Effect of chronic hepatitis C on serum zinc and its relation as a cofactor to cognitive impairment and nutritional status in hemodialysis patients

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Background and aim
The prevalence of hepatitis C virus (HCV) infection among dialysis patients is higher than in the general population. The prevalence of cognitive impairment (CI) is common among hemodialysis (HD) patients. Also patients with end-stage liver disease are vulnerable to cognitive dysfunction. Malnutrition and inflammation are common occurrences in maintenance HD patients. About 40–78% of individuals on HD suffer from hypozincemia. Zinc deficiency has been observed with high prevalence in liver cirrhosis. This study was carried out to assess the effect of chronic HCV on serum zinc level and its relation as a cofactor to CI and nutritional status in HD patients.
Patients and methods
The study involved 80 HD participants who were enrolled into two groups: group I: 40 HCV-positive HD patients (20 without liver cirrhosis and 20 with liver cirrhosis) and group II: 40 HCV-negative HD patients without liver cirrhosis. All participants were evaluated as regards detailed history and clinical examination, standardized mini-mental state examination (MMSE), malnutrition inflammation score (MIS), Child–Pugh classification, complete blood picture (CBP), prothrombin time, international normalized ratio, alanine aminotransferase, aspartate aminotransferase, serum albumin, bilirubin, blood urea, serum creatinine, Na, K, Ca, P, transferrin, ammonia, serum zinc level (predialysis and postdialysis session), virology including anti-HCV Ab, quantitative HCV PCR and hepatitis B surface antigen, *Kt/V*, fibrosis-4 score (FIB-4 score), and abdominal ultrasonography.
Results
We found that MMSE and zinc level were significantly lower and MIS was significantly higher in HCV HD patients with liver cirrhosis when compared with HCV HD patients without liver cirrhosis and HCV-negative HD patients. A positive significant correlation was found between zinc level and MMSE while there was a negative significant correlation between zinc level and MIS.
Conclusion
There may be an association between hypozincemia, CI, and malnutrition in HD patients especially those with chronic hepatitis C associated with liver cirrhosis.

Keywords:
chronic hepatitis C, cognitive impairment, hemodialysis patients, nutritional status, serum zinc level

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Introduction

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% occurring in about 170 million infected persons worldwide and approximately four million people have been newly infected annually [1]. This infection, particularly in its chronic form, is associated with sizable morbidity and mortality. More than 350 000 deaths are attributed to HCV infection each year, most of which are caused by liver cirrhosis and hepatocellular carcinoma [2].

The prevalence of HCV infection among dialysis patients is generally much higher than in the general population [3]. Studies held in dialysis centers from different countries have shown that prevalence ranges from 1 to 84.6% [4].

The incidence of cognitive decline is increasing worldwide, with interventions to delay this decline becoming increasingly important. The prevalence of cognitive impairment (CI) in the UK in the Medical Research Council study was 18% [5]. CI is common

among persons with end-stage renal disease and cognitive functions have been shown to decline in patients on long-term hemodialysis (HD), with 70% of HD patients, aged more than or equal to 55 years, having moderate to severe chronic CI [6].

Patients with end-stage liver disease are vulnerable to cognitive dysfunction. Clinical presentation and pathophysiologic mechanisms of brain injury are dependent on the type of liver failure (fulminant vs. chronic). Chronic liver disease may present with a clinical spectrum ranging from rapidly developing acute confusion and coma to persistent and progressive CIs fully appreciated only on psychometric testing. This CI noted in the presence of a clear sensorium is often referred to as subclinical hepatic encephalopathy which was then called minimal hepatic encephalopathy [7].

Malnutrition and inflammation are common occurrences in maintenance HD patients and they are strong predictors of morbidity and mortality in these patients. These observations, made by different researchers, have led to the coinage of the term malnutrition inflammation complex syndrome [8].

Malnutrition is present in 65–90% of patients with advanced liver disease and in almost 100% of candidates for liver transplantation [9]. Cirrhotic patients who are malnourished not only have a higher morbidity, but also an increased mortality rate [10]. The severity of malnutrition correlates directly with the progression of the liver disease [11].

Zinc is the second most prevalent trace element in the human body and has important antioxidant, anti-inflammatory, and antiapoptotic effects. It is required for cell growth and maturation, and is a cofactor in many metabolic processes [12].

It has been estimated that 20% of the world population are zinc deficient [13]. Zinc deficiency occurs in individuals and populations whose diets are low in sources of readily bioavailable zinc (such as red meat and seafood) and high in substances that limit zinc absorption (such as phytates, oxalates) [14]. These dietary patterns are characteristics that are common in many developing countries [15].

It is a fact that 40–78% of individuals on HD suffer from low serum levels of zinc [16]. This finding may be explained by three different reasons: due to decreased zinc intake, decreased absorption by the gastrointestinal tract and due to increased loss during HD session [17,18].

Zinc deficiency has been observed with high prevalence in liver cirrhosis [19] and this may be attributed to anorexia, changes in this element reservoir in the body, lactulose-induced diarrhea, and use of diuretics to control edema.

Brain growth and development are critically dependent on several micronutrients [20]. Zinc is a key modulator of intracellular and intercellular neuronal signaling-4 that is found in high levels in the brain, particularly the hippocampus, which is considered to be the area involved in learning and memory [21], and in the amygdala, striatum, and the neocortex [22].

In humans, many observational studies have suggested a relationship between zinc deficiency and poor cognition [23,24]. The essential role of zinc in the central nervous systems is marked during brain growth, particularly between 24 and 40 weeks after conception [24], which is the period where the brain goes through extraordinary structural changes, and it is during this critical time that the brain is most sensitive of zinc deficiency, such deficiency will affect the involvement of zinc in various enzymes and neurochemical processes such as synaptic transmission and the release of neurotransmitters [25].

Zinc deficiency may lead to anorexia [26]. Appetite disorders can, in turn, cause malnutrition and inadequate zinc intake, leading to a vicious cycle. This is called 'malnutrition induced malnutrition' [26].

Zinc supplementation has been shown to improve weight gain in patients with anorexia nervosa [27]. In a randomized, double-blind, placebo-controlled trial, a daily supplement of 14 mg zinc from zinc gluconate was found to double the rate of body mass increase compared with patients receiving the placebo control [27].

Aim

This work aimed to assess the effect of chronic HCV on serum zinc level and its relation as a cofactor to CI and nutritional status in HD patients.

Patients and methods

Patients

The study involved 80 HD participants from the Medical Research Institute Hemodialysis Unit and they were enrolled into two groups:

(1) Group I: 40 HCV-positive HD patients [20 without liver cirrhosis (subgroup IA) and 20 with liver cirrhosis (subgroup IB)].
(2) Group II: 40 HCV-negative HD patients without liver cirrhosis.

Participants with any other causes of CI (such as cerebrovascular disease), other causes of nutritional deficiency (such as chronic diarrhea, inflammatory bowel disease, and malignancies), and patients with hepatitis B virus infection were excluded from the study.

Note that patients were diagnosed to have hepatitis C infection by the presence of positive anti-HCV Ab and PCR. Diagnosis of liver cirrhosis was based on US combined with fibrosis-4 score (FIB-4 score). HD session were done three times per week, 4 h per session, and using bicarbonate buffer with a *Kt/V* of more than 1.2 to ensure adequacy of HD.

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and an informed consent was obtained from each patient.

Methods

All participants were evaluated as regards:

(1) Detailed history and clinical examination with special stress on the following:
  (a) Age, sex of patients, and duration of HD.
  (b) Detailed history taking with stress on history of gastrointestinal bleeding and hepatic encephalopathy.
  (c) History of any other causes of CI (such as cerebrovascular disease).
  (d) History of other causes of nutritional deficiency (such as chronic diarrhea, inflammatory bowel disease, and malignancies).
  (e) History of aluminum exposure.
  (f) Clinical manifestations of chronic liver disease including ascites, edema of lower limbs, jaundice, and splenomegaly.
  (g) Measurement of mean blood pressure.
(2) Assessment of cognitive function: using standardized mini-mental state examination (MMSE) [28].
(3) Assessment of nutritional status: using mal-nutrition inflammation score (MIS) [29].
(4) Assessment of the degree of liver cirrhosis: using Child–Pugh classification [30].
(5) Laboratory methods:
  (a) Estimation of complete blood picture, prothrombin time, and international normalized ratio [31].
  (b) Estimation of predialysis serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, blood urea, serum creatinine, sodium, potassium, Ca, P, transferrin [32].
  (c) Estimation of serum ammonia level [32].
  (d) Assessment of adequacy of HD using *Kt/V* [33].
  where ratio=post-BUN/pre-BUN.
  (e) Assessment of virology including anti-HCV Ab, quantitative HCV PCR, and hepatitis B surface antigen [34].
  (f) Assessment of serum zinc level (predialysis and postdialysis session) [32].
  (g) Assessment of liver fibrosis using FIB-4 score [35]:
(6) Radiological investigations:
  Abdominal ultrasonography to assess the presence of liver cirrhosis, portal hypertension, splenomegaly, and ascites [36].

Results

HCV-positive HD patients without liver cirrhosis included eight (40%) women and 12 (60%) men and HCV-positive HD patients with liver cirrhosis included 11 (55%) women and nine (45%) men, whereas HCV-negative patients included 17 (42.5%) women and 23 (57.5%) men with no statistically significant difference between groups as regards sex (P=0.574). The mean age in subgroup IA was 52.10±6.766 years; it was 52.15±9.132 years in subgroup IB; and 47.53±9.964 years in group II with no statistically significant difference among different groups as regards mean age (P=0.083).

The mean duration of HD showed a statistically significant difference between the three groups (P=0.005). As regards clinical manifestations of chronic liver disease, there was no statistically significant difference between both groups as regards the presenting symptoms and signs of the studied cases. As for the MBP, there was a statistically significant difference between group II and subgroup IA and between group II and subgroup IB with no statistical significance between subgroup IA and subgroup IB.

In this study, it was found that 26.4% of patients had CI (4% had severe and 22.4% had mild CI), whereas 73.6% had normal cognitive function using MMSE. The mean value of MMSE showed a statistically significant

Table 1 Comparison between studied groups according to patient's mini-mental state examination and malnutrition inflammation score

	Group I		
	Subgroup IA (*n*=20)		Subgroup IB (*n*=20)
MMSE			
Minimum–maximum	20–29		14–28
Mean±SD	24.95±2.982		20.35±4.196
Median	25		20
P value	P_1=0.156		P_2=0.000*
		P=0.000*	
MIS			
Minimum–maximum	2–22		1–20
Mean±SD	10.25±5.035		14.60±4.684
Median	11		15.5
P value	P_1=0.783		P_2=0.002*
		P=0.011*	

Group IA, HCV-positive hemodialysis patients without liver cirrhosis; group IB, HCV-positive hemodialysis patients with liver cirrhosis; group II, HCV-negative hemodialysis patients; MIS, malnutrition inflammation score; MMSE, mini-mental state examination; P_1, *P* value for comparing between group IA and group II; P_2, *P* value for comparing between group IB and group II; *P*, *P* value for comparing between group IA with group IB. *Statistically significant at $P<0.05$.

difference between subgroup IB and each of the group II and subgroup IA with no statistical significance between subgroup IA and group II (*P*=0.000, 0.000, 0.156, respectively). The mean value of MIS showed a statistically significant difference between subgroup IB and each of group II and subgroup IA with no statistical significance between subgroup IA and group II (*P*=0.002, 0.011, 0.783, respectively) (Table 1).

The quantitative HCV PCR showed no statistically significant difference between subgroup IA, and subgroup IB.

In subgroup IB, by ultrasonographic examination, PHT was detected in nine (45%) patients; ascites was detected in 13 (65%) patients whereas splenomegaly was detected in eight (40%) patients.

The mean of FIB-4 score in subgroup IA was 1.242± 0.513 and in subgroup IB, it was 2.446±1.010 with a statistically significant difference between the two groups.

In HCV patients with liver cirrhosis, seven (35%) patients were in Child A; 13 (65%) patients were in Child B; and no patients (0%) were in Child C (Table 2). Child–Pugh ranged between 5 and 8 with a mean of 6.70±0.979 (Table 3).

The percentage of patients who had zinc deficiency was 53.6% of the studied group. The mean value of zinc level before dialysis showed statistically significant difference between subgroup IB and each of the group II and subgroup IA with no statistical significance between subgroup IA and group II. The

Table 2 Child class in patients with hepatitis C virus and cirrhosis group

Child class (*n*=20)	*n* (%)
A	7 (35)
B	13 (65)
C	0 (0)

Table 3 Distribution of the studied sample according to patient's Child–Pugh in hepatitis C virus positive patients with cirrhosis group

	Child–Pugh
Minimum	5
Maximum	8
Mean	6.70
SD	0.979

mean value of zinc level after dialysis showed a statistically significant difference between subgroup IB and each of the group II and subgroup IA with no statistical significance between subgroup IA and group II. The mean value of zinc level in all groups was significantly higher after HD when compared with its level before HD (Table 4).

There was a positive significant correlation between zinc level (before and after dialysis) and MMSE, while there was a negative significant correlation between age and MMSE and between zinc level and each of MIS and FIB-4 score (before and after dialysis) (Table 5 and Figs. 1–7).

Discussion

HCV infection is one of the main causes of chronic liver disease worldwide [37]. In Egypt, an Egyptian

Table 4 Comparison between the studied groups according to patient's zinc level

Zinc level(μg/dl)	Group I		
	Subgroup IA (n=20)		Subgroup IB (n=20)
Before dialysis			
Minimum–maximum	10–52		11–156
Mean±SD	22.190±11.585		54.820±32.164
Median	18.65		49
P value	P_1=0.435		P_2=0.000*
		P=0.002*	
After dialysis			
Minimum–maximum	15–67		23–189
Mean±SD	35.706±13.115		86.275±35.110
Median	34.5		55.5
P value	P_1=0.495		P_2=0.002*
		P=0.029*	

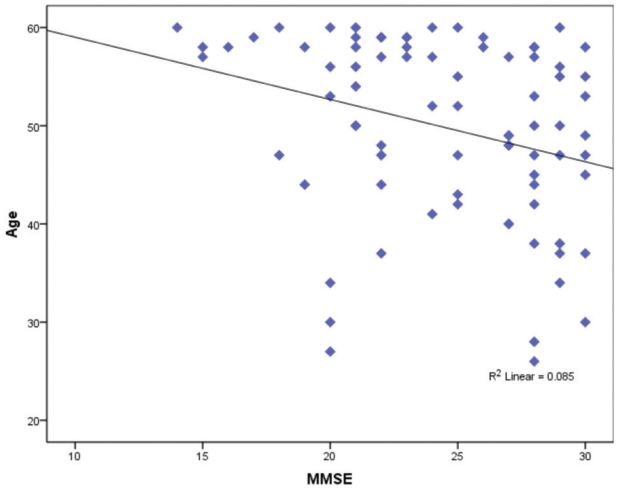
Group IA, HCV-positive hemodialysis patients without liver cirrhosis; group IB, HCV-positive hemodialysis patients with liver cirrhosis; group II, HCV-negative hemodialysis patients; P_1, P value for comparing between group IA and group II; P_2, P value for comparing between group IB and group II; P, P value for comparing between group IA with group IB. *Statistically significant at $P<0.05$.

Table 5 Correlation between different parameters

	Age	Zinc level before dialysis	Zinc level after dialysis	PCR
MMSE				
r	–0.291	0.497	0.426	–0.170
P	0.009*	0.000*	0.000*	0.294
MIS				
r	0.215	–0.558	–0.496	–0.111
P	0.055	0.000*	0.000*	0.497
FIB-4 score				
r	–	–0.466	–0.389	–
P	–	0.002*	0.016*	–
Child class				
r	–	–0.046	–0.198	–
P	–	0.849	0.431	–
PCR				
r	–	–0.197	–0.183	–
P	–	0.223	0.272	–

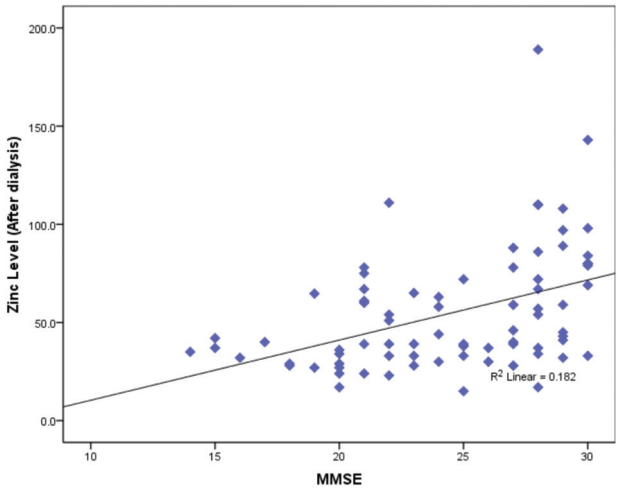
FIB-4 score, fibrosis-4 score; MIS, malnutrition inflammation score; MMSE, mini-mental state examination. *Statistically significant at $P<0.05$.

Figure 1



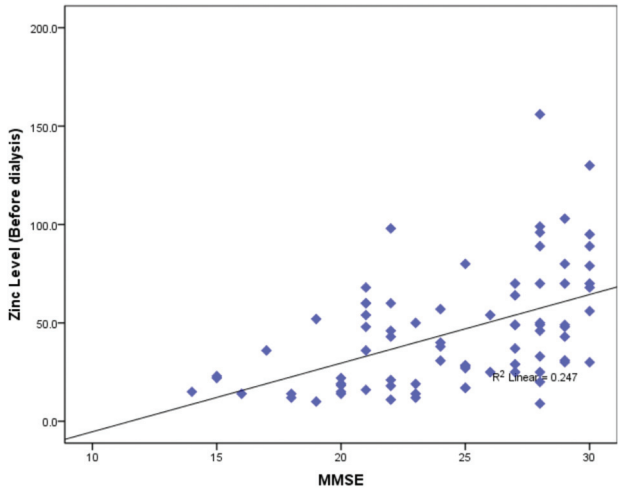
Correlation between age and mini-mental state examination. MMSE, mini-mental state examination.

Figure 2



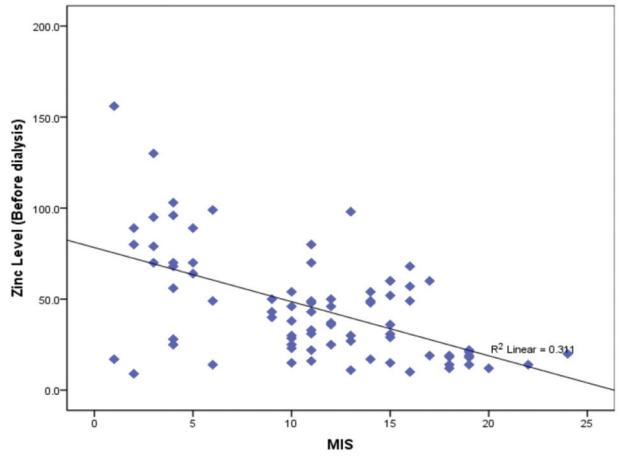
Correlation between zinc level (before dialysis) and mini-mental state examination. MMSE, mini-mental state examination.

Figure 3



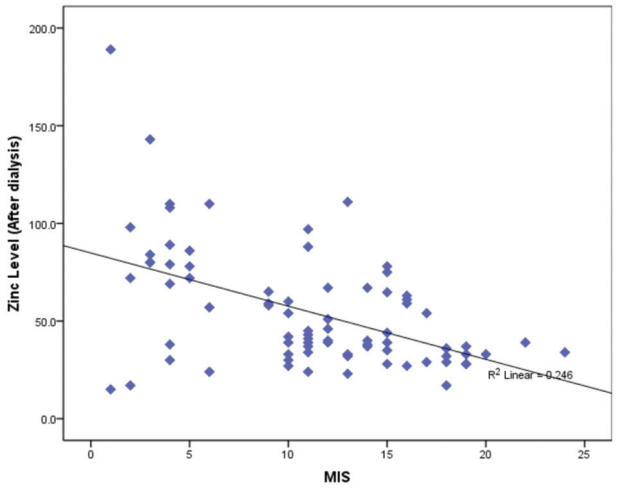
Correlation between zinc level (after dialysis) and mini-mental state examination. MMSE, mini-mental state examination.

Figure 4



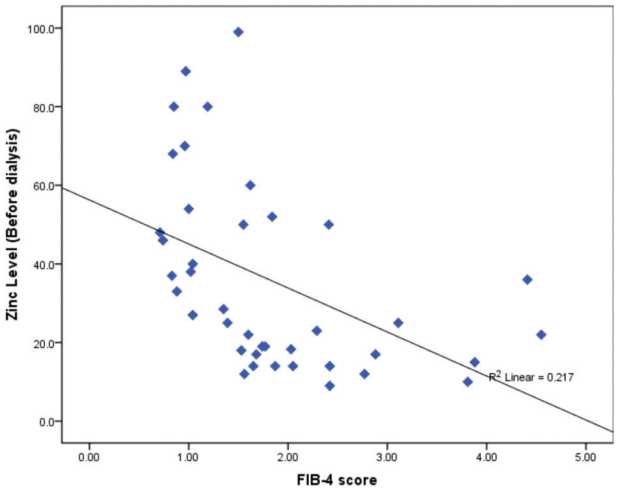
Correlation between zinc level (before dialysis) and malnutrition inflammation score. MIS, malnutrition inflammation score.

Figure 5



Correlation between zinc level (after dialysis) and malnutrition inflammation score. MIS, malnutrition inflammation score.

Figure 6



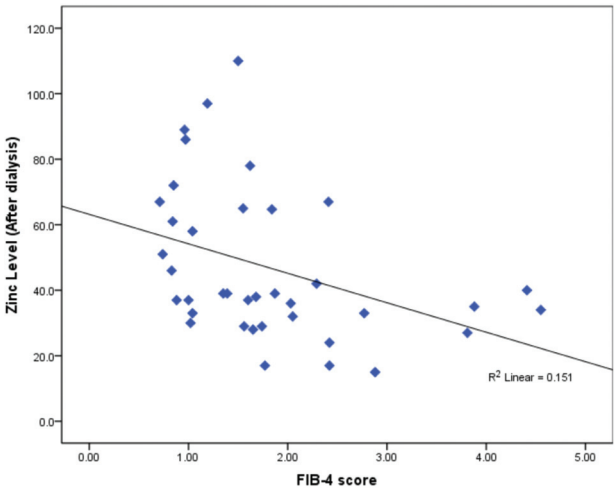
Correlation between zinc level (before dialysis) and fibrosis-4 score. FIB, fibrosis.

demographic health survey conducted in 2008 concluded that 14.7% of the population have been infected, making this the highest prevalence in any population in the world [38]. HCV infection is common and is associated with significant morbidity and mortality among dialysis patients. It is more common in dialysis patients than in healthy populations [39]. Zinc is an essential trace element which is required for the action of many enzymes. Zinc deficiency is a common problem in the world; it is frequently reported in patients with end-stage renal disease undergoing HD [40]. Moreover, zinc deficiency has been observed with high prevalence in liver cirrhosis [41].

The goal of our study is to assess the effect of chronic HCV infection on serum zinc level and its relation as a cofactor to CI and nutritional status in HD patients.

Subgroup IA included eight women and 12 men with a mean age of 52.10±6.766 years and subgroup IB included 11 women and nine men with a mean age of 52.15±9.132 years, whereas group II included 17 women and 23 men with a mean age of 47.53±9.964 years with no statistically significant difference between groups as regards age and sex. The mean duration of HD was 53.75±16.065 months in subgroup IA, 45.20 ±10.739 in subgroup IB, and 58.48±14.884 months in group II with a statistically significant difference between the three groups. In a previous study, it has been reported that the duration of HD is significantly longer in anti-HCV-positive patients than in anti-HCV-negative patients. Further, it has been observed that patients on HD for more than 10 years have an increased incidence of HCV infection.

Figure 7



Correlation between zinc level (after dialysis) and fibrosis-4 score. FIB, fibrosis.

The risk of acquiring HCV infection on HD is estimated at ~10% per year [42].

In our study, the mean value of MMSE score showed a statistically significant difference between subgroup IB and each of the group II and subgroup IA with no statistical significance between subgroup IA and group II. We also found that 4% of participants had severe CI; 22.4% had mild CI; and 73.6% had normal cognitive function using MMSE.

Early studies among HD patients reported moderate rates of CI across multiple cognitive domains [43,44]. One study in 1997 using MMSE found that 30% of 336 HD patients aged 23–93 years had mild to severe CI [45].

Multiple studies reported a cross-sectional, graded relation between estimated glomerular filtration rate level and cognitive function [46–48]. In the heart, estrogen/progesterone study among menopausal women, each 10 ml/min/1.73 m^2 decrement in estimated glomerular filtration rate corresponded to an ~15–25% increase in risk for cognitive dysfunction [49].

In general, the performance of patients with liver disease is found to be worse than that of healthy matched controls across a range of cognitive tests [50]. Further, patients with more severe disease (Child–Pugh stage C) display greater cognitive deficits than patients with less severe disease on tests of immediate memory and processing speed [50].

The mean value of MIS showed a statistically significant difference between subgroup IB and each of group II and subgroup IA with no statistical significance between subgroup IA and group II. This is in accordance with many studies using different methods of nutritional assessment that report the prevalence of malnutrition and inadequate energy and protein intakes in patients with liver cirrhosis [51–53].

Chazot's study [54] assessed the nutritional status of 20 HD patients receiving HD treatment for more than 20 years and was shown that the longer the time spent on HD the more prevalent will be malnutrition [54].

The mean value of hemoglobin showed a statistically significant difference between subgroup IB and group II and between group II and subgroup IA with no statistical significance between subgroup IA and subgroup IB.

Although there have been many previous reports of cases with improvement of red blood cell status after hepatitis infection in patients on maintenance HD [55,56], the mechanisms underlying this improvement are incompletely understood. It was suggested that the liver has some potential to produce erythropoietin (EPO) apart from the kidneys. Thus, stimulation of hepatic EPO production has been considered as an explanation for lessened anemia in HD patients with viral hepatitis [56]. In a recent explanation for the pathogenesis on the molecular level, an increase of hepatic EPO production was suggested to be related to hepatic regeneration during hepatitis and be proportional to increased interleukin-6 level [57].

Lin *et al.* [58], who followed up 80 chronic HD patients (30 HCV-positive and 50 HCV-negative) in Taipei Medical University Hospital for 1 year, concluded that less frequent anemia was observed in chronic hepatitis C. Also, HCV-infected patients needed lower EPO and iron doses than HCV noninfected patients. This data supported by Kranthi *et al.* [59], who reported that lesser EPO and iron requirements in HCV-infected patients compared with HCV noninfected patients.

On the other hand, Sabry *et al.* [60] studied 99 patients (70 HCV-positive and 29 HCV-negative); the result of this study showed comparable hemoglobin and hematocrit levels as well as EPO dose between the two groups. This may be attributed to resistance to EPO action secondary to chronic infection, with impaired iron availability, or perhaps suppressed erythropoiesis by humoral factors, other cytokines, or growth factors.

The small sample size of HCV-infected patients and lack of follow-up were the limitations of our study. Another limiting factor is that endogenous and exogenous EPO levels were not directly measured. Additional clinical variables, such as parathyroid hormone levels, transferrin saturation, and presence of chronic infections and other comorbidities, should be incorporated into future studies because these variables affect responsiveness to EPO therapy. Further, the primary etiology of renal failure for each individual patient should be taken into account, because different forms of renal failure cause varying degrees of EPO deficiency.

The mean value of ALT showed a statistically significant difference between subgroup IB and group IA and between group II and subgroup IA with no statistical significance between subgroup IB and group II. The mean value of AST showed a statistically significant difference between group IB and group II and between subgroup IA and group II with no statistical significance between subgroup IA and subgroup IB.

Several studies have shown that aminotransferase (AST, ALT) levels are low in patients on dialysis and this reduction appears to occur already in patients with advanced chronic kidney disease even before the initiation of renal replacement treatment [61,62]. HD patients with chronic hepatitis C have serum aminotransferase levels which are at the upper limit but still within the normal range, although higher compared with HCV-negative HD patients.

Lemos *et al.* [63] found that HD patients infected with HCV had significantly higher ALT levels compared with the noninfected patients. On the contrary, Fabrizi *et al.* [61] showed that patients on dialysis in general tend to have lower ALT levels. Cotler *et al.* [64] also found that the serum ALT levels were significantly lower in patients with chronic renal failure.

The diminished values of liver enzymes restrict their diagnostic significance, while their use as a tool for hepatitis surveillance and follow-up is unreliable. This might be attributed to liver cell protection by the hepatocyte growth factor, which showed higher concentrations in patients on HD. The lower ALT activity in HD patients might also be a consequence of a smaller serum HCV viral load either due to the adsorption of the virus genome in the dialyzer membrane or due to the induction of endogenous interferon caused by HD [65]. Hepatitis C had a greater tendency toward intermittent exacerbations and remissions, with a very variable and fluctuating ALT profile. Thus, patients with HCV, on dialysis, may have normal ALT levels despite significant histological liver damage [66].

The contribution of hemodilution in the decrease of aminotransferases has also been examined by several investigators. Yasuda *et al.* [62] observed a 15–35% increase in serum ALT/AST after dialysis compared with the predialysis values. Sombolos *et al.* [67] showed that in patients who underwent euvolemic HD, there were no differences in ALT/AST levels before and after the procedure. On the other hand, when HD with fluid removal or isolated ultrafiltration was performed, there was an increase in aminotransferase levels after the procedure.

The mean value of transferrin showed no statistically significant difference between groups. Contreras *et al.* [66] has shown HCV-infected patients to be associated with higher serum iron, ferritin, and transferrin saturation, and this supported the findings of Kalantar-Zadeh *et al.* [68]. Moreover, there was increased red blood cell count, and this was in consistence with a study by Simon *et al.* [69], who found that there was increased endogenous EPO secretion in HCV-infected patients. Patients with chronic hepatitis C infection tended to have a higher ferritin level when compared with non-HCV-infected patients [57].

The mean value of ammonia showed a statistically significant difference between subgroup IB and each of group II and subgroup IA with no statistical significance between subgroup IA and group II, but all cases were in the normal range. In our study, the mean value of *Kt/V* showed no statistically significant difference between groups and it was in the target range to ensure adequate dialysis in all selected patients.

The mean value of quantitative HCV PCR was 986 171.35±2 022 931.146 IU/ml in group IA and 5 448 166.30±1921 IU/ml in group IB with no statistically significant difference between the two groups. Based on the literature data, HCV load in HD patients is usually low. However, in a few studies similar or even higher viral loads were found compared with nonuremic patients [64,70], while fluctuation of HCV-RNA as well as intermittent viremia have also been reported [71].

By ultrasonography in group HCV-positive patients with liver cirrhosis, PHT was detected in nine (45%) patients; ascites was detected in 13 (65%) patients,

whereas splenomegaly was detected in eight (40%) patients. The FIB-4 score showed a statistically significant difference between HCV-positive patients with and without liver cirrhosis.

Child–Pugh in HCV-positive patients with liver cirrhosis ranged between 5 and 8 with a mean of 6.70±0.979; seven (35%) patients were in Child A; 13 (65%) patients were in Child B; and no patients (0%) were in Child C.

In the present study, we found that zinc deficiency was present in 53.6% of HD patients and the mean value of zinc level in all groups was significantly higher after HD when compared with the level before HD. We found that the mean value of zinc level before dialysis showed a statistically significant difference between subgroup IB and each of group II and subgroup IA with no statistical significance between subgroup IA and group II and the mean value of zinc level after dialysis showed a statistically significant difference between group IB and each of group II and subgroup IA with no statistical significance between subgroup IA and group II.

Previous studies have shown that the rate of zinc deficiency in patients undergoing HD has been reported to be between 40 and 78% [72,73]. Moreover, several studies have shown that serum zinc levels were increased after HD [74–76]. The mechanism of elevated zinc after HD is not yet understood it could be related to an increase in transporter protein after HD. It is well known that zinc is transported bound with prealbumin, albumin, and transferrin; thus, zinc increased after HD session as these proteins are not filtered during HD.

Moreover, in the present study; there was a negative correlation between zinc level (before and after dialysis) and FIB-4 score. Mohammed *et al.* [77] studied zinc levels in 42 Egyptian patients with chronic hepatitis C. The results have shown that the levels of zinc in HCV-infected patients were decreased compared with the healthy group. This was in accordance with the result of Qasim *et al.* [78] who compared serum zinc in chronic hepatitis C patients with healthy controls; they found that the serum zinc concentration was significantly lower in HCV patients than controls.

Marchesini *et al.* [41] found that zinc deficiency is common in patients with advanced cirrhosis. Similarly, Pramoolsinsap *et al.* [79] found that serum zinc level decreased significantly in patients with chronic, active hepatitis, cirrhosis, and hepatocellular carcinoma. Kalkan *et al.* [80] and Czuczejko *et al.* [81] also reported that there was a decrease in the level of zinc in the sera of hepatitis cases.

Laguercia *et al.* [82] found that as the disease progresses from chronic hepatitis to liver cirrhosis, serum zinc concentrations decrease. Iwata *et al.* [83] concluded that the mean zinc values decreased with the progression of fibrosis and it was significantly lower in patients with varices. Moreover, the zinc level was significantly lower in patients with a high risk of bleeding than in those with a low risk. In a study by Anber *et al.* [84], there was a progressive decrease of zinc level with progression of HCV disease.

Interestingly, supplementation with zinc has been shown to improve the prognosis of cirrhotic patients, as well as the cirrhosis-related symptoms [85]. In patients receiving oral zinc supplementation, maintenance of serum zinc concentration at more than 80 μg/dl was the most important factor associated with cancer-free survival [86].

On the other hand, Cesur *et al.* [87] compared serum zinc concentrations in chronic HCV patients (n=17) and healthy controls (n=17). They did not find any significant difference between healthy individuals and patients with chronic hepatitis C. This could be related to the small number included in each group.

In our study, there was a positive significant correlation between zinc level (before and after dialysis) and MMSE. Previous studies [88–90] suggested a positive association between zinc intake and measures of cognitive function. Ortega *et al.* [91] indicated a small but a significant correlation between increased zinc intake and MMSE test. Stoecker *et al.* [90] reported a positive correlation between plasma zinc concentration and CI. Two studies examined the association between plasma zinc concentration and cognitive score. One of these has shown that lower plasma zinc was significantly correlated with poor cognitive performance [88], whereas the other study [89] failed to find any association between plasma zinc and CI.

Our study has shown that there was a negative significant correlation between age and MMSE and this is in accordance of what has been found by Rait *et al.* [5] which showed an increasing prevalence of CI by age and this may be explained by a variety of possible causes, including medication side effects, metabolic and/or endocrine derangements, delirium due to

intercurrent illness, depression, and dementia, with Alzheimer's dementia being the most common [5].

In the present study, there was a negative correlation between zinc level (before and after dialysis) and MIS. This is in accordance with many clinical studies that have reinforced the link between zinc deficiency and malnutrition [92–97].

A study in 1995 suggested that the mean serum zinc level varied inversely with the severity of protein energy malnutrition (PEM) [92]. Singla *et al*. [93] conducted a study among Indian children in 1996 and found the serum zinc level to be significantly low in higher grades of malnutrition. A study conducted by Gautam in 2008 showed that the serum zinc levels in Bangladeshi children having PEM were significantly lower than that in the control group [94].

In 2012, Khare *et al*. [95] found low level of zinc in the children having PEM. Another studies done in India and on Sudanese children with PEM showed lower serum zinc levels when compared to the control group [96,97].

Thus, in the present study we showed that MMSE was significantly lower and MIS was significantly higher in HCV HD patients with liver cirrhosis when compared with HCV HD patients without liver cirrhosis and HCV-negative HD patients.

Moreover, zinc level before or after dialysis was significantly lower in HCV HD patients with liver cirrhosis when compared with HCV HD patients without liver cirrhosis and HCV-negative HD patients.

Finally, a positive significant correlation was found between zinc level (before and after dialysis) and MMSE, while there was a negative significant correlation between zinc level (before and after dialysis) and MIS.

The previous findings indicate that there may be an association between hypozincemia, CI, and malnutrition in HD patients especially in those with chronic hepatitis C with liver cirrhosis and that zinc may be an important cofactor in the occurrence of CI and malnutrition in these patients.

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Conflicts of interest
There are no conflicts of interest.

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