Trace elements and flapping tremors in patients with liver cirrhosis

Is there a relationship?

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ABSTRACT

الهدف: مرضى تليف وفشل الكبد معرضون لتغيير في مستويات المصل في العناصر الزهيدة والأملاح التي ممكن أن تكون من الأسباب المؤدية إلى الأمراض العصبية في هؤلاء المرضى. أجريت هذه الدراسة لمعرفة هل توجد علاقة ممكنة بين الرعاش الخافق الكبدي وبين مستويات المنجنيز والحديد والزنك والنحاس في المصل.

الطريقة: أجريت هذه الدراسة في مستشفى جامعة أسيوط في الفترة ما بين يونيو 2006م إلى يونيو 2007م. تم قياس مستويات المنجنيز والحديد الكلى والسعة الترابطية الكلية للحديد والزنك والنحاس باستخدام جهاز الامتصاص الذرى – الإصدار اللهبى مقْياسُ الطَّيف الضَوئِيّ. تم تقييم كل المرضى لبحث وجود الاعتلال المخي باستخدام اختباراتُ القَياسات النَّفْسية اليدوية، والوظائف المعرفية باستخدام اختبارات الحالة المَخية الدنيا. أيضا تم عمل تخطيط كَهْرَبِيَّةِ الدِّماغ و التَّصْويرُ بالرَّنينِ المِغْناطيسِيّ.

النتائج: وجد في المرضى زيادة ذات دلالة إحصائية في مستويات المنجنيز والنحاس وقلة في مستويات السعة الترابطية الكلية للحديد والزنك في المصل مقارنة بالمجموعة الضابطة. 82% من المرضى يعانون من اعتلال دماغي كبدي قليل. وجد في 85% من المرضى زيادة في شدة التصوير بالرنين المغناطيسي بالمادة السوداء و الكرَةُ الشَّاحِبَة في-T1 الصورة الموزونة. وجدت علاقة ايجابية بين الرعاش الخافق وشدة اعتلال الكبد والاعتلال المخي الكبدي الدنيا والنحاس وعلاقة سلبية مع الحديد الكلى والسعة الترابطية الكلية للحديد والزنك.

خاتمة: من هذه الدراسة يستخلص أن الاختلال في الاستتباب للمنجنيز والأملاح الأخرى يؤدى إلى الفيزْيُولُوجْيا المَرَضيَّة في نقيصة المعرفة مع تليف الكبد وليس مع الرعاش الخافق. الدور المرضى المحدد والمقْتَضَيات العلاجية المحتملة تحتاج إلى دراسة.

Objective: To investigate the possible correlation between hepatic flapping tremors and serum manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu).

Methods: This case control study was carried out in Assiut University Hospital, Assiut, Egypt from June 2006 to June 2007. It included 100 patients with liver cirrhosis, 78 had flapping tremor, and 22 had not, and 60 healthy controls. All patients were subjected to assessment of serum Mn, total Fe, total iron binding capacity (TIBC), Zn, and Cu. Assessment of hepatic encephalopathy was carried out using a battery of cognitive function tests. All patients had electroencephalography and MRI of the brain.

Results: Compared to healthy controls, patients showed increase in Mn (p<0.0001), Cu (p<0.05) and decrease in TIBC (p<0.000), Zn (p<0.05). Eighty-two percent of patients had minimal hepatic encephalopathy (mHE). In 85%, MRI-brain showed bilateral hyperintense substantia nigra and globus pallidus on T1-weighted images. A significant positive correlation was present between tremors and severity of liver dysfunction, mHE and serum Cu, and negative correlation with total Fe, TIBC, and Zn.

Conclusion: Altered homeostasis of Mn and other minerals could be responsible for the pathophysiology of cognitive deficits associated with liver cirrhosis, but not with flapping tremors. The exact pathogenic role and possibilities for therapeutic implications need further study.

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The liver and brain interact in numerous ways. The liver supplies nutrients to the brain and removes toxic substances that are harmful to the brain's nerve cells. Liver dysfunction can cause disturbance of brain function and even contribute to brain damage.[1] Hepatic cell dysfunction is commonly associated with impairment of mental functions and motor disturbances.[2,3] Asterixis or flapping tremors, described originally by Adams and Foley,[4] are a common manifestation of liver cirrhosis and failure. It is one type of negative myoclonus.[5] It is an uncontrollable flapping of hands that becomes noticeable on instruction to maintain wrist, and fingers extended on stretching out arms and palms. These intermittent brief lapses result from a sudden cessation of electrical activity in extensor muscles due to an intermittent inhibition of the spinal neuronal system that mediates voluntary tonic extension of the limb.[5] Recently, trace elements have been implicated in the etiology of hepatic encephalopathy and its associated motor neurologic manifestations. Manganese (Mn) is implicated, in part, in the pathogenesis of chronic hepatic encephalopathy.[6] In patients and animals with liver cirrhosis, the Mn concentration was found to be high.[7,8] Abnormal deposits of Mn in the basal ganglia were observed in these patients by MRI.[9] Deposits of Mn in the globus pallidus results in motor symptoms and structural changes of astrocytes that are characteristic of hepatic encephalopathy.[10] Myoclonus and postural tremors have been reported among manifestations of Mn overload.[11,12] Altered homeostasis of trace elements and other minerals has also been reported in several studies of hepatic encephalopathy.[8,13,14] Iron (Fe) plays an essential role as a cofactor for electron transporting enzymes in oxidative metabolism and as an oxygen transporter.[15] In patients with liver cirrhosis, Fe deficiency has been attributed as a factor for manganese-induced neurotoxicity.[16] Zinc (Zn) deficiency and increased serum copper (Cu) levels are common in patients with liver cirrhosis with and without hepatic encephalopathy.[17] Zinc is important for function of more than 200 zinc metalloenzymes. It functions as an antioxidant and prevents hepatocellular injury.[18] Copper (Cu) is required for catalytic activity of enzymes that play essential parts in neurobiology and pathogenesis, including tyrosinase for melanin synthesis, cytochrome-*c* oxidase for electron transport in mitochondrial respiratory chain and antioxidant enzymes.[19] Alteration of Cu/Zn homeostasis in the brain could be related to brain dysfunction and encephalopathy in patients with liver disease, however, the exact pathogenic role has been poorly described. The relationship between serum trace elements and minerals to flapping tremors in patients with liver cirrhosis has not been well established. In this investigation, we aimed to determine if there is a possible association between serum Mn, Fe, Zn, Cu and flapping tremors in patients with liver cirrhosis.

Methods. Included in this case-control study were 100 non-alcohol-induced liver cirrhosis patients, mean age, 47.48 years, and of them 78 patients had flapping tremors and 22 had not. Sixty healthy subjects were included as controls that were matched for age- and gender, their mean age was 44.67 years. The patients were recruited from the Department of Internal Medicine of Assiut University Hospital, Assiut, Egypt from June 2006 to June 2007. The study was approved by the Regional Ethics Committee for Medical Research and informed written consent was obtained from all participants. Excluded from study were patients with other medical, central nervous system diseases, patients with hepatocellular carcinoma, severe non-compensation of cirrhosis at time of study (gastrointestinal bleeding, renal insufficiency, or bacterial infection), alcohol abusers and patients receiving specific other medications. None of the patients had clinically overt hepatic encephalopathy during the previous 3 months. All participants were subjected to clinical assessment including, history taking, and complete medical and neurological examinations. Minimal hepatic encephalopathy (mHE) was diagnosed using manual psychometric tests. Psychometric tests included Number Connection Test part A (NCT-A),[20] Digit Symbol Test (DST) and Block Design Test (BL DES).[21] The NCT-A measures cognitive motor abilities. The DST is a subset of the Wechsler Adult Intelligence Scale (WAIS) and measures motor speed and accuracy. The BL DES describes ability to construct designs or patterns from pictorial models. Minimal hepatic encephalopathy was diagnosed in the presence of at least one abnormal psychometric test, namely, >2 SD for the mean of control subjects. Cognitive functions were assessed using the Mini-Mental State Examination (MMSE).[22] The MMSE consists of a variety of questions, which represents different cognitive domains as orientation, word repetition, attention, calculation, word recall, language, and visual construction. It has a maximum score of 30. Presence of extrapyramidal signs were evaluated by Columbia scale,[23] which scores the following parameters: facial expression, speech disorder, arising from chair, posture, postural stability, gait disturbance, finger dexterity, bradykinesia, and succession movements tested in both hands and feet. The severity of liver failure was graded using standard laboratory parameters and Child-Pugh's classification. All patients had standard electroencephalography (EEG), MRI of brain, and electromyography (EMG) to diagnose asterixis. Laboratory work-up included assessment of the levels of total serum bilirubin, serum alanine (ALT), and aspartate (AST) aminotransferase activities, prothrombin time and activity, ammonia, and complete blood count. Measurement of serum levels of Mn, Fe, total iron binding capacity (TIBC), Zn, and Cu. Venous blood samples were collected into

polypropylene tubes containing EDTA as anticoagulants and stored at -20^{0}C. Serum samples were diluted with deionized water (0.5:4.5 v/v). Trace elements levels were then measured with an atomic absorption/flame emission spectrophotometer (Schimadzu Seisakusho LTD, model AA-630-02, Japan), using an air-acetylene flame and hollow cathode lamps. Standards were obtained from Buck Scientific, New York, United States of America. The lamp current (mA), the wave length (nm), and standard concentration (lot number) were 10 mA, 297.5 nm, 1003 µg/ml Mn in 2% nitric oxide (HNO3) (lot # 9809F), 10 mA, 248.3 nm, 1003 µg/ml Fe in 5% HNO3 (lot # 9809F), 10 mA, 213.9 nm, 999 µg/ml Zn in 2% HNO3 (lot # 9711V) and 10 mA, 324.7 nm, 996 µg/ml Cu in 2% HNO3 (lot # 9805K).

Data were expressed as mean ± SD. Calculations were carried out with the statistical package SPSS for windows, version 12. Qualitative variables were compared with the x^2 test and mean values with Student's t test or Mann-Whitney U test when distribution of data skewed from normal. Association between continuous variables was assessed using Pearson's correlation coefficient. Independent variables associated to flapping tremors were assessed using logistic regression analysis in which a bivariate analysis was performed first to identify individual variables significantly associated with flapping tremors and then significant variables

Table 1 - The demographic, clinical and laboratory characteristics of the studied groups: relationship to presence of flapping tremors.

Characters	Patients (n=100)			Control (n=60)
	Total (n=100)	With tremor (n=78)	Without tremor (n=22)	
Age				
Years	47.48 ± 11.02	47.49 ± 9.79	47.35 ± 15.59	44.67 ± 15.90
Range	(17.5 - 65)	(27 - 65)	(17.05 - 65)	(23 - 70)
Gender				
Men	78 (78%)	58 (74.4%)	20 (90.9%)	40 (66.6%)
Women	22 (22%)	20 (25.6%)	2 (9.1%)	20 (33.3%)
Disease duration				-
Years	3.68 ± 2.16	3.82 ± 2.35	3.15 ± 1.11	
Range	(1 - 14)	(1 - 14)	(2 - 5)	
Child-Pugh's classification				
Grade A	30 (30%)	10 (12.8%)	20 (90.1%)	-
Grade B	48 (48%)	46 (59%)	2 (9.9%)	-
Grade C	22 (22%)	22 (28.2%)	-	-
Total bilirubin				
mg/dl	29.49 ± 9.77*	28.74 ± 9.93*	32.40 ± 8.98*	0.63 ± 0.29
Range	(12 - 52)	(12 - 52)	(20 - 49)	(0.2 - 1.1)
ALT				
IU/L	61.20 ± 89.18*	59.10 ± 92.23	69.40 ± 80.06	29.70 ± 5.72
Range	(15 - 600)	(15 - 600)	(20 -290)	(20 - 40)
AST				
IU/L	89.67 ± 58.66*	85.15 ± 54.94*	107.30 ± 71.93*	25 ± 7.81
Range	(35 - 300)	(35 - 300)	(39 - 280)	(4 - 37)
Prothrombin time				
Seconds	18.67 ± 3.4*	19 ± 3.64*	17.40 ± 2.17*	9.04 ± 1.51
Range	(13 - 31)	(13 - 31)	14 - 20)	(6 - 11)
Prothrombin activity				
%	58.59 ± 17.25*	56.74 ± 18.12*	65.80 ± 11.33*	87.22 ± 8.70
Range	(22 - 100)	(22 - 100)	(50 - 88)	(75 - 100)
Hemoglobin				
mg/dl	7.65 ± 1.94*	7.31 ± 1.59*	8.96 ± 2.56*†	14.05 ± 0.57
Range	(5 - 13)	(5 - 12)	(6 - 13)	(13.40 - 15.20)
Ammonia				
mg/dl	180 ± 50*	200 ± 50*†	100 ± 10	90 ± 15
Range	(75 - 250)	(150 - 250)	(75 - 200)	(75 - 150)

Data are expressed as mean ± SD, number (%), *Significance versus controls, †Significance versus patients without tremors

Table 2 - Serum levels of trace elements and macro-minerals in the studied group.

Measured parameters	Patients (n=100)	Control (n=60)	*P*-value
Manganese			
µg/dl	1.29 ± 0.56	0.68 ± 0.25	<0.000
Range	(0.06 - 2.40)	(0.3 - 1.1)	
Total iron			
µg/	106.92 ± 58.43	119.48±6.89	>0.05
Range	(27 - 271)	(110 - 130)	
Total Iron binding capacity			
µg/L	194.45 ± 94.26	321.48 ± 52.23	<0.000
Range	(50 - 521)	(250 - 400)	
Zinc			
µg/dl	86.43 ± 26	99.44 ± 20.82	<0.05
Range	(60 - 150)	(60 - 120)	
Copper	150.10 ±10.03	120.77±20.24	<0.05
µg/dl	(50 - 170)	(71.34-140)	
Range			

Data are expressed as mean ± SD

Table 3 - Trace elements and macro-minerals in the studied groups: relationship to presence of flapping tremors.

Measured parameters	Patients (n=100)		Control (n=60)
	With tremor (n=78)	Without tremor (n=22)	
Manganese			
µg/dl	1.23 ± 0.56	1.51 ± 0.55	0.68 ± 0.25
Range	(0.06 - 2.4)	(0.4 - 2)	(0.3 - 1.1)
Number of patients (%)(high level)	76 (97.4%)	22 (100%)	-
Significance	*p<0.000	*p<0.000 †p>0.05	
Total iron			
µg/L	112.21 ± 60.48	86.3 ± 46.56	119.48 ± 6.89
Range	(27 - 271)	(32 - 158)	(110 - 130)
Number of patients (%)(low level)	30 (38.5%)	14 (63.6%)	-
Significance	*p>0.05	*p>0.05 p>0.05	
Total iron biding capacity			
µg/L	199.05 ± 99.42	176.50 ± 72.11	321.48±52.23
Range	(50 - 521)	(95 - 280)	(250 - 400)
Number of patients (%)(low level)	52 (66.7%)	20 (90%)	-
Significance	*p<0.000	*p<0.000 †p>0.05	
Zinc			
µg/dl	86.69 ± 28.17	85.40 ± 15.94	99.44 ± 20.82
Range	(60 - 150)	(70 - 115)	(60 - 120)
Number of patients (%)(low level)	12 (15.4%)		
Significance	*p<0.05	-*p<0.05 †p>0.05	-
Copper			
µg/dl	150.26 ± 10.07	150.60 ± 10.09	120.77 ± 20.24
Range	(50 - 170)	(80 - 160)	(71.34 - 140)
Number of patients (%)(high level)	22 (28.2%)	10 (45.5%)	-
Significance	p<0.05	*p<0.05 †p>0.05	

Data are expressed as mean ±SD, *Significance versus controls, †Significance versus patients without tremors.

Table 4 - Correlation between flapping tremors, manganese and other measured trace and macro-minerals.

Measured parameters	Flapping tremors		Manganese	
	r	*P*-value	r	*P*-value
Manganese	0.040	>0.05	-	-
Total iron	- 0.337	<0.01	-0.364	<0.001
Total iron binding capacity	- 0.579	<0.0001	-0.262	<0.01
Zinc	- 0.267	<0.05	-0.065	>0.05
Copper	0.285	<0.05	0.006	>0.05

(p<0.05) were included in multivariate analysis. Results were considered significant at the level of p<0.05.

Results. The demographic, clinical, and laboratory characteristics of the studied groups are demonstrated in **Table 1**. Patients with and without flapping tremor showed high levels of total bilirubin, AST, prolonged prothrombin time and low hemoglobin levels, and prothrombin activities compared with controls. Meanwhile, patients with tremor showed higher ammonia levels than controls. Thirty percent of patients (n=30) demonstrated high levels of ALT. Seventy-eight percent (n=78) of patients had low prothrombin activity. Patients with flapping tremors had higher ammonia levels compared with patients without tremor. Most patients (82%), who appeared normal on bedside examination exhibited different cognitive deficits (in visual-spatial perception, attention, concentration, constructional ability, and so forth) with abnormal scores of the =test in 52% of patients, DST in 12%, and BL DES test in 18%. The MMSE was abnormal in 45% of patients. The mean (±SD) score of the MMSE was 25.5 ± 3.4 (range 17-30), while 22% (n=22) of patients had abnormal EEG. Patients with abnormal test results were diagnosed as having minimal hepatic encephalopathy (mHE). None of the patients demonstrated extrapyramidal signs (data not shown). Compared to the healthy control group, patients with liver cirrhosis showed elevated serum levels of Mn (p<0.000), Cu (p<0.05) and decreased levels of TIBC (p<0.000) and Zn (p<0.05) (**Table 2**). Patients with and without flapping tremors demonstrated significant elevation of Mn (p<0.000 for both) and Cu (p<0.05 for both) and significant decrease in levels of total TIBC (p<0.000 for both) and Zn (p<0.05 for both) (**Table 3**). Eighty-five percent of patients showed striking hyperintensities on T1-weighted images of MRI-brain involving the substantia nigra and globus pallidus bilaterally, including all patients with severe liver disease (Child-Pugh Class C) (data not shown). On bivariate analysis, flapping tremors were positively correlated with severity of liver dysfunction as graded by the Child-Pugh's score (r=0.48, p<0.001), mHE (r=0.472; p<0.01), cognitive impairment as scored by MMSE (r=0.34, p<0.05) and blood ammonia (p<0.000), Cu (r=0.285, p<0.05) and negatively correlated with total Fe (r=-0.337, p<0.01), TIBC (r=-0.579, p<0.0001) and Zn (r=-0.267, p<0.05). Manganese was negatively correlated with Fe (r=-0.364, p<0.001) and TIBC (r=-0.262, p<0.01) (**Table 4**). Whereas in the multivariate analysis, mHE (odd's ratio, 3.46; 95% Cl; 1.23-9.80; p<0.05) and blood ammonia level (odd's ratio, 2.90; 95% Cl, 1.20-6.82; p<0.01) are the independent variables related to flapping tremors.

Discussion. The role of trace elements and several other minerals in the pathogenesis of hepatic encephalopathy in patients with liver cirrhosis has been recently described. However, their relationship with flapping tremors encountered in patients with liver cirrhosis has not been well established. In this study, a significant increase in serum Mn was observed in patients with liver cirrhosis. Manganese is an essential component of enzymes involved in intermediary metabolism, free-radical scavenger system, and various transport proteins.[24] Manganese probably exists in synaptic vesicles of glutamatergic neurons that are present in many connections of basal ganglia and in the whole brain.[25,26] The bodies concentration of Manganese is controlled by the hepatobiliary system.[27] Many human and animal studies demonstrated that with chronic liver diseases, cirrhosis and in hepatic encephalopathy, Mn accumulates in the blood and brain.[6-8,28]

In this study, 85% of patients showed bilateral hyperintense basal ganglionic signals in non-contrast TI-weighted MRIs, possibly due to Mn accumulation in the basal ganglia.[9,10] Several studies have described similar findings in patients with subclinical as well as in overt encephalopathy, though this did not correlate with severity of hepatic encephalopathy.[29] The Mn content of the globus pallidus was reported to be 10 fold higher than in non-cirrhotic individuals.[30] A 2 to 7-folds increase of Mn concentration has been reported in autopsies of the globus pallidus of patients that died from liver cirrhosis.[31]

In this study, flapping tremors were positively correlated with the severity of liver dysfunction as graded according to Child-Pugh's Score and cognitive impairment as scored by MMSE and blood ammonia, but not with blood Mn. It has been found that patients with severe liver failure and portosystemic shunts may develop an extrapyramidal disorder, non-Wilsonian hepatolenticular degeneration, which has been proposed as secondary to Mn accumulation in the basal ganglia.[10,32] Studies revealed that the intensity of pallidal MRI images has been found to correlate with blood Mn and with the presence of extrapyramidal symptoms occurring in the majority of compensated and non-

compensated cirrhotic patients.[3,33] Takeda[26] reported that abnormally high concentrations of Mn in the brain and especially in the basal ganglia are associated with neurological disorders similar to Parkinson's disease. Flapping tremor is one type of negative myoclonus.[5] Action myoclonus and postural tremors are among manifestations of Mn overload. Chelation with EDTA was associated with resolution of myoclonus and abnormal MRI.[11,12] The pattern of selective accumulation of Mn in the globus pallidus is similar to that in Wilson's disease and neurodegeneration with brain iron degeneration, frequently associated mutations in pantothenate kinase 2 (PANK2) gene.[34] The basal ganglia has multiple projections and participates in a number of parallel processes, including cognitive and emotional functions in addition to motor tasks.[31] It belongs to a complex neural network involved in the production of tremors.[35]

In this study, the mean serum iron level of patients was lower than controls but the difference did not reach significant levels. Meanwhile, TIBC was significantly decreased. Also, total Fe and TIBC were negatively correlated with the presence of tremors and with serum Mn. Iron deficiency has been reported in liver cirrhosis and attributed, in part, to malnutrition.[36] The principal receiver and storage site of Fe is the liver.[37] Iron deficiency seems to be frequent among patients showing brain MRI abnormalities compatible with Mn deposits in the basal ganglia.[16] Iron deficiency has been found to competitively increase Mn absorption, which leads to basal ganglia bilateral symmetric hyperintensity on T1-weighted sequences on MRI.[16] This observation suggests that Fe deficiency could be an important risk factor for Mn-induced neurotoxicity and should, therefore, be accurately considered and treated.

In this study, serum Zn in patients with tremor was significantly lowered. Zinc deficiency is common in patients with chronic liver disease with and without hepatic encephalopathy.[17] Various factors like poor dietary intake, impaired intestinal absorption, and excessive urinary loss for which excessive diuretic administration might be responsible for Zn deficiency encountered in patients with liver disease.[38] Also, this study revealed negative correlation between Zn concentrations and presence of tremors and serum Mn. It had been reported that alteration of Zn homeostasis in the brain may be associated with brain dysfunction, and be also involved in many diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and epilepsy.[39]

In this study, the serum Cu level was significantly elevated and its level was positively correlated with tremor. Several studies reported increased serum Cu concentration in patients with liver cirrhosis.[13] Copper is required for catalytic activity of enzymes including Cu/Zn superoxide dismutase for antioxidant defense, dopamine hydroxylase for catecholamine biosynthesis, and ceruloplasmin for brain Fe homeostasis.[40] Disturbed Cu/Zn in the brain due to chronic liver disease might be attributed to altered brain function and encephalopathy. The exact mechanism of nerve damage due to Mn overload is not known. An excess of Mn may be toxic to the brain as this metal has prooxidant activity.[24] Experimental observations suggested that secondary excitotoxic mechanisms play a crucial role in development of Mn-induced neuro-degeneration in the striatum. Manganese toxicity results in extrapyramidal symptoms related to alterations of dopamine functions in the basal ganglia, probably due to a selective increase in Mn concentration in this area.[41] Manganese causes activation of glutamate-gated cation, for example, N-methyl-D-aspartate (NMDA) receptors, which contributes to neuronal degeneration.[42] As the globus pallidus is the output station for glutamate in the basal ganglia, it is vulnerable to Mn toxicity.[43] Direct toxic effects of Mn have been observed in dopaminergic neurons. Normally, dopamine suppresses acetylcholine activity. If dopamine can no longer suppress acetylcholine, the acetylcholine activity will increase, which may be associated with extra pyramidal reactions as in drug-induced Parkinsonism.[44] Takeda[26] demonstrated that Mn modulates release of GABA from neuron terminals of the striatum. The inhibitory action of Mn against GABAergic neuron activity is abnormal excitation of striatal neurons during Mn intoxication with hyperactivity of corticostriatal fibers. Further experimental investigations are necessary to understand the mechanism of action of trace elements in synapses of the brain.

To summarize, the results of this study indicated that homeostasis of trace elements and other minerals, is altered in patients with liver cirrhosis with and without liver cell failure. High Mn levels, whether direct overload caused by liver disease or indirect as caused by alteration of other minerals, were linked with the existence of minimal hepatic encephalopathy and severity of liver disease, but not with the presence of flapping tremors in patients with liver cirrhosis. Further elucidation of the pathophysiologic mechanisms of Mn and other minerals and possibilities of new therapeutic approaches needs future studies.

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