Original Article

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S100 Beta as a Marker of Hepatic Encephalopathy: A Case-control Study

Internal Medicine Section

MANISH CHANDEY[1], PARMINDER SINGH[2], SAHIBA KUKREJA[3], GURINDER MOHAN[4]

ABSTRACT

Introduction: Hepatic Encephalopathy (HE) is a term used to describe a reversible syndrome of impaired brain function involving a complex spectrum of non specific neurological and psychiatric manifestations occurring in patients of severe acute or Chronic Liver Disease (CLD). The current clinical standard employed is psychometric analysis by computing Psychometric Hepatic Encephalopathy Score (PHES), which is cumbersome to perform. Hence, this necessitates the requirement of a serum biomarker which could correlate with the grades of HE. Following a metabolic injury, the earliest response involving the glial response and astrocyte activation results in the secretion of S100 Beta. Hence, estimation of serum S100 beta levels is considered as a strong marker of Central Nervous System (CNS) injury.

Aim: To verify S100 Beta as a marker of HE.

Materials and Methods: This was a case-control study conducted from 1st April 2021 to 31st July 2022. All diagnosed cases of cirrhosis of liver in the Department of General Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amristar, Punjab, India. A total of 40 age and sex-matched healthy controls were recruited after due consent. They were subjected to psychometric analysis (Five pen and paper tests) and on basis of PHES were divided into four groups on the basis of grades of encephalopathy. Serum samples of patients were run for all routine biochemical parameters in line with Child Turcotte Pugh (CTP) score and S100 Beta levels estimation. Statistical analysis was done to find correlation between S100 Beta levels, PHES and CTP score.

Results: A total of 150 patients and 40 controls were recruited in the study. A progressive deterioration of PHES score was found in the various groups of the study population with worsening of grade of HE. S100 Beta levels correlated with the PHES and also Receiver Operating Characteristic (ROC) curve analysis showed that the sensitivity of the marker stood at 92.7% and specificity at 84.8%. S100 Beta levels could fairly differentiate between patients with and without HE. Hence, S100 Beta levels correlated more with grades of HE than with hepatic functional status (CTP Score). At higher grades of HE when it becomes more clinically apparent, S100 Beta levels would help, when other causes of behaviour changes like psychiatric illness coexist or cannot be ruled out. In the diagnosis of low-grade HE whenever neuropsychometric tests are suboptimal, or when competing psychiatric differential diagnoses are in place, S100 beta levels would have a role over and above the PHES score.

Conclusion: Currently, psychometric analysis holds to be the best clinical standard for diagnosis of HE. S100 Beta holds promising results as a marker for establishing a liquid diagnosis of HE.

Keywords: Chronic liver disease, Diagnosis, Psychometric hepatic encephalopathy score

INTRODUCTION

The HE is a term used to describe a reversible syndrome of impaired brain function involving the spectrum of non specific neurological and psychiatric conditions occurring in patients of severe acute or chronic liver insufficiency [1]. Patients with cirrhosis present a spectrum of various neuropsychiatric abnormalities which ranges from clinically imperceptible decline in cognition to clinically obvious alterations of personality, consciousness, cognition and motor function [2,3]. The first step to diagnose HE is to establish that patient has neuropsychiatric dysfunction and carefully exclude other conditions which may mimic the features. Minimal HE is diagnosed on basis of psychometric testing using PHES, a battery of five pen and paper tests and is considered the best clinical standard but these tests consume a lot of time, require training and are affected by the patients' age and education, explaining why Minimal Hepatic Encephalopathy (MHE) is the most under-diagnosed form of HE [4].

The work would be easier if a simple serum biomarker could be done on outpatient basis, which could diagnose and would correlate with the severity of the encephalopathy [5]. S100 Beta is homodimeric in structure, with each monomer having a molecular weight of 10.5 kDa. As a biomarker it is primarily located in the cytoplasm and nucleus of the astrocytes and is excreted by the kidneys [6]. Following a metabolic injury, the earliest response in HE involves the glial response and astrocyte activation results in the secretion of S100 Beta. The path mechanism of HE describes the role of breach in the blood brain barrier, which results in the increase in the concentration of ammonia and other false neurotransmitters in the brain. This breech would also result in the release of the S100 beta protein in the serum and which can hence be estimated [7].

In a meta-analysis published by Machado MV to establish a liquid marker for diagnosis of HE, suggested further investigation into the role of S100 Beta in diagnosis of HE and where various studies gave conflicting results and especially those which were non conclusive or conflicting to this topic had a very small sample size or had no standard to screen the patients on psychometric basis, without which diagnosis of HE cannot be made [8]. Compared to the earlier studies, the current study had a larger study population and a robust psychometric analysis of the study population was done with due comparison to the Indian data [9-13].

The present study aimed to establish S100 Beta as a marker of HE, which is comparable to PHES (The current best clinical standard) and also better than ammonia, which is being used as a serum marker for HE.

MATERIALS AND METHODS

This case-control study was carried out on patients of Department of General Medicine at a tertiary care centre of North India in Punjab

over a period of one year, from 1st April 2021 to 31st July 2022 after taking ethical clearance Vide No.-3189 of 2021.

Inclusion criteria:

- Diagnosed cases of cirrhosis of liver of any aetiology and any grade, as graded by CTP score [14].
- Healthy controls were recruited. Controls were normal on clinical examination, all laboratory parameters as was done for cases and not suffering from any chronic/acute disease.

Exclusion criteria:

- High-grade of encephalopathy (Grade 4 of West Haven criteria [15]).
- Severe malnutrition.
- Neurological diseases/any psychiatric illness.
- Presence of renal failure.
- Respiratory failure.
- Cardiac failure diseases.
- Sepsis.
- History of substance abuse/Alcohol in past two weeks.

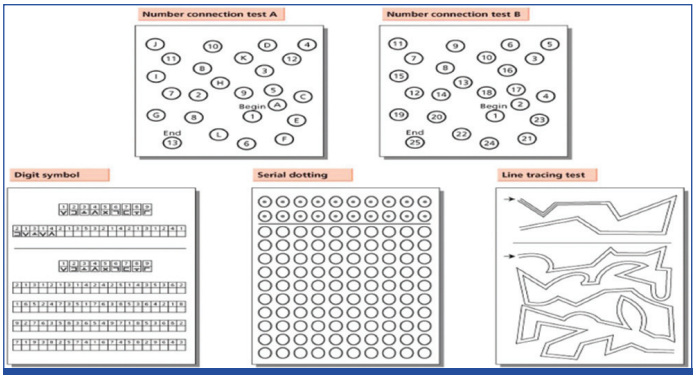
Sample size calculation: It was a time bound study , all the diagnosed patients of liver cirrhosis of any aetiology (recently detected or old patients), presenting in Outpatient Department (OPD)/ Inpatient Department (IPD) of Medicine Department of a tertiary care teaching hospital of North India, after careful history (including presenting complaints, personal history of alcohol intake, history of any other drug abuse, chronic illnesses like diabetes mellitus, any psychiatric illness, medication history), clinical examination, laboratory and radiological investigation, were recruited after taking informed and written consent in vernacular language.

Study Procedure

Healthy controls forty age and sex and matched healthy controls were recruited in the study. A total of 184 cases of cirrhosis of liver were screened and out of which 14 patients had deranged renal function, seven presented in a comatose condition in the emergency, nine patients have history of alcohol intoxication in the last one week, four had evidence of sepsis. Hence, 34 patients were excluded after application of exclusion criteria and 150 cases were recruited in the study. A total 190 adult subjects (both male and females) (cases and controls) of aged ≥18 years were included in this study. Procedure methodology after screening the patients of cirrhosis on the basis of the above exclusion criteria and the controls, they were subjected to psychometric testing by calculating PHES by adopting the below mentioned methodology.

Psychometric analysis [16]: Present study used the following tests to screen patients with HE and recruit in the study: (Psychometric testing) [Table/Fig-1].

1. **The Line Tracing Test (LTT):** For the assessment of the test result, the whole route was divided into small sections, and each touching or crossing the border in a section was counted. The time needed to go through the labyrinth will be the result.
2. **The Serial Dotting Test (SDT):** It was the simplest test of the battery. It was a test of pure motor speed, most prominent positive peaked. The subjects were asked to put a dot in each of the 100 circles given on the sheet after they have prepared by dotting the 20 circles at the top of the sheet first. Test result was the time needed.
3. **Number Connection Test (NCT-A):** Time taken to join numbers and letters in sequence is recorded.
4. **Number Connection Test (NCT-B):** Time taken to join numbers in sequence is recorded.
5. **Digit Symbol Test (DST):** Number of correct symbols inserted into the blank squares below the numbers in 90 seconds was recorded.



[Table/Fig-1]: Showing psychometric tests.

From the above five tests the values were obtained and they were compared with the expected values for these tests [Table/Fig-2] and also took care of the concerns regarding the effects of age and level of education on the expected values, by using values for the North Indian population from a study conducted by a tertiary care centre of North India [16].

Test	Equation	SD
Serial Dotting Test (SDT)	51.471±0.045*Age in years -0.606* formal education in years	10.03
Digit Symbol Test (DST)	32.830-0.312*Age in years ±1.532* formal education in years	9.73
Line Tracing Test (LTT)	70.90-0.12*age in years ±0.44* formal education in years	18.45
Number connection test-B	38.570±0.180*age in years-1.117* formal education in years	8.97
Number connection test-A	53.770±0.380*age in years-2.044* formal education in years	13.73

[Table/Fig-2]: Computing of normal (expected) values of various tests of psychometric analysis, adjusted for age and education years associated with neuropsychological testing.

Calculation of Psychometric Hepatic Encephalopathy Score (PHES): On the basis of the Z score-

Z score is u-x/d

x: observed value

u: calculated value from above equation

d: SD of the population.

Formal education- As per the level of education of the population, 12 years was kept the maximum level in years, for no improvement in psychometric scoring was seen after that. Cut-off for this test battery was set at -5 points. If PHES was ≤-5 points, patient was labeled as having HE and recruited in the study. Covert HE (CHE) will be identified in patients without overt manifestations of HE, when a PHES score was ≤-5 SD.

Diagnostic criteria and group segregation: After screening patients for cirrhosis and evidence of HE, on the basis of PHES score, they will be further divided in the following groups on basis of West Haven criteria [15] as per the clinical symptoms and signs [Table/Fig-3].

A. Cirrhosis without HE;
B. Cirrhosis with CHE and
C. Cirrhosis with overt HE.
D. Forty (40) Age and sex matched healthy controls were included in the study.

Grading of Cirrhosis were done as per CPT score [14]. The patients of cirrhosis, on the basis of the PHES score were separated into Group A; 30.67% (n=46), those with cirrhosis and no evidence of HE. Those with encephalopathy were divided among Group B and C based on the clinical symptoms and West Haven Criteria (15). Group B had 27.33% (n=41) patients and Group C had 42% (n=63).

WHC including MHE	ISHEN/ Group	Description
Unimpaired	No/A	No encephalopathy at all; No history of HE
Minimal/Grade-I	Covert/B	Psychometric or neuropsychological alteration of tests exploring psychomotor speed/executive function or neurophysiologic alterations without clinical evidence of mental change
Grade-II	Overt/C	-Lethargy or apathy -Disorientation for time -Obvious personality changes -Inappropriate behaviour -Dyspraxia -Asterixis
Grade-III	Overt/C	Somnolence to semi stupor -Responsive to stimuli -Confused -Gross disorientation -Bizarre behaviour
Grade-IV	Overt	Coma

[Table/Fig-3]: West Haven Criteria [15].
ISHEN: International society for hepatic encephalopathy and nitrogen metabolism; MHE: Minimal hepatic encephalopathy

Laboratory investigations: A 10 mL of peripheral venous blood was withdrawn from each individual and divided into three aliquots and 2 mL of arterial blood was withdrawn. A 1.8 mL of whole blood was collected in sodium citrate 3.2% (1:9) tube for prothrombin time determination and 2 mL of blood was collected in Ethylenediaminetetraacetic acid (EDTA) Vacutainer for complete blood count estimation. The remaining part and the arterial sample was collected in serum separator tube, centrifuged at 3500 rpm for 10 minutes. Serum was divided into two portions- First one for measurements of liver function tests {(Alanine Transaminases (ALT), Aspartate Transaminase (AST), Albumin (Alb), Total Bilirubin (T.Bili), Alkaline Phosphatase (ALP)} and Renal function tests (Serum Creatinine and Blood Urea Nitrogen estimation), Viral markers (HIV, HBsAg, HCV), HbA1c). Serum from arterial sample was used for estimation of serum ammonia levels. The techniques used in the estimation of the various laboratory parameters are listed in [Table/Fig-4] [17-21].

Techniques	
Parameters assessed	**Test used**
Complete Blood Count	Beckman Counter Coulter LH 780 Analyser
Prothrombin Time (sec)	Tcoag: Destiny Plus set-up
Total Bilirubin Levels (mg/dL)	Jendrassik Grof method [17]
Direct Bilirubin	Dedicated Reagent Diazotisation method [18].
Aspartate Amino Transferase (AST) (IU)	Reagent UV with Phenolsulfophthalien
Alanine Amino Transferase (ALT) (IU)	Reagent UV with Phenolsulfophthalien
Alkaline Phosphatase (ALP) (IU)	p-nitrophenyl phosphate (pNPP), 2-Amino-2-Methyl-1-Propanol (AMP) Buffer
Total Protein	Biuret, reagent blank end point
Serum Albumin (g/dL)	Bromocresol purple
Serum Sodium (mEq/L)	Ion selective electrode indirect
Serum Potassium (mEq/L)	Ion selective electrode indirect
Blood Urea Nitrogen (BUN) (mg/dL)	Urease colorimetric method [19]
Serum Creatinine (mg/dL)	Alkaline picrate kinetic [20]
Arterial Ammonia (ng/dL)	Chemiluminescence method [21]

[Table/Fig-4]: Techniques used in estimation of various laboratory parameters [17-21].

The second part of serum was stored at -20°C for S100 Beta detection. From the serum preserved at -20° in Voltas deep refrigerator, with minimal temperature range up to-30°, S100 Beta levels were estimated on Enzyme Linked Isosorbent Assay (ELISA) kit by Elk Biotechnology Cat: ELK2612. A 96 wells of a precoated microplate with antibody specific to S100 Beta Calcium Binding Protein were used and on principle of sandwich enzyme Immunoassay [22]. The colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of S100 Calcium Binding Protein Beta (S100B) in the samples is then determined by comparing the Optical Density (OD) of the samples to the standard.

Sensitivity: 0.059 ng/mL. The detection range: 0.012-4 ng/mL. (According to the manufacturer supplied kit; Elk Biotechnology Cat:ELK2612, 1312 17th Street #692 Denver, CO 80202 USA).

Specificity: This assay is very sensitive and very specific for finding human S100B. Human S100B and its equivalents did not exhibit any detectable cross-reactivity or interference.

STATISTICAL ANALYSIS

All distribution values among the study population were tested for significance and p-values were calculated. The distribution of parameters among the various groups of the study population, were subjected to Analysis of Variance (ANOVA) for calculation of mean, Standard Deviation (SD), p-value. The significance of correlation among various parameters, the r value was calculated by Pearson's coefficient of correlation. The Receiver Operating Characteristic curve (ROC) analysis was done to obtain the sensitivity and specificity of tests used to establish the encephalopathy.

RESULTS

Distribution of the study population as per sex is depicated in [Table/Fig-5]. The mean age of the cases was 53.34 years and of the control cases was 47.43 years.

[Table/Fig-6] shows alcohol related liver disease as the most common aetiology, present in 53.33% (n=80) of the population followed by viral infection which was present in 22% (n=33).

Variables	Cases	Controls	p-value
Mean age (years)	53.34	47.43	0.01
Number of males	122 (81.33%)	30 (75%)	0.01
Number of females	28 (18.67%)	10 (25%)	0.01

[Table/Fig-5]: Showing distribution of the study population as per age and sex.

Aetiology	N (%)
Alcoholic	80 (53.33)
NASH	21 (14)
HBV	5 (3.33)
HBV±Alcoholic	4 (2.67)
HCV	16 (10.67)
HCV±Alcoholic	8 (5.33)
Others	16 (10.67)
Total	150 (100)

[Table/Fig-6]: Showing the aetiological distribution among the study population.
NASH: Nonalcoholic steatohepatitis; HBV: Hepatitis B virus; HCV: Hepatitis C virus

The mean values of various biochemical parameters showed variation (deterioration) in the pattern of the grades of cirrhossis as comulated by CPT Score. The haematological indices did not show any significant variation in comparison except the decrease in the platelet count and Prothrombin Time (PT). The pattern of lab values which change in the various grades of HE, looks in accordance to the deteriorating grades of cirrhossis [Table/Fig-7] [23-26]. The CTP Score shows worsening in the severity of grade of cirrhossis as depicted by CTP score, with worsening of the grades of encephaopathy [Table/Fig-8]. The study population and the control group were subjected to psychometric analysis and the observed values were compared with the expected for the age and level of education as calculated from [Table/Fig-2].

Groups	Normal value	Group A	Group B	Group C	Controls	p-value
Haemoglobin (g/dL) [23]	12-14	9.46±2.73	9.088±2.93	9.64±2.03	12.59±1.17	0.1
TLC [23]	4000-11000	6813±2443	5878±2611	7172±2952	7270±1612	0.1
Platelet Count (lac/cumm) [23]	2.0-3.5	1.84±0.93	1.57±1.06	1.46±0.90	2.81±0.61	0.01
PT (sec) [24]	14	18.17±4.24	18.46±4.38	24.57±10.77	14±0	0.001
Total Bilirubin (mg/dL) [25]	0.1-1.0	3.48±6.32	3.52±4.65	6.90±8.41	0.80±0.20	0.01
AST (IU) [25]	0-44	109±111.12	126±144.91	113.95±108.79	32±4.4	0.01
ALT (IU) [25]	0-54	72.61±107.44	71.39±79.52	64.44±52.07	28±3.2	0.01
Serum Albumin (g/dL) [25]	3.8-4.4	2.63±0.79	2.49±0.64	2.23±0.51	4.0±0.66	0.01
Serum Creatinine (mg/dL) [26]	0.1-1.3	1.06±1.06	1.07±1.02	1.03±0.26	0.8±0.4	0.01
BUN (mg/dL) [26]	0-20	11.10±4.93	13.93±6.47	16.95±13.59	12±2.8	0.01
Serum Sodium (mEq/L) [26]	135-145	135.93±4.77	136.19±6.17	134.33±6.34	139±4.41	0.01
Serum Potassium (meq) [26]	3.5-5.5	4.16±0.67	4.41±0.61	4.08±0.69	3.8±0.62	0.01

[Table/Fig-7]: The mean values of the biochemical characteristics of the study population [23-26].
TLC: Total leukocyte count; PT: Prothrombin time; AST: Aspartate aminotransferase; ALT: Alanine transaminase; BUN: Blood urea nitrogen

Groups	Number of patients	Child Pugh score	
		Score	SD
Group A	46	7.80±2.10	2.10
Group B	41	9.36±0.64	0.64
Group C	63	12.19±1.46	1.46
Controls	40	5.0±0	0
p-value	0.0001		

[Table/Fig-8]: Distribution of Child Pugh Turcotte (CTP Score) score in the study population.

[Table/Fig-9] shows progressive deterioration of the PHES with worsening of degree of encephalopat.

All the cirrhotic patients were subjected to psychometric analysis, a battery of five pen and paper tests. The distribution of values of the individual tests of PHES is shown in [Table/Fig-10]. All the five constituent tests of the PHES score showed significant deterioration in performance in patient with HE than in patients with cirrhosis without HE and with a statistically significant difference (p-value=0.001) [Table/Fig-10].

Groups	No. of patients	PHES score
		Mean±SD (Range)
Group A	46	-2.39 (-1 to -4)±1.22
Group B	41	-6.41 (-5 to-8)±0.97
Group C	63	-8.69 (-6 to -13)±2.69
Controls	40	2.55±0.99
p-value	0.001	

[Table/Fig-9]: Distribution of PHES values in various groups.

Parameter	Study population			Controls
	Group A	Group B	Group C	
Serial Dot Test (sec)	56.25±4.12	68.01±3.31	74.52±8.13	40.66±4.14
Line Tracing Test (sec)	72.91±3.11	81.04±2.86	85.82±6.17	63.98±2.10
Digit Symbol Test (fig)	29.51±7.17	28.73±5.92	28.25±5.75	31.09±5.54
Number Connection Test-B (sec)	44.01±4.57	51.72±4.24	55.70±6.73	32.36±4.96
Number Connection Test-A (sec)	63.69±7.99	72.82±7.83	77.34±10.61	48.02±8.52
p-value	0.001			

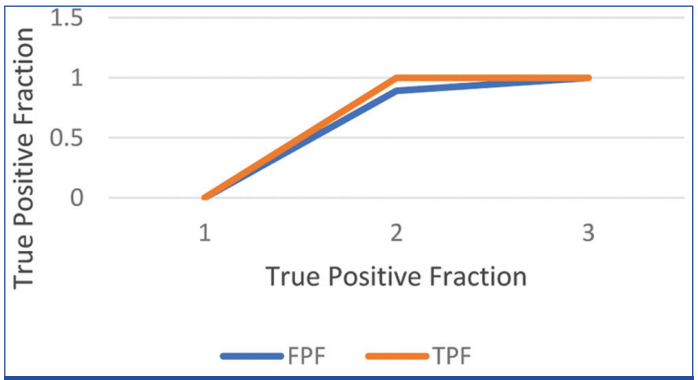
[Table/Fig-10]: Observed values of components of psychometric analysis in various groups of study population.

Serum ammonia levels in the study population groups: In the distribution of mean of arterial ammonia levels (ng/dL) in various groups of the study population. Group A had a mean of 34.69±16.21, Group B had a mean of 51.46±10.12 and Group C had a mean of 85.79±10.76. In the controls the mean value was <15 [Table/Fig-11].

Groups	Number of patients	Arterial ammonia levels
		Mean±SD (ng/dL)
Group A	46	34.69±16.21
Group B	41	51.46±10.12
Group C	63	85.79±10.76
Controls	40	15±0
p-value	0.0001	

[Table/Fig-11]: Arterial ammonia levels in various groups of the study population.

Under the ROC curve analysis for Arterial ammonia levels (At levels of 51 ng/dL) as marker of HE showed better sensitivity (100%) than specificity (10.9%) with area under the curve 0.554 and accuracy of 52.9%, hence establishing that ammonia levels has little role in screening for HE [Table/Fig-12].



[Table/Fig-12]: ROC curve for analysis of arterial ammonia levels as marker for hepatic encephalopthy (compared to PHES score).

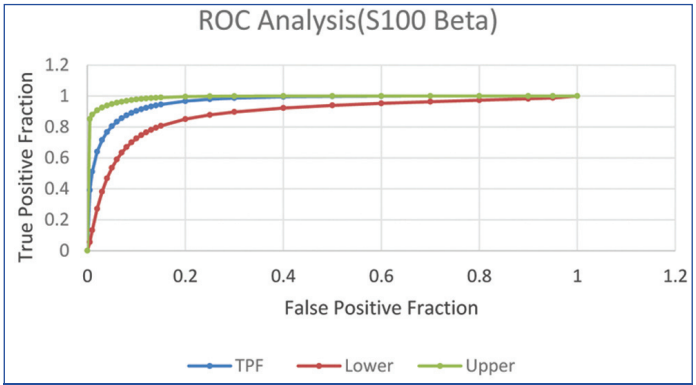
Serum S100 beta levels in the study population groups: A progressive rise in serum values of S100 Beta were seen with increase in the grade of HE [Table/Fig-13].

Groups	Number of patients	S100 Beta levels (ng/mL)
		Mean±SD
Group A	46	0.075±0.019
Group B	41	0.137±0.027
Group C	63	0.196±0.038
Controls	40	0.018±0.089
p-value	0.0001	

[Table/Fig-13]: Serum S100 beta levels in the various groups of the study population.

[Table/Fig-14] shows receiver operating analysis of S100 Beta (At a cut-off value of 0.12 ng/dL) as a diagnostic marker in HE. Values

of S100 beta levels were compared with PHES score criteria of <-5, to diagnose minimal HE The area under the curve was 0.9653 and depicted a accuracy of 88.5%. The sensitivity of the marker stood at 92.7% and specificity at 84.8% [Table/Fig-14].



[Table/Fig-14]: Reciever Operating Analysis (ROC) curve analysis for S100 beta as a marker in Hepatic Encephalopathy (HE).

As shown in the [Table/Fig-15] above, the Serum S100 Beta values showed a significant positive correlation with PHES score in all grades of encephalopathy but a weaker correlation in Group A and B with severity of cirrhosis as gauged by CPT score. In the correlation between PHES score and S100 Beta levels, there was a significant correlation in all the three groups with maximum correlation in patients with no HE and patients with minimal HE. Hence, the role of S100 Beta in diagnosis of HE is significantly appreciated. Patients with cirrhosis and no HE have a r=0.7466, in patients with overt HE, r=0.4426 and in patients with CHE, r=0.3378.

Groups	r (S100 Beta and PHES score)	p-value	r (S100 Beta and CPT score)	p-value
Group A	0.7466	0.001	0.311	0.001
Group B	0.4426	0.001	0.0346	0.01
Group C	0.3378	0.001	0.7611	0.001
Controls	-0.130	0.01	-0.118	0.01

[Table/Fig-15]: S100 beta levels correlation with grades of encephalopathy and severity of cirrhosis.

DISCUSSION

The most important outcome of the patient-oriented trials or research is Health-Related Quality Of Life (HRQOL). In fact, it is considered that the improved quality of life and reduced disability has more impact rather than prolongation of patient's life. HE minimal, covert and overt HE represents a broad spectrum of the neurological manifestations of the liver diseases and has a significant effect on the quality of life [27].

CHE is difficult to diagnose and patients with CHE are more prone to progress to develop overt HE than patients who do not have CHE [28]. So if CHE is diagnosed earlier and is followed by early treatment, this would result in improved quality of life and favourable prognosis of the patient. Currently, though not as a gold standard, psychometric analysis is considered as the best clinical standard to diagnose HE but these tests consume a lot of time, require training and are affected by the patients' age and education, explaining why MHE is the most under-diagnosed form of HE and it can afflict up to 80% of patients with CLD [29]. This necessitates the requirement of a simple serum biomarker that would be accurate, reliable, sensitive/specific and with a high predictive value could identify accurately patients with CHE in an emergency room or in the outpatient department.

[Table/Fig-16] shows the various studies which had been done in this context [9-13,30]. The earlier studies which substantiate S100 Beta in correlation with HE, but have relatively smaller study populations. In present study, authors had used a robust Psychometric analysis to screen for CHE and then patients were included in the study. The study by Strebel H et al., was a negative study; but the study had its limitations [13]. It was a very small pilot study with only 30 patients finishing the study and lacked the control groups. Only 33% of the patients had increased ammonia level, either OHE or MHE, which was much lower than expected and raises concerns on the diagnosis of HE.

The study was conducted on 150 patients of cirrhosis of liver and 40 age and sex matched controls. The study population had a mean of age of 40 years, with the majority of the population were in the age group of (31-60) years. The males constitute 81.33% of the population and females were 18.67%. The demographic profile matched the PREDICT study, which was a nationwide study conducted to study the prevalence of MHE in patients of cirrhosis in which the mean age of study population (n=1114) was 49.5 years and majority of them were males (n=901) (81%) [31].

PHES score and its correlation: The validated expected PHES score for Indian population was used, as done under a study at a tertiary level teaching hospital of North India [16]. The data also took care of the concerns regarding the effects of age and level of education. Overall, this battery of tests assesses motor speed, accuracy, visual construction, visual perception, visuo-spatial orientation, concentration, attention and working memory. All tests were expressed as Z score and the total of the five tests was then calculated. Negative values were suggestive of poorer performance [16]. The mean PHES score values deteriorated from a mean of -2.39±1.22 in patients of cirrhosis without HE. The performance scores deteriorated to -6.41±0.97 in patients with minimal HE to further mean Z score of -8.69±2.69 in patients with overt HE. Values of PHES score performance poorer than five were labelled as having evidence of HE. A clear deterioration in the score status of PHES tests in the group with cirrhosis and cirrhosis with CHE or HE, as well as worsening in the individual PHES tests with a statistically significant difference was seen. PHES score had a weak correlation with the CTP score. The correlation decreased with worsening of

Study	Publication year	Number of patients	Control group	Parameters assessed	Conclusion
Wiltfang J et al., [9]	1999	36	No	S100 Beta, Ammonia levels	S100 B serum level had advantage over ammonia serum level in detecting portal systemic encephalopathy.
Saleh A et al., [10]	2007	43	9	S100 Beta, Neuron-specific enolase, Serum Ammonia	S100 B level had correlation with Hepatic Encephalopathy (HE) (stages I and II), compared to healthy and cirrhotic patients without HE.
Isobe-Harima Y et al., [11]	2008	9	No	S100 Beta	S100 B serum level was higher in HE patients.
Duarte-Rojo A et al., [12]	2016	46	15	S100 Beta	S100 B serum levels were higher in patients with cirrhosis and HE than in healthy subjects.
Strebel H et al., [13]	2020	30	No	S100 Beta, Ammonia	Serum S100 B protein level did not correlate with the presence of HE.
Elgendy NA et al., [30]	2019	60	60	S100 Beta, Ammonia	S100 Beta candidate to be diagnostic serum marker of Minimal Hepatic Encephalopathy (MHE).
Current study	2023	150	40	S100 beta, Ammonia	S100 Beta correlated with early grades of HE and can be used as a marker for HE.

[Table/Fig-16]: Comparison of various studies [9-13,30].

the grades of the encephalopathy. The coefficient of correlation in Group A was 0.454 which decreased to 0.169 and 0.179 in Group B and C, respectively. This finding correlated with a study done in a tertiary care hospital of North India done by Dhiman RK et al., where PHES score correlated weakly with CTP score and had a coefficient of correlation (r)=-0.272 [16]. Hence, suggesting PHES score has no correlation with grades of cirrhosis as graded by CTP Score.

Arterial ammonia levels (ng/dL) and its correlation: Arterial ammonia levels in the various groups showed rise in the mean value. The value in Group A was 34.69±16.21 in Group B with minimal HE was 51.46±10.12 and in patients with overt HE were 85.79±10.76. The values at extreme of the spectrum in control Group and Group C had significant difference but in Group A and Group B showed a significant overlapping. This finding was in correlation with earlier studies which failed to establish the role of ammonia levels as marker of screening for HE [32]. In the correlation between ammonia levels and PHES score, there was a weak correlation in patients with minimal/ CHE (Group B) (r=0.0917), than in patients with overt HE (Group C) (r=1.000). Hence, maintaining little role for ammonia levels as marker for diagnosis of minimal encephalopathy. Similarly in a study by Miller KE done on 121 patients, though the ammonia levels increased with increase in severity of HE, but did not correlate with levels of HE [33]. Also, in a study of review of records done on 1200 patients with cirrhosis and HE by Haj M and Rockey DC they also concluded that increase in ammonia levels do not add diagnostic, staging, or prognostic value for HE patients [32]. The ROC curve analysis established little role of serum ammonia levels as marker of HE. The finding correlated with a study by Mahmoud RAK et al., which concluded ammonia level as a weak marker to diagnose HE [34].

Serum S100 beta levels (ng/mL) and its relevance as a marker: With an aim to establish a liquid diagnosis of HE, as was discussed in a review article by Machado MV S100 Beta was considered a worth investigating marker for diagnosis of HE [8]. S100 Beta levels showed a progressive rise in patients with HE than in patients without HE. The control group had S100 Beta levels (ng/mL) of 0.018±0.089 ng/mL, while in patients with Cirrhosis without HE had raised levels to 0.075±0.019, which increased to almost double the value to 0.137±0.027 in patients with minimal HE and to 0.196±0.038 in patients with overt HE. The rise in value of S100 beta in patients of cirrhosis without HE than in control group is supporting the role of S100 Beta as marker of blood brain barrier breach and hence is suggestive that even in patients with underlying cirrhosis there is level of dysfunction of blood brain barrier.

S100 beta correlated more with PHES score than CTP score: These findings matched with study of Duarte-Rojo A et al., which showed significant correlation between levels of S100 Beta and PHES score in the total population (r=0.624) as well as in patients with cirrhosis and CHE and non CHE. (r=-0.413, p=0.019) [12]. The study also showed a positive correlation of S100 Beta and CTP score (r=0.515, p<0.001). Similarly in a study by Saleh A et al., a significant positive correlation was found between S100 beta levels and the level of cognition (r=0.70, p<0.001) [10]. It was also concluded that though S100 Beta levels show some correlation with CTP score, but it more strongly related to cognitive impairment than the score. As tendency to develop HE is greater in higher grades of cirrhosis, correlation in Group C between S100 Beta levels and CPT score is significant. Hence, it is established that S100 Beta levels predict HE rather than varies with severity of cirrhosis.

The ROC curve analysis determines S100 Beta to be a better marker for diagnosis of HE, when compared to ammonia levels or PHES score computation. In a study by Duarte-Rojo A et al., ROC curve showed matching pattern with sensitivity of 83.3% and specificity of 63.6% and area under the ROC curve was 0.801 [12]. Similarly in a study by Wiltfang J et al., ROC curve analysis at a (>77.5 ng/mL) serum level of S100 Beta, the best cut-off for diagnosis of HE had a sensitivity of 80% and specificity of 85% [9]. The findings also matched with a study by Saleh A et al., in which sensitivity and specificity for each value of S100 Beta levels were calculated and ROC curve at a value of 0.198 ug/L, showed specificity at 91.3% and sensitivity at 51.7% [10]. In a similar study by Elgendy NA et al., to assess the S100 Beta levels in MHE, showed that S100 Beta levels had a specificity of 65% and sensitivity of 90% in diagnosis of CHE and a specificity of 85% and sensitivity of 80% in diagnosis of overt HE [30].

Limitation(s)

There were certain limitations in the study. Patients in various grades of HE who were admitted in IPD, were serially evaluated with daily assessment of psychometric analysis and check progress of treatment. However, S100 Beta levels could have been also done daily to monitor correlation in their levels with improvement in patients PHES score and clinical condition. Multiple challenges made psychometric testing cumbersome. Difficulty in the understanding of patients of the tests who were of low education status, multiple tests in the outpatient assessment. Though at times repeat testing were performed once patient understood the procedure better and the best observed values were then documented. A larger data set, specifically with more sample-to-sample overlap, would be desirable to demonstrate a stronger correlation and hence clinical usefulness of the marker.

CONCLUSION(S)

At a time when diagnosis of HE is standardised on clinical criteria's, the current study establishes a direction in the shift towards liquid diagnosis of the condition. The present study concluded the presence of a strong association of S100 Beta levels with the earlier grades (I and II) of HE and is a sensitive marker in detection of HE, with its role defined comparable to psychometric analysis which forms the current clinical standard for diagnosing minimal HE. However, there is a need to establish its role in disease progression and improvement.

REFERENCES

[1] Sharma K, Akre S, Chakole S, Wanjari MB. Hepatic encephalopathy and treatment modalities: A review article. Cureus. 2022;14(8):01-16.
[2] Weissenborn K. Hepatic encephalopathy: definition, clinical grading and diagnostic principles. Drugs. 2019;79(1):05-09.
[3] Jalan R, Rose CF. Heretical thoughts into hepatic encephalopathy. J Hepatol. 2022;77(2):539-48.
[4] Morgan MY. Hepatic encephalopathy in patients with cirrhosis. Sherlock's Diseases of the Liver and Biliary System. 2018:151-79.
[5] El Khiat A, El Hiba O, Aimrane A, Draoui A, Jayakumar AR, Norenberg MD. Hepatic encephalopathy: An overview of basic concepts and mechanisms. Front Med Case Rep. 2022;3(3):01-19.
[6] Yardan T, Erenler AK, Baydin A, Aydin K, Cokluk C. Usefulness of S100B protein in neurological disorders. JPMA-J Pak Med Ass. 2011;61(3):276-81.
[7] Pisarek W. S100 Beta protein as a marker of hepatic encephalopathy: A breakthrough in diagnostics or a false trail? Review of the literature. Postępy Higieny i Medycyny Doświadczalnej. 2022;76(1):128-31.
[8] Machado MV. Liquid diagnosis of hepatic encephalopathy: Are we there already? GE-Portuguese J Gastroenterol. 2020;27(6):378-82.
[9] Wiltfang J, Nolte W, Otto M, Wildberg J, Bahn E, Figulla HR, et al. Elevated serum levels of astroglial S100β in patients with liver cirrhosis indicate early and subclinical portal-systemic encephalopathy. Metabolic Brain Disease. 1999;14(4):239-51.
[10] Saleh A, Kamel L, Ghali A, Ismail A, El Khayat H. Serum levels of astroglial S100-beta and neuron-specific enolase in hepatic encephalopathy patients. EMHJ-Eastern Mediterranean Heal J. 2007;13(5):1114-23.
[11] Isobe-Harima Y, Terai S, Segawa M, Uchida K, Yamasaki T, Sakaida I. Serum S100b (Astrocyte-Specific Protein) is a useful marker of hepatic encephalopathy in patients with fulminant hepatitis. Liver International: Official Journal of the International Association for the Study of the Liver. 2008;28(1):146-47.
[12] Duarte-Rojo A, Ruiz-Margáin A, Macias-Rodriguez RU, Cubero FJ, Estradas-Trujillo J, Muñoz-Fuentes RM, et al. Clinical scenarios for the use of S100β as a marker of hepatic encephalopathy. World J Gastroenterol. 2016;22(17):4397-402.
[13] Strebel H, Haller B, Sohn M, Schepp W, Gundling F. Role of brain biomarkers S-100-beta and neuron-specific enolase for detection and follow-up of hepatic encephalopathy in cirrhosis before, during and after treatment with L-ornithine-L-aspartate. GE-Portuguese Journal of Gastroenterology. 2020;27(6):391-403.

[14] Tsoris A, Marlar CA. Use of the Child Pugh Score in Liver Disease. [Updated 2021 Mar 22]. Stat Pearls [serial online][cited 2021 February 21]. Treasure Island (FL): StartPearls Publishing. 2022.
[15] Karanfilian BV, Park T, Senatore F, Rustgi VK. Minimal hepatic encephalopathy. Clinics in Liver Disease. 2020;24(2):209-18.
[16] Dhiman RK, Kurmi R, Thumburu KK, Venkataramarao SH, Agarwal R, Duseja A, et al. Diagnosis and prognostic significance of minimal hepatic encephalopathy in patients with cirrhosis of liver. Digestive Diseases and Sciences. 2010;55(8):2381-90.
[17] Choosongsang P, Bodhikul A, Musigavon P, Pocathikorn A, Prasongsab T, Musaw A, et al. Modified Jendrassik-Grof method for measurement of direct bilirubin: An improvement of in-house method. Songklanagarind Med J. 2011;29(1):19-26.
[18] Guirguis N, Bertrand AX, Rose CF, Matoori S. 175 years of bilirubin testing: Ready for point of care? Advanced Healthcare Materials. 2023;5023:2203380.
[19] Özbek O, Berkel C, Isildak Ö, Isildak I. Potentiometric urea biosensors. Clinica Chimica Acta. 2022;524:154-63.
[20] Dissanayake RK, Ranaweera KKPT, Priyadarshani AMB. A narrative review on laboratory investigations of serum creatinine and solutions to problems therein. J Health Sci and Innovative Res. 2022;3(2):25-34.
[21] Chen H, Li H, Lin JM. Determination of ammonia in water based on chemiluminescence resonance energy transfer between peroxymonocarbonate and branched NaYF4:Yb3+/Er3+nanoparticles. Analytical Chemistry. 2012;84(20):8871-79.
[22] Jordan B. How to set up an ELISA. Diagnostic and Therapeutic Antibodies. 2000:373-79.
[23] Qamar AA, Grace ND. Abnormal hematological indices in cirrhosis. Canadian Journal of Gastroenterology. 2009;23(6):441-45.
[24] O'Leary JG, Greenberg CS, Patton HM, Caldwell SH. AGA clinical practice update: Coagulation in cirrhosis. Gastroenterology. 2019;157(1):34-43.
[25] Sharma P. Value of liver function tests in cirrhosis. J Clin Experi Hep. 2022;12(3):948-64.
[26] Montagnese S, Bajaj JS. Impact of hepatic encephalopathy in cirrhosis on quality-of-life issues. Drugs. 2019;79(1):11-16.
[27] Ridola L, Nardelli S, Gioia S, Riggio O. Quality of life in patients with minimal hepatic encephalopathy. World J Gastroenterol. 2018;24(48):5446-53.
[28] Bajaj JS, Lauridsen M, Tapper EB, Duarte-Rojo A, Rahimi RS, Tandon P, et al. Important unresolved questions in the management of hepatic encephalopathy: An ISHEN consensus. Official Journal of the American College of Gastroenterology| ACG. 2020;115(7):989-1002C.
[29] Francoz C, Glotz D, Moreau R, Durand F. The evaluation of renal function and disease in patients with cirrhosis. J Heptol. 2010;52(4):605-13.
[30] Elgendy NA, ELBialy R, El Sayed Mohammed E. Assessment of S100β in patients with minimal hepatic encephalopathy. Am J Biochem. 2019;9(2):45-55.
[31] Rathi S, Chopra M, Chouduri G, Sharma P, Madan K, Chhabra M, et al. Prevalence of minimal hepatic encephalopathy in patients with liver cirrhosis: A cross-sectional, clinicoepidemiological, multicenter, nationwide study in India: The PREDICT study. J Clin Experimental Hepatol. 2019;9(4):476-8N.
[32] Haj M, Rockey DC. Ammonia levels do not guide clinical management of patients with hepatic encephalopathy caused by cirrhosis. Official J Ame Coll Gastroenterol ACG. 2020;115(5):723-28.
[33] Miller KE. Do ammonia levels correlate with hepatic encephalopathy? American Family Physician. 2003;68(7):1408.
[34] Mahmoud RAK, Roshdy M, Lashin S, Bakr YM. Blood ammonia, S100B and neuron-specific enolase levels in patients with hepatic encephalopathy. Arab J Lab Med. 2005;31(2):227-40.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
2. Junior Resident, Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
3. Professor and Head, Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.
4. Professor and Head, Department of Medicine, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Parminder Singh,
H. No. 106, Chinar A2, Omaxe Parkwoods, Baddi,
District Solan-173205, Himachal Pradesh, India.
E-mail: ppinka113@gmail.com

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