



ORIGINAL RESEARCH ARTICLE

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Diagnostic role of serum brain-derived neurotrophic factor in HCV cirrhotic patients with minimal hepatic encephalopathy with and without schistosomiasis

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Abstract

Background Liver cirrhosis (LC) advances from an asymptomatic phase (compensated cirrhosis) to a symptomatic phase (decompensated cirrhosis). Up to 80% of patients with LC may experience minimal hepatic encephalopathy (MHE), which is the first stage of hepatic encephalopathy (HE). Due to the lack of serum indicators, the diagnosis of MHE is frequently based on neuropsychometric tests. Therefore, this study aimed to evaluate the role of brain-derived neurotrophic factor (BDNF) as a diagnostic marker for MHE in HCV cirrhotic patients with or without hepatic schistosomiasis.

Patients and methods The study consisted of 60 patients with divided into 3 groups (20 patients with HCV-related LC with overt HE, 20 patients with HCV-related LC without overt HE, and 20 patients with HCV-related LC and hepatic schistosomiasis co-infection without overt HE) as well as 20 healthy controls. Patients without overt HE were evaluated for MHE by psychometric tests (trail making tests A and B). Serum BDNF was measured in all patients as well as healthy controls.

Results Serum BDNF was found to be significantly lower in patients with LC regardless of etiology than in healthy controls; however, no statistically significant difference was found between patients with and without overt HE. Upon subdivision of patients without overt HE into “normal” and “deficient” using psychometric tests, serum BDNF was found to be significantly lower in patients with overt as well as those with “deficient” psychometric tests (have MHE). Serum BDNF had a sensitivity of 65.85% and specificity of 84.62%, and positive predictive value (PPV) was 82.0%, and negative predictive value (NPV) was 70.0% for diagnosis of MHE.

Conclusion Serum BDNF concentration was found to be significantly lower in patients with deficient psychometric tests having either overt or covert HE which suggests that serum BDNF can be used as a diagnostic marker for MHE.

Keywords HCV, Schistosomiasis, Liver cirrhosis, Minimal hepatic encephalopathy, Serum brain-derived neurotrophic factor, Psychometric tests

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Introduction

Liver cirrhosis (LC) is a chronic, diffuse, and irreversible liver disease that is the end stage of various conditions, including hepatitis B or C infection, a high rate of alcohol use, non-alcoholic fatty liver disease, autoimmune disorders, cholestatic illnesses, and an excess of iron or copper. After a prolonged time of inflammation, normal liver tissue is eventually replaced by tissue that is fibrotic and regenerating nodules form, which leads to LC [1], eventually leading to portal hypertension and consequently liver failure [2].

Clinically, LC starts first with a compensated phase followed by the stage of decompensation, characterized by complications that frequently require hospitalization and eventually lead to a reduction in the quality of life and a high death rate [3].

Schistosomiasis and HBV/HCV co-infection worsen the condition and produce advanced liver disease, which raises the mortality rate [4] and are frequently linked to countries where schistosomiasis is endemic as in Egypt, where patients with HCV may have a co-infection rate of up to 50% [5].

In cirrhotic livers, the number of active hepatocytes decreases because of the active liver inflammation, which also impairs the liver's detoxifying functions. Moreover, portal hypertension leads to the opening of portosystemic shunts (PSS) by-passing the liver. As a result, the blood's concentration of toxins is raised, which causes them to more easily accumulate in the central nervous system (CNS) [6], eventually leading to hepatic encephalopathy (HE) [7].

HE is described as a distinctive functional alteration of the mental state that is reversible and caused by decreased liver function and/or enhanced PSS shunting [8]. Not only does HE lower the patient's quality of life, but it also increases mortality [7]. Many cirrhotic patients can develop minimal hepatic encephalopathy (MHE), which is the earliest type of HE. MHE lacks overt clinical manifestations [9] and screening serum markers, and can be solely diagnosed by abnormal psychometric tests [10].

An important neurotrophic factor is the brain-derived neurotrophic factor (BDNF). It is mostly released from the cerebral cortex and hippocampus [11]. Neuronal development, differentiation, and repair are linked to BDNF expression [12]. BDNF promotes dopaminergic activity, synaptic function, and neurogenesis and is essential for the growth of the nervous system [13].

BDNF protein is found in most areas of the brain and in the blood and is known to decrease in patients with decompensated LC; however, its role as a potential serum marker for screening of MHE remains unclear [14].

Aim of the work

This study aimed to evaluate the potential diagnostic role of serum BDNF as a marker of MHE in hepatitis C virus cirrhotic patients with or without hepatic schistosomiasis.

Patients and methods

This study is a case-control study which included 80 participants who were divided into 60 patients with HCV-related LC and 20 healthy controls. Inclusion criteria were patients aged between 18 and 70 with HCV-related LC. Patients were selected from the Department of Tropical Medicine, Alexandria University Hospital, during the period from September 2022 till August 2023. The study was conducted following permission from the local ethical committee of Alexandria University, according to the Declarations of Helsinki (IRB No.: 00012098). All patients had provided informed consent for both their inclusion in the research and the publication of their data.

Patients with HCV-related LC were further divided into three groups. Group 1 included 20 patients with HCV-related LC who had overt HE, group 2 included 20 patients with HCV-related LC without overt HE, while group 3 included 20 patients with HCV-related LC and hepatic schistosomiasis co-infection without overt HE. Group 4 consisted of 20 age and gender-matched healthy controls.

Patients with LC due to an etiology other than HCV; metabolic, ischemic, or toxic causes of encephalopathy; a known history of previous neuropsychiatric diseases, hepatocellular carcinoma (HCC), or any other malignancies; and hypertensive and diabetic patients were not included in the study.

Thorough history and meticulous clinical examination were obtained to all patients. Blood samples were obtained for complete blood count (CBC), liver function tests (LFTs) including serum albumin, total serum bilirubin, prothrombin activity (PA) and international normalized ratio (INR), alanine transaminase (ALT), aspartate transaminase (AST), fasting blood glucose (FBG), hemoglobin A1C test (HBA1C), total cholesterol, serum triglycerides, blood urea nitrogen (BUN), serum creatinine, serum sodium and potassium. HCV infection was diagnosed by HCV antibody (Ab) using an enzyme-linked immunosorbent assay (ELISA) kit under standardized procedures.

LC was diagnosed based on combined clinical, laboratory, and radiological evidence by ultrasonography (US) as well as Fibrosis Index Score (FIB-4) of greater than 3.24. Other causes of LC were excluded by hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), immunoglobulin electrophoresis, antinuclear antibody (ANA), anti-smooth muscle antibody

(ASMA), and serum ferritin in addition to 24 urinary copper excretion. Computed tomography (CT) scan was done on day 1 as well as 48 h post encephalopathy to all patients with overt HE to exclude hemorrhagic as well as ischemic stroke. Sera of patients were also obtained to evaluate serum ammonia level [15], indirect hemagglutination test (IHAT) was used for schistosomiasis [16], and hepatic schistosomiasis was confirmed by presence of periportal fibrosis demonstrated by abdominal ultrasonography. Serum (BDNF) was detected by ELISA kit using the standardized protocol and procedures.

All participants were subjected to psychometric tests: Trail making test (TMT) A and TMT B, [17], and were divided into “normal” and “deficient” accordingly. In TMT A, the participant connects a string of 25 ringed numbers in ascending order with a pencil. In TMT B, the participant alternates between connecting 25 encircled numbers as well as letters in numerical and alphabetical sequence. The numbers and letters are arranged in a set, semi-random arrangement to prevent the examinee’s lines from crossing each other. The total time needed to finish sections A and B is the main aspect of concern [18]. The TMT is assessed according to the duration it requires to finish. Correction of errors made by the examiner is included in the time. TMT-A scores are classified as “normal” or “deficient” if they score 29 or 78 s, respectively. A “normal” score for the TMT-B is 75 s, while a “deficient” score is higher than 273 s [19]. A “deficient” TMT score indicates the diagnosis of MHE.

Statistical analysis of data

Data were uploaded to the computer and statistically analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Shapiro–Wilk test was used to test the normality of the distribution of variables; comparisons between groups for categorical variables were evaluated using the chi-square test, (χ^2) and Monte Carlo test (MC). Student *t*-test (*t*-test) was used to compare two groups for normally distributed quantitative variables. Mann Whitney test (*U*) as well as Kruskal–Wallis were used to compare between the two groups for not normally distributed quantitative variables. The diagnostic performance of the serum BDNF was measured using the Receiver Operating Characteristic Curve (ROC); an area of more than 50% indicates an acceptable performance, and an area of roughly 100% is the optimum performance for the test. Linear regression was performed to identify the factor that influenced serum BDNF level. The significance of the acquired results was assessed at the 5% level.

Results

Clinical, laboratory, and demographic criteria of studied patients

The clinical, laboratory, and demographic data of the studied participants are summarized in Table 1.

To evaluate serum levels of ammonia and BDNF according to the etiology of LC

Serum level of ammonia was statistically significantly highest in group 1 patients with overt HE, with a mean level of 114.35 ± 17.48 mg/dl, followed by group 3 patients with HCV/schistosomiasis co-infection-related LC without overt HE, with a mean level of 84.70 ± 17.13 mg/dl, followed by group 2 patients with HCV-related LC without overt HE, with a mean level of 76.85 ± 18.96 mg/dl ($p < 0.001$). However, the difference between serum levels of ammonia in groups 2 and 3 was not significant. Serum ammonia was statistically significantly higher in groups 1, 2, and 3 than in group 4, the control group, where the mean level of ammonia was 49.90 ± 10.09 mg/dl ($p < 0.001$).

The median level of serum BDNF was 126.69 pg/ml, 121.87 pg/ml, and 175.65 pg/ml in groups 1, 2, and 3, respectively, and all were statistically significantly lower than group 4 with a median level of BDNF = 261.72 pg/ml ($p < 0.001$). Serum BDNF level was found to be least in group 1 with overt HE, followed by group 3, followed by group 2; however, the difference between the three groups did not reach the level of significance, as shown in Table 2.

To establish relation between serum BDNF and HE grade

The number of patients with a “deficient” TMT test was statistically significantly higher in group 3 with HCV-related LC and schistosomiasis co-infection than in group 2 with HCV-related LC ($p < 0.001^*$). Moreover, within group 3, serum BDNF was statistically significantly lower in patients with deficient than average psychometric tests, as shown in Table 3.

The median BDNF level was found to be least in group 1 as well as those with “deficient” TMT in groups 2 and 3; however, the difference between both groups did not reach the level of significance. It was found to be significantly lower in both groups than those with “average” TMT in groups 2 and 3 ($p < 0.001$). Median BDNF was statistically significantly highest in group 4 than the three groups ($p < 0.001$), as shown in Table 4.

To establish relation between serum ammonia and HE grade

Serum ammonia was found to be statistically significantly higher in patients with “deficient” TMT than in those with “normal” TMT within group 3; however, although higher in patients with “deficient” TMT than those with

Table 1 Comparison between the different studied groups according to different parameters

	Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)	Group 4 (n = 20)	Test of sig	p
Gender						
Male	13 (65%)	10 (50%)	14 (70%)	13 (65%)	$\chi^2 = 1.920$	0.589
Female	7 (35%)	10 (50%)	6 (30%)	7 (35%)		
Age (years)						
Min. – Max	47.0–69.0	42.0–70.0	54.0–68.0	44.0–65.0	F = 2.058	0.113
Mean ± SD	57.20 ± 7.03	59.80 ± 7.72	58.40 ± 3.27	55.10 ± 5.87		
ALT (U/L)						
Min. – Max	24.0–120.0	19.0–120.0	26.0–150.0	13.0–27.0	H = 43.953*	< 0.001*
Median (IQR)	40.0(35.50–68.50)	36.50(26.0–44.0)	43.50(36.0–74.0)	18.0 (16.0 – 22.0)		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.183, p ₂ = 0.902, p ₃ = 0.146					
AST (U/L)						
Min. – Max	49.0–151.0	31.0–167.0	38.0–211.0	11.0–38.0	H = 46.696*	< 0.001*
Median (IQR)	98.5(60.5–111.5)	57.5(47.50–81.50)	75.5(59.0–117.50)	22.0 (20.50–31.0)		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.106, p ₂ = 0.812, p ₃ = 0.168					
Albumin (g/dl)						
Min. – Max	1.90–3.20	2.20–3.40	2.10–3.60	3.40–4.80	F = 68.614*	< 0.001*
Mean ± SD	2.53 ± 0.36	2.95 ± 0.30	2.83 ± 0.35	4.01 ± 0.38		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.001*, p ₂ = 0.035*, p ₃ = 0.698					
Total bilirubin (mg/dl)						
Min. – Max	1.70–6.20	1.0–3.60	0.90–5.70	0.40–1.0	H = 55.513*	< 0.001*
Median (IQR)	3.25 (2.20–4.80)	1.45 (1.30–1.95)	2.0 (1.65–2.55)	0.70 (0.50–0.80)		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.001*, p ₂ = 0.048*, p ₃ = 0.160					
PT (seconds)						
Min. – Max	14.80–22.40	13.20–21.80	14.20–19.70	11.50–13.0	F = 32.941*	< 0.001*
Mean ± SD	18.44 ± 2.23	16.33 ± 2.80	16.47 ± 1.85	12.25 ± 0.46		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.008*, p ₂ = 0.015*, p ₃ = 0.997					
INR						
Min. – Max	1.30–2.20	1.10–2.10	1.20–1.80	0.90–1.10	F = 30.103*	< 0.001*
Mean ± SD	1.69 ± 0.26	1.47 ± 0.31	1.46 ± 0.20	1.03 ± 0.06		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	p ₁ = 0.012*, p ₂ = 0.008*, p ₃ = 0.999					
Psychometric tests						
Average	0 (0%)	12 (60%)	7 (35%)	20 (100%)	$\chi^2 = 42.577^*$	< 0.001*
Deficient	20 (100%)	8 (40%)	13 (65%)	0 (0%)		
p₀	< 0.001*	^{FE} p = 0.003*	< 0.001*			
Sig. bet. grps	p ₁ < 0.001*, ^{FE} p ₂ = 0.008*, p ₃ = 0.113					

ALT Alanine aminotransferase, AST Aspartate aminotransferase, PT Prothrombin time, INR International normalized ratio, IQR Interquartile range, SD Standard deviation, χ^2 , chi-square test, H Kruskal–Wallis test, pairwise comparison bet. each 2 groups was done using post hoc test (Dunn’s for multiple comparisons test); F, one-way ANOVA test, pairwise comparison bet. each 2 groups was done using post hoc test (Tukey); p, p value for comparing between the different studied groups; p₀, p value for comparing between group 4 and each other group; p₁, p value for comparing between group 1 and group 2; p₂, p value for comparing between group 1 and group 3; p₃, p value for comparing between group 2 and group 3. *Statistically significant at p ≤ 0.05

“average” TMT in group 2 patients, the difference did not reach the level of significance, as shown in Table 5 (p < 0.05*).

Serum ammonia level was found to be highest in group 1 patients, followed by “deficient” TMT patients in groups 2 and 3, followed by “deficient” TMT patients

Table 2 Comparison between the different studied groups according to ammonia and BDNF

	Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)	Group 4 (n = 20)	Test of sig	p
Ammonia (mg/dl)						
Min. – Max	87.0–153.0	52.0–114.0	60.0–121.0	28.0–67.0	F = 53.036*	< 0.001*
Median (IQR)	114.35 ± 17.48	76.85 ± 18.96	84.70 ± 17.13	49.90 ± 10.09		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	$p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.428$					
BDNF (pg/ml)						
Min. – Max	36.34–252.65	59.09–475.06	101.46–317.47	211.80–408.31	H = 34.254*	< 0.001*
Median (IQR)	126.69 (91.63–202.92)	121.87 (102.33–156.74)	175.65 (121.42– 237.38)	261.72 (247.46–282.67)		
p₀	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps	$p_1 = 0.001^*, p_2 = 0.035^*, p_3 = 0.698$					

χ^2 chi-square test, SD Standard deviation, p_3 , p value for comparing between group 2 and group 3; p_2 , p value for comparing between group 1 and group 3; p_1 , p value for comparing between group 1 and group 2; p_0 , p value for comparing between group 4 and each other group; p , p value for comparing between the different studied groups; IQR, interquartile range; H, Kruskal–Wallis test, pairwise comparison bet. each 2 groups was done using post hoc test (Dunn’s for multiple comparisons test); FE Fisher exact, F one-way ANOVA test, pairwise comparison bet. each 2 groups was done using post hoc test (Tukey); BDNF, brain-derived neurotrophic factor

* Statistically significant at $p \leq 0.05$

Table 3 Relation between psychometric tests and BDNF in 2 and 3 groups

BDNF (pg/ml)	Psychometric tests		U	p
	Deficient	Average		
Group 2	(n = 8)	(n = 12)		
Min. – Max	59.09–238.85	85.42–475.06	33.0	0.270
Mean ± SD	124.64 ± 56.53	165.54 ± 108.13		
Median	109.28	135.80		
Group 3	(n = 13)	(n = 7)		
Min. – Max	101.46–252.26	180.10–317.47	7.0*	0.001*
Mean ± SD	145.90 ± 46.37	241.31 ± 43.55		
Median	128.47	244.11		

U, Mann–Whitney test; p, p value for comparing between average and normal

* Statistically significant at $p \leq 0.05$

in groups 2 and 3, followed by group 4, the difference being significant between all groups as shown in Table 6 ($p < 0.05$).

Diagnostic validity of serum BDNF in diagnosing patients with deficient psychometric tests

The obtained results showed that BDNF has a sensitivity of 65.85% and specificity of 84.62%, and positive predictive value (PPV) was 82.0%, negative predictive value (NPV) was 70.0%, and area under curve (AUC) was 0.837, with cutoff point ≤ 137.88 pg/ml in discriminating patients with “deficient” TMT from those with “normal” TMT as shown in Table 7 and Fig. 1.

Factors affecting serum BDNF level in patients with HCV-related LC

Univariate analysis revealed that all factors, ALT, AST, albumin, total bilirubin, PT, INR, ammonia, and psychometric tests, correlated positively to serum BDNF level; however, psychometric tests were the only independent factor affecting serum BDNF level as shown in Table 8.

Table 4 Relation between psychometric tests and BDNF

BDNF (pg/ml)	Group 1 (n = 20)	Psychometric test (group 2 + 3)		Group 4 (n = 20)	H	p
		Deficient (n = 21)	Normal (n = 19)			
Min. – Max	36.34 – 252.65	59.09–252.26	85.0–475.06	211.80–408.31	36.456*	< 0.001*
Median (IQR)	126.69 (91.63–202.92)	123.24 (103.15–171.20)	163.01 (126.17–249.58)	261.72 (247.46–282.67)		
p₀	< 0.001*	< 0.001*	0.002*			
Sig. bet. Grps	$p_1 = 0.923, p_2 = 0.046^*, p_3 = 0.034^*$					

IQR Interquartile range, SD Standard deviation, H Kruskal–Wallis test, pairwise comparison bet. each 2 groups was done using post hoc test (Dunn’s for multiple comparisons test); p , p value for comparing between the different studied groups; p_0 , p value for comparing between group 4 and each other group; p_1 , p value for comparing between group 1 and deficient; p_2 , p value for comparing between group 1 and normal; p_3 , p value for comparing between deficient and normal

* Statistically significant at $p \leq 0.05$

Table 5 Relation between ammonia and psychometric tests in group 2 and group 3

Psychometric tests	N	Ammonia (mg/dl)			U	p
		Min. – Max	Mean ± SD	Median		
Group 2 (n = 20)						
Deficient	8	60.0–114.0	85.50 ± 19.67	84.50	25.0	0.082
Average	12	52.0–105.0	71.08 ± 16.86	66.50		
Group 3 (n = 20)						
Deficient	13	68.0–121.0	91.08 ± 16.73	87.0	17.0*	0.024*
Average	7	60.0–89.0	72.86 ± 10.88	74.0		

U, Mann–Whitney test; p, p value for comparing between average and normal

* Statistically significant at $p \leq 0.05$

Table 6 Relation between psychometric tests and ammonia

Ammonia (mg/dl)	Group 1 (n = 20)	Psychometric tests (group 2 + 3)		Group 4 (n = 20)	H	p
		Deficient (n = 21)	Normal (n = 19)			
Min. – Max	87.0 – 153.0	60.0–121.0	52.0–105.0	28.0–67.0	59.278*	< 0.001*
Median (IQR)	108.0 (103.50–126.0)	86.0 (80.0–104.0)	67.0 (61.50–78.0)	50.0 (43.0–57.50)		
p_0	< 0.001*	< 0.001*	0.005*			
Sig. bet. Grps	$p_1 = 0.015^*$, $p_2 < 0.001^*$, $p_3 = 0.034^*$					

IQR Interquartile range, SD Standard deviation, H Kruskal–Wallis test, pairwise comparison bet. each 2 groups was done using post hoc test (Dunn's for multiple comparisons test); p, p value for comparing between the different studied groups; p_0 , p value for comparing between group 4 and each other group; p_1 , p value for comparing between group 1 and deficient; p_2 , p value for comparing between group 1 and normal; p_3 , p value for comparing between deficient and normal

* Statistically significant at $p \leq 0.05$

Table 7 Validity (AUC, sensitivity, specificity) for BDNF to discriminate positive (n = 41) from negative psychometric tests (n = 39)

	AUC	p	95% C.I	Cutoff ^a	Sensitivity	Specificity	PPV	NPV
BDNF (pg/ml)	0.837	< 0.001*	0.750 – 0.925	≤ 137.88	65.85	84.62	82.0	70.0

AUC Area under a curve; p value Probability value, CI Confidence intervals, NPV Negative predictive value, PPV Positive predictive value

* Statistically significant at $p \leq 0.05$

^a Cutoff was chosen according to the Youden index

Discussion

HE is a brain impairment precipitated by liver failure and affects thirty to forty percent of LC patients over the course of their illness. Clinically undetectable MHE, which is a cognitive disturbance directly linked to loss of quality of life, frequently precedes clinically evident HE [20].

Ammonia has an essential role in the pathophysiology of hepatic encephalopathy (HE) in patients with LC. Serum ammonia concentrations have been linked to immunological dysfunction, hepatotoxicity, and sarcopenia, and may be used as a prognostic indicator [21]. Ammonia levels do not accurately diagnose HE in patients with chronic liver disease.

In the present study, the mean level of ammonia in patients with LC due to HCV (group 2) and due to

combined HCV and schistosomiasis (group 3) was within the normal range; however, they were significantly higher than the healthy control group (group 4), while patients with overt HE showed the highest level of ammonia which was statistically significantly higher compared to all other studied groups. This indicates that ammonia may be a marker of overt HE and worsening of liver disease. Regarding the etiology of LC, patients with combined HCV and schistosomiasis infection had significantly higher serum ammonia level than patients with HCV infection only. This indicates that serum ammonia level increases with the progression of chronic liver disease as well as with HCV and hepatic schistosomiasis co-infection.

Gundling et al. identified 47.2% and 78.3%, respectively, as the sensitivity and specificity of

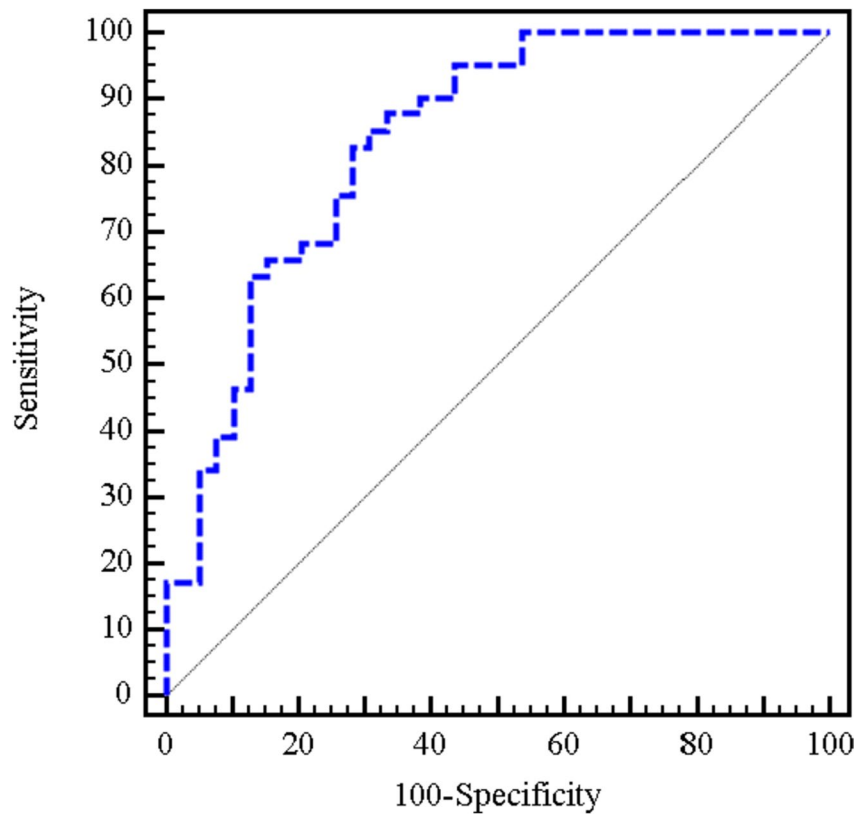


Fig. 1 ROC curve for BDNF to discriminate positive ($n=41$) from negative psychometric tests ($n=39$)

Table 8 Univariate and multivariate linear regression analysis to detect the most independent factors affecting for BDNF in the total sample ($n=80$)

	Univariate		^a Multivariate	
	<i>p</i>	<i>B</i> (LL – UL 95% C.I)	<i>p</i>	<i>B</i> (LL – UL 95% C.I)
ALT	0.001*	– 1.124 (– 1.756 to – 0.492)	0.997	– 0.002 (– 1.251–1.247)
AST	<0.001*	– 0.827 (– 1.226 to – 0.427)	0.703	– 0.182 (– 1.132–0.768)
Albumin	<0.001*	62.680 (37.369–87.990)	0.147	30.554 (– 11.018–72.127)
Total bilirubin	<0.001*	– 23.335 (– 35.629 to – 11.042)	0.829	– 2.129 (– 21.690–17.431)
PT	<0.001*	– 11.693(– 17.450 to – 5.935)	0.706	– 9.187 (– 57.482–39.108)
INR	<0.001*	– 105.161(– 158.749 to – 51.572)	0.931	19.584 (– 432.404–471.572)
Ammonia	<0.001*	– 1.174 (– 1.800 to – 0.548)	0.087	1.331 (– .0198–2.859)
Psychometric tests (deficient)	<0.001*	– 93.940 (– 125.456 to – 62.424)	0.005*	– 78.741(– 132.566 to – 24.916)

B, unstandardized coefficients, *C.I* Confidence interval, *LL* Lower limit, *UL* Upper limit

^a All variables with $p < 0.05$ were included in the multivariate. *Statistically significant at $p \leq 0.05$

venous ammonia levels ≥ 55 mol/L to diagnose HE. Ammonia had positive and negative predictive values that were 77.3% and 48.6%, respectively [22]. Nicolao et al. investigated how ammonia and HE are related. Despite obvious neurological improvements from their HE, they noticed that several patients with chronic

liver illness had levels of ammonia that were either unaffected or rising [23].

MHE is a condition that frequently affects LC patients and involves impaired neuropsychological or neurophysiological functioning [24]. Neuropsychiatric tests include examining problems in visuospatial

functioning, attention, speed of processing, and response inhibition with paper and pencil tests and computer tests and have a high sensitivity and specificity in detecting MHE in clinically apparent patients [25]. The TMT is one of the most used neuropsychological tests for assessing executive functions (EFs). It has a number of advantages, including that it is sensitive to executive abnormality, is simple to comprehend, and is rapid to use [26].

In the present study, the psychometric tests used were TMT A and TMT B. These tests revealed deficient results in all patients with overt hepatic encephalopathy (100%), 40% HCV LC ($n=8$), and 65% in cirrhotic patients due to combined HCV and schistosomiasis ($n=13$) which suggests that higher prevalence of MHE among patients with combined infection.

BDNF is a member of the neurotrophic protein group, which is essential for the development, survival, and functionality of neurons as well as for the healthy development of the central and peripheral nervous systems [27].

In this study, serum BDNF was statistically significantly lower in patients with LC with and without overt HE than in the control group. No statistically significant difference was found between groups 2 and 3, showing that according to this study, schistosomiasis co-infection has no detectable effect on serum level of BDNF.

Patients were further subdivided according to the TMT into “normal” and “deficient,” with patients with “deficient” TMT representing patients with MHE. The median level of BDNF was least in group 1 patients with overt HE, as well as in patients with a “deficient” TMT (MHE) in groups 2 and 3, with no significantly detected statistical difference between both groups. However, BDNF was found to be significantly higher in patients with “normal” TMT (no MHE) in groups 2 and 3, and was significantly highest in group 4, the healthy controls.

Thus, BDNF was found to differentiate between patients with HE whether overt or minimal, from those without, and therefore, can detect MHE in apparently normal individuals.

This means that patients with combined HCV and schistosomiasis infections median BDNF level was statistically significantly lower in those with deficient psychometric tests in comparison to patients with normal psychometric tests.

Our results went in concordance with Stawicka et al., who assessed serum BDNF in 78 patients with LC. Forty-three people completed thorough psychometric testing to assess MHE. Patients with LC had serum BDNF levels that were two times lower than those of healthy individuals (13.6 (7.8–22.6) vs. 33.0 (24.1–40.7) ng/ml, p 0.001, respectively), and this difference was due to a level of

liver insufficiency determined by the model for end-stage liver disease (MELD) [28].

In addition, our results went in agreement with Shu et al., who studied the serum BDNF level in patients with HBV-induced LC. At first, ELISA was used to detect serum BDNF levels, combined with the evaluation of other indices related to the reflection of liver functions. It was found that serum BDNF level was significantly lower in the patients with LC than those without [29].

Conclusion

In conclusion, serum BDNF is a potentially inexpensive, noninvasive marker for the diagnosis of MHE in patients with LC. Serum level of BDNF is not affected by HCV and hepatic schistosomiasis co-infection.

Limitations of study

The most important limitation of the study was that group 1 patients with overt HE who could not perform the test as they were comatose or disoriented were considered “deficient” TMT without being able to conduct the psychometric test. Moreover, further studies with a larger sample size are needed for better validation of the screening as well as diagnostic roles of serum BDNF in diagnosing MHE.

Abbreviations

AB	Antibody
ALT	Alanine transaminase
ANA	Antinuclear antibody
ASMA	Anti-smooth muscle antibody
AST	Aspartate transaminase
AUC	Area under curve
BDNF	Brain-derived neurotrophic factor
BUN	Blood urea nitrogen
CBC	Complete blood count
CNS	Central nervous system
CT	Computed tomography
EF	Executive function
ELISA	Enzyme-linked immunosorbent assay
FBG	Fasting blood glucose
FIB-4	Fibrosis-4
HbA1C	Hemoglobin A1C
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HE	Hepatic encephalopathy
IHAT	Indirect haemagglutination test
INR	International normalized ratio
LC	Liver cirrhosis
LFTs	Liver function tests
MELD	Model of end-stage liver disease
MHE	Minimal hepatic encephalopathy
NPV	Negative predictive value
PA	Prothrombin activity
PPV	Positive predictive value
PSS	Portosystemic shunts
ROC	Receiver operating characteristic
TMT	Trail making test
US	Ultrasound

Authors' contributions

Essam S. Bedewy: conceptualization, planning study design, revision of the manuscript. Abeer El Hadidi: laboratory work, statistical analysis of results, and revision of the manuscript. Naglaa Fathi: contributed to study design and planning, parasitological diagnosis of schistosomiasis as well as revision of the manuscript. Yossra T. El Zawawy: patient's data collection and major writing of the manuscript. Amany N. Abbasy: contributed to study design and planning, follow up of the work as well as revision of the manuscript. All authors revised and approved the manuscript.

Funding

The authors declare that they did not receive any funding for this study.

Availability of data and materials

All data analyzed and generated during this study are available upon request from the valued editors. The corresponding author "Yossra T. El Zawawy" will oversee sending data when required.

Declarations

Ethics approval and consent to participate

This study has been approved by the Faculty of Medicine, Alexandria University Ethics Committee. All procedures were strictly conducted in accordance with the Declaration of Helsinki. The IRB number for this study is 00012098. All patients had given an informed written consent stating the title, procedure, and purpose of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 5 November 2023 Accepted: 26 January 2024

Published online: 19 February 2024

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