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**CLINICAL INVESTIGATION**

# Determination of Alkaline Phosphatase Activity in Patients with Different Zinc Metabolic Disorders

Zine KECHRID, Rabah KENOUZ

Department of Biochemistry, Faculty of Sciences, University of Annaba, PO BOX 12, Annaba 23000, ALGERIA.

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**Abstract:** Our purpose was to investigate the effect of different zinc metabolic disorders on alkaline phosphatase activity. Serum zinc, glucose and alkaline phosphatase activity were studied in 32 patients with liver cirrhosis, 30 with chronic renal failure and 42 with insulin-dependent diabetes mellitus (IDDM) compared to 42 healthy volunteers. Serum glucose concentration was significantly higher ($P < 0.001$) in IDDM and liver cirrhosis patients ($P < 0.05$) compared to the controls. Serum zinc concentrations tended to be lower in all different diseases but did not reach statistical significance. Alkaline phosphatase activity was higher in IDDM ($P < 0.05$) and liver cirrhosis patients ($P < 0.01$) compared with the controls. No positive correlation between serum zinc and alkaline phosphatase activity was recorded in any disease. In conclusion, the investigated diseases did not affect the level of serum zinc; however, there was an alteration in alkaline phosphatase activity in patients suffering from diabetes and liver cirrhosis. Consequently, this may be attributed to the functional disturbance that occurred in these patients. Finally, zinc concentration did not follow alkaline phosphatase activity as indicated by the weak correlation obtained.

**Key Words:** Zinc, liver cirrhosis, diabetes, chronic renal failure, alkaline phosphatase

## Introduction

Zinc is recognised as essential for the activity of a wide range of enzymes, and the first demonstration that zinc had a special biological function in relation to enzyme function came with the discovery that carbonic anhydrase contained significant amount of zinc which appeared to be required for normal activity (1). Zinc participates in either the activation of enzyme systems forming a metallo-enzyme complex or non-specifically as a constituent of enzyme forming metallo-enzymes including lactic dehydrogenase, malate dehydrogenase, RNA polymerase, sorbitol dehydrogenase, alkaline phosphatase, and angiotensin I (2-6). A reduction in tissue zinc concentrations may lead to a reduction in the activity of a wide range of enzymes, which are dependent on this element for normal function. Carbonic anhydrase and alcohol dehydrogenase are two examples of enzymes that are particularly sensitive to reductions in tissue zinc concentration (7,8) Zinc deficiency in humans may result from inadequate dietary zinc intake, especially during pregnancy (9), lactation (10) and periods of rapid growth when requirements are high, or as a result of other factors which affect zinc availability. Zinc depletion in humans may also occur as a result of unusual circumstances, which result in increased zinc loss from the body. These include excessive sweating (11), chronic blood loss, the development of disease states such as diabetes (12,13), liver cirrhosis (14,15), chronic renal failure (16) and gastrointestinal disorders (17), and heart failure (18). On the basis of all this previous informations, the present study was undertaken to investigate the effect of these diseases on alkaline phosphatase activity in humans.

## Materials and Methods

The experimental protocol consists of:

### Patients

Thirty-two adults (18 men and 14 women) with liver cirrhosis, with a mean age of

51.4 ± 6.1 years (range 24-61 years). No patient had any other diseases which might influence the results.

Thirty patients (17 men and 13 women) with chronic renal failure and a mean age of

27 ± 4.1 years (range 22-60 years).

Forty-two subjects (20 men and 22 women) with type I diabetes mellitus (IDDM). The subjects ranged in age from 25 to 45 years (mean age 32.5 ± 4.6 years).

### Controls

Healthy volunteers (biology students and members of the staff were used as controls

(22 men and 20 women) with an average age of 24 ± 7.3 (range 20-52 years).

### Methods

Venous blood was collected in the morning at 8.00 after an overnight fast from patients and controls for measurements of whole blood glucose, serum zinc, and alkaline phosphatase activity.

### Analysis

Zinc concentration was measured in duplicate after 20-fold dilution of serum in double distilled water by flame atomic absorption spectrophotometer (Pye Unicam SP 9000) at 213.8 nm. Zinc standards were prepared from a 1 mg/ml zinc nitrate standard solution (BDH) using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10% v/v) for 16 h and rinsed with double distilled water (19).

Blood glucose was measured in 10 ml samples of fresh whole blood by the glucose oxidase (EC 1.1. 3. 4) method, using a YSI Model 27 glucose analyser and a kit comprising phosphate buffer containing the enzymes (GOD, POD) and D-glucose (Sigma).

Alkaline phosphatase activity was determined using commercial test kits comprising 1 mol/l diethanolamine HCl buffer pH 9.8, 0.5 mmol/l magnesium chloride and 10 mmol/l of the substrate p-nitrophenylphosphate (20).

### Statistical Analysis

The results are given as the mean and standard error of the mean. Comparisons between groups are assessed using Student's t-test. The data were also analysed for correlations between the serum zinc and alkaline phosphatase activity in all diseases.

(The number of samples used in this statistical analysis was 15 from each disease). The results were considered significant when $P < 0.05$.

## Results

Glucose concentrations were significantly higher in IDDM ($P < 0.001$) and liver cirrhosis patients ($P < 0.05$) compared to the control group. Serum zinc concentrations in all different diseases were lower than those in the control group, but were not significant. Serum alkaline phosphatase activity was higher in IDDM ($P < 0.05$) and liver cirrhosis patients ($P < 0.01$) compared with the controls (Table). The data were analysed to see if any correlation could be found between serum zinc and alkaline phosphatase activity in all diseases: diabetes ($r = 0.252$), liver cirrhosis ($r = 0.387$), and chronic renal failure ($r = 0.266$). According to the results reported in the Figure, a negative correlation between serum zinc and alkaline phosphatase activity was established.

## Discussion

Zinc is essential for the activity of more than 100 enzymes that participate in the major metabolic pathways. Over 40 metallo-enzymes exist in which zinc is bound to the apoenzyme in specific stoichiometric ratios, where it serves as one or more structural, regulatory or catalytic functions (1). A number of studies have indicated that changes in the concentration of zinc in tissues and body fluids follow the course of some diseases such as diabetes, liver cirrhosis, chronic renal failure and gastro-intestinal disorders, according to the relationships between zinc and alkaline phosphatase and the effect of the diseases mentioned above. The present investigation reveals the net effect of these diseases on alkaline phosphatase activity. In this experiment, serum glucose concentrations in patients with IDDM are much higher than those of the controls. This confirms the diabetic state. Serum glucose concentrations of liver cirrhosis

Table. Glucose (mg/100ml), serum zinc (µg/100 ml) and alkaline phosphatase activity (UI/l)in patients with liver cirrhosis, diabetes mellitus (IDDM) type I, chronic renal failure and controls.

| Sample | | Glucose concentration | | Serum Zinc | | Alkaline phosphatase | |
|---|---|---|---|---|---|---|---|
| | | Mean ± SE | | Mean ± SE | | Mean ± SE | |
| Control group | (n = 42) | 80 | 8 | 81 | 3.7 | 79 | 4 |
| Liver cirrhosis | (n = 32) | 132* | 10 | 61 | 14 | 152** | 10 |
| Diabetes mellitus | (n = 42) | 284*** | 14 | 77 | 2.4 | 117* | 14 |
| Chronic renal failure | (n = 30) | 88 | 15 | 68 | 12 | 87 | 8 |

* ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$): Significantly different from control;
n: number of samples.

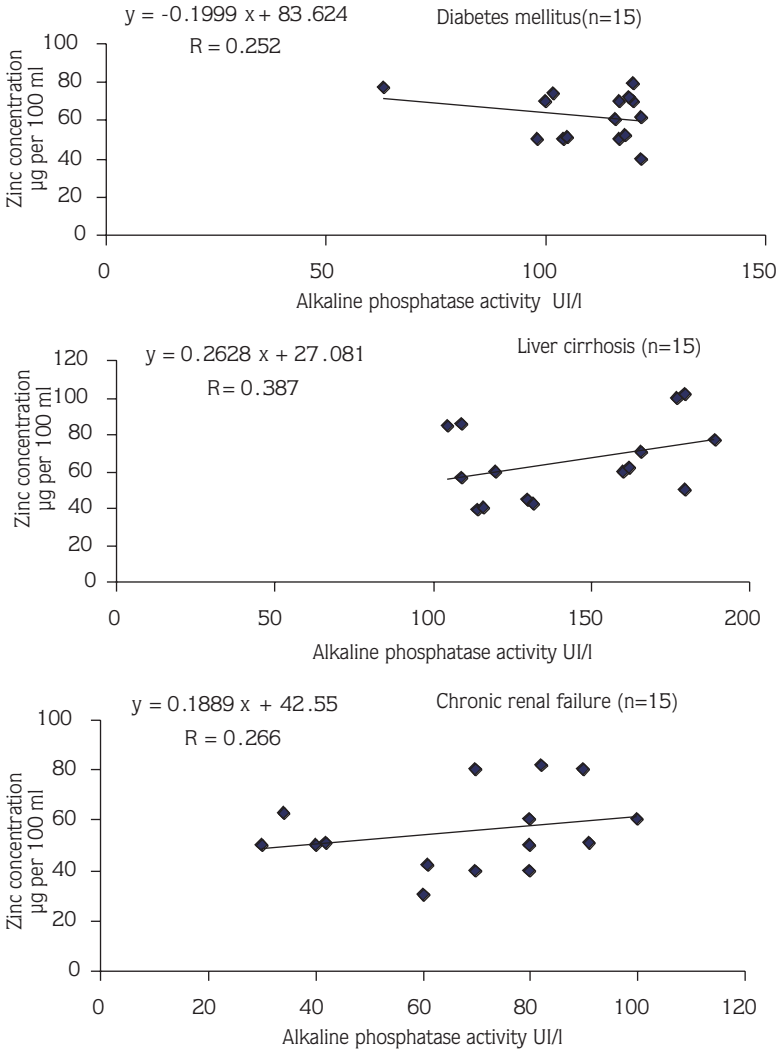


Figure. Showing the correlation between serum zinc and alkaline phosphatase activity in liver cirrhosis, diabetes mellitus (IDDM) and chronic renal failure, n is the number of samples (The number of samples used in this correlation was 15 from each disease).

patients were also higher than those of healthy people; this is likely related to liver dysfunction (21,22). There are non-significant differences in serum zinc concentrations between patients with all the different diseases and the control group. This result clearly demonstrated the ability of these patients to reduce zinc losses from the body. For example, they may have had decreased endogenous zinc secretion into the gastro-intestinal tract (23,24) or increased efficiency of absorption of zinc from their diet (25,26), or the patients had a high efficiency to retain zinc in their bodies (27). In our study, there was a significant rise in serum alkaline phosphatase activity in patients with IDDM and liver cirrhosis compared with the controls. The results are in total agreement with some previously published reports (2,28,29). The increase in serum alkaline activity in the diabetic state could be as a result of an increased call for energy through alkaline phosphatase activity rather than the glycolytic and oxidative pathway of glucose 6 phosphate, and in cirrhosis disease it might be attributed to the cytolysis and leakage out of necrotic or damaged cells in the liver. In addition, the statistical evaluation showed there was not a good correlation between serum zinc concentration and alkaline phosphatase activity.

In conclusion, these different diseases did not affect the level of serum zinc. There was also an alteration in alkaline phosphatase activity in patients with diabetes and liver cirrhosis. This may be due to the pathological changes which occurred in these diseases. However, there was in general no positive correlation between serum zinc and alkaline phophatase activity.

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*Corresponding author:*

*Department of Biochemistry,*

*Faculty of Sciences,*

*University of Annaba,*

*PO Box 12, Annaba 23000, ALGERIA.*

*E-mail: zinekechrid@yahoo.fr*

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