls.

| | TOTAL LD | | | LD-1±SEM | | LD-5±SEM | | $LD_2+LD_3+LD_4$±SEM | |
|---|---|---|---|---|---|---|---|---|---|
| No.of sampges | Range (U/L) | Mean activity ±SEM (U/L) | Mean specific activity±SEM (U/G protein) | (U/L) | (% of LD) | (U/l) | (% of LD) | (U/L) | (% of LD) |
| Patients:23 | 336-2184 | 860±95.5[a] | 13.±1.65[a] | 316±22.9[a] | 40±2.4[a] | 257±45.8[a] | 28±5[a] | 287±38.3[a] | 32±2.4[a] |
| Controls:14 | 144-384 | 240±19.7[b] | 3.0±0.28[b] | 148±11.4[b] | 62±2.5[b] | 40±5.3[b] | 17±1.3[b] | 52±8.2[b] | 21±2.6[b] |

* Meanse denoted by different superscripts are significantly different ($P<0.005$).

**Table III- Hematocrit and G6PD activity in erythrocyte hemolysates of patients with visceral leishmaniasis and in controls.**

| | Hamatocrit (%) | | G6PD(U/L hemolysate) | |
|---|---|---|---|---|
| No. of samples | Range | Mean±SEM | Range | Mean±SEM |
| Patients: 24 | 18-36 | $29\pm1.0^{a*}$ | 336-2,208 | $1,044\pm110^{a}$ |
| Controls: 15 | 35-45 | $41\pm0.8^{b}$ | 1920-2,640 | $2,267\pm61^{b}$ |

* Means denoted by different superscripts are significantly different ($p<0.005$).

variation in the activities of AST, ALT (Table I) and LD (Table II) in the sera of patients with visceral leismaniasis as demonstrated by the broader range of the activities and the higher SEM of the respective means may suggest different levels of liver involvement in different patients.

Depressed activity of G6PD in the erythrocytes of such patients is probably acquired during the course of the disease due to derangements in the cell membrane of the erythrocytes.[3] Also a wider variation in the activity of G6PD in patients as demonstrated by a broader range and a higher SEM (Table III) might reflect anaemias with different levels of severity in different patients.

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