Predictor biomarkers for cognitive impairment in Temporal Lobe Epilepsy in adults

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Abstract

Background: The most frequent cause for focal seizures is temporal lobe epilepsy (TLE). The temporal lobe is primarily responsible for speech, memory, learning, and affective behavior. The previously mentioned functions would be disrupted if the lobe's internal structures were damaged.

Aim: To determine cognitive impairment in TLE in adults and to investigate if there was an association between serum levels of HMGB1 and S100B expressions with cognitive impairment in TLE for the possibility of detecting predictive biomarkers for cognitive impairment in TLE.

Subjects and methods: This study was a case control study of 200 subjects at the Neurology Department at Mansoura University and Kafrelsheikh University Hospitals. They were divided into two groups: **Group A:** 100 patients with TLE and **Group B:** 100 healthy controls.

Results: A statistically significant higher mean MoCA score was discovered among the control than the cases group (28.66±0.794 versus 17.27±7.21, respectively). A statistically significant higher median serum concentration of HMGB1and S100B was discovered among cases compared to the control group (p-value<0.001*). A statistically significant higher median serum concentration of HMGB1 and S100B was discovered among cases with impaired cognitive than normal cognitive functions.

Conclusion: People with TLE reported lower MoCA scores in several cognitive domains, mainly language and naming, comparing with the control group. Elevated serum concentrations of S100B and HMGB1 are associated with low MoCA scores in patients with TLE, so they can be considered as promising predictive biomarkers for the identification of cognitive impairment in TLE. For a more accurate evaluation, future investigations of large numbers of cases with long-term monitoring are required.

Key words: HMGB1, temporal lobe epilepsy, cognitive impairment

Introduction

The most frequent cause for focal seizures is temporal lobe epilepsy (TLE). The temporal lobe is primarily responsible for speech, memory, learning, and affective behavior. The previously mentioned functions would be disrupted if the lobe's internal structures were damaged. Individuals with TLE frequently suffer from depression, anxiety, and memory impairment. Mesial temporal sclerosis is the most prevalent epileptogenic lesion in this form of epilepsy, and the hippocampus is frequently severely impacted [1].

The hippocampus is considered to be essential for episodic memory, whereas other regions of the temporal lobe are more significant for semantic memory. A substantial cognitive impairment, especially in the memory domain, is frequently correlated with severe hippocampal atrophy, which is caused by more frequent and longer seizures [2].

Patients with mesial TLE have a pattern of distributed cognitive deficits that affect not only memory but also a wide range of cognitive domains, such as executive functions, intelligence quotient, language, and sensorimotor skills. A cumulative literature has emerged,

which establishes a correlation between structural alterations and cognitive performance [3].

Therefore, there is an unmet clinical need for the development of biomarkers that have the ability to predict and evaluate the disease condition in addition to comorbidities, such as cognitive impairment. This could help in developing novel treatments for epilepsy that reduce related comorbidities, such as cognitive impairment, while also delaying the onset of seizures. [4, 5].

Although there hasn't been much progress in this area, reliable biomarkers are required for the prediction of early cognitive impairment. Therefore, research was done on the relationship between proinflammatory pathways and epileptogenesis and cognitive impairment as well as how they may be used as biomarkers for early cognitive impairment diagnosis [6].

HMGB1 acts as an inflammatory cytokine in response to epileptogenic insults [7]. It acts as a pathogenic inflammatory response to mediate wide ranges of circumstances such as epilepsy [8], septic shock, ischemia, traumatic brain injury [9], Parkinson's disease [10], Alzheimer's disease [11], and multiple sclerosis [12].

Memory impairment was associated with HMGB1 through the mediation of RAGE and TLR4. HMGB1 demonstrates pro-excitatory impacts in the hippocampus through raising the phosphorylation of NR2B-NMDA receptors, consequently elevating the conductance of the receptor calcium channel [13].

A calcium-binding protein called S100B is mostly produced by astrocytes. Cell cycle progression and signal regulation are two of the many biological functions that S100B proteins control. These proteins act as stimulators of cell migration and proliferation and inhibitors of differentiation and apoptosis [14, 15].

Micromolar concentrations of S100B cause RAGE to become persistently activated, which increases the generation of oxygen radicals and may cause apoptosis and mitochondrial malfunction. Additionally, its signaling pathways trigger the transcription of genes that promote apoptosis, which in turn affects memory [16]. In other words, it has neurotoxic or neurotropic effects that vary with dose. These effects happen through the brain's RAGE receptor [17].

This study aimed to detect cognitive impairment in TLE in adults and to investigate if there was an association between serum levels of HMGB1 and S100B expressions with cognitive impairment in TLE for the possibility of detecting predictive biomarkers for cognitive impairment in TLE.

Subjects and methods

This research was case-control research of 200 subjects at the Neurology Department at Mansoura University and Kafrelsheikh University Hospitals. They have been classified into two groups: Group A: 100 cases with TLE, and Group B: 100 healthy controls whose age, sex, and education matched with group A. This study has been accepted by the

Institutional Ethics Committee of Mansoura Faculty of Medicine. Written informed consents have been taken from all recruited cases or their relatives and from healthy controls. Studied patients were selected in the period between April 2022 and April 2024.

Inclusion criteria: Patients with TLE who were above the age of 18 years and had documented electroencephalogram results.

Exclusion criteria: Patients less than 18 years, symptomatic epilepsy, patients with other serious developmental disorder or intellectual disability, patients with demyelinating conditions, dementia, and Parkinson's illness, patients with metabolic disorders, patients with severe psychiatric conditions and substance abuse disorders, and patients receiving glucocorticoid or immunosuppressive agents.

Methods:

All patients and controls have been subjected to a complete history taking as regard age, sex, and educational level. Patients were evaluated about their history of TLE with regard to age at onset, frequency of seizures, duration of epilepsy and quantity of AEDs. AEDs used by the patients during the study were sodium valproate, carbamazepine, lamotrigine, topiramate, levetiracetam, and lacosamide. Complete general and neurological examination and measurement of serum levels of HMGB1 and S100B by ELISA kit for both patients and controls.

Sample collection:

Three ml of fasting venous blood were withdrawn in plain tubes from all participants (epilepsy patients and healthy subjects); blood samples have been allowed to clot for fifteen minutes at room temperature and then centrifuged at 3000 round per minutes for twenty minutes to obtain serum. Subsequently, the collected serum was stored at -80 Celsius until ELISA assay.

Detection of serum concentrations of HMGB1

Serum concentrations of HMGB1 have been evaluated by a commercial enzyme-linked immunosorbent assay kit after the instructions of the manufacturer (SinoGeneClon Biotech Co.,Ltd, China). Catalog No : SG-10384 for the quantitative determination of Human HMGB1 concentrations. (Detection method: sandwich, Double antibody), with kit's ideal detection range (5 μg/L-100 μg/L). Serum levels of HMGB1 were obtained via TECAN infinite F50 - ELISA Reader SN. 1303004564 (TECAN, Austria).

Detection of serum levels of S100B

Serum concertation of S100B have been measured by a commercial enzyme-linked immunosorbent assay kit after the instructions of the manufacturer (SinoGeneClon Biotech Co.,Ltd, China). Catalog No : SG-11708 for the quantitative determination of Human S100B concentrations. (Detection method: sandwich, Double antibody), with kit's ideal detection range (35 pg/ml -2000 pg/m). Serum levels of S100B were obtained via TECAN infinite F50 - ELISA Reader SN. 1303004564 (TECAN, Austria)

MRI brain

The Radiology Department at Kafrelsheikh University Hospitals used a 3 Tesla scanner (Acheiva; Philips Medical Systems, Best, Netherlands) to perform the brain MRI examination. A head coil was utilized for examining all cases and controls in the supine position. Routine images (T2, T1, FLAIR) have been have been obtained for all cases. The following parameters were used: repetition time (TR) = 9583 seconds, inversion time (TI) = 3400 seconds, echo time (TE) = twenty-five seconds, echo train length (ETL) = 17, 50 contiguous axial slices, IR delay = 325 seconds, thickness = three millimetres, matrix size = 240× 142 millimetres square, field of view (FOV) = 230× 184 millimetres square.

Evaluation of cognitive functions using:

Montreal Cognitive Assessment (MoCA) (Arabic version) [18].

MoCA was an efficient and precise method to screen for cognitive impairment. Attention, short-term memory, executive function, verbal expression, calculation, orientation, abstract thinking, and visual-spatial ability were all evaluated in the cognitive domain. The total score was 30 points. A normal cognitive function was indicated by a score of 26 points or higher.

Electroencephalogram (EEG)

EEG recordings were performed in the Neurology department using Nihon Kohden (model EEG 1200K), 21 EEG channels, using hyperventilation and photic stimulation as provocative procedures. EEGs were recorded while the subjects were awake with their eyes closed. The electrode placement was done in accordance with the international standard 10-20.

Statistical analysis and data interpretation:

- The data analysis has been carried out utilizing SPSS software, version 25 (SPSS Inc., PASW Statistics for Windows version 25). SPSS Inc., Chicago. Percentages and numbers have been utilized for presenting qualitative data. The median (minimum and maximum) has been utilized to express quantitative data for non-normally distributed data, while the mean± has been utilized. The standard deviation of normally distributed data is determined following the Kolmogorov-Smirnov test has been conducted to determine its normality. The outcomes have been assessed for significance at the 0.05 level.
- Mann Whitney U test have been used for comparing among two examined groups for not normally distributed data.
- Monte Carlo, Chi-Square tests have been utilized for comparing qualitative data among groups as suitable
- Student t test has been utilized for comparing 2 independent groups for normally distributed data.
- The Spearman's rank-order association assesses the direction and strength of the linear correlation among 2 non-normally distributed continuous parameters and/or ordinal parameters. The test value indicated as r; values are represented as the following:
- Negative correlation indicated inverse association.
- Positive value indicated direct association.
- r from (0.3: 0.6) or (-0.3: -0.6) …… moderate association.
- r from (0: 0.3) or (0: -0.3) …… weak correlation.
- r from (0.6: 1) or (-0.6: -1) …… strong association
- The receiver operating characteristic curve (ROC curve) has been utilized to assess the validity (specificity and sensitivity) of continuous parameters, determining the optimal cut-off point. Accuracy and predictive values are evaluated through cross tabulation.

Results

The age, sex, as well as educational level of the research subjects, are presented in Table 1. Insignificant statistical differences have been discovered among the examined groups regarding mean age, sex and educational level (p = 0.775, 0.478, & 0.334, respectively).

Table (1): Comparison of general characteristics among examined groups

	Cases group (N=100)	Control group (N=100)	Test of significance	P value
Age / years Mean ±SD	31.33±9.77	30.94±9.52	t=0.286	0.775
Sex Male Female	49(49.0) 51(51.0)	43(43.0) 57(57.0)	χ^2=0.725	0.478
Educational level Primary	13(13.0)	18(18.0)	χ^2=2.19	0.334

Technical **High education**	53(53.0) 34(34.0)	43(43.0) 39(39.0)		

t: Student t test, χ^2=Chi-Square test

Table 2 shows that the median age at onset of disease was 10; the median disease duration was 15.5 years. The frequency of epileptic attacks per month was as follows: 32% had one attack, 32% had two attacks, 23% had three attacks, and 13% had four attacks. Among studied cases, 57% were drug-resistant epilepsy. As regard the number of antiepileptic drugs, 49% were on three AEDs, 30% were on monotherapy, and 21% were on two AEDs. As regard the EEG findings among studied cases, they were as follows: 25% were normal 75% had abnormal EEG findings.

Table (2): Disease characteristics among studied patients

	Cases group (N=100)	
Age at onset of disease (years) Median (min-max)	10(3-36)	
Duration of disease (years) Median (min-max)	15.5(1-40)	
Frequency of epileptic fits/ month One attack Two attacks Three attacks Four attacks	N 32 32 23 13	% 32.0 32.0 23.0 13.0
Drug responsiveness Responsive Resistant	N 43 57	% 43.0 57.0
Number of AED One drug (monotherapy) Two drugs Three drugs	N 30 21 49	% 30.0 21.0 49.0
EEG Normal Right temporal epileptiform activity Right temporal with 2ry generalization Left temporal epileptiform activity Left temporal with 2ry generalization	N 25 6 36 10 23	% 25.0 6.0 36.0 10.0 23.0

Table 3 demonstrates that a statistically significant higher mean MoCA score was discovered among the control than the cases group (28.66±0.794 versus 17.27±7.21, respectively). For cases, 78% had impaired cognitive function (severe cognitive impairment 20%, moderate cognitive impairment 50%, and mild cognitive impairment 8%) and 22% had normal cognition versus 100% normal cognition among the control group.

Table (3): Comparison of MoCA total score among examined groups

	Cases group (N=100)	Control group (N=100)	Test of significance	P value
MoCA	17.27±7.21	28.66±0.794	t=15.71	0.001*

Impaired	78(78.0)	0	χ^2=127.87	0.001*
Normal	22(22.0)	100(100.0)		

Data expressed as mean± SD / number (%),*statistically significant

Table 4 demonstrates that a statistically significant difference was discovered among cases and the control group in all MoCA domains. Median MoCA domains were higher among control than cases group. Among the cases group, the most common domains that affect the MoCA score were language and naming.

Table (4): Comparison of MoCA main domains between studied groups

MoCA domains	Cases group (N=100)	Control group (N=100)	Test of significance	P value
Visuospatial and executive function	2(0-5)	5(4-5)	Z=9.320	0.001*
Naming	2(1-3)	3(3-3)	Z=8.32	0.001*
Attention	3(0-6)	6(6-6)	Z=10.58	0.001*
Language	2(0-3)	3(3-3)	Z=10.97	0.001*
Abstraction	0(0-2)	2(2-2)	Z=10.67	0.001*
Delayed Recall	0(0-4)	4(3-5)	Z=10.41	0.001*
Orientation	6(0-6)	6(5-6)	Z=2.12	0.018*

Data expressed as median (min-max), Z: Mann Whitney U test, *statistically significant

Table 5 demonstrates that a statistically significant positive correlation was detected between MoCA score and age at onset of disease (r = 0.762) and with educational level (r = 0.267), and a statistically significant negative association was detected between MoCA score and disease duration (r = -0.900), frequency of attacks (r = -0.798), and number of AEDs (r = -0.868).

Table (5): Correlation between MoCA and disease characteristics among studied patients

	MoCA	
	R	**P**
Age at onset of disease (years)	0.762	<0.001*
Duration of disease (years)	-0.900	0.001*
Frequency of epileptic fits / month	-0.798	0.001*
Number of AED	-0.868	0.001*
Educational level	0.267	0.001*

*statistically significant, r: Spearman correlation coefficient

Table 6 shows that a statistically significant lower median MoCA score was discovered among medication-resistant compared to medication-responsive epilepsy (p-value <0.001). Similarly, a statistically significant association has been detected between EEG results and MoCA score, with a higher median MoCA score among normal EEG.

Table (6): Relation between MoCA score and drug responsiveness, EEG findings among studied patients

	MoCA	Test of significance
Drug responsiveness Responsive Resistant	 27(17-29) 14(2-17)	 Z=8.33 P<0.001*
EEG Normal Abnormal	 23(16-29) 14(2-29)	 Z=5.09 P<0.001*

Data expressed as median (min-max), Z: Mann Whitney U test, *statistically significant

Table 7 shows that a statistically significant higher median serum level of HMGB1 and S100B was discovered among cases than the control group (p-valueless than 0.001*).

Table (7): Comparison of serum level of HMGB1 and S100B among examined groups

	Cases group (N=100)	Control group (N=100)	Test of significance	P value
Serum level of HMGB1(µg /L)	43(6.1-89.0)	11.8(6.1-60)	Z=12.01	<0.001*
Serum level of S100B (pg/ml)	787(46-2355)	207(46-820)	Z=12.24	<0.001*

Data expressed as median (min-max), Z: Mann Whitney U test, *statistically significant

Table 8 demonstrates that a statistically significant negative correlation has been detected between MoCA score and serum level of HMGB1 (r = -0.938) and between MoCA score and serum level of S100B (r = -0.916) among studied patients (Fig. 1, 2).

Table (8): Correlation between MoCA and serum level of HMGB1, S100B among studied patients

	MoCA	
	R	P
HMGB1 (µg /L)	-0.938	0.001*
S100B (pg/ml)	-0.916	0.001*

r: Spearman correlation coefficient, *statistically significant

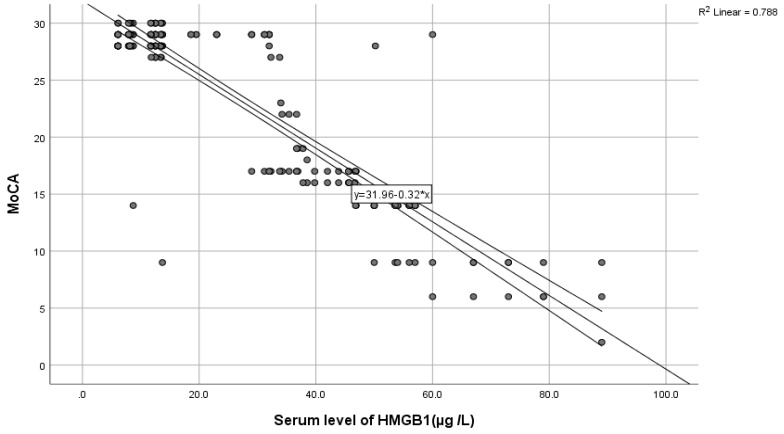

Fig. 1 Correlation between MoCA score and serum level of HMGB1 among studied cases

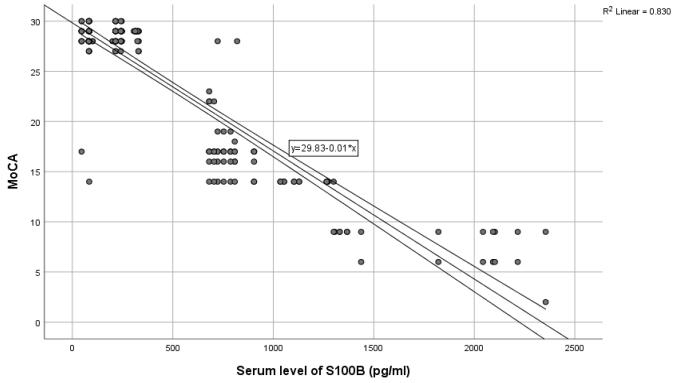

Fig. 2 Correlation between MoCA scores and serum level of S100B among examined cases

Table 9 demonstrates that the area under the curve for serum levels of HMGB1 in differentiating cases from control groups was excellent (area under the curve = 0.960), 26 µg /L was the most accurately determined cut-off point from the curve, yielding a specificity of 80% and a sensitivity of 90% (Fig. 3).

Table (9): Validity of serum level of HMGB1(µg /L) in differentiating cases from control groups

Area	Std. Error[a]	P value	95% Confidence Interval		Cut- off point	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
.960	.018	.001*	.925	.994	26	90.0	80.0

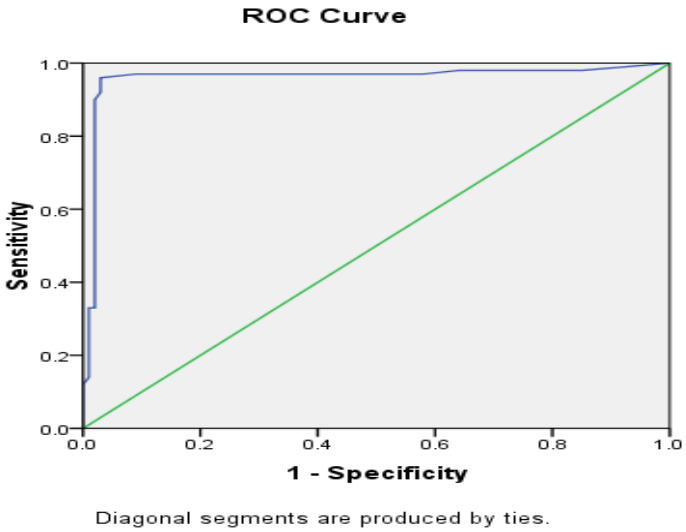

Fig. 3 ROC Curve of serum level of HMGB1 (µg /L) in differentiating cases from control groups

Table 10 demonstrates that the area under the curve for serum level of S100B in differentiating cases from control groups was excellent (area under the curve = 0.975), resulting in a specificity of 86% and a sensitivity of 98%, with the best determined cut-off point from the curve being 243 pg/ml (Fig. 4).

Table (10): Validity of serum level of S100B (pg/ml) in differentiating cases from control groups

Area	Std. Error[a]	P value	95% Confidence Interval		Cut- off point	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
.975	.013	.001*	.950	1.000	243	98	86

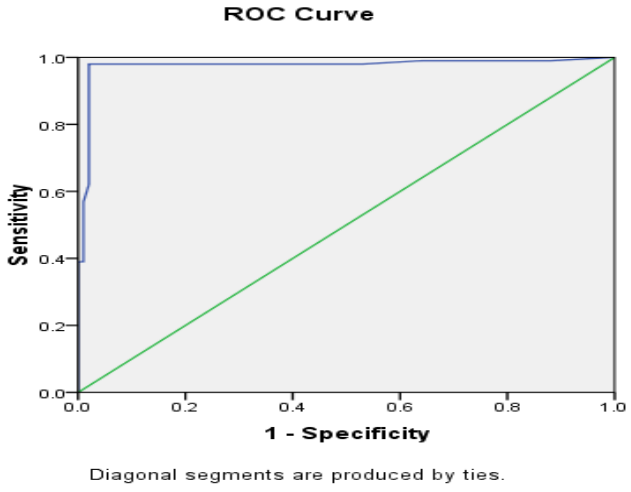

Fig. 4 ROC Curve of serum level of S100B (pg/ml) in differentiating cases from control groups

Table 11 demonstrates that a statistically significant greater median serum level of HMGB1 and S100B is found among cases with impaired cognitive than normal cognitive functions.

Table (11): Relation between serum level of HMGB1, S100B and cognitive dysfunction among studied cases

	Cognitive dysfunction		Test of significance
	Impaired	**Normal**	
HMGB1(µg /L) Mean ±SD	49.35±15.65	26.05±8.25	Z=6.53 P<0.001*
HMGB1(µg /L) Median (min-max)	46.9(8.7-89)	30.1(6.1-33.8)	P<0.001*
S100B (pg/ml) Mean ±SD	1077.94±514.38	320.18±7.67	Z=6.78 P<0.001*
S100B (pg/ml) Median (min-max)	856(46-2355)	320.5 (306-330)	P<0.001*

Z: Mann Whitney U test, *statistically significant

Table 12 demonstrates that the area under curve for serum concentration of HMGB1 in differentiating cases with cognitive dysfunctions from normal cognition was excellent (the area under curve = 0.957); 33.05 µg/L was the most accurately determined cut-off point from the curve, yielding a specificity of 95.5% and a sensitivity of 91% (Fig. 5).

Table (12): Validity of serum level of HMGB1 in differentiating cases with cognitive dysfunctions from normal cognition

Area	Std. Error[a]	P value	95% Confidence Interval		Cut- off point	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
.957	.020	.001*	.918	.995	33.05	91.0	95.5

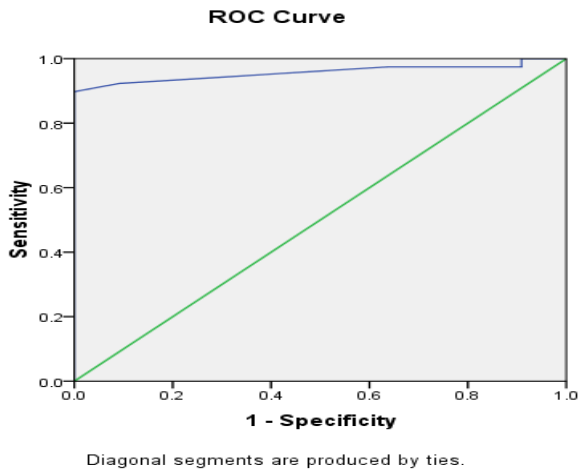

Fig. 5 ROC Curve of serum level of HMGB1 in differentiating cases with cognitive dysfunctions from normal cognition

Table 13 demonstrates that the area under the curve for the serum level of S100B in differentiating cases with cognitive dysfunctions from normal cognition was excellent (area under the curve = 0.974), The cut-off point that was most accurately determined from the curve was 326.5 pg/ml, yielding a specificity of 72.7% and a sensitivity of 97.4% (Fig. 6).

Table (13): Validity of serum level of S100B in differentiating cases with cognitive dysfunctions from normal cognition

Area	Std. Error[a]	p value	95% Confidence Interval		Cut- off point	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
.974	.018	.001*	.939	1.009	326.5	97.4	72.7

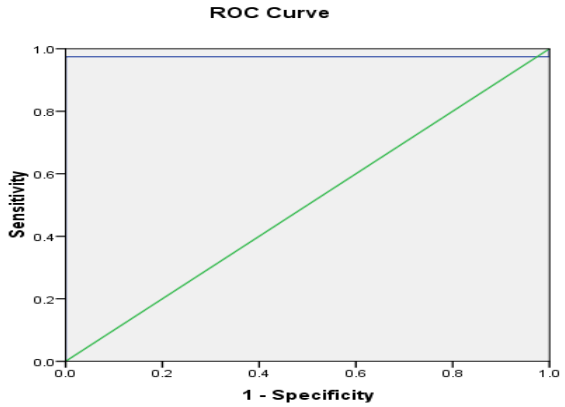

Fig. 6 ROC Curve of serum level of S100B (pg/ml) in differentiating cases with cognitive dysfunctions from normal cognition

Discussion

TLE, which is thought to affect 40% of epileptic patients, is the most common and likely to be the most resistant type of focal epilepsy [19].

While the temporal lobe, specifically the hippocampus, is the site of epileptic focus in the majority of TLE cases, nearly all cases experience focal unawareness seizures with or without generalized seizures [20].

TLE has the potential to cause structural damage and a decrease in cognitive abilities as a progressive condition. Patients with TLE have been shown to have impairments in cognitive function outside of the memory domain, and a connection between structural alterations and cognitive impairments has been suggested [20].

When the CNS is damaged by vascular injury, neurodegenerative lesions, and inflammation, it results in rapid release of HMGB1 that controls the inflammatory response,

which prevents synaptic plasticity. This makes learning, memory loss, and cognitive dysfunction worse by aggravating the pathological destruction to injured tissues [21].

Furthermore, in this context, another marker should be taken into account. S100B is a small protein that is located in the neurvous system. According to previous studies, nanomolar concentrations of S100B promote neurite outgrowth, protect neurons from apoptotic triggers, and increase astrocyte proliferation. However, at micromolar concentrations, it triggers pro-inflammatory cytokines, stress-induced inflammatory enzymes, and neuronal death, all of which have an impact on cognitive processes [22, 23].

This study aimed to determine cognitive impairment in TLE in adults and to investigate if there was an association between serum levels of HMGB1 and S100B expressions with cognitive impairment in TLE trying to detect predictive biomarkers for cognitive impairment in TLE.

The present study reported statistically insignificant difference among examined groups regarding mean age, sex and educational level, which was in line with Bjørke and his colleagues [20] who reported that age and education level within the case and the control groups have been comparable, verifying an effective matching technique.

This study demonstrated that patients with TLE exhibited significantly lower scores than healthy control subjects in all MoCA domains, including visuospatial and executive function, attentive function, language, naming, orientation, delayed recall, in addition to abstract thinking. For people with TLE, these cognitive disorders can have a major impact on their normal functioning and quality of life.

Our findings were consistent with Tani and Adali [24]; Gangemi and his colleagues [25] who found that individuals with temporal lobe epilepsy had a considerably lower average MoCA score than those in the control group.

In our results, the most common domains that affect the MoCA score among the cases group were language and naming, which were in line with Tani and Adali [24] who revealed that individuals with temporal lobe epilepsy performed worse on particular subtests that focused on language, executive function, memory, and attention than the control group.

TLE is known to cause neuronal hyperactivity through abnormal voltage-gated ion channel and transmitter function [26]. Changes in brain morphology and function may result from this abnormal neural activity, which could exacerbate cognitive and neuropsychological diseases [27].

Our results demonstrated that a statistically significant positive correlation has been found between MoCA score and age at onset of seizures ($r = 0.762$) and with educational level ($r = 0.267$), and a statistically significant negative correlation was discovered between MoCA score and disease duration ($r = -0.900$), frequency of attacks ($r = -0.798$), and number of AEDs ($r = -0.868$).

Our findings were supported by Wang and his colleagues [28] who reported that a statistically significant positive correlation has been discovered between MoCA score and educational level (r = 0.499) and a statistically significant negative correlation was discovered between MoCA score and frequency of attacks (r = -0.299) and number of AEDs (r = -0.299), while they reported that there was no significance between MoCA and illness duration and age of onset.

Also, in line with Tani and Adali [24] who reported that a statistically significant positive correlation has been discovered among MoCA score and educational level (r = 0.62) and a statistically significant negative association among MoCA score and disease duration (r = -0.343) and frequency of attacks (r = -0.364), while they reported that there was no significance between MoCA and age of onset.

From that, we demonstrated that the frequency and duration of seizures can lead to cognitive loss in cases with TLE, as exposure to seizure activity can result in cumulative brain damage that affects cognitive processes gradually [29].

The current study found that more frequent seizures had greater negative impacts on cognitive functioning. This is explained by the fact that epileptic seizures result from excessive synchronized firing of brain neurons. Hence, hypoxia within the neuronal membrane and electrical failure lead to permanent neuronal damage, and recurrent seizures lengthen the duration of abnormal electrical discharge in the cortex, thus impacting function of brain and leading to a loss in cognitive function. The loss of cognitive function was demonstrated to be correlated with abnormal gray matter and white matter [30].

The prolonged duration of the condition may cause neuronal death or injury, which could have a compounding impact and cause abnormal cerebral metabolism and morphological structure, ultimately exacerbating the cognitive impairment [28].

Cognitive impairment decreased with increasing educational attainment, and a high level of education emerged as a protective factor for epileptic patients' cognitive performance. People with higher levels of education have greater intellectual reserves and are better able to comprehend and complete various cognitive function tests. Additionally, individuals with higher levels of education tend to be better self-disciplined and more knowledgeable about the illness, which leads them to adhere to the treatment plan more closely. This improves the treatment's effectiveness and therefore lowers cognitive impairment [28].

The cognitive function of cases was significantly impaired as the number of AEDs increased, while those who received monotherapy experienced the least cognitive impairment. Pharmaceutical facts may reasonably interpret such a connection: the majority of AEDs have cognitive negative consequences, including inattention, sleepiness, dizziness, and insomnia which accumulate with the increasing number of AEDs. The combined neurotoxicity might elevate the probability of cognitive decline if the medications are metabolized by the liver, despite the fact that their blood levels are in the normal range. [28].

Cognitive impairment was higher when seizures began at an earlier age. It was caused by the underlying abnormality of the brain and genetics. Additionally, an extended course of illness has been correlated to an earlier age at onset; the susceptibility to cognitive impairment is

exacerbated by the presence of more medications, elevated comorbidity, more medication interactions, and an altered metabolic rate [28].

In our study, we observed that there was a statistically significant lower median MoCA score among drug-resistant than drug-responsive epilepsy ($p<0.001$). Similarly, a statistically significant association was detected between MoCA score and EEG findings, with a higher median MoCA score among normal EEG ($p<0.001$).

Our study is similar to Gavrilovic and his colleagues [31] who reported that there was a significantly lower MoCA score among drug-resistant epilepsy than drug-sensitive epilepsy ($p<0.001$) and also that there was a statistically significant association between cognitive impairment and abnormal EEG findings. Also, drug responsiveness had a significant impact on the presence of cognitive impairment.

The cognitive impairment in cases with TLE may be caused by a variety of factors, as indicated by the previous results. Age at onset of seizures, frequency of seizures, duration of epilepsy, resistance to treatment, and quantity of AEDs in epileptic cases are the critical factors that could potentially impact cognitive status, either individually or in combination.

HMGB1 is one of the well-characterized and is a ubiquitous nuclear protein that is released by glia and in response to neuron inflammation. It induces the activation of TLR4 and RAGE in the target cells. The HMGB1/TLR4 axis that results is a significant cause of neuroinflammation, which consequently leads to increased seizure recurrence and cognitive impairment [32].

S100B produces its neurotoxic impact by causing apoptosis. Micromolar levels of S100B interface with RAGE, resulting in an elevation of reactive oxygen species. Consequently, cytochrome-C is released, causing the caspase cascade and leading to apoptotic neuronal cell death, which results in memory loss and cognitive impairment [17].

The current research demonstrated a statistically significant higher median serum level of HMGB1and S100B among cases than the control group (p-value less than 0.001).

Our findings were in accordance with Kan and his colleagues [33]; Kamaşak and his colleagues [34]; Huang and his colleagues [35] who found that in comparison to healthy controls, epilepsy cases showed elevated HMGB1 expressions.

Our findings are in agreement with Chang and his colleagues [36]; Lu and his colleagues [37] who reported that compared with the controls, the cases with TLE had higher S100B concentration (p-value < 0.01).

In our investigation, we stated a statistically significant negative correlation between MoCA score and serum concentration of HMGB1 ($r = -0.938$) and between MoCA score and serum concentration of S100B ($r = -0.916$).

This came in accordance with Wang and his colleagues [38] who determined a statistically significant negative association among MoCA score and serum level of HMGB1.

This was an explanation for the knowledge that HMGB1 was implicated in memory impairment through the mediation of TLR4 and RAGE. In the hippocampus, HMGB1

demonstrates pro-excitatory impacts through elevating the phosphorylation of NR2B-NMDA receptors, thus elevating the conductance of the receptor calcium channel [13].

The GluR1 subunit of AMPA receptor, which is essential for both synaptic plasticity and memory, is prevented from being expressed on the cell surface by NR2B-containing NMDA receptors. NR2B could additionally contribute to memory by decreasing the span of retrovirus-correlated DNA sequences/extracellular signal-regulated kinases activation pathway in the adult brain. It is remarkable that the same mechanism that modulates the seizure-facilitating impact of HMGB1 may also be responsible for the facilitation of learning deficits [32].

Also, our results, confirmed by Zhang and his colleagues [39] who showed that hydrolysis rates of S100B levels were negatively correlated with the total MoCA score in epileptic patients ($P<0.05$).

Our results were in accordance with Liang and his colleagues [40] who concluded that the most valuable biomarker of epilepsy that aids in both clinical diagnosis and prognosis is serum S100B.

The 'Hyde side' of S100B is expected to contribute to the clarification of the role(s) played by the S100B protein, which activates a RAGE-dependent autocrine loop in astrocytes, resulting in a pro-inflammatory/neurodegenerative phenotype [41]. Therefore, S100B can be correlated with danger/damage-associated molecular pattern (DAMP) molecules, or alarmins. These molecules are released in the endogenous microenvironment to initiate tissue reactions to damage, leading to pro-excitatory impacts in the hippocampus that in turn lead to a loss of memory and impaired synaptic plasticity [42].

In the present study, concerning the validity of serum levels of HMGB1 (µg /L) in differentiating cases from control groups, our results showed that the area under the curve was excellent (area under the curve = 0.960), with the best determined cut-off point from the curve being 26 µg /L, yielding a specificity of 80% and a sensitivity of 90%.

Our findings were consistent with Kan and his colleagues [33] who found HMGB1 prediction values for epilepsy risk. HMGB1 had a good predictive value of epilepsy risk with an area under the curve of 0.905 (95% confidence interval, 0.864–0.946).

In the present study, concerning the validity of serum levels of S100B (pg/ml) in differentiating cases from control groups, our results showed that the area under the curve was excellent (area under the curve = 0.975), with the best determined cut-off point from the curve was 243 pg/ml, yielding a specificity of 86% and a sensitivity of 98%.

Our findings were consistent with Khamis and his colleagues [43] who found predictive value of S100B in the diagnosis and prognosis of epileptic conditions. S100B had a good predictive value of epilepsy risk with an area under the curve of 0.981 yielding a specificity of 93.33 % and sensitivity of 95.56 %.

With respect to the relation between serum levels of HMGB1, S100B, and cognitive dysfunction among studied cases, we demonstrated a statistically significant higher median

serum level of HMGB1, S100B among cases with impaired cognitive functions than normal cognitive functions ($p<0.001$).

This came in accordance with Wang and his colleagues [38] who found among the studied patients, HMGB1 was significantly more likely to occur in the group with cognitive impairment than in the group without ($P < 0.05$).

These findings are in concordance with those of Zhang and his colleagues [39] who reported that patients with cognitive impairment had considerably greater serum S100B levels than those without epilepsy ($P < 0.05$). Additionally, those epileptic patients with and without cognitive impairment had significantly greater serum S100B levels than the control group ($P < 0.05$).

Our study is similar to Chang and his colleagues [36] who stated that pediatric cases who had greater frequency of seizures and poorer cognitive performance showed elevated concentration of S100B.

In our results, regarding validity of serum level of HMGB1 in differentiating cases with cognitive dysfunctions from normal cognition, we reported that area under the curve was excellent (area under the curve =0.957) with the best determined cut-off point from the curve was 33.05 µg /L yielding a specificity of 95.5% and a sensitivity of 91%.

This came in accordance with Wang and his colleagues [38] who found predictive value of HMGB1 for the progress of cognitive impairment in their cases, with the area under the ROC curve was 0.807 (95% confidence interval:0.683- 0.931, $P < 0.001$),with a specificity of 69.6% and a sensitivity of 86.8%.

In our findings, according to the validity of serum level of S100B in differentiating cases with cognitive dysfunctions from normal cognition, the area under the curve for the serum level of S100B in differentiating cases with cognitive dysfunctions from normal cognition was excellent (area under the curve = 0.974), with the best determined cut-off point from the curve was 326.5 pg/ml, yielding a specificity of 72.7% and sensitivity of 97.4%.

We suggest that S100B and HMGB1 proteins could act as promising non-invasive, universal biomarkers for epilepsy, neuroinflammation, and cognitive dysfunction, as they fulfill numerous criteria to qualify as ideal biomarkers. They show relative stability in blood and may be measured quickly and cost-effectively in blood samples.

Conclusion

In comparison to the control group, people with TLE had lower MoCA scores in a number of cognitive domains, primarily language and naming. HMGB1 and S100B serum levels are thought to be promising predictive indicators for identifying cognitive impairment in TLE. Future research including a large number of patients with long-term follow-up is required for a more precise assessment.

List of abbreviations

- TLE: Temporal Lobe Epilepsy

- HMGB1: High-mobility group box-1
- MoCA: Montreal Cognitive Assessment
- AEDs: Anti-epileptic drugs
- MRI: Magnetic resonance imaging
- EEG: Electroencephalogram
- SPSS: Statistical Package for Social Science
- ROC: Receiver operating characteristic
- SD: Standard deviation
- RAGE: Receptor for advanced glycation end-products
- TLR4: Toll-like receptor 4
- DAMP: Damage-associated molecular patterns
- CNS: Central nervous system

Declarations

Ethics approval and consent to participate

Institutional review board (IRB) approval was obtained prior to the study (MD.22.02.606); and written informed consent was taken from all participants enrolled in the study before taking blood samples and answering any questions.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors read and approved the final manuscript.

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