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The Relationship Between Trace Elements and Hepatic Encephalopathy in Egyptian Patients With Liver Cirrhosis

KEYWORDS

Ahmed Samir Abo Halima
MD Internal medicine, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Ossama Ashraf Ahmed
MD Internal medicine, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Hanan Mahmoud Badawy
MD Internal medicine, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Amal Shawky Bekir
MD Internal medicine, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

ABSTRACT *Introduction : Hepatic encephalopathy is a complex and potentially reversible neuropsychiatric syndrome complicating acute or chronic liver disease. Clinical manifestations are multiple and varied, ranging from minimal neurological changes to coma. Ammonia is the main toxic substance involved in the pathogenesis of hepatic encephalopathy, although other mechanisms, such as modifications of the blood-brain barrier, disruptions in neurotransmission and abnormalities in GABAergic and benzodiazepine pathways may also play a role. The identification and treatment of precipitating factors is crucial in the management of patients with hepatic encephalopathy (Bismuth M et al., 2011).*

Apart from increased blood ammonia, alterations in various other substances have been implicated in the pathogenesis of hepatic encephalopathy (HE). The role of trace elements like zinc and manganese has been described recently (Chetri K and Choudhuri G, 2003).

Aim of the Study : The purpose of this case control study is to investigate the possible relationship between hepatic encephalopathy and serum levels of manganese (Mn), zinc (Zn), and copper (Cu) in egyptian cirrhotic patients compared with age- and sex-matched control subjects and correlate them with various clinical and laboratory parameters.

Patients and methods : This case control study was carried out in Sohag General Hospital from January to March 2012. It included 30 cirrhotic patients and 10 healthy subjects as controls that were matched for age and gender. Cirrhotic patients were classified into 2 groups, Group I: which included 15 cirrhotic patients with hepatic encephalopathy. Group II: which included 15 cirrhotic patients without hepatic encephalopathy. All participants were subjected to full history taking, thorough clinical examination, abdominal ultrasonography and routine laboratory investigations. Serum levels of manganese (Mn), zinc (Zn), and copper (Cu) and also blood ammonia levels were estimated in all subjects. All patients were classified according to modified Child-Pugh's classification to asses the severity of liver cirrhosis. Severity of hepatic encephalopathy was assessed according to West Haven (W-H) criteria.

Results : In this study, serum manganese levels were highly significant increased in cirrhotic patients with or without HE compared to controls (median 2.5 µg/dl in cirrhosis with HE and 2.2 µg/dl in cirrhosis without HE vs. 0.75 µg/dl in controls, p<0.001 for both)

Whereas, serum manganese levels were not significantly different between cirrhotic patients with HE and cirrhotic patients without HE (median 2.5 µg/dl in cirrhosis with HE vs. 2.2 µg/dl in cirrhosis without HE, p>0.05)

In this study, serum zinc levels showed highly significant decrease in child C patients in comparison to child B patients in both cirrhosis with HE group (median 47.5 µg/dl in child B vs. 26.5 µg/dl in child C, p>0.01) and in all cirrhotic patients (both with and without HE) (median 45.0 µg/dl in child B vs. 28.5 µg/dl in child C, p>0.001)

In this study, serum copper levels showed significant increase in child C patients in comparison to child B patients in cirrhosis with HE group (median 92.25 µg/dl in child B vs. 165.5 µg/dl in child C, p>0.05)

Conclusion; serum levels of studied trace elements differ significantly in patients with liver cirrhosis compared to healthy subjects as there was significant increase in serum levels of manganese and copper, whereas, there was significant decrease in serum zinc levels in cirrhotic patients (regardless presence or absence of HE) compared to control subjects. However, there were no significant differences in serum levels of studied trace elements in cirrhotic patients with HE compared to those without HE.

Introduction

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 **(Butterworth RF, 2003a)**.

Hepatic encephalopathy is a complex and potentially reversible neuropsychiatric syndrome complicating acute or chronic liver disease. Clinical manifestations are multiple and varied, ranging from minimal neurological changes to coma. Ammonia is the main toxic substance involved in the pathogenesis of hepatic encephalopathy, although other mechanisms, such as modifications of the blood-brain

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barrier, disruptions in neurotransmission and abnormalities in GABAergic and benzodiazepine pathways may also play a role. The identification and treatment of precipitating factors is crucial in the management of patients with hepatic encephalopathy **(Bismuth M et al., 2011)**.

Manganese (Mn) is an essential mineral that is a cofactor for many enzymes required in the synthesis of proteins, carbohydrates, and lipids. Because hepatic clearance is essential in Mn homeostasis, conditions in humans resulting in hepatic insufficiency including cirrhosis and both acquired and congenital portosystemic shunting have been reported to result in increased blood Mn concentrations and increased Mn content in the central nervous system. Because Mn toxicity causes neurologic disturbances, increased Mn concentrations have been implicated in the pathogenesis of hepatic encephalopathy **(Gow AG et al., 2010)**.

Zinc plays an important role in human physiologic processes being cofactor of many enzymes. With the rising of class of liver impairment and developing liver encephalopathy, level of zinc in blood drops. Treatment with diuretic therapy results in increase of discharge of zinc with urine and prevents recovery of its level in blood **(Shaposhnikova NA et al., 2007)**.

Copper (Cu) is required for catalytic activity of enzymes that play essential parts in neurobiology and pathogenesis of neuro-degenerative diseases; including tyrosinase for melanin synthesis, cytochrome-c oxidase for electron transport in mitochondrial respiratory chain and antioxidant enzymes **(Waggoner DJ et al., 1999)**.

Aim of the Study

The purpose of this case control study is to investigate the possible relationship between hepatic encephalopathy and serum levels of manganese (Mn), zinc (Zn), and copper (Cu) in egyptian cirrhotic patients compared with age- and sex-matched control subjects and correlate them with various clinical and laboratory parameters.

Patients and methods

This case control study was carried out in Sohag General Hospital from January to March 2012. It included 30 cirrhotic patients and 10 healthy subjects as controls that were matched for age and gender and with no other comorbidities or medications that affect the results. Cirrhotic patients were classified into 2 groups, **Group I:** which included 15 cirrhotic patients with hepatic encephalopathy. **Group II:** which included 15 cirrhotic patients without hepatic encephalopathy. All participants were subjected to full history taking, thorough clinical examination, abdominal ultrasonography and routine laboratory investigations. Serum levels of manganese (Mn), zinc (Zn), and copper (Cu) and also blood ammonia levels were estimated in all subjects. All patients were classified according to modified Child-Pugh's classification to asses the severity of liver cirrhosis. Severity of hepatic encephalopathy was assessed according to West Haven (W-H) criteria. IBM SPSS statistics (V. 23.0, IBM Corp., USA, 2012) was used for data analysis.

Results

This case control study was carried out in Sohag General Hospital from January to March 2012. It included 30 cirrhotic patients and 10 healthy subjects as controls that were matched for age and gender. Cirrhotic patients were classified into 2 groups:

Group I: which included 15 cirrhotic patients with hepatic encephalopathy. Their gender was 8 males (53.3%) and 7 females (46.7%). Their mean age was (54.467±7.2984) years. Their HE grades according to West Haven criteria were (1/2/3=9/4/2). 6 patients without ascites, 2 with mild ascites and 7 with moderate ascites. Their median child score was (11±2.4689) and their child classes were (A/B/C=0/6/9).

Group II: which included 15 cirrhotic patients without hepatic encephalopathy. Their gender was 9 males (60.0%) and 6 females (40.0%). Their mean age was (52.867± 8.1404) years. 1 patient without ascites, 3 with mild ascites, 5 with moderate ascites and 6 patients with massive ascites. Their median child score was (10±2.2824) and their child classes were (A/B/C=1/6/8).

Control group (Group III): which included 10 healthy subjects. Their gender was 5 males (50.0%) and 5 females (50.0%). Their mean age was (52.1± 9.6546) years.

Table 1. Comparison between the three studied groups as regards the trace elements and ammonia.

	Cirrhosis with HE group (n=15)		Cirrhosis without HE group (n=15)		Control group (n=10)		H	P–value	Sig.
	Median	SD	Median	SD	Median	SD			
Serum manganese (Mn) (µg/dl)	2.5	0.8353	2.2	0.2086	0.75	0.6154	17.56	0	HS
Serum Zinc (Zn) (µg/dl)	31.5	13.2474	40	12.462	90.5	19.579	22.194	0	HS
Serum Copper (Cu) (µg/dl)	117.5	43.2433	96	20.535	73.25	6.918	17.177	0	HS
Blood ammonia (NH_3) (µg/dl)	185	49.3093	160	62.6849	39	7.6768	21.341	0	HS

There were highly significant differences between the studied groups as regards the trace elements and ammonia where manganese, copper and ammonia show marked increase and zinc shows marked decrease in patients with liver cirrhosis compared with control group.

Table 2. Comparison between cirrhosis with HE and without HE groups as regards the trace elements and ammonia.

	Cirrhosis with HE group (n=15)		Cirrhosis without HE group (n=15)		Z	P–value	Sig.
	Median	SD	Median	SD			
Serum manganese (Mn) (µg/dl)	2.5	0.8353	2.2	0.2086	-0.728	0.467	NS
Serum Zinc (Zn) (µg/dl)	31.5	13.2474	40	12.462	-1.452	0.146	NS
Serum Copper (Cu) (µg/dl)	117.5	43.2433	96	20.535	-1.681	0.093	NS
Blood ammonia (NH_3) (µg/dl)	185	49.3093	160	62.6849	-1.348	0.178	NS

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There was no significant change (p>0.05) in comparison between cirrhosis with HE and without HE groups as regards the trace elements and ammonia.

Table3. Correlation between blood ammonia and trace elements in cirrhosis with HE group.

Cirrhosis with HE group (n=15)	Blood ammonia (NH_3) (µg/dl)		
	r–value	P–value	Sig.
Serum manganese (Mn) (µg/dl)	0.625	0.013	S
Serum Zinc (Zn) (µg/dl)	-0.646	0.009	HS
Serum Copper (Cu) (µg/dl)	0.736	0.002	HS

There was a significant (p<0.05) positive relation between blood ammonia and serum manganese (serum manganese increases as blood ammonia increases and vice versa). There was a highly significant (p<0.01) negative relation between blood ammonia and serum zinc (blood ammonia increases as serum zinc decreases and vice versa). There was a highly significant (p<0.01) positive relation between blood ammonia and serum copper (serum copper increases as blood ammonia increases and vice versa).

Table 4. Correlation between blood ammonia and trace elements in cirrhosis without HE group.

Cirrhosis without HE group (n=15)	Blood ammonia (NH_3) (µg/dl)		
	r–value	P–value	Sig.
Serum manganese (Mn) (µg/dl)	-0.251	0.367	NS
Serum Zinc (Zn) (µg/dl)	-0.52	0.047	S
Serum Copper (Cu) (µg/dl)	-0.24	0.389	NS

There was a non significant (p>0.05) relation between blood ammonia and serum manganese and copper. There was a significant (p<0.05) negative relation between blood ammonia and serum zinc (blood ammonia increases as serum zinc decreases and vice versa).

Table5. Correlation between serum zinc and serum manganese and copper in the three studied groups.

r–value		Serum Zinc (Zn) (µg/dl)		
		P–value	Sig.	
Serum manganese (Mn) (µg/dl)	Cirrhosis with HE	-0.404	0.136	NS
	Cirrhosis without HE	0.123	0.662	NS
	Control	-0.766	0.01	S
Serum Copper (Cu) (µg/dl)	Cirrhosis with HE	-0.459	0.085	NS
	Cirrhosis without HE	0.467	0.079	NS
	Control	-0.705	0.023	S

(By Ranked Spearman Correlation Test)

There was a non significant (p>0.05) relation between serum zinc and serum manganese and copper in cirrhosis with and without HE groups. Whereas there was a significant (p<0.05) negative relation between serum zinc and serum manganese and copper in control group (serum zinc increases as serum manganese and copper decrease and vice versa).

Table6. Correlation between grade of HE and trace elements and blood ammonia in cirrhosis with HE group.

Cirrhosis with HE group (n=15)	Grade of HE		
	r–value	P–value	Sig.
Serum manganese (Mn) (µg/dl)	0.404	0.135	NS
Serum Zinc (Zn) (µg/dl)	-0.328	0.232	NS
Serum Copper (Cu) (µg/dl)	0.527	0.044	S
Blood ammonia (NH_3) (µg/dl)	0.588	0.021	S

There was a non significant (p>0.05) relation between grade of HE and serum manganese and zinc. Whereas there was a significant (p<0.05) positive relation between grade of HE and serum copper and blood ammonia (grade of HE increases as serum copper and blood ammonia increase and vice versa).

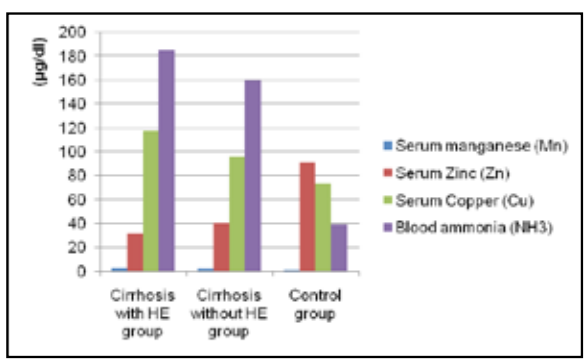

Figure 1. Comparison between the three studied groups as regards the trace elements and ammonia.

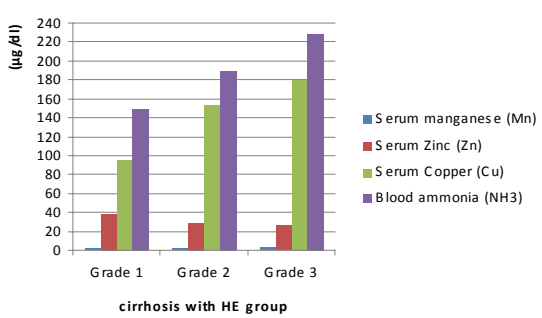

Figure2. Comparison between different HE grades of cirrhosis with HE group according to West-Haven criteria as regards the trace elements and ammonia.

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Discussion

In this study, serum manganese levels were highly significant increased in cirrhotic patients with or without HE compared to controls (median 2.5 µg/dl in cirrhosis with HE and 2.2 µg/dl in cirrhosis without HE vs. 0.75 µg/dl in controls, p<0.001 for both) (Figure 4.1). This result agrees with **Rahelić D et al., 2006** and **Hamed SA et al., 2008**. Higher serum levels of manganese in research of **Krieger D et al., 1995** as well as in research of **Layrargues GP et al., 1998** were also found in cirrhotic patients.

Whereas, serum manganese levels were not significantly different between cirrhotic patients with HE and cirrhotic patients without HE (median 2.5 µg/dl in cirrhosis with HE vs. 2.2 µg/dl in cirrhosis without HE, p>0.05). This result agrees with **Rahelić D et al., 2006**. But disagrees with the researches of **Hauser RA et al., 1996** and **Krieger D et al., 1995**. They found increased concentrations of manganese and suggested a beneficial effect of prevention of accumulation or decreasing manganese concentration in patients with liver cirrhosis.

In this study, serum zinc levels were highly significant decreased in cirrhotic patients with or without HE compared to controls (median 31.5 µg/dl in cirrhosis with HE and 40.0 µg/dl in cirrhosis without HE vs. 90.5 µg/dl in controls, p<0.001 for both) (Figure 4.1). This result agrees with **Rahelić D et al., 2006**, **Hamed SA et al., 2008** and **Ramzy I et al., 2008**. They explained low zinc levels with low ingestion due to protein reluctance, increased loss in gastroenterological system due to diarrhea or intestinal malabsorption and increased urinary losses for which excessive diuretic administration might be responsible for Zn deficiency encountered in patients with liver disease.

.Although there was a slight decrease in serum zinc levels of cirrhotic patients with HE compared to those without HE but these differences in this research were not statistically significant(median 31.5 µg/dl in cirrhosis with HE vs. 40.0 µg/dl in cirrhosis without HE, p>0.05). This result disagrees with **Rahelić D et al., 2006** and **Ramzy I et al., 2008**. They found significantly lower zinc levels in cirrhotic patients with HE as compared to cirrhotic patients without HE and suggested a beneficial effect of zinc supplementation in prevention of HE by activating glutamine synthetase, which can decrease the level of ammonia and improve HE.

This research did not confirm significant lower concentrations of zinc in cirrhotic patients with HE in comparison to cirrhotic patients without HE. That partially could be explained with small number of patients included in this study. In this study, serum copper levels were highly significant increased in cirrhotic patients with or without HE compared to controls (median 117.5 µg/dl in cirrhosis with HE and 96.0 µg/dl in cirrhosis without HE vs. 73.25 µg/dl in controls, p<0.01 for both) . This result agrees with **Rahelić D et al., 2006** and **Hamed SA et al., 2008**.

Also **Halifeoglu I et al., 2004** reported that serum copper increased whereas serum zinc decreased significantly in cirrhotic patients compared to controls.

Whereas, serum copper levels were not significantly different between cirrhotic patients with HE and cirrhotic patients without HE (median 117.5 µg/dl in cirrhosis with HE vs. 96.0 µg/dl in cirrhosis without HE, p>0.05) . This result agrees with **Rahelić D et al., 2006**.

In this study, blood ammonia levels were highly significant increased in cirrhotic patients with or without HE compared to controls (median 185.0 µg/dl in cirrhosis with HE and 160.0 µg/dl in cirrhosis without HE vs. 39.0 µg/dl in controls, p<0.001 for both) .This result agrees with **Hamed SA et al., 2008**.

Whereas, blood ammonia levels were not significantly different between cirrhotic patients with HE and cirrhotic patients without HE (median 185.0 µg/dl in cirrhosis with HE vs. 160.0 µg/dl in cirrhosis without HE, p>0.05) This result disagrees with **He Y et al., 2011**. They found significantly higher levels of venous ammonia in patients with hepatic encephalopathy than in cirrhotic patients without hepatic encephalopathy.

ALSO **Nicolao F et al., 2003** also found that in patients with encephalopathy, each form of ammonia was higher than in both controls and patients without encephalopathy

This research did not confirm significant higher concentrations of ammonia in cirrhotic patients with HE in comparison to cirrhotic patients without HE. That partially could be explained with small number of patients included in this study.

In this study, serum manganese, zinc and copper did not show significant differences in comparison between cirrhotic patients with different HE grades in cirrhosis with HE group . Whereas, there was remarkable increase of blood ammonia levels as the grade of hepatic encephalopathy increased (median 150.0 µg/dl in grade I, 190.5 µg/dl in grade II and 229.5 in grade III, p>0.05) but these differences in this research were not statistically significant. This result agrees with **He Y et al., 2011** who found significant increase of both venous ammonia levels as the grade of hepatic encephalopathy increased. ALSO **Ong JP et al., 2003** found that venous total ammonia levels correlated with the severity of hepatic encephalopathy as well as arterial total ammonia levels.

There was also highly significant negative correlation between serum zinc level and CTP–score in both cirrhotic patients with HE (r = -0.75, p<0.01) and cirrhotic patients without HE (r = -0.683, p<0.01). This result agrees with **Somi MH et al., 2007**, but disagrees with **Ramzy I et al., 2008** and **Yang SS et al., 2004** as they found that serum zinc levels did not correlate with Child-Pugh score. This result confirms that there is a stepwise decline in serum zinc level with worsening of child class in cirrhotic patients.

The above data indicate that serum levels of zinc and copper are affected by the degree of hepatic decompensation according to Child-Pugh classification rather than presence or absence of HE. Serum manganese level, on the other hand, shows no significant change along the course of liver cell failure.

In this study, there was significant negative correlation between blood ammonia level and serum zinc level in both cirrhotic patients with HE (r = -0.646, p<0.01) and cirrhotic patients without HE (r = -0.52, p<0.05). This result agrees with **Yoshida Y et al., 2001** who found significant inverse correlation between fasting serum zinc and fasting venous ammonia levels.

conclusion

this research confirmed that serum levels of studied trace elements differ significantly in patients with liver cirrhosis

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compared to healthy subjects as there was significant increase in serum levels of manganese and copper, whereas, there was significant decrease in serum zinc levels in cirrhotic patients (regardless presence or absence of HE) compared to control subjects. However, there were no significant differences in serum levels of studied trace elements in cirrhotic patients with HE compared to those without HE.

There were significant differences in serum levels of zinc and copper with more severe clinical state of liver cirrhosis according to Child-Pugh classification in cirrhotic patients with HE as there was significant decrease in serum zinc levels, whereas, there was significant increase in serum copper levels in patients with child C class compared to those with child B class. However, serum manganese levels did not differ significantly with progression of liver cirrhosis according to Child-Pugh classification.

In conclusion, decreased serum concentrations of zinc and increased levels of manganese and copper in patients with liver cirrhosis could have an important role in the pathogenesis of liver cirrhosis and its complications, especially in hepatic encephalopathy. The supplementation of zinc could improve hepatic encephalopathy. The decrease in manganese and copper levels could also have a beneficial effect on the neurological status in patients with liver cirrhosis and hepatic encephalopathy.

Considering all that, the correction of serum trace elements concentrations would have a beneficial effect on some complications of liver cirrhosis and may be on progression of the disease, so it would be recommendable to provide laboratory analysis of trace elements as a routine.

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