Evaluation of Serum Zinc Status and Serum Alkaline Phosphatase Activity in Alcoholic Liver Disease Patients - A Hospital Based Study from Chennai, Tamil Nadu

Uma T.[1], Nirmaladevi P.[2], Shanthi R.[3], Mahalakshmi R.[4]

[1, 2] Department of Biochemistry, Madras Medical College, Chennai, Tamil Nadu India.
[3]Department of Biochemistry, Government Omandurar Medical College, Chennai, Tamil Nadu, India. [4]Department of Biochemistry, Stanley Medical College, Chennai, Tamil Nadu, India.

ABSTRACT

BACKGROUND

Alcoholism remains to be the major cause of morbidity and mortality throughout the world. Consuming alcohol is the potent etiological factor for the development of alcoholic liver diseases (ALD), ranging from fatty liver to hepatocellular carcinoma with varying rates of development in both genders depending on the quality, quantity, and duration of the drink. Zinc deficiency has been documented with the progression of alcoholic liver disease. It is also a well-known fact that zinc is a co-factor for enzyme alkaline phosphatase. This study aims to assess the zinc status and alkaline phosphatase activity in patients with various stages of alcoholic liver disease, correlate zinc with alkaline phosphatase activity, albumin, gamma glutamyl transferase activity, MELD score and duration of alcohol intake and analysing the need for evaluating zinc in these patients.

METHODS

This comparative observational study involves group I healthy controls and group II patients diagnosed to have ethanol related decompensated liver disease with or without portal hypertension for more than three years from the Department of Medical Gastroenterology, Government Medical College Hospital. 5 ml of venous blood in fasting state was collected from both groups and assayed for serum zinc, and serum alkaline phosphatase activity. The data was statistically analysed.

RESULTS

The study results demonstrate that higher percentage of patients with alcoholic liver disease have low serum zinc levels than healthy controls. Zinc when compared with variables like serum albumin, duration of alcohol intake, MELD score, serum gamma glutamyl transferase and alkaline phosphatase in the case and control groups were found to be statistically significant.

CONCLUSIONS

There is decrease in serum zinc level and increased alkaline phosphatase activity in patients with alcoholic liver disease. The statistically significant data is a strong rationale for evaluating the zinc status and thereby supplementing zinc to patients with alcoholic liver disease.

KEYWORDS

Alcoholic Liver Disease, Zinc, Alkaline Phosphatase, MELD Score

Corresponding Author:
Dr. Uma T.,
 # 59/143-P, Bangaru Street,
Near Sayani Complex,
Ayanavaram, Chennai - 600023,
Tamil Nadu, India.
E-mail: bhas_2001in@yahoo.com

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BACKGROUND

Alcohol consumed by all strata of society remains to be a major cause of morbidity and mortality worldwide. Unquestionably ethanol is a hepatotoxic compound that leads to serious forms of alcoholic liver disease. Alcohol acts as a potential hepatotoxin for the disease development depending on the existence of cofactors like gender, polymorphism of alcohol metabolizing enzymes, immunity, infection, nutrition, and drug status. Alcohol attributable injuries and negative impacts are now becoming the major concern to the health of the public. Chronic alcoholism results in changes of the intestinal epithelial barrier, increase in pro-inflammatory cytokine production. The micro tubular function is disturbed due to the binding of acetaldehyde to alpha – tubulin. This leads to fat accumulation in Golgi apparatus of perivenular hepatocytes. There is down regulation of microsomal triglyceride transfer protein (MTP) which does packaging of TAG (Triacylglycerols) and apo B into VLDL. Acetaldehyde inhibits PPAR – ALPHA a transcription factor, which regulates mitochondrial, microsomal and peroxisomal fatty acid oxidation systems in liver so that there is increase in free fatty acids in liver. The overall effect of alcohol metabolism is the altered redox state, the increase in NADH / NAD ratio which impairs gluconeogenesis, decrease in the substrate flow to TCA cycle, inhibition of fatty acid oxidation and increase in TAG synthesis and generation of reactive oxygen species which are the key factors in mediating alcoholic liver disease.[1]

Zinc deficiency is one of the most consistent nutritional/biochemical observations in alcoholic liver disease. Zinc is an essential trace element involved in various biological functions. It serves as a catalytic cofactor for hundreds of enzymes like alkaline phosphatase and alcohol dehydrogenase. Zinc is also required for stabilizing the zinc figure motif of a large number of zinc proteins, including critical transcription factors. Several mechanisms underlying alcohol-induced zinc deficiency have been suggested, including reduced dietary zinc intake and intestinal absorption, disturbed hepatic zinc metabolism, and increased urinary zinc excretion. Generation of reactive oxygen species in association with alcohol metabolism not only alters the expression of zinc transporters, but also releases zinc from zinc proteins. Dietary zinc supplementation has been shown to prevent and also reverse alcohol-induced liver injury in animal models and clinical studies. The beneficial effects of zinc are achieved by both hepatic actions and extrahepatic actions. Inhibition of oxidative stress and restoration of alcohol-inactivated zinc finger transcription factors, hepatocyte nuclear factor 4α and peroxisome proliferation activator α, represent important molecular mechanisms of zinc actions. Most of the plasma zinc is bound to serum albumin (75 - 85 %). As albumin level decreases in liver disease there is a decline in the level of serum zinc.

Role of zinc in liver function is significant and in liver disease there is decrease levels of serum zinc, particularly in alcoholic liver disease with increased loss of urinary zinc. Zinc suppresses alcohol induced oxidative stress, apoptosis of hepatocytes and TNF – alpha production. Intestinal integrity is maintained by zinc. Supplementation of zinc also attenuates fibrotic changes.

The documentation of Zinc deficiency in alcoholic liver disease also correlates well with the progression of liver disease.[2] It is also a well-known fact that zinc is a co-factor for enzyme alkaline phosphatase and alcohol dehydrogenase.[3,4]

Alkaline phosphatase is group of enzymes that take part in hydrolysis of phosphates at higher pH. Contribution of this enzyme is mostly from liver, bone, placenta, least from intestinal epithelium and kidney. Hepatic alkaline phosphatase is most densely represented near the canalicular membrane of the hepatocyte. An experimental study shows one of the functions of enzyme alkaline phosphatase in liver cell membrane is to hydrolyse phosphorylcholine and so that choline gets across the canalicular membrane into the bile.

When cholestasis happens due to intrahepatic origin, bile secretion from hepatocyte to canaliculi is impeded which leads to regurgitation of the enzyme into plasma which is reflected as increased activity.

Increased activity of alkaline phosphatase with increased gamma glutamyl transferase enzyme in serum always reflects the etiology of hepatobiliary origin

Accordingly, diseases that predominantly affect hepatocyte secretion (e.g., obstructive diseases) will be accompanied by elevations of alkaline phosphatase levels. Bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis (PBC) are some examples of diseases in which elevated alkaline phosphatase levels are often predominant over transaminase level elevations.[5]

Chronic alcoholism, one of the etiological factors for chronic liver failure leads to multi organ dysfunction and early death. Many studies have showed increased morbidity and mortality in patients with chronic liver failure undergoing surgical or interventional procedures. In view of categorizing and predicting the outcome of such procedures various scoring systems were devised. Of which Child Pugh (CP) classification was more prevalently used. The parameters used were serum bilirubin, prothrombin time, serum albumin with two more clinical parameters like encephalopathy and ascites. Points were given for each index and based on the cumulating points of risk, stratification is done. Even though Child Pugh classification is useful in cirrhotic patient undergoing medical management and as well predicting postoperative outcome it has been not sensitive in predicting short term outcome of morbidity (within 30 days) in cirrhotic individuals.

To overcome this deficit in scoring system MELD (Model for End-stage Liver disease)[6] score was introduced. Initially it was used to predict morbidity outcome in patients with cirrhosis undergoing TIPS (Trans Jugular Intra Hepatic Porto systemic Shunt) procedure. Three easily measurable values i.e., serum bilirubin, International Normalised Ratio and serum creatinine are used. These values are entered in a formula and MELD score arrived. Later MELD score was extended to measure mortality risk in hospitalized and ambulatory patients with cirrhosis. Its usefulness appears due to scoring irrespective of underlying disease etiology. In 2002 UNOS (United Network of Organ sharing) has

introduced a modified MELD scoring system for organ allocation in patients with liver failure awaiting liver transplant. MELD scoring is done for patients with age 12 and above. For patients with age less than 12 PELD score is used.

METHODS

This is a comparative observational study conducted for a period of six months, April to September 2016. Prior approval was obtained from the Institutions Ethical Committee. The study involves two groups, Group – I with 50 healthy members as control, recruited from master health checkup at a Government Medical College.

Group – II with 50 cases of pre-diagnosed alcoholic liver disease with a duration of more than 3 years from Medical Gastroenterology Department, Government Medical College Hospital. Written consent with explained protocol was obtained from both groups.

Inclusion Criteria

Patients diagnosed to have ethanol related decompensated liver disease with or without portal hypertension more than three years.

Exclusion Criteria

Patient with non-alcoholic liver disease, Patients on zinc supplementation, Patients with malabsorption syndromes, Patients who had undergone intestinal resection procedures, Patients with co-morbid conditions like diabetes mellitus, malignancy, renal failure, known viral etiology and on treatment with steroids and hormones.

Sample Collection and Preparation

Laboratory Assessment of Zinc

Sample recommended is serum or plasma 5 ml of venous blood from ante-cubital vein (overnight fasting sample) was collected under strict aseptic precautions. Serum samples were separated by centrifugation at 2000 -2500 rpm for I5 minutes and used for analysis. Samples were stored at - 20 ^{0}C until analysis.

Estimation of Zinc, creatinine and Liver function tests for cases and controls were performed in Beckman coulter, the fully automated random access clinical chemistry analyzer.

A reference interval for serum zinc is 80 -120 µg / dl or 12 -18 µmol / L. For urine zinc 0.2 to 1.3 mg / 24 h.

Circadian changes include high values in the mornings than evening and post prandial decrease. Prothrombin time by clot-based assay was performed in fully automated coagulation analyzer. The maximum serum creatinine value in MELD score was set to 4.0 and values automatically interpreted according to recent dialysis. The MELD score calculated as follows:

MELD Score = 0.957 x Log e (creatinine mg / dL) + 0.378 x Log e (bilirubin mg / dL) + 1.120 x Log e (INR) + 0.6431

the score multiply by 10 and round to the nearest whole number. Laboratory values less than 1.0 were set to 1.0 for the purposes of the MELD score calculation.

Statistical Analysis

We enrolled in a total number of 100 subjects. The sample size for the study was calculated with acceptable level of significance ($p < 0.05$) and keeping the power of study at 95 %. The data was collected tabulated and analyzed. The collected data were analysed with IBM.SPSS statistics software 23.0 Version. Armonk, NY : IBM Corp. To describe about the data descriptive statistics mean & S.D were used. To find the significant difference between the bivariate samples in Independent groups the Mann-Whitney U test was used. In the above statistical tool the probability value 0.05 is considered as significant level. Comparison of mean values of zinc and alkaline phosphatase were done. Pearson correlation analysis was done to find out the relationship among different variables. Graphical representation was done using scatter diagrams.

RESULTS

Demographic data was compared between the groups, the cases, and controls. The distribution of age among Group I & II analysed and p value was > 0.05. Hence the difference between the two groups was not statistically significant.

Variable	Mean ± S.D.		P Value
	Cases	Control	
T. Bilirubin	16 ± 7.4	0.5 ± 0.2	p < 0.005
Albumin	2.3 ± 0.8	4.8 ± 6.2	p < 0.001
Globulin	3.2 ± 0.8	2.5 ± 0.4	p < 0.005
AST	160 ± 55.4	18.6 ± 11.1	p < 0.005
ALT	71 ± 62	10.8 ± 5.8	p < 0.005
GGT	130.2 ± 112	9.6 ± 5.4	p < 0.005
Alk. Phosphatase	234 ± 191.6	89.2 ± 28.4	p < 0.005
Creatinine	1.2 ± 1.02	0.7 ± 0.1	p < 0.005
INR	1.4 ± 0.4	0.9 ± 0.06	p < 0.005
Prothrombin time	17.5 ± 3.9	11.2 ± 0.6	p < 0.005
Zinc	47.5 ± 19.7	98.8 ± 26	p < 0.005

Table 1. Mean and Standard Deviation of Variables in Groups

In table 1, biochemical parameters were compared between the case and control groups using Mann-whitney U test and found to be statistically significant.

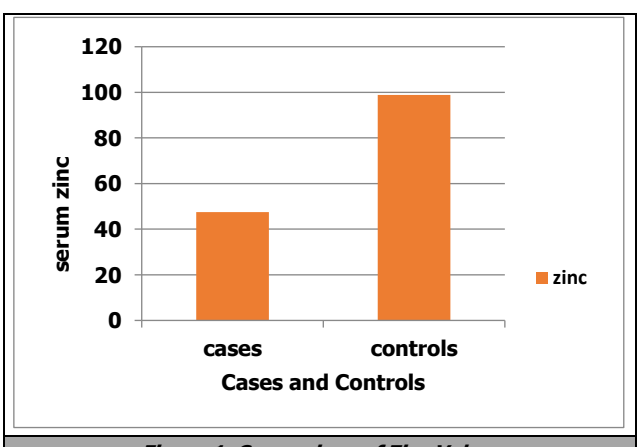

Figure 1. Comparison of Zinc Values between Cases and Controls

Serum zinc in µg / dl, was statistically significant with a p – value of less than 0.005 between the cases and control group. Zinc when compared with variables like serum albumin, duration of alcohol intake, MELD score, serum gamma glutamyl transferase and alkaline phosphatase in the case and control groups were found to have a p - value of less than 0.05 and was statistically significant. Pearsons correlation coefficient was used to find out the correlation between variables. Serum zinc values had negative correlation with duration of alcohol intake, MELD score and serum gamma glutamyl transferase while serum zinc values had positive correlation with serum albumin and significant and negative correlation with serum alkaline phosphatase.

Variables	Pearson's Correlation Coefficient (r)	Significance (p)	Interpretation
Zinc vs Albumin	0.174	<0.05	Significant & positive correlation
Zinc vs Duration of alcohol intake	-0.603	< 0.05	Significant & negative correlation
Zinc vs MELD	-0.185	< 0.05	Significant & negative correlation
Zinc vs GGT	-0.301	<0.05	Significant & negative correlation
Zinc vs Alkaline phosphatase	-0.06	<0.05	Significant

Table 2. Correlation of Serum Zinc with Albumin, Duration of Alcohol Intake, MELD Score, Gamma Glutamyl Transferase and Alkaline Phosphatase in Alcoholic Liver Disease Patients

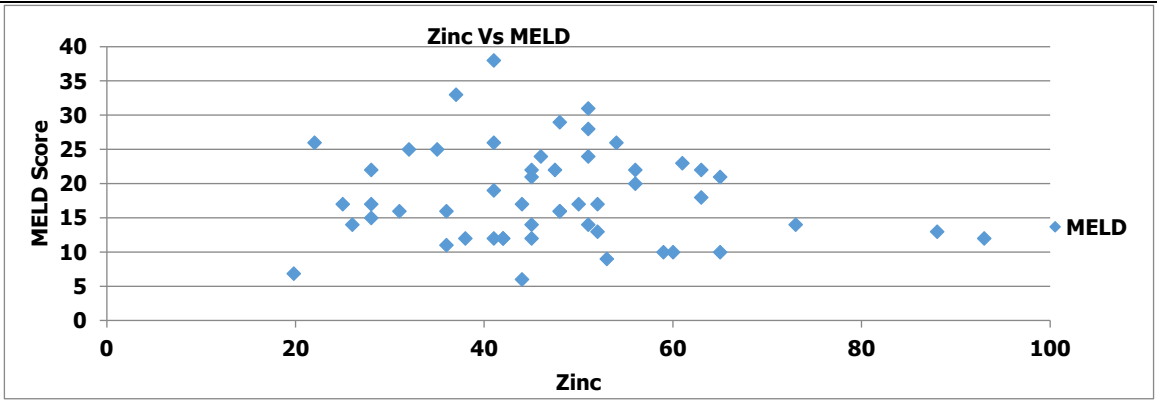

Figure 2. Relationship between Zinc Values and MELD Score

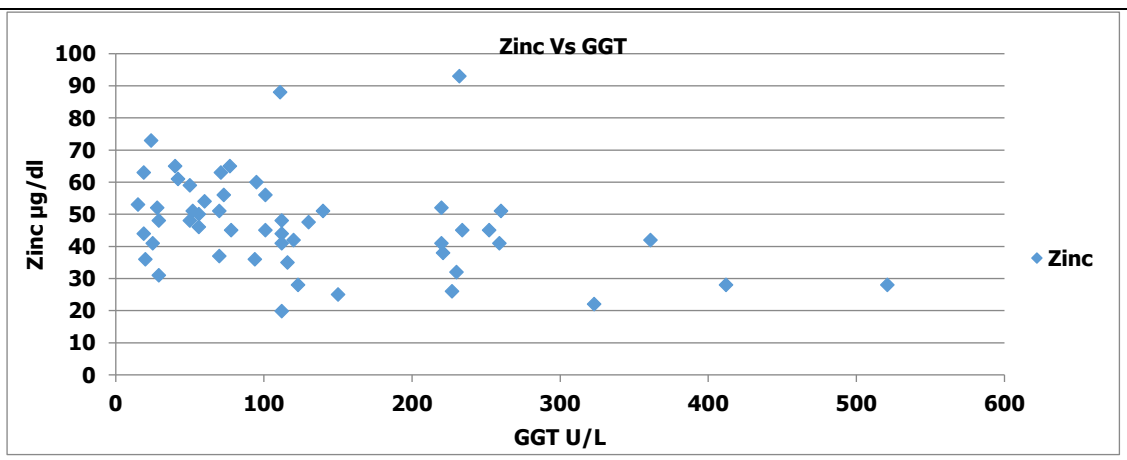

Figure 3. Relationship between Zinc Values and GGT

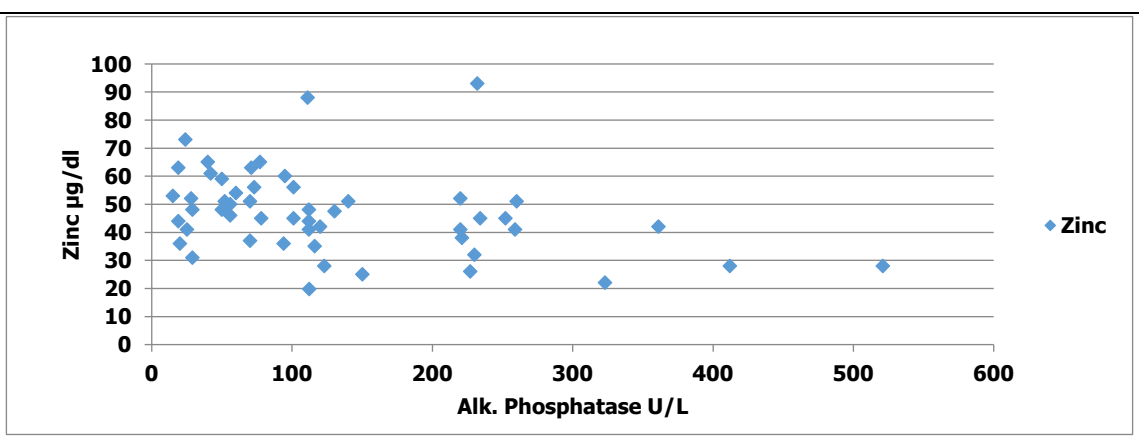

Figure 4. Relationship between Zinc Values and Alkaline Phosphatase

Variables	Correlation coefficient (r)	Significance (p) value	Interpretation
MELD Vs Duration of alcohol	0.275	< 0.05	Significant & positive correlation

Table 3. Correlation of MELD and Duration of Alcohol in Alcoholic Liver Disease Patients

MELD score was compared with the duration of alcohol intake in the cases and control groups and was found to be statistically significant and there was a positive correlation between the two.

DISCUSSION

The present study results demonstrate that higher percentage of patients with alcoholic liver disease have low serum zinc levels compared to normal subjects. Ferdousi et al[8] has evaluated the zinc status in patient with liver cirrhosis and has shown significant lower plasma zinc levels. A study by Fatia et al[7] also showed data with low serum zinc level which was very significant. Low levels of zinc in alcoholic liver diseases can be attributed to albumin bound zinc fraction, which is decreased in liver disease, the anorectic effect of alcohol which decreases the zinc uptake and also the diuretic effect of alcohol which can cause increase loss of zinc in urine. According to Ana CR Schneider et al[8] there is an observation of decreased plasma zinc even in paediatric patients with cirrhosis.

In the present study comparison between the parameters of liver function tests like albumin, globulin, show uniform significance between cases and controls. Azam et al[9.10] has analysed various enzyme panel in liver disease and has found increase of the liver enzymes in various stages of liver disease.

In our present study there is a significant increase in the levels of GGT and ALP among cases. This increase can be related to chronic alcoholism and obstruction. According to RpPerillo et al[11] patients who consume alcohol for longer duration and present with alcoholic liver disease had marked elevation of serum alkaline phosphatase. It is observed from this study that intrahepatic cholestasis occurs secondary to alcoholic cirrhosis. In the present study there is an increased alkaline phosphatase level in spite of hypozincemia among cases and this can be due to obstruction in the later stages of cirrhotic liver. Weismann et al[12] has analysed ALP status in zinc deficient patients of acrodermatititis enteropathica and elderly since zinc is the cofactor for ALP and has found ALP levels decreased when zinc levels went down. The karl Pearson's correlation coefficient between serum zinc and alkaline phosphatase showed negative association and trivial correlation. A study by JM Pekarthy et al[13] discusses the function of alkaline phosphatase and the increase of the enzyme in obstruction. Few cases show abnormal coagulation parameters like prothrombin and INR. In the study there is a decrease in serum albumin levels among cases when compared to controls and this is explained by the derangement of synthetic functions in chronic liver disease. Creatinine analysed in cases and controls also showed significant p value.

A significant and negative correlation was found between zinc and duration of the alcohol intake. A significant and negative correlation was seen between zinc and MELD score.

This explains that the decrease in the trace element zinc is related to the duration of alcohol intake and progression of the disease. A significant and positive correlation was found between serum zinc and serum albumin levels. The study by Kaushik Kar et al[14] has analysed the same and had stated a significant p value < 0.001. This attributes to the bound nature of albumin and zinc. A significant and positive correlation was analysed between duration of alcohol intake and MELD score. This clearly shows the contribution of alcohol as a hepatotoxic agent to worsen the condition of the patient.[15]

Further study with larger sample size, estimation of urinary levels of zinc, tissue zinc levels, use of gold standard AAS for estimation of zinc are needed to overcome the limitations of the study and exactly evaluate the status of zinc in these patients and trial with supplementation of zinc to bring down the severity of the disease.

CONCLUSIONS

There is decrease in serum zinc level and increased alkaline phosphatase activity in patients with alcoholic liver disease. Zinc deficiency is seen in patients with increased MELD score. The statistically significant data of correlating zinc with variables like albumin, duration of alcohol, liver enzymes provide us a strong rationale for evaluating the zinc status and thereby supplementing zinc to patients with alcoholic liver disease.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
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