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Original Research

Correlation of MMP-9 and HMGB1 expression with the cognitive function in patients with epilepsy and factors affecting the prognosis

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Abstract: This study was designed to investigate the expressions and roles of MMP-9 and HMGB1 in peripheral blood of patients with epilepsy and their relationship with the cognitive function and to explore factors affecting the prognosis of epilepsy patients. A total of 127 patients with epilepsy were collected in the study group and 120 healthy subjects receiving a physical examination at the same time were collected in the control group. The MMP-9 and HMGB1 expressions and their diagnostic value for epilepsy were compared between the two groups. The relationship between MMP-9 and HMGB1 expression levels and the clinical-pathological features and the Mini-mental State Evaluation Scale (MMSE) of patients from the study group were also analyzed. The serum levels of MMP-9 and HMGB1 in the study group were significantly higher than those in the control group (P< 0.001), and were greatly decreased after the treatment (P<0.001). The ROC curve showed that MMP-9 and HMGB1 combined detection had a good diagnostic efficiency for epilepsy. MMP-9 was much related to the type and disease duration of epilepsy (P< 0.05). HMGB1 was significantly associated with disease duration, seizure, and previous treatment history of epilepsy (P< 0.050). According to the Pearson correlation coefficient analysis, the expressions of MMP-9 and HMGB1 were negatively correlated with MMSE scores of the study group (P< 0.001). Logistic regression analysis showed that the duration of disease, seizures, MMP-9, and HMGB1 were independent risk factors for the prognosis of epilepsy. The expression levels of MMP-9 and HMGB1 in peripheral blood of patients with epilepsy are significantly increased, and negatively correlated with neurological function scores. They have potential involvement in the occurrence and development of epilepsy, which makes them significant for the diagnosis and treatment of epilepsy in the future.

Key words: MMP-9; HMGB1; MMS; Epilepsy.

Introduction

Epilepsy is a transient brain dysfunction caused by sudden abnormal discharge of brain neurons (1). Sudden and repetitive, an epileptic seizure usually terminates automatically after a period of time (2). Data show that the prevalence of active epilepsy is about 6.38 per 1,000 people, and the lifetime prevalence rate is about 7.60 per 1,000 people. The annual cumulative incidence of epilepsy is 67.77/100,000, and the incidence rate is 61.44/100,000 (3). The pathogenesis of epilepsy is complex, which may be caused by genetic factors, brain diseases, and systemic diseases (4). Epilepsy attacks varying age groups, from the newborns to the middle-aged and elderly people (5). Generally manifesting as wart, convulsions, screaming, bruising, urinary incontinence, tongue bite, foaming or foaming of the mouth, and dilated pupils, epileptic seizure causes no fatal harm to the human body but can deprive patients of their self-awareness and body control, leading to terrible consequences (6). At present, epilepsy is mainly treated by drug therapy, which takes a long treatment period. About 50% to 60% of patients are reported to be completely cured after 2 to 5 years of regular treatment (7).

Scholars at home and abroad are striving to find out a new treatment for epilepsy that can effectively improve the clinical cure rate and shorten the treatment cycle (8,9). With the advancement in epilepsy study, the important role of matrix metalloproteinases (MMPs) in epilepsy has been observed. MMPs are closely related to many physiological and pathological processes in the human body, including inflammatory response, tumor growth and neurological diseases (10). Matrix metalloproteinase-9 (MMP-9) is extremely important in the MMPs family and has strong biological activity. It has been discovered to be closely related to post-ischemic nerve injury (11), but its role in epilepsy is not clear. High mobility group protein B1 (HMGB1) is a non-histone chromosomal binding protein widely distributed in various organs and tissues of the human body. HMGB1 has been proved by many studies to be involved with intracranial infection, brain injury, and reperfusion injury, and to be of high regulation for neuroinflammation diseases (12, 13).

In the clinic, the pathogenesis of epilepsy remains unverified and controversy worldwide. We hypothesize that MMP-9 and HMGB1 may be associated with the seizure and progression of epilepsy, so we conducted this study to explore the role of MMP-9 and HMGB1 in epilepsy and their relationship with the cognitive function of patients. This study also analyzed the risk factors affecting the prognosis of patients, aiming to provide effective reference and guidance for future clinical diagnosis and treatment of epilepsy.

Materials and Methods

General Information

A prospective analysis was performed on 127 patients with epilepsy admitted to our hospital and 120 healthy subjects receiving a physical examination at the same time. Patients with epilepsy were included in the study group, including 72 males and 55 females, aged from 34 to 71 years, with an average age of (60.4±7.6) years. Healthy subjects were included in the control group, including 69 males and 51 females, aged from 35 to 70 years, with an average age of (59.8±8.0) years. This experiment has been approved by the ethics committee of our hospital and obtained informed consent from all subjects.

Inclusion and exclusion criteria

The inclusion criteria for the study group were as follows: Patients meeting the diagnostic criteria for epilepsy (14); patients aged from 18 to 79 years; patients with complete medical record and good cooperation with the medical staff; patients who themselves or whose immediate family member signed the informed consent form. The exclusion criteria for the study group were as follows: patients with tumors, other neurological disorders such as Alzheimer's disease and Parkinson's syndrome; patients with cerebral stroke and abnormal cognitive function, organ failure, organ dysfunction, infectious diseases, or vascular disease. The inclusion criteria for the control group were as follows: People with normal physical examination results and good cooperation with the medical staff; people with no previous neurological and psychiatric diseases; people who themselves or whose immediate family member signed the informed consent form.

Methods

After admission and before the treatment, 4 ml of fasting venous blood was taken from all subjects in the two groups and placed at room temperature for 30 minutes. Centrifuged for 10 minutes at 4000 rpm/min, the supernatant liquid was collected to measure the serum levels of MMP-9 and HMGB1 using enzyme-linked immunosorbent assay (ELISA). The MMP-9 kit was purchased from Tecan (Shanghai) Trading Co., Ltd. (BE59491). The HMGB1 kit was purchased from Shanghai Jingkang Bioengineering Co., Ltd. (JK-(a)-5185). All operations were in strict accordance with the kit instructions in a sterile environment. Patients in the study group were treated according to the clinical guidelines for epilepsy (15) after admission. For grand mal seizures, phenobarbital was prescribed at 90-300 mg/d, sodium valproate at 0.6-1.2 mg/d, and carbamazepine at 600-1200 mg/d. For complex partial seizures, phenytoin was prescribed at 0.2-0.6 mg/d, carbamazepine at 0.2-1.2 mg/d. For absence seizures, clonazepam was prescribed at 5-25 mg/d, diazepam at 7.5-40 mg/d. For status epilepticus, an intravenous injection of diazepam at 10-20 mg each time is preferred. The treatment outcome was evaluated according to the epilepsy rehabilitation guide (16). Complete recovery without seizures was defined as a controlled disease, a reduction of 75 to 99% in seizure frequency as a marked response, a reduction of 50 to 74% as a moderate response, a reduction of less than 50% as no response, an increase of at least 25% as an aggravated disease.

Outcome measures

The serum levels of MMP-9 and HMGB1 in the two groups were measured. The binary logistic analysis of MMP-9 and HMGB1 was performed using SPSS to calculate the combining predictor of the two proteins, and then the ROC curve analysis was conducted to calculate the diagnostic value of the combined detection of two proteins for epilepsy. The relationship between MMP-9 and HMGB1 expression levels and the clinical-pathological features was explored. The cognitive function of patients in the study group was assessed according to the Mini-mental State Evaluation Scale (MMSE) (17). The total score was 30 points, and higher scores indicated better cognitive function. The correlation between MMP-9 and HMGB1 with the cognitive function of patients was analyzed. The serum levels of MMP-9 and HMGB1 in the study group before and after treatment were monitored. Risk factors affecting the prognosis of patients in the study group were analyzed.

Statistical analysis

Statistical calculations were conducted by SPSS24.0 (Beijing Strong Vinda Information Technology Co., Ltd.). All the data were visualized using Graphpad8 (SOFTHEAD Inc.). The results were checked twice. Counting data such as patient gender, smoking habits were expressed in terms of (rate) and compared between the two groups by the chi-square test. Measurement data such as MMP-9 and HMGB1 levels were expressed in the form of (mean±standard deviation) and compared between two groups by the independent t-test. The comparison between multiple groups was performed by one-way ANOVA and LSD post-hoc test. The diagnostic value was analyzed by the ROC curve. The binary logistic regression analysis was performed on the ROC curve of the combined detection to obtain the constants and various coefficients of the regression equation. Logit (P) = - 2.167 + 0.126 Marker1 + 0.088 Marker2. After removing the constant term, the right term of the equation was divided by the coefficient of Marker1 to obtain the combining predictor. Combining predictor = Marker1 + Marker 2 × 0.088/0.126. The combining predictor was calculated using the Transform and Compute Variable functions in SPSS, and the ROC curve analysis was performed. The correlation analysis used the Pearson correlation coefficient. The risk factors were analyzed by logistic regression. A statistical difference was recognized when $P< 0.050$.

Results

Comparison of general information

No significant differences were detected between the two groups in age, BMI, duration of disease, gender, living environment, ethnicity, education level, marital status, smoking, and drinking ($P> 0.050$). The number of subjects with a family history in the study group was notably more than that in the control group ($P< 0.001$). More details are shown in Table 1.

Table 1. Comparison of general information.

	Study group (n=127)	Control group (n=120)	t or $\chi 2$	P
Age			0.605	0.546
	60.4±7.6	59.8±8.0		
BMI (KG/CM2)			0.457	0.648
	22.89±2.69	23.05±2.81		
During of disease (year)			0.572	0.568
	3.72±1.08	3.80±1.12		
Gender			0.016	0.898
Male	72 (56.69)	69 (57.50)		
Female	55 (43.31)	51 (42.50)		
Living environment			0.670	0.413
Urban area	68 (53.54)	58 (48.33)		
Rural area	59 (46.46)	62 (51.67)		
Ethnicity			1441	0.230
Han nationality	120 (94.49)	117 (97.50)		
Minority nationality	7 (5.51)	3 (2.50)		
Education level			1.266	0.261
< high school	89 (70.08)	76 (63.33)		
≥ high school	38 (29.92)	44 (36.67)		
Marital status			1.467	0.226
Married	59 (46.46)	65 (54.17)		
Unmarried	68 (53.54)	55 (45.83)		
Smoking			0.023	0.880
Yes	75 (59.06)	72 (60.00)		
No	52 (40.94)	48 (40.00)		
Drinking			0.758	0.384
Yes	48 (37.80)	39 (32.50)		
No	79 (62.20)	81 (67.50)		
Family history			28.783	< 0.001
Yes	42 (33.07)	7 (5.83)		
No	85 (66.93)	113 (94.17)		
Epilepsy type				
Primary	19 (14.96)			
Secondary	108 (85.04)			
Seizure activity				
Grand mal seizure	42 (33.07)			
Petit mal seizure	16 (12.60)			
Psychomotor seizure	36 (28.35)			
Focal seizure	15 (11.81)			
Complex partial seizure	18 (14.17)			
Previous treatment history				
Yes	49 (38.58)			
No	78 (64.42)			

Comparison of MMP-9 and HMGB1 levels between the two groups

Before the treatment, the MMP-9 level in the study group was 1.42±0.39 pg/ml, significantly higher than that of the control group (1.04±0.26 pg/ml) (P< 0.00). Before the treatment, the HMGB1 level in the study group was 5.01±1.37 pg/ml, significantly higher than that of the control group (3.24±0.82 pg/ml) (P< 0.001) (Figure 1)

Diagnostic value of MMP-9 and HMGB1 for epilepsy

Seen from the ROC curve, the sensitivity and specificity of MMP-9 in the diagnosis of epilepsy were 55.12 and 84.25% when the cut-off value was 1.307pg/ml. The sensitivity and specificity of HMGB1 in the diagno-

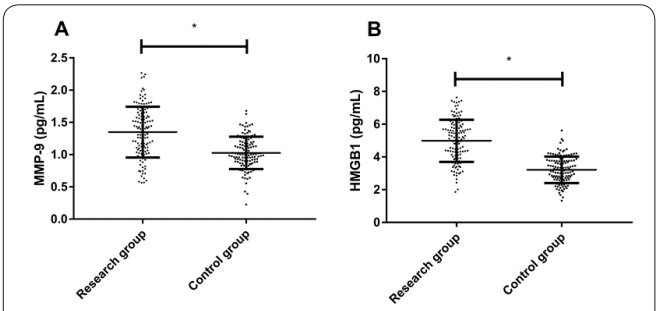

Figure 1. Comparison of MMP-9 and HMGB1 levels between the two groups. **(A)** Comparison of serum MMP-9 levels between the two groups. * indicates P< 0.001 when compared with serum MMP-9 level in the study group. **(B)** Comparison of serum HMGB1 levels between the two groups. * indicates P< 0.001 when compared with serum HMGB1 level in the study group.

Table 2. Diagnostic value of MMP-9 and HMGB1 for epilepsy.

	MMP-9 (pg/ml)	HMGB1 (ng/ml)	Combined diagnosis
Cut-off	1.307	4.260	0.531
AUC	0.732	0.870	0.903
Std.Error	0.032	0.022	0.020
95%CI	0.670~0.795	0.826~0.914	0.865~0.942
P	< 0.001	< 0.001	< 0.001
Sensitivity (%)	55.12	71.65*	79.17*#
Specificity (%)	84.25	92.50*	89.17*

Note: * indicates that P< 0.050 when compared with the sensitivity and specificity of MMP-9 single diagnosis. # indicates that P< 0.050 when compared with the sensitivity and specificity of HMGB1 single diagnosis.

Table 3. Relationship between MMP-9 and HMGB1 expression levels and the clinical-pathological features.

	N	MMP-9 (pg/ml)	t or F/P	HMGB1 (ng/ml)	t or F/P
Age			1.817/0.072		0.597/0.551
< 60	84	1.32±0.30		5.31±1.14	
≥60	43	1.42±0.28		5.45±1.20	
BMI (KG/CM2)			1.016/0.311		0.264/0.792
< 22	35	1.44±0.26		5.13±1.42	
≥22	92	1.38±0.31		5.20±1.30	
During of disease (year)			15.172/0.001		2.675/0.009
< 3	64	1.20±0.40		5.08±1.27	
≥3	63	2.11±0.26		5.69±1.30	
Gender			0.708/0.376		0.081/0.936
Male	72	1.35±0.26		5.40±1.30	
Female	55	1.37±0.34		5.42±1.49	
Family history			0.857/0.181		0.154/0.878
Yes	42	1.43±0.28		5.26±1.32	
No	85	1.42±0.30		5.30±1.40	
Epilepsy type			4.011/0.001		0.099/0.921
Primary	19	1.17±0.92		5.42±1.17	
Secondary	108	1.84±0.62		5.39±1.22	
Seizure activity			0.105/0.981		2.878/0.026
Grand mal seizure	42	1.34±0.25		5.92±1.20	
Petit mal seizure	16	1.30±0.30		4.76±1.39	
Psychomotor seizure	36	1.32±0.24		5.88±1.37	
Focal seizure	15	1.35±0.30		5.22±1.22	
Complex partial seizure	18	1.32±0.28		5.60±1.57	
Previous treatment history			0.909/0.365		3.512/0.006
Yes	49	1.54±0.32		5.07±1.20	
No	78	1.49±0.29		5.97±1.52	

sis of epilepsy was 71.65 and 92.50% when the cut-off value was 4.260 pg/ml. The sensitivity and specificity of MMP-9 and HMGB1 combined detection in the diagnosis of epilepsy was 79.17 and 89.17% when the cut-off value was 0.531pg/ml. More details are shown in Table 2 (Figure 2).

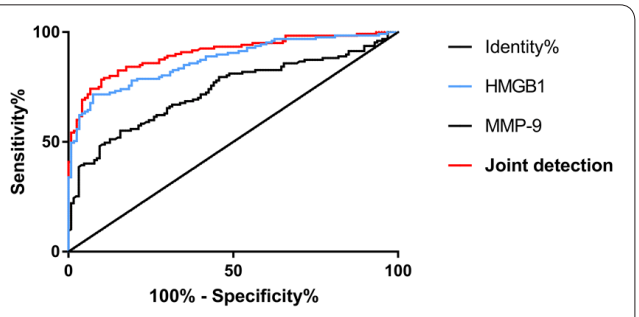

Figure 2. Diagnostic value of MMP-9 and HMGB1 for epilepsy according to the ROC curve.

Relationship between MMP-9 and HMGB1 expression levels and the clinical-pathological features

MMP-9 level in the study group was not significantly related to age, BMI, gender, family history, seizure status, and previous treatment history (P> 0.050), but greatly related to the type and duration of epilepsy (P< 0.050). HMGB1 level in the study group was not significantly related to age, BMI, gender, family history, and seizure type (P> 0.050), but greatly related to the duration of epilepsy, seizure status, and previous treatment history (P< 0.050). More details are shown in Table 3.

Table 6. Different factor and assignment in current research.

	MMP-9	HMGB1
r	-0.809	-0.732
95%CI	-0.862~-0.739	-0.804~-0.639
R squared	0.655	0.536
P	< 0.001	< 0.001

Correlation of MMP-9 and HMGB1 levels with the MMSE score

According to the Pearson correlation coefficient analysis, the MMP-9 was negatively correlated with the MMSE score, with MMSE scores at 10.24±3.44 points in the study group (r=-0.809, P< 0.001). The HMGB1 was also negatively correlated with the MMSE scores in the study group (r=-0.732, P< 0.001). More details are shown in Table 4 and Figure 3.

Serum levels of MMP-9 and HMGB1 before and after treatment

The MMP-9 and HMGB1 levels after the treatment were in the study group were 1.18±0.30pg/ml and 3.86±0.53 ng/ml, respectively, significantly lower than those before treatment (P< 0.001). (Figure 4)

Treatment outcomes in the study grou

The treatment outcome was controlled disease in 27 patients, marked response in 39, the moderate response in 22, no response in 35, and aggravated disease in 4. Patients in the study group were divided into the excellent prognosis subgroup (88 patients whose outcomes were controlled disease, marked response, and moderate response) and the poor prognosis subgroup (39 patients whose outcomes were no response and aggravated disease) by the treatment outcome. According to the univariate analysis for the two subgroups of the study group, factors affecting the prognosis of epilepsy included age, duration of disease, family history, seizure activity, and serum levels of MMP-9 and HMGB1 (P0.050). More details are shown in Table 5.

Multivariate analysis for factors affecting epilepsy prognosis

The univariate analysis indicators (age, duration of disease, family history, seizure activity, serum levels of MMP-9 and HMGB1) were assigned as shown in Table 6. Then SPSS was employed to perform multivariate regression analysis with Forward: LR method. The results revealed that the independent risk factors for the prognosis of epilepsy included the duration of disease (OR: 1.096, 95% CI: 1.024 to 1.274), seizure activity (OR: 6.842, 95% CI: 2.622 to 27.652), MMP-9 level (OR: 3.511, 95% CI: 1.678 to 7.622), and HMGB1 level (OR: 3.027, 95% CI: 1.241 to 5.627) (Table 7).

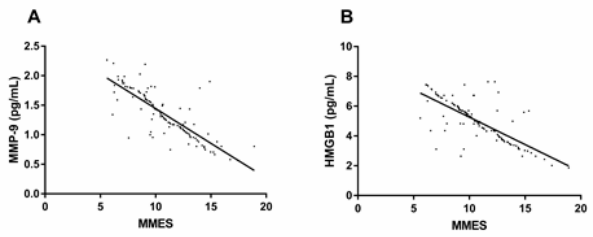

Figure 3. Correlation of serum MMP-9 and HMGB1 levels with the MMSE score before treatment in the study group. **(A)** Correlation of serum MMP-9 level with the MMSE score before treatment in the study group. According to the Pearson correlation coefficient analysis, the expression of MMP-9 was negatively correlated with MMSE scores of the study group (r=-0.809, P< 0.001). **(B)** Correlation of serum HMGB1 level with the MMSE score before treatment in the study group. According to the Pearson correlation coefficient analysis, the expression of HMGB1 was negatively correlated with an MMSE score of the study group (r=-0.732, P<0.001).

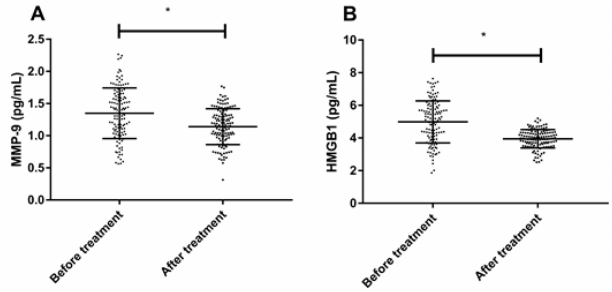

Figure 4. Serum levels of MMP-9 and HMGB1 in the study group before and after treatment. **(A)** Serum MMP-9 levels before and after treatment. * indicates that P< 0.001 when compared with MMP-9 level before the treatment. **(B)** Serum HMGB1 levels before and after treatment. * indicates that P< 0.001 when compared with HMGB1 level before the treatment.

Discussion

Epilepsy is a common neurological dysfunction disease in the clinic, with very high morbidity all over the world (18). Clinically, epilepsy is mainly divided into primary (functional) epilepsy and secondary (symptomatic) epilepsy according to the etiology or is divided into grand mal seizures, petit mal seizures, psychomotor seizures, focal seizures, complex partial seizures according to the seizure activity (19). The sudden seizure of epilepsy, if not rescued timely, may not only affect the body's memory, cognition, and motor function due to the organic changes caused by the abnormal discharge in the synaptic link of the nerve cells in the brain but may also confront patients' life with the threat from external disturbances (20). Therefore, the search for new methods for the diagnosis and treatment of epilepsy is crucial. This study analyzed the roles of MMP-9 and HMGB1 in epilepsy, which is helpful for future clinical screening of epilepsy and for sparking new ideas for potential therapeutic targets for epilepsy.

In this study, MMP-9 and HMGB1 were significantly increased in patients with epilepsy, suggesting that MMP-9 and HMGB1 may be involved in the occurrence and development of epilepsy. Such results are consistent with those of studies by Bronisz et al. (21) and Yam et al. (22), which can hence support the results of this

Table 5. Univariate analysis for factors affecting epilepsy prognosis.

	Excellent prognosis subgroup (n=88)	Poor prognosis subgroup (n=39)	c2	P
Age			64.752	< 0.001
< 60	78 (88.64)	6 (15.38)		
≥60	10 (11.36)	33 (84.62)		
BMI (KG/CM2)			0.566	0.452
< 22	26 (29.55)	9 (23.08)		
≥22	62 (70.45)	30 (76.92)		
During of disease (year)			31.792	< 0.001
< 3	59 (67.05)	5 (12.82)		
≥3	29 (32.95)	34 (87.18)		
Living environment			0.002	0.964
Urban area	47 (53.41)	21 (53.85)		
Rural area	41 (46.59)	18 (46.15)		
Ethnicity			0.514	0.474
Han nationality	84 (95.45)	36 (92.31)		
Minority nationality	4 (4.55)	3 (7.69)		
Education level			0.492	0.483
< high school	60 (68.18)	29 (74.36)		
≥ high school	28 (31.82)	10 (25.64)		
Marital status			0.186	0.666
Married	42 (47.73)	17 (43.59)		
Unmarried	46 (52.27)	22 (56.41)		
Smoking			0.001	0.990
Yes	52 (59.09)	23 (58.97)		
No	36 (40.91)	16 (41.03)		
Drinking			0.250	0.617
Yes	32 (36.36)	16 (41.03)		
No	56 (63.64)	23 (58.97)		
Family history			48.912	<0.001
Yes	12 (13.64)	30 (76.92)		
No	76 (86.36)	9 (23.08)		
Epilepsy type			0.008	0.929
Primary	13 (14.77)	6 (15.38)		
Secondary	75 (85.23)	33 (84.62)		
Seizure activity			13.012	0.011
Grand mal seizure	26 (29.55)	16 (41.03)		
Petit mal seizure	14 (15.91)	2 (5.13)		
Psychomotor seizure	30 (34.09)	6 (15.38)		
Focal seizure	6 (6.82)	9 (23.08)		
Complex partial seizure	12 (13.64)	6 (15.38)		
Previous treatment history			0.171	0.679
Yes	35 (39.77)	14 (35.90)		
No	53 (60.23)	25 (64.10)		
MMP-9 (pg/ml)			59.513	< 0.001
< 1.18	76 (86.36)	6 (15.38)		
≥1.18	12 (13.64)	33 (84.62)		
HMGB1 (ng/ml)			78.492	< 0.001
< 3.86	80 (90.91)	4 (10.26)		
≥3.86	8 (9.09)	35 (89.74)		

Table 6. Different factor and assignment in current research.

Factor	Assignment
Age	<60=0, ≥60=1
Duration of disease	<3=0, ≥3=1
Family history	No=0, Yes=1
Seizure activity	Grand mal seizure=0, petit mal seizure=1, psychomotor seizure=2, focal seizure=3, complex partial seizure=4
MMM--9 (pg/ml)	<1.18=0, ≥1.18=1
HMGB1 (ng/ml)	<3.86=0, ≥3.86=1

Table 7. Multivariate regression analysis between studied variable in this research.

	B	S.E.	Wald	P	OR	95%CI
Duration of disease	0.087	0.036	5.047	0.019	1.096	1.024~1.274
Seizure activity	1.862	0.842	5.367	0.027	6.842	2.622~27.652
MMM--9	1.267	0.441	8.622	0.007	3.511	1.678~7.622
HMGB1	1.124	0.632	3.892	0.043	3.027	1.241~5.627

study. MMP-9, with the maximum relative molecular mass in the MMPs family, is mainly from neutrophils, neutropenia macrophages, and T/B lymphocytes and mainly acts on gelatin, type IV and V collagen (23). Our speculation is that the significant increase in MMP-9 level in this study is promoted by the role of MMP-9 as an inflammatory factor in the nervous system. Studies have shown that MMP-9, through the degradation of the extracellular matrix and the up-regulation of tumor necrosis factor-α (TNF-α) as a pro-inflammatory protein, can destroy the normal structure of the human blood-brain barrier and hence lead to inflammation of the nervous system (24). De Vries et al (25) found in their study that TNF-α level increases significantly in patients with epilepsy. Therefore, we hypothesize that MMP-9 relies on TNF-α to affect epilepsy. But the TNF-α level was not detected in this study, so this hypothesis needs to be verified by further study. HMGB1, as a member of the high mobility group protein, enjoys different functions due to its various forms in the body.

For example, intracellular HMGB1 mainly acts on gene transcription, while extracellular HMGB1 acts as an inflammatory factor (26). So far, HMGB1 has been frequently mentioned in studies on neurological diseases. Many researchers at home and abroad believe that HMGB1 regulates the inflammation of nervous system mainly through the receptor for advanced glycation end products, Toll-like receptor 2, and Toll-like receptor 4 (TLR4) (27). We speculate that its regulation on epilepsy may be related to TLR4. MyD88 is a very important transcriptional protein in the TLR4 signaling pathway. In the study of Wen et al (28), the TLR4/MyD88 pathway in the hippocampus of rats with epilepsy was significantly up-regulated, inducing neuronal apoptosis and autophagy. Therefore, a conjecture is drawn that HMGB1 may affect the proliferation of downstream pro-inflammatory factors through its influence on TLR4 to up-regulate MyD88, resulting in the occurrence and onset of epilepsy. HMGB1 is proved to be closely related to the seizure activity and duration of disease, which suggests that the HMGB1/TLR4 signaling pathway may be a potential therapeutic target for epilepsy.

Correlation analysis shows that MMP-9 and HMGB1 were negatively correlated with the cognitive function scores, indicating the close relationship between MMP-9 and HMGB1 level and neurological disorders in patients with epilepsy. According to the ROC analysis, the combined detection of MMP-9 and HMGB1 has a good diagnostic value for epilepsy. Patients in this study are mostly with secondary epilepsy, the causes of which have been studied in detail for many times (29). However, the true etiology of primary epilepsy is not clear. It is possible that MMP-9 and HMGB1 detection may not be effective in the diagnosis of primary epilepsy, which requires further verification. Multivariate regression analysis showed that the duration of disease, seizures activity, MMP-9, and HMGB1 are independent risk factors for the prognosis of epilepsy, suggesting that close attention should be paid to the treatment progress of epilepsy patients with long duration of disease and severe seizure activity (30-44).

This study aims to investigate the role of MMP-9 and HMGB1 in epilepsy, but it is defective due to limited experimental conditions. The lack of basic experimental support makes the mechanism of MMP-9 and HMGB1 in epilepsy remain an unverified conjecture. The limited case number and epilepsy type are shortcomings, too. We brought up the idea that the HMGB1/TLR4 signaling pathway may be a potential therapeutic target for epilepsy but we failed to verify the drug resistance of HMGB1 in epilepsy, leaving it a new study direction for domestic and foreign scholars. This study will be improved and more subjects will be included to get more accurate results.

In summary, the expression levels of MMP-9 and HMGB1 in peripheral blood of patients with epilepsy are significantly increased, and negatively correlated with neurological function scores. They have potential involvement in the occurrence and development of epilepsy, which makes them significant for the diagnosis and treatment of epilepsy in the future.

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Availability of data and materials
The datasets used and/or analyzed during the present

study are available from the corresponding author on reasonable request.

Authors' contributions

QH and JL conceived and designed the study. QH, JL, ZS and XZ were responsible for the collection, analysis and interpretation of the data. ZS drafted the manuscript. JL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jiaxiang People's Hospital. Signed written informed consent was obtained from the patients and/or guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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