ALKALINE PHOSPHATASE ACTIVITY ASSOCIATED WITH SERUM ZINC , AND CALCIUM LEVELS IN PATIENTS WITH RENAL DISEASES

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Abstract

This study was done to investigate the relationship between serum Zinc(Zn) and Calcium(Ca) levels with Alkaline phosphatase(ALP) activity in patients with different renal diseases(renal stone, acute nephritis, chronic renal failure(CRF) undergoing hemodialysis(HD). Serum (Zn),(Ca) , urea and (ALP) activity were investigated in (38) patients with different renal diseases compared to control group (38 healthy subjects in Hilla teaching hospital . Serum (Zn) concentration tended to be lower in all patient groups , but not reach statistical significance($P>0.05$) in patients with renal stone and acute nephritis . (ALP) activity was low in patients with(CRF) undergoing (HD) ($P<0.05$) as compared with control. Serum (Ca) concentration was significantly low($P<0.05$) than control group in all patients with (HD), but was in the normal range in other groups(renal stone and acute nephritis). A positive correlation between serum (Zn) and (ALP) activity was recorded in patients with(CRF) undergoing (HD).

Statistically , we did not find any significant difference in serum (Zn), (Ca) and (ALP) levels between long- term and short term dialyzed patients($P>0.05$).

In Conclusion the investigated diseases (CRF) with (HD) affect the levels of serum (Zn) , (Ca)and (ALP). However there was an alteration in (ALP) activity in patients suffering from type diabetes
mellitus and liver cirrhosis consequently, this may be attributed to the functional disturbance that occurred in these patients.

Finally, (Zn) concentration follows (ALP) activity as indicated by a correlation obtained. We recommend that (Zn) and (Ca) should be given to patients with (CRF) undergoing (HD).

Introduction

The essential nature of trace elements is widely accepted. The indispensable feature of the most prominent of these trace elements rests on their role as functional or structural components of their crucial metalloenzymes and metalloprotein(1). Disorders of essential elements arise from inadequate intake, genetic defects , excessive exposure , or impaired elimination. Sever deficiency is rare , but specific symptoms and reduced protection against risk factors have been a described to suboptimal intake of trace elements(2,3).

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Zinc(Zn) is recognized as essential for the activity of a wide range of enzymes ,and first demonstration that zinc had a special biological function in relation to enzyme came with the discovery that carbonic anhydrase contained significant amount of (Zn) which appeared to be required for normal activity (4). Zinc participates in either the activation of enzyme systems forming a metalloenzyme complex or non specifically as a constituent of enzyme forming a metalloenzyme including lactic dehydrogenase , metate dehydrogenase , RNA polymerase , sorbitol dehydrogenase , alkaline phosphatase , and angiotensin(5,6,7). A reduction in the activity of a wide range of enzymes, which are dependent on this element for
Function. Carbonic anhydrase and alcohol dehdrogenase are two examples of enzymes that are particularly sensitive to reduction in tissue Zinc (Zn) concentration (8, 9).

Calcium (Ca) is the most abundant action in the body the principle mineral of the human skeleton. It has a key role in the composition of skeletal tissue and also involved in a series of regulatory process that are vital for life. Among the principle functions in which(Ca) participates are the maintenance and function of cell membranes , neuromuscular excitability, transmission for nerve impulses , enzymatic reactions , regulation of secretion of parathyroid hormone(10). The major regulator of extracellular(Ca) balance is parathyroid hormone.

The changes in the plasma or serum concentration of trace elements in patients with renal insufficiency depended on many factor. However the degree of renal insufficiency is an important determinant of the plasma or serum concentrations of trace elements. In Patients on renal replacement therapy(dialysis, transplantation) changes in concentration of trace elements in tissue may differ markedly from those found in plasma or serum . Such patients have a greater risk of developing toxicity or deficiency of trace elements, due to decreased excretion in the urine , concentration of the dialysate by trace elements due to decreased excretion in the urine, contamination of dialysate by trace element, or loss of trace elements during dialysis(11)Zinc depletion in human may occur as a result of unusual circumstances , which result in increase zinc loss from the body. These included excessive sweating(12), chronic blood loss, the development of disease states such diabetes(13,14), liver cirrhosis(15,16),chronic renal failure(17) and gastrointestinal disorders(18) and heart failure(19). On the basis of all this previous informations , the present study was undertaken to investigate the effect of renal disease on alkaline phosphatase activity in human associated with serum levels of zinc and calcium.

Materials and methods

The study was carried out in Hilla teaching hospital for period between(October 2004 – March 2005). The experimental protocol consist of :

Patients

Thirty – eight adults (26 men , 12 women) with different renal disease , with mean age (51.4 ± 6.1 yr), range (30 – 61 yr) consist of :

-Ten adults (8 men and 2 women) with renal stone disorders, the mean age for this patient group (40.3 ± 5.6 yr) (range 28 to 48 yr)

- Ten patients (6 men and 4 women) with acute nephritis and a mean age of (30 ± 4.1 yr) (range 22 – 60 yr).
- Eighteen patients (12 men and 6 women) who hemodialyzed patients (HD) and received chronic maintenance hemodialysis (approximately 4 hours per treatment, 3 times per week). The patients ranged in age from (25 to 65 yr) (mean age 32.5 ± 3.5 yr).
- The control group consist of 38 health subjects (25men and 13 women). The mean age of those in the control group (40 ± 6.8 yr).

The study subjects were all in the same socioeconomic class and had similar nutritional habits. Medical history was obtained from each subjects regarding any other disease and complication. Those with sever illness and acute medical events as well as a drinkers of alcohol , were excluded.

Methods

Venous blood was collected in the morning after an overnight fast from patients and controls for measurements of serum Zn , Ca , ALP and urea levels.

Analysis

Zn concentration was measured in duplicate after (1:5) (v/v) dilution of serum in double distilled water by flame atomic absorption spectrophotometer(Pye Unicome SP 2900 at 218 nm) (20).Ca concentration was measured by Electrolytmeter. Urea concentration was measured by enzymatic method using a kit from Rondox- France)(21). ALP activity was determined using commercial kit comprising 1 mol/L diethanolamine. Hcl buffer pH 9.8, 0.5 mmol/L magnesium chlorid and 10 mmol/L of substrate P- nitro phenyl phosphate (22)

Statistical analysis

The results are given as mean ± SD (standard deviation). Comparison between groups are assessed using students (t – test). The data were also analyzed for correlations between serum Zn, Ca, and ALP activity in patients with renal disease P- value ($P < 0.05$) was considered to be statistically significant

Results

Urea concentrations were significantly higher in patients with different renal diseases ($P<0.05$) as compared with control group. *Table (1)*There were no significant differences in age between patients groups and controls. Serum Zn concentrations in HD patients were lower than those in control group ($P <0.05$), but non- significant change will be show in other patient groups(acute nephritis , renal stone) . Also serum Ca

concentrations were lower in HD patients than in control group *Table (2)*.Serum ALP activity was significantly lower (P<0.05) in HD patients than in the control group. *Table(2)*. The data were analyzed to see if any correlation could be found between serum Zn , Ca and ALP activity in patients with renal stone , acute nephritis, and CRF undergoing HD (r= -0. 44, - 0.61 , 0.25) respectively , *Fig (1)*. According to the results reported in this figure a significant positive correlation (P<0.05) between serum Zn and ALP activity was established in HD group.

Table (3) Shows mean serum Zn , Ca ,and ALP in both HD and control groups in relation to other diseases and complications . The result was significant in case of diabetes mellitus and non significant in liver cirrhosis . *Table (4)* Shows serum Zn, Ca , and ALP levels according to HD duration . 12 patients(had been on hemodialysis < 9 month . 6 patients (had been on hemodialysis for between (9 to 60 month). The mean HD duration was 12.4 month. There was no significant difference in serum Zn , Ca and ALP levels among patients based on dialysis duration(P>0.05).

Table (1) Clinical characteristics of the patients with renal disease (n =38) and of the control group (n= 38) expressed as the mean ± SD. *(P<0.05) significantly different from control.

Characteristics	Control n=38	Renal stone n= 10	Acute nephritis n= 10	CRF n= 18
Age (yr)				
Mean	40 ± 8.7	40.3 ± 5.6	30 ± 4.1	32.5 ± 3.5
Range	18 – 67	28 – 48	22 - 60	25 – 65
Urea(mg/dl)	40 ± 6.1	83.1 ± 22.1*	90.5 ± 37.5*	173 ± 53.4*

Table (2) Serum ZN, Ca, ALP levels of renal diseases patients (n= 38) and of the control group (n = 38) expressed as the mean ± SD. *(P<0.05) significantly different from control.

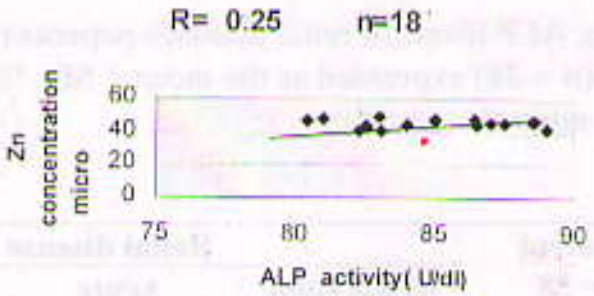
Parameters	Control n= 38	Renal disease		
		Renal stone n= 10	Acute nephritis n= 10	CRF n= 18
Zn(µg/dl)	71.9± 24.7	58.51±24.1	54. 41±18.57	45.04±25.7*
Ca(mmol/L)	2.28±0.20	1.95±0.18	1.75±0.14	84.8±26.65*
ALP(U/L)	122.28±41.8	197.3± 48.1	102.36±37.8	84.8± 26.3*

Table(3)Serum Zn, Ca, and ALP levels of HD by hemodialysis duration expressed as the mean ± SD.

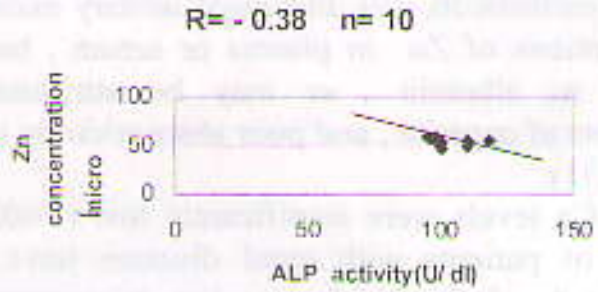
Dialysis duration(month)	Zn (µg/dl)	Ca (mmol/L)	ALP (U/L)
<9 month(n=12)	43.4 ± 8.4	0.76 ± 0.3	87.3 ± 21.3
≥9 month(n= 6)	41.5 ± 6.1	0.73 ± 0.21	85.2 ± 18.4

Table (4) Patients diseases and complications in relation to Zn, Ca, ALP levels *(P<0.05) significantly different from control.

Diseases	Zn(µg/dl)	Ca(mmol/L)	ALP(U/L)
Type 2 diabetes mellitus(n= 6)	70.8 ± 24.8	0.94 ± 0.4	132.3 ± 44.1*
Liver cirrhosis n= 4	61.3 ± 15.7	1.8 ± 0.2	134 ± 50.3*
Without any complications or other disease (n = 22)	75.1 ± 20.2	2.0 ± 0.11	100.5 ± 34.5



(a)



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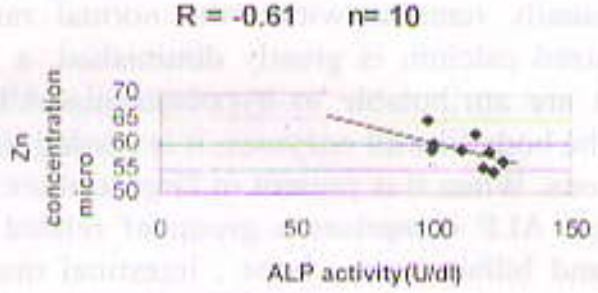

(c)

Fig(1) Showing the correlation between Zn concentration and ALP activity in groups:(a) CRF undergoing HD ; n=18, (b) acute nephritis; n=10 and (c) renal stone ; n=10 .

Discussion

Zn is essential for the activity of more 100 enzymes that participate in the major metabolic pathways. Over 40 metalloenzymes exist in which zinc is bound to the apoenzyme in specific stiochiometric ratios, where it serves one or more structural, regulatory or catalytic function(4). A number of studies have indicated that changes in the concentration of zinc in tissues and body fluids follow the course of some disease such as diabetes , liver cirrhosis , chronic renal failure and gastrointestinal 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 renal diseases on alkaline phosphatase activity. In this experiment serum urea levels in patients with renal diseases are higher than those of controls. This provide a crude measurement of the decrease in glomerular filtration rate(GFR). Serum Zn concentration was low ($P<0.05$) in HD patients than those in the control. This confirm the decline plasma levels of Zn which have been reported in patients with renal insufficiency and in patients with end – stage renal diseases undergoing HD or continuous ambulatory peritoneal

dialysis(CAPD)(23,24,25) . However, other authors have reported normal or elevated levels of Zn in uremic patients(26, 27). Increased urinary excretion of Zn may account for the lower concentrations of Zn in plasma or serum , because of decrease in Zn binding protein such as albumin , or may be attributed to protein – calory malnutrition(28,29). Loss of appetite , and poor absorption by intestinocyts may also be contributing factors(30,31).

In our study , serum Ca levels were significantly low ($P<0.05$) in HD patients than control. The majority of patients with renal diseases have hypocalcaemia , which accounts in part for the development of secondary hyperparathyoidism(11). The most common cause of hypocalcaemia are renal failure , endocrine disorders, nutritional and metabolic disorders (32).In addition to clinical syndromes characterize by either a deficiency of parathyroid hormone or an end organ resistance to parathyroid hormone. hypocalcaemia is also observed in uremia , malabsorption , acute pancreatitis , hypoalbuminemic conditions such as nephrotic syndrome and cirrhosis are mild , since the ionized calcium usually remains within the normal range. However, in those condition in which ionized calcium is greatly diminished, a series of symptoms may frequently be seen that are attributable to hypocalcemia(.Alkaline phosphatase is an enzyme found thought the body like all enzymes, it is needed in small amounts to trigger specific chemical reactions. When it is present in large amounts , it may signify bone or liver disease or a tumor. ALP comprises a group of related enzymes found in high concentration in liver and biliary tract , bone , intestinal mucus and placenta(33). A decreased serum ALP may be due to Zn deficiency, hypothyroidism , Vit – C deficiency , folic acid deficiency , Vit- D deficiency, low phosphorus levels, malnutrition with low protein assimilation and insufficiency parathyroid gland function(34). This is in a agreement with our study , which show a positive correlation between serum Zn level and ALP activity, this suggested low levels of Zn account of a decreased in ALP activity due to Zn participates in the activation of enzyme systems forming a metallo enzyme complex or non – specific as constituent of enzyme forming a metallo enzyme including lactic dehydrogenase , RNA polymerase , ALP(4,6), so a reduction of Zn concentration may lead to a reduction in the activity of these enzymes which are dependent on this element for their function . There are non - significant differences in serum Zn concentrations between patients with all the different (type 2 diabetes mellitus , liver cirrhosis) and control group. This results demonstrated the ability of these patients to reduce Zn loses from the body . For example they may have had decreased endogenous Zn section into the gastro intestinal tract(36) or increased efficiency of absorption of Zn from their diet(37)or the patients had a high efficiency to retain Zn in their bodies (38). In our study , there was a significant rise in serum ALP activity in patients with type 2 diabetes mellitus and liver cirrhosis compared with control group. The results are in total agreement with some previously published reports (39). The increase in ALP activity in the diabetic state could as a result of an increased call for energy through ALP activity rather than glycolytic and oxidative pathway of glucose – 6 – phosphate and in cirrhosis it might be attributed to the cytolysis and leakage out of necrotic or damage cell in the liver(40).

Conclusion

In patients with CRF undergoing HD there were a significant decrease in Zn and Ca levels. There was also a significant decreased in ALP activity. However, there was a positive correlation between serum Zn and ALP activity. Diseases like type 2 diabetes mellitus and liver cirrhosis did not affect the level of serum Zn or Ca , but there was also alteration in ALP activity in patients with these diseases. This may be due to pathological changes which occurred in these diseases. Zn and Ca supplementation recommended for patients with CRF undergoing HD who proven Zn deficiency.

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