Original Article

Serum zinc level in liver cirrhosis with hepatic encephalopathy and its correlation with different stages of hepatic encephalopathy

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Abstract

Background: Hepatic encephalopathy (HE) severe complication of liver cirrhosis with high mortality. Few studies have found zinc deficiency in liver cirrhosis and HE patients and found it as a precipitating factor for the development of HE. This study was done to measure the serum zinc level in patients with liver cirrhosis with HE and a correlation was obtained between serum zinc level with grades of HE. **Material and Methods:** A cross-sectional observational study was done on 150 patients with liver cirrhosis with HE at a tertiary care center in Jharkhand. All cases were evaluated by history taking, clinical examination, and a questionnaire and classified into different WHC grades of HE and CPC classes of cirrhosis. Routine blood investigations, imaging studies, and morning serum zinc levels were done for all patients. **Results:** Majority of patients with liver cirrhosis with HE had zinc deficiency. There was a statistically highly significant ($P < .00001$) association between low serum zinc levels and WHC grades of HE. The serum zinc levels in different classes of cirrhosis showed highly significant differences ($P < .00001$). The mean serum zinc level was significantly low in patients who died (35.56 ± 11.65 vs 48.36 ± 10.91, $P < .0001$). The study revealed a strong positive correlation (r = .88, $P = .048$) between serum zinc and serum albumin levels. **Conclusion:** Serum zinc is deficient in patients with liver cirrhosis and HE. Zinc deficiency is significantly associated with higher severity of cirrhosis and higher grades of HE. All patients with liver cirrhosis with HE and hypoalbuminemia should be evaluated for zinc deficiency.

Keywords: Cirrhosis of liver, hepatic encephalopathy, serum zinc level, West Haven classification

Introduction

The burden of liver disease and cirrhosis is increasing worldwide. In India, there is a serious burden of liver disease because it accounted for 18.3% of two million global liver disease-related deaths in 2015.[1] Chronic liver disease is a progressive deterioration of liver functions, which includes the production of clotting factors and other proteins, detoxification of harmful products of metabolism, and excretion of bile for more than 6 months.[2] This is a continuous process of inflammation, destruction, and regeneration of liver parenchyma leading to fibrosis to cirrhosis.[3] Cirrhosis is a final stage of chronic liver disease that results in

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disruption of liver architecture, formation of widespread nodules, vascular regeneration, neo angiogenesis, deposition of extracellular matrix, and variable degree of vascular (portosystemic shunting).[4] Progression of liver disease and fibrosis, from fibrosis to cirrhosis and decompensation and HE and critical illness is a major cause of mortality in this population. viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and hepatocellular carcinoma are some of the major primary diseases of the liver.[5] Clinically liver diseases generally present in a few distinct patterns and are usually classified as hepatocellular, cholestatic, or mixed pattern of hepatocellular and cholestatic.[6] In liver diseases of hepatocellular patterns, such as ALD and viral hepatitis, features of inflammation predominate. In liver diseases of cholestatic pattern such as gallstone with obstruction, primary biliary cholangitis, or malignant obstruction, features of bile flow inhibition predominate, while in a mixed pattern of liver disease, features of both hepatocellular and cholestatic pattern are present.

Most liver disease onset and progression are insidious and it may take weeks, months, or years for clinical detection and symptoms of hepatic decompensation to appear after the onset of injury, while some liver diseases have acute or rapid onset and may manifest as acute liver failure.[5]

Liver failure is the most severe clinical consequence of liver disease. It may be either acute liver failure (occurs within 26 weeks), which is a result of sudden and massive hepatic destruction, or chronic liver failure, which is more frequent and occurs due to insidious and progressive liver injury over years or decades.[5] Viral hepatitis (hepatitis A, B, C, E), drug-induced liver injury, malaria, leptospirosis, and ALD are some of the most common causes of acute liver disease in India.[7,8] Hepatitis B, hepatitis C, and ALD are some of the most common causes of chronic liver disease in India. Hepatitis C is the predominant cause of chronic liver disease, in general, while ALD is the most common cause of cirrhosis.

Etiology of chronic liver disease across the country has significant regional variation. Hepatitis C is the most common cause in the northern region. Hepatitis B is predominant in the east and south and the second most frequent etiology in the rest of the country. NAFLD is predominant in the western and central regions of the country. ALD is predominant in northeastern regions of the country, closely followed by hepatitis B and hepatitis C.[9]

The child Pugh classification of cirrhosis is a system of distinguishing cirrhosis in different classes and its utility lies in that it helps in monitoring the decline of patients on the path to chronic liver failure.[5] It distinguishes cirrhosis between class A (well compensated), class B (partially compensated), and class C (decompensated), which correlates with different morphologic features histologically.

Hepatic encephalopathy (HE) is a serious complication of liver disease that occurs due to complex and variable changes in neuropsychiatric status and is defined as an impairment in brain function due to liver insufficiency and/or portosystemic shunt which consequently requires frequent hospital admission and leads to poor quality of life.[10] HE is classified into different types based on different parameters. It is divided into type A, type B, or type C according to liver pathology.[10] Type A HE is due to acute liver failure, while type B HE and type C HE are due to portosystemic shunt and cirrhosis, respectively.[10] Other criteria for classification are the presence or absence of precipitating factors, West Haven classification, and duration of HE. Based on time duration HE is classified into episodic, recurrent, or persistent HE.[10] The pathogenesis of HE is largely unclear. Portal systemic shunting and hepatocellular failure are the main reasons behind the development of HE. Ammonia is the main gut-derived toxin, which is thought to be responsible for the development of HE. Ammonia escapes hepatic detoxification because of liver disease and it impinges on the brain and it leads to the development of low-grade cerebral oedema, which ultimately impacts neuronal function.[11] In the early stages of HE clinical signs are often subtle and easily missed, which makes it difficult to diagnose in early stages.[8] The majority of patients with liver cirrhosis and HE (approximately 90%) have elevated ammonia levels in the plasma.[12]

Zinc is an essential trace element that has many functions in human body. It acts as a cofactor for many enzymes and proteins involved in anti-inflammatory, antioxidant and apoptotic effects. It plays very important role in cellular integrity and biological functions related to cell development, division and growth. More than 300 enzymes have zinc ions within their catalytic domains. Zinc binding proteins represent approximately 10% of human proteomes. The liver plays a very crucial role in maintaining systemic zinc homeostasis.[13] Zinc deficiency is diagnosed when serum zinc level is <70 mcg/dl (<12 mmol/l). Normal serum zinc level is 70-150 mcg/dl. Altered metabolism of zinc and zinc deficiency is observed in ALD, viral liver disease, and many other types of liver disease. Decreased dietary intake, induction of hepatic metallothionein, activation of certain zinc transporters, and increased urinary excretion are some of the mechanisms for altered zinc metabolism and zinc deficiency.[14] Patients with cirrhosis of liver with HE has high prevalence of zinc deficiency.[15] Zinc deficiency results in impairment of nitrogen metabolism by reducing the activity of urea cycle enzyme, ornithine transcarbamylase in the liver and of glutamine synthetase in the muscle.[16,17] Increased blood ammonia level was inversely associated with serum zinc level in patients with cirrhosis of liver.[18] Various grades of HE in decompensated liver disease patients and serum zinc level may have a direct correlation. Primary objective of this study is to estimate serum zinc level in all patients with liver cirrhosis with HE and to find out association between serum zinc level and various grades of HE. The secondary objective is to find out association between serum zinc level and severity of liver cirrhosis determined by child Pugh classification, to find out correlation between serum zinc and serum albumin level, correlation between serum zinc and serum bilirubin level, and difference between serum zinc level in patients who survived and who did not survive.

Materials and Methods

Study design This was a descriptive prospective observational study.

Study site and duration This study was done on admitted patients in department of medicine at a tertiary care centre of an eastern state of India where cases are referred from different referral centers. Study was done over 1 year period after approval granted by institutional committee.

The study population and sampling study population comprised all consecutive patients who met the inclusion criteria and were diagnosed with liver cirrhosis with HE and were admitted to the Department of Medicine during the study period. HE and liver cirrhosis were diagnosed on the basis of clinical examination, laboratory measurements and ultrasound of the abdomen. The Cochrane formula was used for sample size calculation using a Z score of 1.96 at a confidence interval of 95%, a significance level of 0.05, and a 9% prevalence of cirrhosis on the basis of a previous study.[19] This formula gave a required sample size of 126. During the 1 year study period, we recruited 164 patients out of which 14 patients could not be included in the study due to various reasons. so the final sample size was 150.

Inclusion and Exclusion Criteria Patients of both sexes, aged >18 years who had given written informed consent and were diagnosed as a case of liver cirrhosis with HE were included in the study. Cases with metabolic encephalopathy, psychiatric disorders, altered sensorium due to head injury or stroke, and those in alcohol withdrawal or alcohol-intoxicated state were excluded from the study.

Data Collection and Measurements All patients were explained about the study in detail, and data were collected using a questionnaire, which included sociodemographic variables, clinical profile, past and present history and other relevant parameters. Patients were enquired and examined for sleep pattern, anxiety, depression, euphoria, disorientation, confusion, abdominal distension, pedal oedema, and jaundice. History was also taken for precipitating factors such as upper gastrointestinal (UGI) bleeding, fever, constipation, diarrhoea, vomiting, pain abdomen, abdominal distension, malnutrition, and high protein intake. Detailed clinical examination for signs of liver cirrhosis, such as icterus, pallor, spider naevi, palmar erythema, clubbing, ascites, pedal oedema, asterixis, constructional apraxia, confusion, coma, impairment of addition/subtraction, orientation, and signs of dehydration, was done. Personal history of alcohol intake and duration of alcohol and drug abuse was taken. HE was diagnosed by the presence of neurological abnormalities and impaired consciousness in the absence of other causes of neurological deterioration. HE was graded into grade 0 (MHE) to grade 4 (coma) according to west haven classification (WHC). Cirrhosis of liver was diagnosed on the basis of clinical symptoms and signs, laboratory results and ultrasound features. It was classified into class A, class B, and class C according to child Pugh classification (CPC). CPC classification was done using values of serum albumin, bilirubin, prothrombin time (PT), international normalized ratio (INR), presence/absence and severity of ascites, and HE. Each variable was scored 1, 2, or 3. Decompensation was defined as child Pugh score ≥7 (CPC B) or presence of ascites, HE, or UGI bleeding. Investigations, such as CBC, LFT, RFT, blood sugar fasting and post prandial, serum albumin, PT/INR, serum electrolyte, serum iron profile, serum and urinary copper estimation, serum ceruloplasmin, autoimmune markers of liver disease (ANA, AMA, SMA, anti-LKM antibody), ultrasound of abdomen, and chest x ray, were done in patients to ascertain the etiology and status of patient in case to case basis. Serum zinc level estimation was done in morning blood samples for all patients.

Principle and Procedure of Serum Zinc Estimation It is based on the principle of formation of yellow colour complex when zinc tris is mixed with dithizone, centrifuged serum and trichloroacetate. Procedure involves taking 3 ml samples each of standard zinc solution, distilled water, and sera in three test tube marked as standard, blank, and unknown. These samples were mixed and centrifuged at 3600 rpm and kept for 30 min. Supernatant of mixed centrifuged samples were later mixed with 0.3 ml of 10 N NaOH, 0.5 ml of HCC, and 0.5 ml of dithizone reagent. Yellow color complex formed and read at 555 nm after 5 min.

Ethical Approval and Informed Consent The institutional ethics committee of our institution gave ethical approval for this study via memo no MN 59/2019 dated February 16, 2019. Written informed consent was taken from each patient or their relative after explaining in detail about the study.

Statistical Analysis Data collected were either categorical or quantitative. Categorical data were presented in a table by frequency and percentages while quantitative data were presented as mean ± standard deviation and median. The Chi-square test was used to compare categorical variables while the Student *t*-test and ANOVA were used for comparing quantitative variables, which were normally distributed. Pearson's correlation test was used to find the correlation between different variables. $P \leq 0.05$ was considered significant. Data were stored in the Microsoft Excel sheet and were analyzed using the statistical package for social sciences (SPSS) version 22.0, IBM, Chicago, Illinois, USA.

Observation and Result

This descriptive observational study included 150 cases of liver cirrhosis with HE. Cases of liver cirrhosis with HE were more common in the age group of 30-50 years and least common in the <30 years of age group (68% vs 10%) [Table 1]. Males were more commonly affected than females, comprising 129 (86%) of all cases 150 (100%) [Table 2]. The mean age of patients was 44.6 ± 6.34 years.

Out of 150 patients, 129 (86%) patients recovered, while 21 (14%) patients died during the course of treatment resulting in a mortality rate of 14%. Mortality was higher in higher grades of HE in comparison to lower grades of HE (OR = 9.72, $P < 0.0001$).

Chronic alcoholism was the most common cause of liver cirrhosis in 92 (61.33%) cases followed by hepatitis B virus in 18 (12%), NAFLD in 8 (5.33%), hepatitis C in 6 (4%), autoimmune in 5 (3.33%), both alcohol and hepatitis B virus in 5 (3.33%), Wilson disease in 2 (1.33%), and hemochromatosis in 1 (0.66%). A mixed etiology of alcohol, hepatitis B, and hepatitis C was found in 1 (0.66%) case, while in 12 (8%) cases, no etiology can be found [Table 3]. Most patients with alcohol as etiology of liver cirrhosis had a history of >15 years of alcohol consumption.

Patients presented with varied clinical manifestations, most of them having multiple symptoms and signs. The most common presenting complaint was abdominal distension in 108 (72%) cases followed by pedal oedema in 99 (66%), icterus in 92 (61.33%), fever in 77 (51.33%), constipation in 73 (48.66%), disorientation in 71 (47.33%), altered sleep in 69 (46%), asterixis in 66 (44%), melena in 64 (42.66%), hematemesis in 60 (40%), pain abdomen in 59 (39.33%), nausea/vomiting in 36 (24%), confusion in 24 (16%), and diarrhea in 6 (4%). 12 (8%) cases were in coma, and all of them were in WHC grade 4. Seizure was observed in 2 (0.75%) cases [Table 4].

In this study, we found multiple precipitants responsible for the development of HE in cases of liver cirrhosis. In the majority of patients, one precipitant was identified, some had more than one precipitant, while in a few no precipitant could be identified. The most common precipitating factors of HE was infection in 79 (52.66%) cases, which included lower respiratory tract infection in 33 (22%), spontaneous bacterial peritonitis in 28 (18.66%), sepsis in 11 (7.33%), and urinary tract infection in 7 (4.66%). Other precipitating factors were dyselectrolytemia in 40 (26.66%) [hypokalemia in 21 (14%) hyponatremia in 19 (12.66%)], gastrointestinal bleeding in 36 (24%), constipation in 27 (18%), diuretics, dehydration, acute gastroenteritis, paracentesis, and excessive protein intake [Table 5]. In 12 (8%) patients, no precipitant cause could be identified.

Patients were classified into different grades of severity of HE using West Haven criteria out of 150 patients, 18 (12%) patients were in grade 0, 48 (32%) in both grades I and II, 24 (16%) in grade III, and 12 (8%) in grade IV [Table 6].

As per this study, almost all patients of HE had low serum zinc level and higher WHC grade (grades III and IV) of HE had lower serum zinc level (<40 mcg/dl) compared to lower WHC grade of HE (grades 0, I, and II) [Table 7]. There was a highly significant ($P < 0.00001$) association between low serum zinc level and higher grades of encephalopathy. Results gives an inference that low serum zinc concentration may lead to development of higher grades of HE or as the level of zinc in serum decreases severity of HE increases.

Patients were classified by Child-Pugh classification of cirrhosis in different classes (classes A, B, and C). Maximum patients were in Group C (78 out of 150), followed by 60 cases in class B, and 12 cases in class A. Serum zinc level was measured for each patient in different CPC class of cirrhosis, and it was seen that patients with liver cirrhosis had low a serum zinc level. It was

Table 1: Age distribution of cases

Age	Frequency	Percentage
<30 years	15	10
30–50 years	102	68
>50 years	33	22
Total	150	100

Table 2: Sex distribution of cases

Sex	Frequency	Percentage
Male	129	86
Female	21	14
Total	150	100

Table 3: Etiology of liver cirrhosis

Etiology	Frequency	Percentage
Alcohol	92	61.33
Hepatitis B	18	12
Hepatitis C	6	4
NAFLD	8	5.33
Autoimmune	5	3.33
Wilson's disease	2	1.33
Hemochromatosis	1	0.66
Alcohol + hepatitis B + hepatitis C	1	0.66
Alcohol + hepatitis B	5	3.33
Idiopathic	12	8

NAFLD: Nonalcoholic fatty liver disease

Table 4: Clinical presentation of patients

Clinical Profile	Frequency	Percentage
Icterus	54	36
Pain abdomen	59	39.33
Fever	77	51.33
Ascites	108	72
Black stool	64	42.66
Constipation	73	48.66
Nausea/vomiting	36	24
Asterixis	66	44
Altered sleep	69	46
Hematemesis	60	40
Disorientation	71	47.33
Diarrhea	6	4
Pedal oedema	99	66
Confusion	24	16
Seizure	2	0.75
Coma	12	8

also seen that patients in CPC class C (patients with advanced liver dysfunction and poor prognosis) has lowest serum zinc level compared to classes A and B [Table 8]. There was a highly significant ($P < .00001$) association between low serum zinc level and higher class of liver cirrhosis. These results show a negative association between serum zinc level and CPC class of cirrhosis and it can also be inferred that as the serum zinc level decreases, severity of liver cirrhosis increases.

Table 5: Precipitating factors of Hepatic encephalopathy in liver cirrhosis patients

Precipitants	Frequency	Percentage
Infection	79	52.66
i) LRTI	33	22
ii) UTI	7	4.66
iii) Sepsis	11	7.33
iv) Spontaneous bacterial peritonitis	28	18.66
Constipation	27	18
Electrolyte abnormalities	40	26.66
i) Hyponatremia	19	12.66
ii) Hypokalaemia	21	14
GI Bleed	36	24
Diuretics	5	3.33
Dehydration	5	3.33
Acute gastroenteritis	3	2
Paracentesis	3	2
Excessive protein intake	2	1.33
Unidentified	12	8

Table 6: West Haven grading of patients

Grading	Frequency	Percentage
Grade 0	18	12
Grade I	48	32
Grade II	48	32
Grade III	24	16
Grade IV	12	8
Total	150	100

The mean serum zinc level was estimated and compared between patients of different WHC grades of HR. A significant difference in the mean serum zinc level was found between WHC grade 4 vs WHC grade 3 (28.23 vs 35.82, $P = 0.0251$), WHC4 vs WHC2 (28.23 vs 46.31, $P = 0.0000$), WHC4 vs WHC1 (28.23 vs 53.28, $P = 0.0000$), WHC4 vs WHC0 (28.23 vs 55.96, $P = 0.0000$), WHC3 vs WHC2 (35.82 vs 46.31, $P = 0.0005$), WHC3 vs WHC1 (35.82 vs 53.28, $P = 0.0000$), WHC3 vs WHC0 (35.82 vs 55.96, $P = 0.0000$), WHC2 vs WHC1 (46.31 vs 53.28, $P = 0.0498$), and WHC2 vs WHC0 (46.31 vs 55.96, $P = 0.0018$), while there was no significant difference in mean serum zinc level between WHC1 vs WHC0 (53.28 vs 55.96, $P = 0.0826$) [Table 9].

Mean serum zinc level was compared between patients of different CPC class of cirrhosis, where significant difference was found between CPC class C vs CPC class B (41.43 vs 50.78, $P = 0.0054$), CPC class C vs CPC class A (41.43 vs 58.58, $P = 0.0000$), while there was no significant difference between CPC class B vs CPC class A (50.78 vs 58.58, $P = 0.2522$) [Table 10].

Data analysis showed a gradual decline in mean serum zinc level with the increase in severity of WHC grade of HE and also with the increase in severity of CPC class of cirrhosis, which suggested a negative correlation between these entities. The mean serum zinc level and mean serum albumin in WHC grades of HE showed a significantly high positive correlation (Pearson's correlation coefficient $r = 0.88$, $P = .048$) [Table 11]. While the mean serum zinc level and mean serum bilirubin in WHC grades of HE showed no significant correlation (Pearson›s correlation coefficient $r = 0.442$, $P = .455$) [Table 11]. Serum zinc level was also significantly low in patients who died in comparison to patients who survived (35.56 ± 11.65 vs 48.36 ± 10.91, $P < 0.0001$) [Table 12]. A comparison of mean serum zinc, mean serum albumin, and mean serum bilirubin levels in liver cirrhosis and HE patients is given in Table 13, and linear regression

Table 7: Hepatic encephalopathy grades and corresponding serum zinc level

WHC Grade	Serum zinc level (in mcg/dl) No. and % of cases within WHC grade					Total
	>60	50–59	40–49	30–39	<30	
Grade 0	12 (66.66%)	3 (16.67%)	1 (5.55%)	1 (5.55%)	1 (5.55%)	18 (100%)
Grade I	6 (12.50%)	15 (31.25%)	24 (50.00%)	1 (2.08%)	2 (4.16%)	48 (100%)
Grade II	3 (6.25%)	3 (6.25%)	18 (37.50%)	24 (50.00%)	0 (0.00%)	48 (100%)
Grade III	1 (4.16%)	1 (4.16%)	2 (8.33%)	14 (58.33%)	6 (25.00%)	24 (100%)
Grade IV	0 (0.00%)	0 (0.00%)	1 (8.33%)	2 (16.66%)	9 (75.00%)	12 (100%)
Total	22 (14.66%)	22 (14.66%)	46 (30.66%)	42 (28%)	18 (12%)	150 (100%)

Table 8: Child-Pugh class (CPC) and corresponding serum zinc level

CPC	Serum zinc level (in mcg/dl) No. and % of cases within CPC					Total
	>60	50–59	40–49	30–39	20–29	
Class A	3 (25.00%)	3 (25%)	4 (33.33%)	1 (8.33%)	1 (8.33%)	12 (100%)
Class B	9 (15%)	18 (30%)	27 (45%)	3 (5%)	3 (5%)	60 (100%)
Class C	9 (11.54%)	1 (1.28%)	11 (14.10%)	42 (53.85%)	15 (19.23%)	78 (100%)
Total	21 (14%)	22 (14.66%)	42 (28%)	46 (30.66%)	19 (12.66%)	150 (100%)

Table 9: Comparison of serum Zinc level in WHC grades of encephalopathy

WHC Grade	Mean±SD serum zinc level	*P*
WHC 4: WHC 3	M 4=28.23±8.63 M 3=35.82±7.89	0.02518
WHC 4: WHC 2	M 4=28.23±8.63 M 2=46.31±7.61	0.0000
WHC 4: WHC 1	M 4=28.23±8.63 M 1=53.28±6.61	0.0000
WHC 4: WHC 0	M 4=28.23±8.63 M 0=55.96±13.99	0.0000
WHC 3: WHC 2	M 3=35.82±7.89 M 2=46.31±7.61	0.0005
WHC 3: WHC 1	M 3=35.82±7.89 M 1=53.28±6.61	0.0000
WHC 3: WHC 0	M 3=35.82±7.89 M 0=55.96±13.99	0.0000
WHC 2: WHC 1	M 2=46.31±7.61 M 1=53.28±6.61	0.0498
WHC 2: WHC 0	M 2=46.31±7.61 M 0=55.96±13.99	0.0018
WHC 1: WHC 0	M 1=53.28±6.61 M 0=55.96±13.99	0.0826

WHC: West Haven grade of encephalopathy, M: Mean serum zinc

Table 10: Pairwise comparison of serum zinc level in different CPC classes of liver cirrhosis

Child-Pugh class of cirrhosis (CPC)	Mean±SD serum zinc level	*P*
CPC A: CPC B	M_A=58.58±10.29 M_B=50.78±10.05	0.2522
CPC A: CPC C	M_A=58.58±10.29 M_C=41.43±11.40	0.0000
CPC B: CPC C	M_B=50.78±10.05 M_C=41.43±11.40	0.00547

Table 11: Pearson correlation (*r*) between serum zinc level and other parameters

Parameters	*r*	*P*
MSZL and MSAL	0.8807	0.048
MSZL and MSBL	0.4422	0.455

MSZL: mean serum zinc level, MSAL: mean serum albumin level, MSBL: mean serum bilirubin level

Table 12: Comparison of serum zinc level in survivors and nonsurvivors

	Mean±SD zinc level	No.	*P*
Survived	48.36±10.91	21	<0.0001
Not survived	35.56±11.65	129	

among mean serum zinc, albumin, and bilirubin is shown in Figures 1 and 2.

Discussion

This study was a cross sectional observational study done on 150 patients with liver cirrhosis with HE at a tertiary care centre from Jharkhand. Males were more common in numbers 129 (86%) than females 21 (14%) and patients of 30-50 years of age group was most commonly involved. Study by Aftab Ahmad Soomro *et al.*[20] in 2009 also had more numbers of males with male: female ratio of 49 (55.75%):39 (44.3%). A study by Rajesh k Meena *et al.*[21] had most patient in 30-50 year age group in similar to current study. Higher number of male percentages in our study is due to the fact that males are more commonly alcoholic than females in Jharkhand state. Mean age of patient in our study was 44.6 ± 6.34 which is comparable to mean age of 42.75 ± 15.88 in study by Aftab Ahmad Soomro *et al.*[20] Chronic alcohol consumption in 92 (61.33%) cases and hepatitis B virus infection in 18 (12%) of cases were the most common etiology for liver cirrhosis in current study and duration of alcohol intake was more than 15 years in most patients where alcohol was found as etiology. Meena *et al.*[21] also found chronic alcohol consumption of >10 years and hepatitis B infection as the most common etiology similar to our study. In contrast to their study, where other etiology were Wilson disease current study had diverse etiology of liver cirrhosis, which included hepatitis c virus infection, NAFLD, autoimmune liver disease, Wilson disease, hemochromatosis, and other mixed cause. Liver cirrhosis develops in a sequence of events, in case of chronic alcohol consumption it is steatosis, steatohepatitis, fibrosis then cirrhosis whereas in case of hepatitis virus infection it is inflammation, necrosis, regeneration, and then cirrhosis.[22,23] Abdominal distension, pedal oedema, jaundice, fever, constipation, disorientation, altered sleep, hematemesis, and pain abdomen were the most common presenting complaints. Similar presenting complaints in slightly different frequencies were noted in their study by Vansh Deep *et al.*[24] and Rajesh K Meena *et al.*[21] Most common precipitating factor for HE was infection in 79 (52.66%) cases followed by gastrointestinal bleed in 36 (24%), constipation in 27 (18%), hypokalemia in 21 (14%), hyponatremia in 19 (12.66%), and diuretics in 5 (3.33%). Among infection, lower respiratory tract infection and spontaneous bacterial peritonitis were predominantly responsible for decompensation, whereas in some cases urinary tract infection and sepsis were responsible. Alike to present study, infection in 64.2%, gastrointestinal bleeding in 13.2%, hypokalemia in 11.4%, hyponatremia in 7.5%, dehydration in 7.5%, and constipation in 3.8% were the most common precipitating factor for HE in study by Amoako Duah *et al.*[25] While in contrast to present study, most common precipitating factor was constipation in 41% of cases in study by Rajesh K Meena *et al.*[21] This study had maximum number of patients 48 (32%) in both grades I and II of West Haven grading of HE which is complemented by results of study by Vansh Deep *et al.*[24] (35% and 37% in WHC grades 1 and 2) and Rajesh K Meena *et al.*[21] (34.7% and 32% in WHC grades 1 and 2). In a study by Ghada M. Galal *et al.*,[26] there were approximately similar number of patients in all WHC grades of HE. Higher number of patients in WHC grades 1 and 2 may be due to the fact that in these grades initial symptoms and signs of HE appears which can be appreciated by the patients or their attendants for which they consult a doctor or get admitted in a hospital. Results of this study revealed low serum zinc level in almost all patients of HE having lowest zinc level in higher WHC grade of HE and

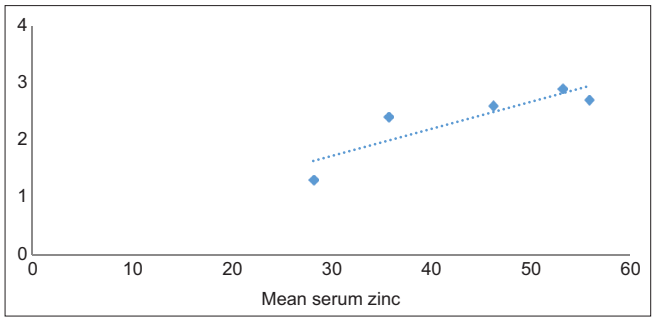

Figure 1: Linear regression of mean serum zinc and mean serum albumin

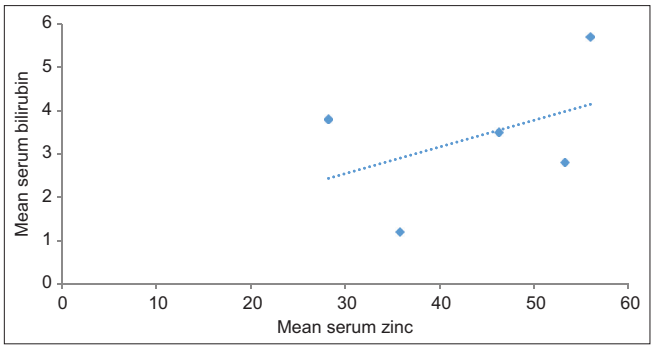

Figure 2: Linear regression of mean serum zinc and mean serum bilirubin

Table 13: Mean serum zinc, mean serum albumin and mean serum bilirubin levels in liver cirrhosis and hepatic encephalopathy patients

	Mean serum zinc level	Mean serum albumin	Mean serum bilirubin
M1	28.23	1.3	3.8
M2	35.82	2.4	1.2
M3	46.31	2.6	3.5
M4	53.28	2.9	2.8
M5	55.96	2.7	5.7

comparatively higher level in lower WHC grade of HE which shows a negative correlation between serum zinc level and WHC grade of HE. There was a statistically significant association between low serum zinc level and WHC grades of HE ($P < .00001$). In concurrence with this study, Rajesh K Meena *et al.*[21] also found low serum zinc level in higher grade of HE in statistically significant manner ($P = .000$) in his study. Similarly Vansh Deep *et al.*[24] in his study found low mean serum zinc level in higher WHC grade of HE ($P = .000$). Another study by Hiwarkar SR[27] revealed negative correlation between mean serum zinc level and WHC grade of HE (Spearman's correlation coefficient = −0.8967, $P < .0001$) supporting our result. The main culprit behind development of HE in liver cirrhosis is raised ammonia level.[12] Low serum zinc level impairs the function of ornithine transcarbamylase and of glutamine synthetase in the muscle.[17,28] Urea cycle is responsible for conversion of toxic ammonia into urea and the leftover ammonia is converted into urea in muscle, which involves glutamate synthetase enzyme. So impairment in function of these enzymes leads to raised ammonia level and development of HE. The low serum zinc level also increases intestinal permeability by oxidative stress which increases endotoxemia leading to HE.[29] Comparison of serum zinc level among different WHC grades of HE in current study showed statistically significant difference ($P < .05$) except between WHC grades 1 and 0 ($P = .0826$), which is supported by the results in study by Ghada M Galal *et al.*[26] where there were significant difference ($P = .021$) in mean serum zinc level between early (grades 1 and 2) and advanced (grades 3 and 4) grades of HE. In parallel with the results of study by Rajesh K Meena *et al.*,[21] results of present study had low serum zinc level in more severe or higher class of cirrhosis (CPC class C) in comparison to less severe or lower class of cirrhosis (CPC classes A and B). Comparison of serum zinc level in different class of cirrhosis showed significant difference ($P < .00001$) and pairwise comparison of serum zinc level among individual class of cirrhosis revealed highly significant difference ($P < .01$) except between CPC classes A and B ($P = 0.2522$). Similar to results of this study, Vansh Deep *et al.*[24] also found highly significant difference in zinc level between CPC classes B and C ($P = .000$). Zinc deficiency in liver cirrhosis occurs due to reduced synthesis of albumin and relative increase in alpha 2 macroglobulin that binds more strongly to zinc, which leads to substantial increase in urinary excretion of zinc.[30,31] Decreased intake of zinc and reduced absorption of zinc from small intestine due to portosystemic shunt also results in zinc deficiency.[32] Apart from this altered zinc transport and decreased hepatic content of zinc along with diuretics induced inhibition of reabsorption of zinc from kidney tubules causes hypozincemia in chronic liver disease.[33,34] Strong positive correlation was seen between mean serum albumin level and mean serum zinc level in different grades of HE (Pearson's correlation coefficient (r) = 0.8807, $P = .048$). Rajesh K Meena *et al.*[21] also found statistically significant ($P = .029$) association between low serum zinc and serum albumin. Alike to this study, Vansh Deep *et al.*[24] also found a strong positive correlation between serum albumin and serum zinc level (r = 0.8939, $P < .00001$). Zinc, which is not bound to albumin, is excreted in urine in hypoalbuminemic state is considered to be the main reason behind state of hypozincemia due to hypoalbuminemia in advanced liver disease.[35] No significant correlation (r = .4422, $P = .455$) was obtained between mean serum zinc level and mean serum bilirubin level in this study. Ghada M Galal also found no significant correlation between serum zinc and bilirubin level (r = −0.149, $P = .277$).[26] The serum zinc level was significantly high in group of patients who survived in comparison to group of patients who did not survived (48.36 ± 10.91 vs 35.56 ± 11.65, $P < .0001$), which is supported by results in study of Hiwarkar SR *et al.*[27] where he found highly significant difference in zinc level between survivors and nonsurvivors (57.67 ± 10.89 vs 32.30 ± 11.69, $P < .0001$). Takuma *et al.*[36] in his randomized control trial in 2010 came to the conclusion that zinc is effective in management of HE significantly reducing the WHC HE grade ($P = .03$) and ammonia level in blood ($P = .01$) in the group, which were supplemented

by zinc. A meta-analysis by Chavez Tapia *et al.*[37] in 2013 found that zinc supplementation improved number connection test in HE and reduced rate of recurrence of HE. Another meta-analysis by Diglio *et al.*[38] in 2020 found an improvement in HE in cirrhotic patients receiving zinc supplementation.

Conclusion

An observational cross-sectional study was done on 150 patients with liver cirrhosis with HE and morning serum zinc level was estimated in each patient. It was concluded from this study that serum zinc level was low in patients with liver cirrhosis with HE and significantly lower level in higher grades of HE. Zinc deficiency can be a precipitating factor for development of HE in liver cirrhosis. Serum zinc level is low in liver cirrhosis and significantly low with increased severity in higher class of cirrhosis. Patients with liver cirrhosis with HE who had low serum albumin also had low serum zinc indicating that low serum albumin can be a surrogate marker for low serum zinc. All patients with liver cirrhosis with HE and hypoalbuminemia should be evaluated for zinc deficiency. Hypozincemia is significantly associated with mortality in HE so it can also be used as a prognostic marker. Early screening for serum zinc level in patients with liver cirrhosis with HE and its replacement could result in prevention of worsening of HE and can also be used in treatment of HE, which can be proved by larger study particularly a case control study or randomised control trial.

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Authors' contributions

D. K conceived the manuscript and P.K revised it. D.K, P.K, and M.K.P done the statistical analysis, D.K, P.K, M.K.P, R.S, R.T.G, M.L.P, S.K, A.K, V. V, S.K, T.A wrote the manuscript. P.K, R.S, R.T. G, A.K, S.K prepared tables and figures. Supervision was done by V. and T.A. All authors have read and approved the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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