Role of Zinc Deficiency in Development of Minimal Hepatic Encephalopathy among HCV Induced Compensated Cirrhotic Patients

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Background and study aim: Minimal hepatic encephalopathy is a term used to describe cirrhotic patients who are clinically normal but show abnormalities of neurophysiological variables and/or cognition. Implicated theories of pathogenesis are similar to those of overt hepatic encephalopathy. Hypozincaemia was studied as a theory for the pathogenesis of overt hepatic encephalopathy. The aim of the present study is to evaluate the role of hypozincaemia in development of minimal hepatic encephalopathy among HCV induced compensated cirrhotic patients.

Patients and Methods: 30 patients with HCV induced compensated liver cirrhosis with minimal hepatic encephalopathy were studied versus 30 patients with HCV induced compensated liver cirrhosis without minimal hepatic encephalopathy. Diagnosis of minimal hepatic encephalo-pathy was based on positivity of both Number Connection Tests A and B. Serum zinc was evaluated for all subjects. Zinc deficient minimal hepatic encephalopathy patients were supplemented with zinc sulphate heptahydrate 220 mg daily for 1 month before HCV treatment and scores of Number Connection Tests A and B were redetermined.

Results: Serum zinc is significantly lower in patients with minimal hepatic encephalopathy than in those without minimal hepatic encephalopathy. Scores of Number Connection Tests A and B among zinc deficient patients with minimal hepatic encephalopathy were significantly improved after zinc supplementation.

Conclusion: Zinc deficiency may play a role in development of minimal hepatic encephalopathy among patients with HCV induced compensated liver cirrhosis. Zinc supplementation is recommended for the treatment of minimal hepatic encephalopathy among those patients.

INTRODUCTION

Zinc is an important trace element and is a constituent of many metal-protein complexes such as metalloenzymes. It has a pivotal role in the regulation of protein metabolism, immune responsive-ness and membrane integrity. A group of disorders have been reported in patients with chronic liver disease which may reflect hypozincaemia. These abnormalities include taste and smell abnormalities, photoreceptor dys-function, immune dysfunction and hypogonadism [1]. Hypozincaemia affects nitrogen metabolism by impairing the activity of urea cycle enzymes in liver [2] and by reducing the activity of glutamine synthetase in muscles [3]. It has also been reported

that hypozincaemia may have a role in the pathogenesis of overt hepatic encephalopathy as serum zinc concentrations are decreased in patients with this condition and are inversely correlated with arterial concentrations of blood ammonia [4].

Minimal hepatic encephalopathy is a term used to describe cirrhotic patients who are clinically normal but show abnormalities of neurophysiological variables and/or cognition [5]. It negatively affects health related quality of life and the ability to perform complex tasks such as driving [6]. It also increases the risk of developing overt hepatic encephalopathy [7].

Impaired psychometric performance defines the presence of minimal hepatic

encephalopathy in cirrhotic patients who appear clinically normal. Patients with minimal hepatic encephalopathy show deficits in attention, fine motor skills and working memory while other cognitive abilities are relatively preserved. Patients with overt hepatic encephalopathy show additional disturbances in psychomotor speed, executive function and concentration [8].

Promising results have been obtained using a group of paper and pencil neuropsychological tests; namely Number connection tests A and B, Figure connection test, Line racing test, Circle dotting, Serial-dotting test and Digit symbol test. This battery of tests is called the Psychometric Hepatic Encephalopathy Score (PHES) and is used to assess the required domains of attention, concentration, memory, visual perception, visuospatial orientation and visuoconstructive abilities. They are easily applicable and have been shown to have a high specificity for the diagnosis of minimal hepatic encephalopathy [5,8].
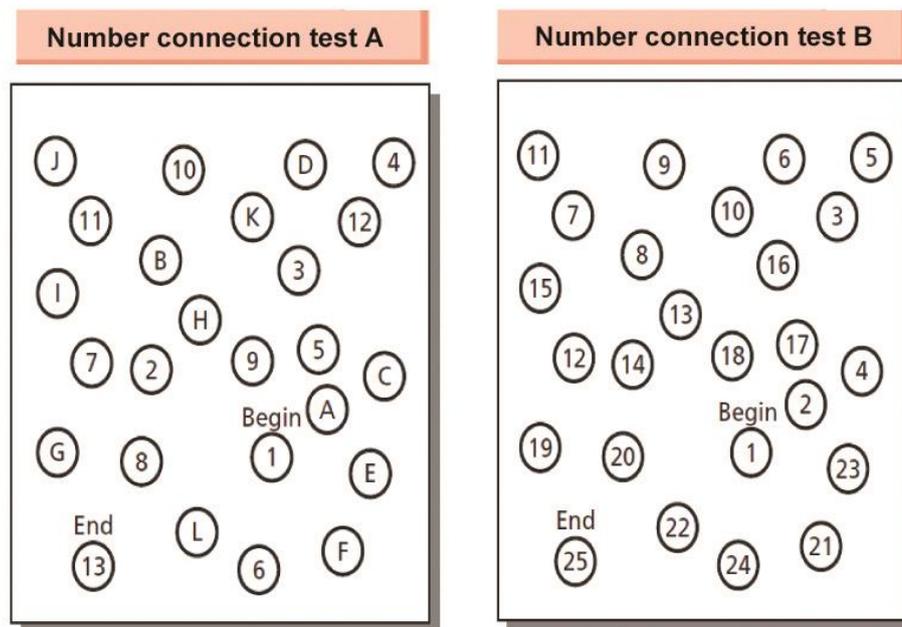
PATIENTS AND METHODS

This case-control study was conducted in Tropical Medicine and Clinical Pathology departments, Zagazig University hospitals in the period from January to June, 2017. Cases and controls were recruited from the HCV treatment unit affiliated to Tropical Medicine Department, Zagazig University hospitals. The study included 60 patients classified into two groups; first group (GI) of 30 patients with HCV induced compensated liver cirrhosis with minimal hepatic encephalopathy and second group (GII) of 30 patients with HCV induced compensated liver cirrhosis without minimal hepatic encephalopathy. Categorization of patients into either group was based upon the scores of both Number Connection Tests A and B. Positivity of both tests defines MHE. All participants gave a written consent to be included in the study.

All compensated cirrhotic patients in G I and G II had no evident clinical abnormalities, were on average dietary constitution with no intentional protein restrictions, had no constipation and not intentionally taking medical, herbal nor dietary laxatives and were not taking diuretics.

Patients with history of overt hepatic encephalopathy, those taking alcohol, those with history of a neurologic disease (eg, previous stroke), those with extremes of BMI and those with history of diuretic intake within one week prior to estimation of their serum zinc were excluded from the study.

All study participants were subjected to complete history taking and clinical examination. Routine laboratory investigations in the form of CBC, LFT, PT and INR and pelviabdominal ultrasonography were done for all study participants. Diagnosis of liver cirrhosis was based upon laboratory parameters (low platelet count, elevated AST/ALT ratio) and sonographic parameters (small right lobe, enlarged caudate lobe, undulated surface, coarse echopattern, irregular hepatic veins, dilated portal vein, collaterals and splenomegaly) [9]. Diagnosis of compensation was based upon clinical (absent symptoms and signs of liver disease), laboratory (normal serum albumin, bilirubin and prothrombin concentrations) and sonographic (absent ascites) grounds.

Two psychometeric tests (Number Connection Tests A and B) were applied to all cirrhotic patients included in the study to detect minimal hepatic encephalopathy and to give a score for each patient [10]. Minimal hepatic encephalopathy among cirrhotic patients was diagnosed by positivity of both Number connection tests A and B; both of which are tests of visuo-spatial orientation and psychomotor speed. The subject is given a sheet of paper with 25 circles which are randomly spread over the paper. In NCT-A, the task is to connect the circles 1-A-2-B-3-C and so on until L-13 as quick as possible. In NCT-B, the task is to connect circles 1 through 25 as quick as possible. Numbers and letters are written in arabic and the patient is shown a demonstration. Test result is the time needed by the patient to complete the test including error correction time. In this work, NCT is considered abnormal when the time taken by the patient to complete the test is greater than mean + 2SD from that of healthy controls (more than 47 seconds).

Serum zinc level estimation was performed for all study individuals by colorimetric method using commercially available kit (zinc assay kit, Segma-Aldrich, Germany). Minimum of two ml of venous blood was collected by venipuncture on a sterile red-capped vacutainer (Becton Dickinson Laboratories, Franklin Lakes, NJ, USA). Blood was incubated at 37°C for 10-20 min till complete clot formation. Serum was separated in blood samples by centrifugation at 1800–2000 rpm for 15 minutes. Serum was deproteinized using 7% TCAA. Aliquots from supernatant were stored at −70°C for later analysis. Normal serum zinc is 80-150 µg/dL. Zinc deficiency was defined when serum zinc concentration <80 µg/dL.

Zinc deficient patients with HCV induced compensated liver cirrhosis and minimal hepatic encephalopathy were supplemented with zinc sulphate heptahydrate 220 mg/day equivalent to 50 mg/day of elemental zinc divided into two doses for one month before HCV treatment and Number Connection tests A and B were repeated and test scores were compared with those before zinc supplementation.

Data were checked, entered and analyzed using SPSS version 19 for data processing and statistics. Continuous data were expressed as mean ± SD and categorical data were expressed as number (percentage). Continuous data were checked for normality by using Kolmogorov-Smirnov test. Student t-test was used to compare two groups of normally distributed continuous data and Mann-Whitney U (MW) test was used to compare two groups of non-normally distributed continuous data. Categorical data were compared using the Chi-square ($\chi2$) test. For the previously mentioned statistical tests, the threshold of significance was fixed at 5 % level (P value). P value of >0.05 indicates non-significant results. P value of <0.05 indicates significant results. P value of <0.001 indicates highly significant results. The smaller the P value obtained, the more significant are the results.

RESULTS

Table (1): Baseline characteristics of the studied groups

	G1 Patients with MHE	G2 Patients without MHE	P value	Significance
Age	54.5 ± 9.6	55.8 ± 9.2	0.6	NS
Sex (M / F)	19 / 11	18 / 12	0.92	NS
BMI	27.1 ± 3.5	26.7 ± 2.8	0.61	NS
Hb (gm / dL)	12.8 ± 2.7	13.1 ± 2.5	0.77	NS
WBC (x 10^3 cells/ mL)	6.3 ± 1.8	6.1 ± 1.9	0.71	NS
Plt (x 10^3 cells/ mL)	92 ± 37	85 ± 40	0.8	NS
ALT (U / liter)	69 ± 20	68 ± 18	0.82	NS
AST (U / liter)	70 ± 22	67 ± 20	0.65	NS
Albumin (g / dL)	3.4 ± 0.4	3.7 ± 0.4	0.11	NS
Bilirubin (mg / dL)	1.38 ± 0.4	1.4 ± 0.4	0.89	NS
Prothrombin time (seconds)	12.5 ± 1.6	12.1 ± 1.5	0.85	NS
Liver span (cm)	16.4 ± 2.3	16.5 ± 2.1	0.9	NS
Splenic axis (cm)	15.1 ± 3.5	14.8 ± 3.1	0.78	NS
Portal vein diameter (mm)	13.6 ± 0.8	13.5 ± 0.7	0.9	NS
Portosystemic collaterals	18 / 30 (60 %)	5 / 30 (16.7 %)	0.04	S
NCT-A score (seconds)	70 ± 5	41 ± 6	< 0.001	HS
NCT-B score (seconds)	64 ± 11	35 ± 10	< 0.001	HS
Serum zinc (mcg / dL)	66 ± 5	85 ± 4	< 0.001	HS

Table (2): Number of zinc deficient patients in both groups

	G1 Patients with MHE (30 patients)	G2 Patients without MHE (30 patients)	P value	Significance
Low serum zinc	23 / 30 (76.7 %)	4 / 30 (13.3 %)	< 0.001	HS
Normal serum zinc	7 / 30 (23.3 %)	26 / 30 (86.7 %)	< 0.001	HS

Table (3): Number Connection Test results before and after zinc supplementation in zinc deficient patients (23 patients) with HCV induced compensated liver cirrhosis and minimal hepatic encephalopathy

NCT	Score before zinc supplementation (seconds)	Score after zinc supplementation (seconds)	P value	Significance
A	69 ± 4	50 ± 5	< 0.001	HS
B	60 ± 7	45 ± 6	< 0.001	HS

DISCUSSION

Minimal hepatic encephalopathy is not uncommon complication of liver cirrhosis. Dhiman et al. [11] reported that the prevalence of minimal hepatic encephalopathy among compensated cirrhotic patients varied between 22% and 74% depending on the time and the number of psychometric tests used and the severity of the liver disease.

Many studies proposed the role of zinc deficiency in the pathogenesis of overt and minimal hepatic encephalopathy [12,13,14]. Zinc acts as a coenzyme for urea cycle enzymes and may be deficient in patients with liver cirrhosis especially if associated with protein malnutrition, maldigestion and malabsorption [2].

Previous studies assessing the role of zinc deficiency on development of minimal hepatic encephalopathy were performed on patients not specifically complicating chronic HCV infection and included cirrhotic patients of all grades of liver cirrhosis. The aim of the present work is to study the role of zinc deficiency in development of minimal hepatic encephalopathy among a group of patients with HCV induced compensated liver cirrhosis to assess non-traditional factors of the pathogenesis helping to give a chance for a new line for treatment of such a problem which negatively affects the quality of life of cirrhotic patients and puts them at risk of accidents and of developing overt hepatic encephalopathy.

In this study, there was a highly significant statistical difference between both studied groups as regard scores of Number Connection Tests A and B. This reflects that selection of patients in either group was based upon scores of both tests. We adopted positivity of both tests to define MHE. Weissenborn et al. [15] reported that combination of both NCT A and B is a sensitive method for diagnosis of early HE.

Mean age of patients with MHE is 54.5 years compared with a mean age of 55.8 years for patients without MHE with a non-significant difference between both means. This result indicates the fact that age is not an included risk factor for the development of MHE.

In this study, 19 patients with MHE were males and 11 were females while 18 patients without MHE were males and 12 were females. This non-significant difference in male-to-female ratio between both groups indicates that gender is not an included risk factor for the development of MHE. Male-to-female ratio of HCV induced liver cirrhosis is approximately 1.5:1. This is in agreement with Abd El Wahab et al. [16] who stated a ratio of 1.25:1. These figures reflect the fact that males are more commonly exposed to infection than females and to the fact that females may clear viremia better than males [17].

As regard to routine laboratory parameters, there was a non-significant difference between both groups as regard CBC and liver function tests. Serum albumin was higher in the group of HCV induced compensated liver cirrhosis without MHE compared with those with MHE but the statistical difference did not reach the level of significance. The relative hypoalbuminemia among patients with MHE reflects protein malnutrition among these patients. This is in agreement with Van der Rijt et al. [18] who reported a similar state of protein malnutrition among a group of patients with overt HE.

As regard to sonographic parameters, there was a non-significant difference between both groups as regard liver span, splenic axis and portal vein diameter. This non-significant difference can be explained by the fact that patients in both groups were HCV induced compensated liver cirrhotic patients. As regard to portosystemic collaterals, there was a significant difference between both

groups being more common among the group of MHE. This finding is in agreement with Tarantino et al. [19] who reported a strong relationship between portosystemic collaterals and ammoniacal encephalopathy.

The difference in both serum zinc concentration and number of zinc deficient patients between the two groups are statistically highly significant. Serum zinc concentration in the group of compensated liver cirrhosis with MHE is lower than that in the group of compensated liver cirrhosis without MHE (mean 66 µg/dL vs 85 µg/dL). Number of zinc deficient patients in the group of compensated liver cirrhosis with MHE is higher than that in the group of compensated liver cirrhosis without MHE (23/30 vs 4/30).

Postulated pathogenetic mechanisms of hypozincaemia in a sector of cirrhotic patients include inadequate intake of zinc-rich protein diet, alcohol-induced malabsorption and the influence of cytokines espicially interleukin-6 which is well known to decrease zinc metabolism [20]. Increase urinary zinc excretion in patients with cirrhosis seems related to the impairment of albumin synthesis. The decrease in serum albumin and the increase of free amino acid concentration in cirrhosis cause displacement of zinc bound from the macromolecular ligand that result in the increase of zinc filtration in the renal glomerulus [21].

In 2004, Yang et al. [13] studied the role of hypozincaemia on development of subclinical hepatic encephalopathy among 20 non-alcoholic cirrhotic patients most of them were HBV induced and some of them had decompensated liver disease. Their findings agree with the results of the present study namely the presence of a correlation between hypozincaemia and development of MHE among HCV induced compensated cirrhotic patients.

On supplementing zinc-deficient minimal hepatic encephalopathy patients with zinc sulphate heptahydrate 220 mg/day (equivalent to 50 mg / day elemental zinc), mean scores of NCT A and B improved with a highly significant statistical difference. This finding is similar to that obtained by Mousa et al. [22] who supplemented a group of compensated and decompensated liver cirrhotic patients with zinc and antioxidants for 3 months and found a significant improvement of scores of psychometric tests they adopted after supplementation.

In conclusion, the lowered serum zinc among HCV induced compensated cirrhotic patients with minimal hepatic encephalopathy and the improvement of scores of NCT A and B of zinc deficient patients after zinc supplementation suggest a role of hypozincaemia in development of minimal hepatic encephalopathy.

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