Association of serum zinc with markers of liver injury in very heavy drinking alcohol dependent patients

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Abstract

Zinc deficiency is a frequent complication of alcohol abuse for multiple reasons including poor intake, increased excretion, internal redistribution, and altered transporters. Zinc deficiency has been postulated to play a role in the development/progression of alcoholic liver disease (ALD). This study aimed to relate serum zinc levels with alcohol intake, serum albumin concentration, and markers of inflammation and liver injury. One hundred and eight male and female very heavy drinking (10 drinks/day) individuals without clinical evidence of alcoholic liver disease (ALD)

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were grouped by serum zinc concentration: normal zinc group (zinc level > 71 mcg/dL) included 67 patients, and low zinc group (zinc level <71 mcg/dL) included 41 patients. Data were collected on demographics, drinking history in last 90 days (heavy drinking days, HDD90 and total drinks, TD90) & Lifetime Drinking history (LTDH), and clinical/laboratory assessments. Our data show that in a very well-characterized, chronically heavy-drinking population without clinical evidence of liver disease, about 40% of subjects had low serum zinc levels. Frequency of heavy drinking days (HDD90) was significantly higher in the low zinc group. Total drinks in past 90 days, LTDH, and HDD90 showed significant associations with low zinc levels. The group with the low serum zinc had a higher AST/ALT ratio (good marker of alcoholic liver disease). Those in the low-zinc group had the lower albumin levels, a marker of hepatic synthetic function, and the highest CRP level, a biomarker of inflammation.

1. Introduction

Alcoholic liver disease (ALD), a significant medical problem worldwide, manifests itself as a spectrum of disease, namely steatosis, inflammation, fibrosis, cirrhosis and, in some cases, hepatocellular carcinoma, and it has been attributed to heavy and chronic alcohol consumption [1, 2]. Several clinical events and molecular mechanisms participate in the onset of the ALD [3–5] and many of these mechanisms of liver injury depend upon nutritional status (including minerals) and nutrient interactions with chronic or heavy alcohol drinking [6–11].

Chronic alcohol intake can lead to the deficiency of several micronutrients including zinc [10, 12] that could contribute to ALD development and progression. This has been shown in experimental animals [13]. Reports on altered zinc metabolism in liver disease, linked with organ/tissue/cell metabolic dysfunction, such as abnormal dark adaptation in ALD, have been documented for over a half-century [14–17].

Several proteins synthesized in the liver could be affected by alcohol-induced liver injury. C-reactive protein (CRP) is an hepatic acute phase protein that increases with infections or inflammation [18]. Reductions in CRP levels have been observed with light and moderate alcohol drinking [19]; however the relation of CRP and heavy drinking has not been investigated thoroughly. Albumin is a major binding protein for zinc, and albumin levels usually decrease with liver disease due to decreased synthesis and/or vascular leakage with inflammation [20].

Drinking profile and nutrition are risk factors that are thought to be involved in ALD development and progression [21–24]. Heavy drinking is primarily estimated as an historical measure assessed by collecting drinking history [25], and can be estimated by the use of tests such as serum Carbohydrate-Deficient Transferrin (CDT) [26].

In this study, we evaluated the interactions of drinking patterns and serum zinc, albumin and CRP concentrations on the development of liver injury (as demonstrated by ALT, AST, AST:ALT ratio, and bilirubin levels) in a cohort of very heavy drinkers without evidence of clinical ALD.

2. Patients and Methods

2.1 Patient population and enrollment

This study was conducted under a larger protocol study approved by the Institutional Review Board of the NIAAA (ClinicalTrials.gov identifier # NCT00106106), and all patients signed Informed Consent documents prior to entering the study. One hundred and eight male and female patients, aged 21 – 65, were included in this study (Table 1). Patients had a diagnosis of alcohol dependence according to the DSM-IV, based on the alcohol dependence module of the SCID I-interview, and they had alcohol withdrawal symptoms. Patients were excluded from the study if they were diagnosed with severe psychiatric illness, were suicidal or violent, or had agitation requiring immediate clinical treatment. Patients with other clinically significant psychiatric illnesses (unless stable, and not requiring medication such as antidepressants, lithium, neuroleptics, naltrexone, acamprosate, disulfiram, benzodiazepines or antiepileptic compounds within the last four weeks) were also excluded. Illnesses such as advanced lung disease, unstable cardiovascular disease, renal failure (creatinine clearance < 30 ml/min), advanced liver disease (hepatocellular carcinoma, clinically evident alcoholic hepatitis, cirrhosis), or HIV were other exclusionary criteria. Day of assessment exclusionary criteria included: (1) pregnancy (negative test required) or ongoing breastfeeding; and/or (2) a positive urine screen for any illicit drug. Importantly, these patients had no clinical signs of alcoholic liver disease.

2.2 Specimen sampling and clinical data

On the day of evaluation, blood samples were collected for serum trace metals (including zinc), as well as a comprehensive metabolic chemistry panel (including albumin concentration). Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), AST:ALT ratio, and total bilirubin levels were used to assess liver injury. Samples were also assayed for the marker of inflammation, C-reactive protein (CRP), and carbohydrate deficient transferrin (CDT), a biomarker of alcohol intake.

Heavy drinking is defined as 15 drinks or more per week for males and eight drinks or more per week for females (https://www.cdc.gov/alcohol/faqs.htm). All patients in this study qualified as heavy drinkers per the Center of Disease Control recommendation. Time-line follow-back past 90 days (TLFB90) questionnaire [23] is a validated and well-established instrument to collect self-reported data on total number of drinks for each day in the past 90 days. Other recent drinking measures that were derived from the TLFB90 questionnaire include: Total drinks in the past 90 days (TD90), Number of drinking days in the past 90 days (NDD90), Drinks per drinking day in the past 90 Days (DPD90), Average drinks per drinking day in the past 90 days (AvgDD90), and Heavy drinking days in the past 90 Days (HDD90). We also used the lifetime drinking history (LTDH) questionnaire and the number

of years as other drinking measures of evaluation in this study (https://pubs.niaaa.nih.gov/publications/AssessingAlcohol/measures.htm).

The standard lower limit of normal for serum zinc is 71 mcg/dL. Patients were grouped by serum zinc levels, 71 mcg/dL as normal; and <71 mcg/dL as the zinc deficient/low group. To evaluate the relevance of the degree of zinc deficiency in the clinical determination of ALD, we further separated the low zinc patients into two sub-groups; borderline (61-70 mcg/dL) and deficient <61 mcg/dL). We used "Controlling Nutritional Status Test" (CONUT) data to establish nutritional status [27]. Patients did not show any clinical evidence of liver disease, and overt clinical liver disease was an exclusion criteria in the study.

2.3 Statistical Analysis

Two-way analysis of variance (ANOVA) was used to assess whether there were significant differences between males and females within each zinc group, as well as whether there were significant differences between the normal zinc and low zinc groups for each sex. One-way ANOVA was used to examine significant differences between the normal zinc and low zinc groups when sex was not a significant variable. Multiple regression models were used to examine which drinking markers were significantly associated with zinc levels, and which laboratory markers were associated with zinc level when controlling for drinking history. Step-down variable selection was applied to obtain a parsimonious model for interpretation. Drinking history measures were included as covariates, as applicable. SPSS 24.0 (IBM Chicago, IL) and statistical software R (https://www.r-project.org/) and Microsoft Excel 2016 (MS Corp, Redmond WA) were used for data analyses. A test was considered significant if p<0.05 was observed. Levels of markers are shown as Mean±SD in the tables and as Mean±SE in the figures.

3. Results

3.1 Patient description

An approximately equal number of male (n=21) and female (n=20) patients were in the low zinc group (41 patients); however, there were more males than females in the normal zinc group (67 patients). Mean age was significantly higher in low zinc group; in particular, the age for males in normal zinc group was significantly lower than for those in the low zinc group. BMI was not different between the two groups, nor between males and females (Table 1). We did not find any statistically significant differences between males and females in the demographic and drinking profiles for the patients within or across the groups. Patients were not clinically malnourished as assessed by the CONUT.

3.2 Association of serum zinc and drinking markers

HDD90, a measure of heavy drinking history over the previous 90 days, was significantly higher in the low zinc group. However, there were no significant differences between groups on other drinking measures (see Table 1). When we used a multiple regression model to examine which drinking markers were associated with zinc level, we found that drinking markers impacted zinc level differently in the low zinc group and the normal zinc group.

Step-down variable selection procedure was used, and NDD90 was the only drinking marker left in the model which was significantly associated with zinc level in both the low and normal zinc groups. We also found that NDD90 was highly correlated with HDD90 (correlation coefficient = 0.90), moderately correlated with TD90 (correlation coefficient = 0.56), and not correlated with LTDH (correlation coefficient = 0.08). In the low zinc group, serum zinc level was significantly associated with NDD90, HDD90, TD90, and LTDH (Fig. 1). On the other hand, in the normal zinc group, serum zinc was modestly, but significantly associated with NDD90 and HDD90, but not with TD90 or LTDH. Sex and BMI were not significantly associated with zinc level. There were no other significant associations between zinc and the TLFB drinking history marker or carbohydrate deficient transferrin (CDT) in either group.

3.3 Association of serum zinc and albumin

Serum albumin in the low zinc group was significantly lower than in the normal zinc group (Fig. 2a). When we evaluated the association of albumin with zinc, we found a highly significant association in the overall population as well as in the normal zinc group where both albumin and zinc were within the normal ranges, and a less robust association in the low zinc group (Fig. 2b-d). There was a significant lowering in albumin (p=0.027) in females compared to their male counterparts in the low zinc group. With LTDH as a covariate, the significance in this difference further augmented to p=0.014.

3.4 Association of serum zinc and C-reactive protein (CRP)

CRP was significantly elevated in the low zinc group (Table 2), and there was a significant association between serum zinc and CRP levels in the low zinc group (Fig. 3). However, no such association between serum zinc and CRP levels was found in the normal zinc group, even with adjusting for the drinking makers, HDD90 or NDD90 as independent variables. When we ran the analysis with log transformed data, the results were not changed. In the low zinc group, we further established the association between CRP and liver injury markers. CRP had a significant association with the AST:ALT ratio, adjusted R^2=0.216, p=0.001, and the association was augmented to an adjusted R^2=0.267, p=0.004 with HDD90 as an additional independent variable. Total bilirubin and CRP showed a significant association as well, adjusted R^2=0.229, p=0.001 in low zinc patients.

3.5 Evaluation of liver injury markers in relation to zinc and drinking profile

AST levels were numerically greater in the low zinc group than in the normal zinc group, but this did not reach statistical significance. However, when we evaluated the AST:ALT ratio (a robust marker of ALD), we found a significant elevation of this ratio in the low zinc group, p=0.016 (Fig. 4a). In a multiple regression analysis, AST, total bilirubin, and CRP together showed a highly significant main effect with zinc, p < 0.001.

We then evaluated the association of zinc and the AST:ALT ratio by zinc group, and we found that there was no significant association between AST:ALT and zinc in the normal zinc group. However, there was a significant association between zinc and the AST:ALT ratio (Fig. 4b) in the low zinc group, and that association became stronger when co-varied with NDD90. There was a significant association found between zinc level and total

bilirubin in the low zinc group (Fig. 4c), which was not observed in the normal zinc group. We further evaluated other markers of liver injury–the acute inflammatory marker, CRP, and drinking history markers–by the level of zinc deficiency (borderline low versus deficient [61-70 mcg/dL vs. <61 mcg/dL]) that might show augmented liver injury (Table 3). We found that there were statistically significantly higher AST:ALT ratio, higher CRP levels, and higher drinking history markers in the zinc deficient group vs. the borderline group (Table 3).

4. Discussion

In this study, we showed that 38% (41 subjects) of the 108 heavy drinking subjects admitted to a treatment program for alcohol use disorder (AUD) without clinical evidence of alcoholic hepatitis had low serum zinc concentrations. Drinking history profile has been validated and used extensively to understand the variability in alcohol intake by the rate and level of drinking; however correlations of drinking measures and zinc status have not been reported to date. TLFB90 has been shown to be a highly reliable measure to assess drinking history (high convergent validity), with responses from different groups of the drinking population (quantity-frequency) and various markers of drinking (grid measures) [28], as well as satisfactory reliability (consistency in responses over a longer period of time) [29]. HDD90, a measure of heavy drinking history, was significantly higher in the low zinc group. However, there were no significant between group differences in other drinking measures. This suggests that episodic heavy drinking (as assessed by the HDD90) may be important in the development of zinc deficiency. When zinc was in the normal range, markers of drinking history were either not significantly associated with zinc level, or their association was very weak. However, we showed significant associations between low serum zinc and specific markers of heavy alcohol intake, namely with LTDH, TD90, HDD90 and NDD90, and these results support the concept that specific patterns of drinking and their independent and collective presentation (primarily NDD90, HDD90) are associated with zinc deficiency.

A decrease in the serum zinc concentration is well documented after systemic inflammation or infection as part of the acute-phase response [30, 31]. During the acute-phase or stress response, zinc is redistributed to specific organs and cellular compartments, especially the liver, leading to a decrease in the serum zinc concentration [32]. CRP is an acute phase protein that is elevated with infection/inflammation. Interestingly, moderate alcohol consumption has been associated with a lowering of CRP [33, 34], suggesting that moderate drinking may have an anti-inflammatory effect. In this study, patients in the normal zinc group also had normal concentrations of CRP, and there was no association between zinc and CRP. However, in the low-zinc group, CRP concentrations were elevated, and there was a strong inverse correlation between elevated CRP levels and decreased serum zinc levels. This finding suggests that the decrease in the serum zinc concentration may be due, at least in part, to systemic inflammation and the acute-phase response.

Serum albumin is a negative acute-phase reactant, and serum albumin level decreases with acute inflammation [20]. Serum albumin is the major zinc binding protein in the blood. Two main mechanisms for hypoalbuminemia are decreased hepatic production of albumin and vascular leakage [35]. Zinc has been shown to attenuate vascular permeability in *in vitro*

studies [36]. We showed significant correlations between albumin and zinc in the total population (as has been also reported previously [37]), as well as in the normal zinc group in this study. The less robust correlation in the low zinc group could reflect a dissociation of zinc from serum albumin and redistribution of zinc due to inflammation in the acute-phase response. Females tended to have lower albumin levels compared to their male counterparts in the low zinc group.

Serum AST and ALT were numerically but not statistically higher in the low zinc group, but importantly, the AST:ALT ratio was significantly elevated in the low zinc group. The AST:ALT ratio of 2 is often used as a biomarker of alcoholic hepatitis [38]. In our study, the AST:ALT ratio may indicate the development/progression of liver injury and it has a relation to the level of serum zinc (Fig. 4a & 4b) in these heavy drinking patients. Increased liver injury in the low zinc group and an association between bilirubin and serum zinc is also shown in Fig. 4c with low serum zinc correlating strongly with increases in the serum bilirubin. These translational human data are consistent with animal studies showing that zinc deficiency occurs in early experimental ALD.

There are limitations to the study. While we had a moderately large study population of 108 patients, we could not identify sex-based differences between groups based on zinc levels. This may be due to inadequate sample size. Effect sizes in this study were mostly moderate, again indicative of the patient sample size in our study. This was not a longitudinal study and we could not correlate changes in zinc status with improvement or worsening of liver injury. Zinc status was only assessed by serum zinc levels, but this is the main indicator of zinc status used clinically. Our results clearly support the involvement of zinc in ALD development. Drinking history markers showed significant correlations, but with mild effect, suggesting that they may play an important role but that there may be other cofactors involved. These other cofactors could include metabolic factors, genetics, ethnicity, etc. Determining these cofactors was beyond the scope of this study.

In conclusion, this study showed that almost 40% of very heavy drinking alcohol-dependent patients had low serum zinc concentrations. The low-zinc group had more evidence of liver injury (AST, ALT, AST:ALT, bilirubin), lower serum albumin, and a strong inverse correlation between low zinc and C-reactive protein. To our knowledge, this is the first study evaluating zinc status in a large cohort of subjects with AUD who had very well characterized drinking histories and no clinical evidence for advanced liver disease. These data provide further support for a role of zinc deficiency in the early stages/development of human ALD.

Acknowledgments

ABBREVIATIONS

AD	Alcohol Dependent
ALD	Alcoholic Liver Disease
ALT	Alanine Aminotransferases
AST	Aspartate Aminotransferases
CDT	Carbohydrate-deficient transferrin
CRP	C - reactive protein
Gr. 1	Group 1 with normal serum zinc level
Gr. 2	Group 2 with low zinc level
LTDH	Lifetime Drinking History
TLFB	Timeline Followback (Total drinks in the past 90 days [TD90], Number of drinking days in the past 90 days [NDD90], Drinks per drinking day in the past 90 Days [DPD90], Average drinks per drinking day in the past 90 days [AvgDD90], and Heavy drinking days in the past 90 Days [HDD90])

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Highlights

Markers of very heavy drinking and zinc deficiency were associated with liver injury

A close association of zinc deficiency and low albumin was observed in subjects with liver injury (as defined by AST, ALT and bilirubin)

Elevated C-reactive protein was significantly associated with zinc deficiency in subjects with liver injury (as defined by AST, ALT and bilirubin)

The acute phase inflammatory response is one possible mechanism for the observed hypozincemia in alcohol abuse

The degree of zinc deficiency correlated with markers of progressive alcoholic liver disease
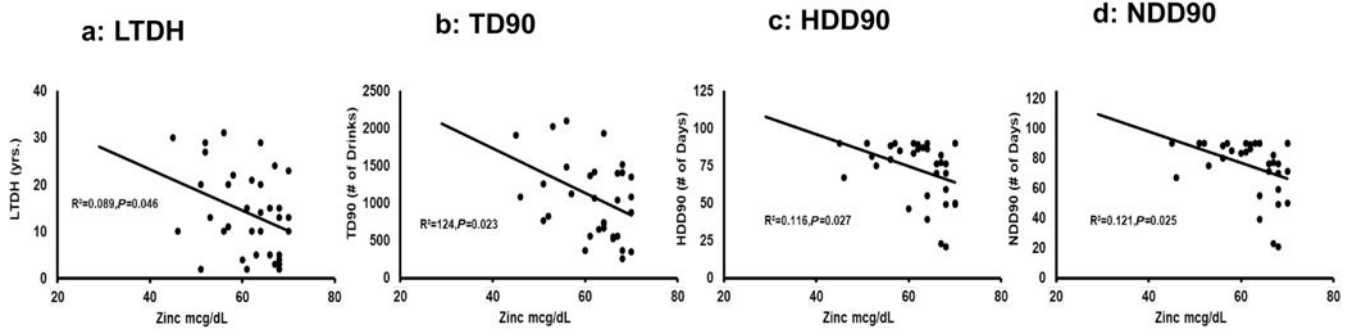
Figure 1.
Association of zinc level with drinking markers in low zinc group. Significant correlations were noted among these variables in individuals with low serum zinc levels (Figures 1a-1d). Limited or no significant associations were found between serum zinc levels and drinking markers among subjects with normal serum zinc levels (not shown). Statistical significance was set at p   0.05.
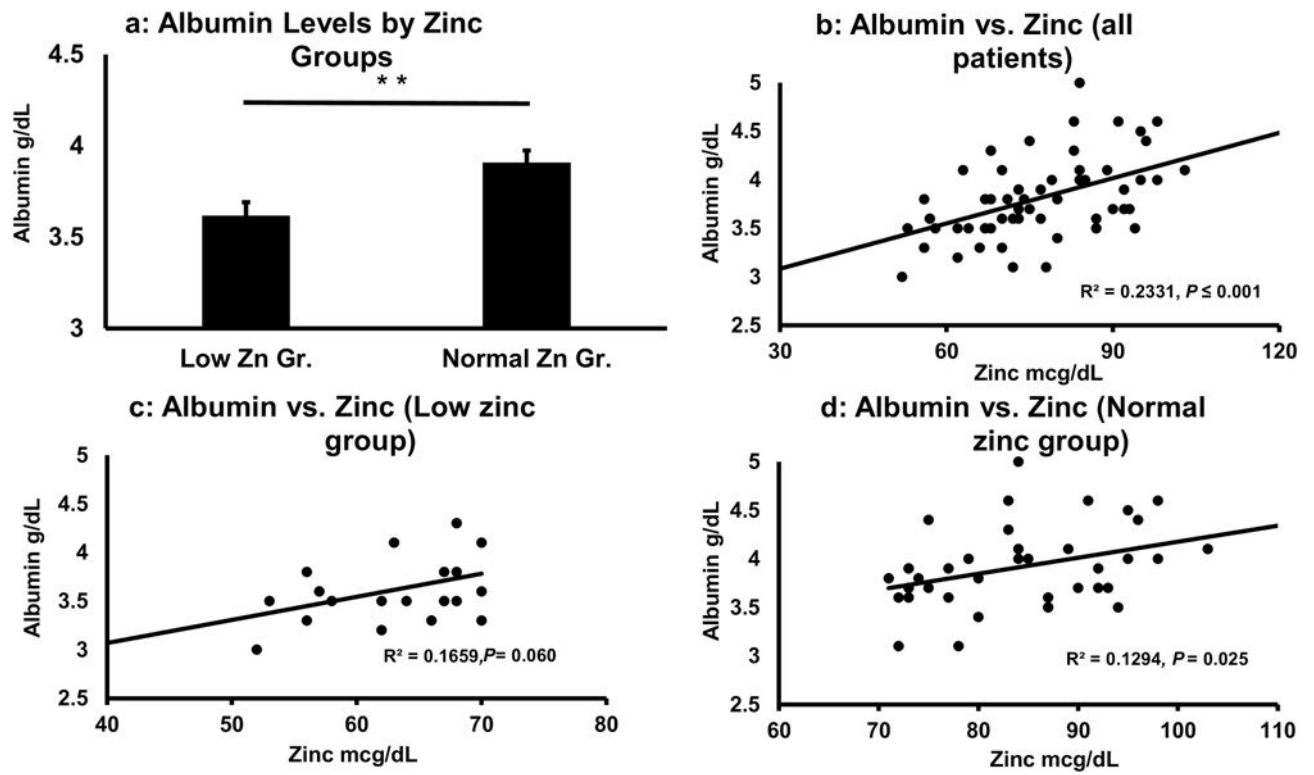
Figure 2.
Serum albumin by zinc level. Fig. 2a: Significant lowering of albumin in the low zinc group, p = 0.007 compared to the normal zinc group. Fig. 2b: Overall zinc vs. albumin across all the patients shows a close association of albumin and zinc. Fig. 2c: albumin correlation in the low zinc group. Fig. 2d: Albumin correlation in the normal zinc group. Statistical significance was set at p   0.05; ** p   0.01.
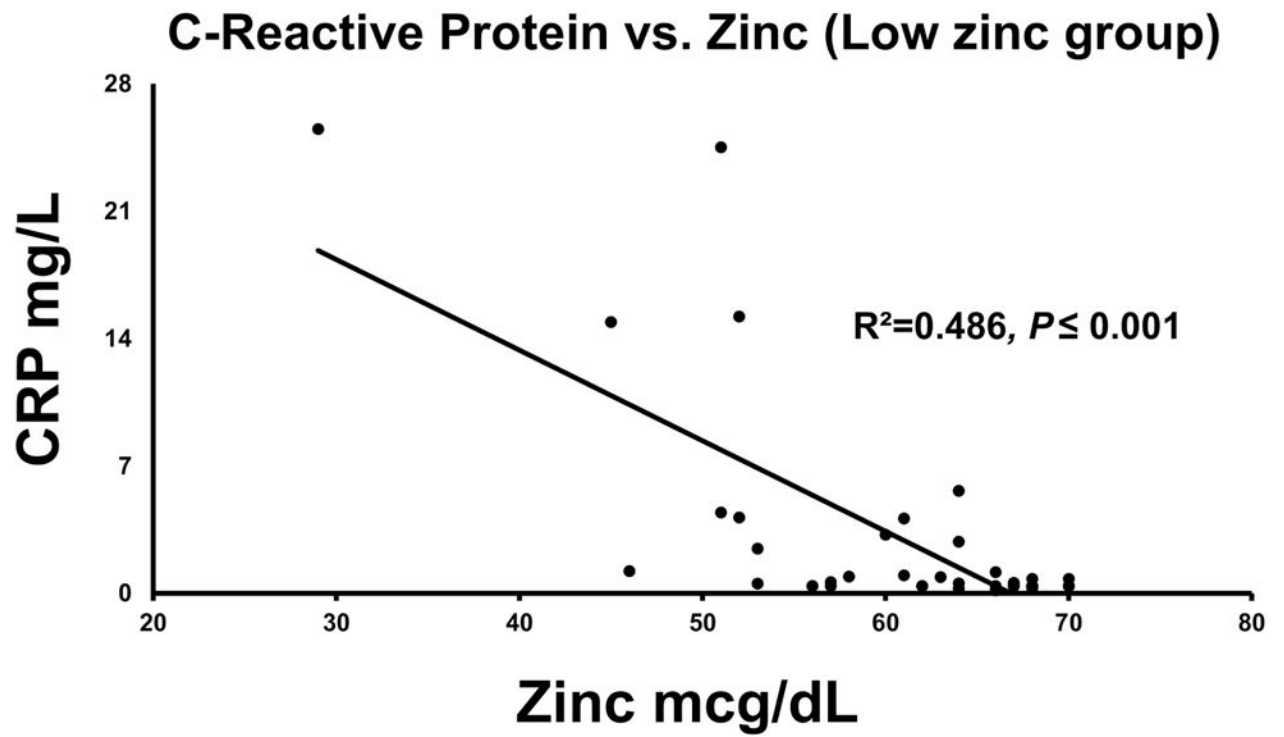
Figure 3.
Association of zinc levels with CRP levels in the low zinc group, with a high effect of the association. Statistical significance was set at p   0.05.

Figure 4.
Association of zinc level with AST:ALT ratio and bilirubin levels. Fig. 4a: AST:ALT ratio was significantly higher in low zinc group, p = 0.016. Fig. 4b: Association of zinc and AST:ALT ratio in low zinc group. Fig. 4c: Association of Total bilirubin and zinc were significantly associated in low zinc group. Statistical significance was set at * p   0.05.

Table 1

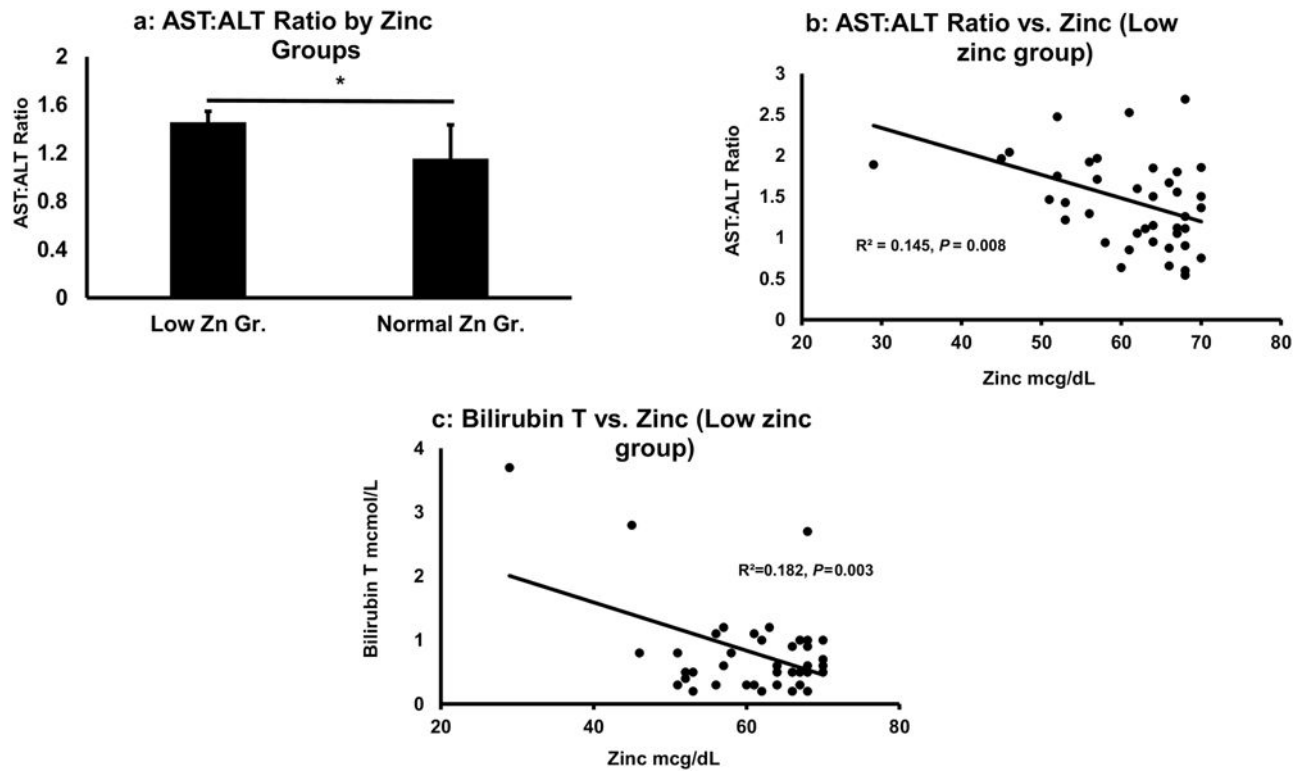Demographic and drinking history assessment in AD patients by zinc level and sex. P-value in the last column resulted from the comparison between normal zinc and low zinc groups. Data presented as Mean±SD. Statistical significance set at P ≤ 0.05.

Measures	Normal Zinc			Low Zinc			p-value for normal vs. low zinc
	Males (50)	Females (17)	Total (67)	Males (21)	Females (20)	Total (41)	
Age (years)	38.9 ± 11.1[#]	40.8 ± 12.0	39.4 ± 11.3	44.5 ± 9.3[#]	45.3 ± 8.3	44.9 ± 8.8	0.025
BMI (kg/m²)	26.5 ±3.5	25.1 ± 5.7	26.1 ± 4.2	26.2 ± 4.9	26.7 ± 7.1	26.4 ± 6.0	NS
Drinking Profile (History/Lab)							
TD90	1076±585	901±688	1029±614	1187±529	990±510	1066±573	NS
HDD90	67.5 ± 26.5	64.7±17.6	66.7 ± 24.3	71.6 ± 19.7	74.1 ± 20.9	72.6 ± 19.9	0.030
AvgDPD90	14.5 ± 6.9	12.4 ± 7.1	13.9 ± 7.0	15.3 ± 7.2	13.1 ± 6.1	14.4 ± 6.8	NS
NDD90	72.5 ± 23.0	68.7 ± 17.4	71.4 ± 21.6	74.2 ± 19.4	75.9 ± 19.8	74.9 ± 19.3	NS
LTDH	14.2 ± 9.6	13.0 ± 9.8	13.9 ± 9.6	15.9 ± 9.5	11.8 ± 8.1	14.0 ± 9.0	NS
CDT	0.102 ± 0.14	0.072 ± 0.05	0.094 ± 0.12	0.124 ± 0.09	0.084 ± 0.08	0.104 ± 0.09	NS

Note:

[#] significant difference between normal versus low zinc for males, p=0.050; BMI: Body mass index; TD90: Total drinks in 90 days; HDD90: heavy drinking days in 90 days; AvgDPD90: Average drinks per drinking day in last 90 days; NDD90: number of drinking days in last 90 days; LTDH: lifetime drinking history; CDT: Carbohydrate Deficient Transferrin (Unit in ratio: 0.00 – 0.010 normal [for alcohol abuse]).

Table 2

Serum zinc, laboratory markers of Liver injury, and markers of nutritional state in alcohol dependent patients. Data are presented as Mean±SD. Statistical significance set at P   0.05. NS: Not significant.

Measures	Normal Zinc			Low Zinc			p – value for normal vs. low zinc
	Males	Females	Total	Males	Females	Total	
Zinc	84.1 ± 9.9	86.8 ± 26.0	84.8 ± 15.4	61.4 ± 8.02	60.3 ± 9.4	60.9 ± 8.6	Not Applicable
Laboratory markers of Liver-injury							
AST	69.8 ± 79.8	68.7 ± 84.4	69.5 ± 80.3	86.8 ± 59.0	117.6 ± 122.2	101.8 ± 95.3	*0.062*
ALT	57.1 ± 39.9	49.3 ± 55.3	55.1 ± 44.0	63.7 ± 35.3	70.1 ± 77.1	66.8 ± 58.8	NS
AST:ALT ratio	1.1 ± 074	1.3 ± 0.4	1.15 ± 0.6	1.3 ± 0.5	1.6 ± 0.6	1.45 ± 0.6	0.016
INR	0.989 ± 0.17	0.966 ± 0.04	0.984 ± 0.16	1.003 ± 0.12	0.999 ± 0.08	1.001 ± 0.10	NS
Bilirubin Total	0.808 ± 0.74	0.547 ± 0.18	0.742 ± 0.66	0.781 ± 0.70	0.825 ± 0.76	0.802 ± 0.72	NS
Albumin	3.9 ± 0.4	3.8 ± 0.5	3.9 ± 0.4	3.8 ± 0.3 [@]	3.5 ± 0.3 [@]	3.6 ± 0.3	0.007
CRP	1.5 ± 2.3	0.5 ± 0.2	1.3 ± 2.1	2.6 ± 4.3	3.5 ± 7.7	3.1 ± 6.1	0.037
Nutritional Evaluation (CONUT)							
CONUT	1.06 ± 0.9	0.88 ± 1.1	1.01 ± 0.9	1.24 ± 1.5	1.45 ± 1.2	1.34 ± 1.3	NS

Note:

[@] significant lowering in albumin level in females compared to males in low zinc group, p=0.027. Serum zinc: normal > 70 [Unit: mcg/dL]; AST: Aspartate Aminotransferase, normal 40 [Unit: U/L]; ALT: Alanine Aminotransferase, normal 41 [Unit: U/L]; INR: international normalized ratio, normal range: 0.8 – 1.2 (in absence of anticoagulation therapy); Bilirubin Total: normal 1.2 [Unit: mcmol/L]; CRP: C - reactive protein, normal < 5.0, [Unit: mg/L]; Albumin: normal 3.5 – 5.2 [Unit: g/dL]. Units and levels derived from NIH department of Laboratory Medicine guidelines.

Table 3

Sensitivity of zinc level with liver injury progression and inflammation in AD patients who exhibit liver injury. Significant differences were identified in liver injury and drinking markers in zinc deficient group of AD patients.

Markers	Borderline (70-61)	Deficient (60)	*Effect & p*-value
Liver injury progression and inflammation			
AST/ALT Ratio	1.3±0.5 (26)	1.7±0.6 (15)	R^2=0.118; p=0.028
CRP	0.954±1.3 (25)	6.6±8.9 (15)	R^2=0.206; p=0.003
Drinking Markers			
LTDH	11.86±7.9 (22)	17.61±9.9 (13)	R^2=0.097; p=0.068
TD90	904.1±456.7 (23)	1403±661.5 (11)	R^2=0.171; p=0.015
NDD90	70.4±21.6 (23)	84.4±7.6 (11)	R^2=0.119; p=0.046

Serum zinc: normal > 70 [Unit: mcg/dL];]; CRP: C - reactive protein, normal < 5.0 [Unit: mg/L]; LTDH: lifetime drinking history;TD90: Total drinks past 90 days; NDD90: number of drinking days in last 90 days.