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ORIGINAL ARTICLE

Diagnostic Value of Matrix Metalloproteinase-2 in Children with Refractory Epilepsy

Mohamed Ali Abdou Mohamed[1], Doaa M. Abdelmonem[2], Asmaa Farag Mohammed Elghareeb[1] *, Mohammed Ragab Abdellatif[1]
[1] Pediatrics Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[2] Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

***Corresponding author:**
Asmaa Farag Mohammed Elghareeb

E-mail address:
fasmaa997@gmail.com

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ABSTRACT

Background: Epilepsy is a common neurological condition that causes disruptions in the brain's electrical activity for a number of reasons. Resistant epilepsy is an uncontrolled seizures in spite of two well-tolerated, suitably chosen antiepileptic medications (AEDs) given either in combination or as monotherapies. This is the first investigation at Zagazig University Hospitals to find out if refractory epilepsy and matrix metalloproteinase-2 (MMP-2) are related. The purpose of this study is to evaluate the diagnostic utility of Matrix Metalloproteinase-2 in children receiving treatment at Zagazig University Hospitals for refractory epilepsy.

Methods: Forty three children with refractory epilepsy and Forty three healthy children were examined for matrix metalloproteinase-2 by Enzyme Linked Immunosorbent Assay (ELISA).

Results: The mean MMP-2 concentration exhibited a substantial decrease in the patient group compared to the control group, with a highly significant p-value of <0.001.

Conclusions: This study supports that MMP-2 could play a crucial role in timely and precise clinical interventions for children with refractory epilepsy.

Keywords: Matrix Metalloproteinase-2; Children; Refractory Epilepsy.

INTRODUCTION

Epilepsy is a common neurological condition, characterized by aberrant electrical activity in the brain. It includes a range of seizure types, each with unique symptoms, etiology, outcomes, and therapeutic approaches [1].

The principal treatment for epilepsy is antiepileptic medications (AEDs); nevertheless, some patients have refractory epilepsy, in which the use of well-tolerated and carefully chosen AEDs does not control seizures [2]. Throughout the course of the condition, drug regimen adjustments are frequently necessary for patients with refractory epilepsy. It has a significant effect on patients, their families, and at all society. It can result in further brain damage as well as long-term neurobehavioral and neuropsychiatric issues.

Comprehending the fundamental processes of epileptogenesis is essential for formulating efficacious therapeutic approaches [1]. The pathophysiology of epilepsy has been linked to inflammatory molecules and humoral mediators, such as cytokines and matrix metalloproteinases (MMPs) [2]. Among these, MMPs are important for remodeling the extracellular matrix in both healthy and diseased states. They have a role in a number of processes, including the development of epileptogenesis, the progression of epilepsy, brain remodeling following seizures, cell death brought on by seizures, disruption of the blood-brain barrier (BBB), neuroinflammation, and abnormal synaptic plasticity [3].

The two most prevalent MMPs expressed in the brain are MMP-2 and MMP-9. MMP-2 has been linked to neurodevelopmental processes, and it has been suggested that downregulating it will prevent inflammatory cells from migrating into the central nervous system and disrupting the blood-brain barrier [4]. According to recent research, seizures cause the BBB's MMPs to increase, which deteriorates tight junctions and allows barrier leakage. It is imperative to comprehend the exact

function of MMP-2 in the etiology of epilepsy, as it could potentially function as a biomarker for epilepsy in human serum [3].

METHODS

This case control study was performed at the pediatrics neurology unit, pediatric outpatient clinic and clinical pathology department at Zagazig University Hospitals. Children attending Zagazig University Hospitals was classified into: Patient group: Children aged between 2 and 16 years with refractory epilepsy, meeting ILAE task force criteria for drug-resistant epilepsy and control group: Healthy children age and sex-matched with the patient group.

Inclusion criteria included children aged 2 to 16 years with refractory epilepsy, both sexes and healthy children aged 2 to 16 years. Exclusion criteria included children younger than 1 year old or older than 16 years old, children with other neurological diseases and refusal of parents of children participation in the study.

All subjects underwent comprehensive history taking, a thorough clinical examination, and routine laboratory investigations were carried out. These included a complete blood count on an automated cell counter (XN330 Sysmex, Japan) with a differential count on peripheral Leishman stained slides, electrolytes (Na, K, Ca, and CL) on a Sensa core ST200 plus, liver function tests, serum urea nitrogen, and creatinine on a Cobas8000, and enzyme-linked immunosorbent detection of MMP-2 level in plasma.

Specimen collection and storage:

Each patient had a vein puncture to obtain three milliliters of venous blood, which were then placed in a sterile, spotless separator tube for serum isolation and allowed to clot. To extract the serum, centrifugation was performed for 20 minutes at a speed of 2000–3000 rpm. After that, the serum was transferred to an eppendorf tube and kept cold (-4o C) until the day of the study. Samples that show signs of precipitation are centrifuged once more.

Measurement of MMP-2:

ELISA was used to quantify MMP-2 in serum samples. Matrix metalloproteinases (MMP-2) ELISA Kits (Catalogue No 201-12-0905) were supplied by the Chinese company BioKets. The kit measures the amount of MMP-2 in samples using an Enzyme-Linked ImmunoSorbent Assay (ELISA) that is a double-antibody sandwich method. After pre-coating the enzyme well with MMP-2 monoclonal antibody and incubating it, MMP-2 antibodies are labeled with biotin and combined with streptavidin-HRP to form an immune complex. This is followed by further incubation and washing to remove any remaining uncombined enzyme. After adding Chromogen Solution A and B, the liquid's hue first turns blue and then, eventually, turns yellow due to the acid's effects. There was a positive correlation between the sample's Human Substance MMP-2 content and color chroma.

Sample Size:

Assuming a mean ± SD of MMP-2 in epileptic patients vs. controls (14.6 ± 9 vs. 6 ± 18), the sample size was calculated as 86 patients (43 in each group) with 80% power and 95% confidence using OpenEpi.

Ethical approval

The Institutional Review Board (IRB) of Zagazig University's Faculty of Medicine authorized the submission of the study protocol (IRB#:9589-26-6-2022). Parents provided written, informed consent. This study followed the guidelines [the World Medical Association's Code of Ethics (Declaration of Helsinki)] for human studies.

Statistical Analysis

All data were gathered, tabulated, and statistically evaluated using Microsoft Office Excel 2010 for Windows (Microsoft Corp., Redmond, WA, USA), SPSS 22.0 for Windows (IBM Inc., Chicago, IL, USA), and MedCalc 13 for Windows (MedCalc Software bvba, Ostend, Belgium). Mann-Whitney U test, Kruskal Wallis H test, Fisher's exact test, Chi-square test, correlation coefficient (r) and Receiver Operating Characteristic (ROC) curve analysis were performed.

RESULTS

There were no statistically significant differences in gender distribution, age, weight, and weight percentile between the patient and control groups (Table 1). Consanguinity was reported in 60.5% in the patient group family history, and 60.5% had no family history of epilepsy. 44.2% of the cases had febrile convulsion, while 11.6% of our cases had positive family history of febrile convulsion. The GTC epilepsy was the commonest type of fits among the case group (60.5%). The average duration of epilepsy was 9.16±5.92 years and (62.8%) had no loss of consciousness. The daily frequency was the highest (41.9%), 25.6% had once status epilepticus, and stress was the commonest risk factor (18.6%), 42.9% of cases had three AEDs as treatment (the most common) (Table 2).

34.9% of cases had normal MRI results, while 65.1% showed abnormalities. Generalized epileptic activity was the commonest EEG finding (27.9%) followed by epileptogenic focus (18.6%) and post-ischemic changes (16.3%). Decrease in cerebral brain fluid (CBF) which is represented by ischemia lead the neuron to lose their membrane gradients so cell death causing many abnormal changes in the pattern of EEG (Table 3).

Regarding complete blood count, highly significant differences were observed in hemoglobin levels in the patient group compared to the control group. However, no significant differences were found in red blood cells, white blood cells, and platelet count between the two groups. Regarding liver and kidney function tests, significant differences were observed in total serum bilirubin, direct serum bilirubin and total serum protein which were significantly lower in the patient group compared to the control group, while SGOT and SGPT levels were higher in the patient group. However, no significant differences were found in albumin, creatinine, and blood urea nitrogen levels between the two groups. Regarding serum electrolyte levels, no significant differences were found in calcium, sodium, potassium, and magnesium

levels between the two groups. The mean CRP level was markedly elevated in the patient group compared to the control group. The mean MMP-2 concentration exhibited a substantial decrease in the patient group compared to the control group, with a highly significant p-value of <0.001 (Table 4).

In the correlation analysis between the serum level of MMP-2 and study variables, several significant correlations were identified. There was a positive correlation between MMP-2 levels and [age (years), weight (kg), hemoglobin (Hb) levels, partial thromboplastin time, PT concentration, total and direct serum bilirubin levels, and total serum protein levels] among the studied subjects (Table 5).

No significant differences in MMP-2 levels were observed based on (the type of epilepsy, duration of epilepsy, seizure frequency, previous status epilepticus, different lines of treatment, the history of previous status epilepticus) (Table 6).

MMP-2 concentration had high sensitivity (100%), specificity (88.37%), positive predictive value (89.6%), negative predictive value (100%) and accuracy (94.19%) (Table 7, Figure 1S).

Table 1: Comparison between patient group and control group regarding basic characteristics.

Basic characteristics	Patient group (N=43)		Control group (N=43)		Test	p-value (Sig.)
	No.	%	No.	%		
Gender						
Male	22	51.2%	21	48.8%	0.047[a]	0.829
Female	21	48.8%	22	51.2%		(NS)
Age (years)						
Mean±SD	6.30±3.69		7.42±3.74		-1.484[b]	0.138
Median (Range)	6 (1 – 15)		7 (1.50 – 15)			(NS)
Weight (kg)						
Mean±SD	20.18±6.99		22.02±5.57		-1.818[b]	0.069
Median (Range)	19 (10 – 43)		21 (14 – 35)			(NS)
Weight percentile						
Mean±SD	38.81±26.71		38.13±33.69		-0.368[b]	0.713
Median (Range)	34 (1 – 95)		33 (1 – 99)			(NS)

Categorical variables were expressed as number (percentage); Continuous variables were expressed as mean ± SD & median (range); a: Chi-square test; b: Mann Whitney U test; p-value<0.05 is significant; Sig.: Significance.

Table 2: History of patient group (N=43).

History	Patient group (N=43)	
	No.	%
Consanguinity		
Negative	26	60.5%
Positive	17	39.5%

History	Patient group (N=43)	
	No.	%
Family History of epilepsy		
Absent	26	60.5%
Present	17	39.5%
Family history of febrile convulsion		
Absent	38	88.4%
Present	5	11.6%
Previous febrile convulsion		
Absent	24	55.8%
Present	19	44.2%
Clinical data		
Type of epilepsy		
Focal	13	30.2%
Peti mal	4	9.3%
GTC	26	60.5%
Duration of epilepsy (years)		
Mean±SD	3.24±2.19	
Median (Range)	2.50 (1 – 9)	
Aggravating factors		
Absent	26	60.5%
Fever	5	11.6%
Stress	8	18.6%
Sadness	4	9.3%
Seizure frequency		
Daily	18	41.9%
1-5 times/week	10	23.3%
1-5 times/month	11	25.6%
≤Once a month	4	9.3%
Loss of consciousness		
Absent	27	62.8%
Present	16	37.2%
Lines of treatment		
Two lines	3	7%
Three Lines	20	46.5%
Four Lines	10	23.3%
Five Lines	10	23.3%
Previous status epilepticus		
No	13	30.2%
Once	11	25.6%
Twice	8	18.6%
Three times	6	14%
Four times	2	4.7%
Five times	3	7%

Categorical variables were expressed as number (percentage).
Continuous variables were expressed as mean ± SD & median (range).

Table 3: Radiological and EEG findings of patient group (N=43).

Investigations	Patient group (N=43)	
	No.	%
MRI		
Normal	15	34.9%
Abnormal	28	65.1%
CP	3	7%
Cerebral atrophy	6	14%
Mid-brain atrophy	3	7%
Corpus callosum agenesis	2	4.7%
Agenesis	1	2.3%
Micro-lizenchephally	4	9.3%
Hydrocephalus	3	7%
Enlarged subarachnoid space	1	2.3%
Sub-dural Hygroma	1	2.3%
Focal lesions	1	2.3%
Encephalomalcia	1	2.3%
Hypoxic injury	2	4.7%
EEG		
Normal	16	37.2%
Abnormal	27	62.8%
Epileptogenic focus	8	18.6%
Generalized epileptic activity	12	27.9%
Post-ischemic changes	7	16.3%

Categorical variables were expressed as number (percentage).

Table 4: Comparison between patient group and control group regarding complete blood count and coagulation profile.

Complete blood count & Coagulation profile	Patient group (N=43)	Control group (N=43)	Test[b]	p-value (Sig.)
Hb (g/dl)				
Mean±SD	11.07±1.22	13.10±1.23	-5.958	<0.001
Median (Range)	11.10 (7.70 – 14.50)	13.50 (10.20 – 14.60)		(HS)
RBCs ($x10^6/mm^3$)				
Mean±SD	4.22±0.62	4.42±0.67	-1.376	0.169
Median (Range)	4.10 (2.90 – 6.50)	4.50 (3.10 – 5.50)		(NS)
WBCs ($x10^3/mm^3$)				
Mean±SD	10.67±4.32	9.88±1.48	-0.052	0.958
Median (Range)	9.60 (4.20 – 23)	11 (6.20 – 11)		(NS)
Plt count ($x10^3/mm^3$)				
Mean±SD	371.62±127.50	348.97±101.12	-0.377	0.707
Median (Range)	356 (126 – 854)	346 (167 – 466)		(NS)
PT (sec.)				
Mean±SD	12.50±2.92	13.53±1.35	-1.857	0.063
Median (Range)	12.60 (8.10 – 19.40)	13.50 (11.50 – 16)		(NS)
PTT (sec.)				
Mean±SD	38.82±5.43	40.81±3.63	-1.352	0.176
Median (Range)	39.80 (24.80 – 47.40)	40.50 (35.10 – 46.50)		(NS)
PT concentration				
Mean±SD	119.08±26.02	101.05±13.28	-3.541	<0.001
Median	115.60	97.60		(HS)

Complete blood count & Coagulation profile	Patient group (N=43)	Control group (N=43)	Test[b]	p-value (Sig.)
(Range)	(74.20 – 119.10)	(80.50 – 123.40)		
INR				
Mean±SD	1.07±0.15	1.04±0.22	-1.651	0.099
Median (Range)	1.03 (0.88 – 1.48)	0.97 (0.82 – 2.22)		(NS)
TSB (mg/dl)				
Mean±SD	0.37±0.26	0.53±0.25	-3.038	0.002
Median (Range)	0.30 (0.11 – 1.23)	0.54 (0.12 – 1.01)		(S)
DSB (mg/dl)				
Mean±SD	0.17±0.15	0.20±0.07	-3.148	0.002
Median (Range)	0.12 (0.01 – 0.73)	0.20 (0.10 – 0.44)		(S)
Total serum protein (g/dl)				
Mean±SD	6.36±1.54	7.42±0.66	-3.796	<0.001
Median (Range)	6.35 (3.15 – 9.50)	7.33 (6.18 – 8.77)		(HS)
Albumin (g/dl)				
Mean±SD	4.43±0.84	4.31±0.44	-0.380	0.704
Median (Range)	4.33 (3.11 – 6.19)	4.24 (3.66 – 6.01)		(NS)
SGPT (IU/L)				
Mean±SD	20.93±16.91	13.96±10.71	-2.933	0.003
Median (Range)	14.70 (4 – 78.20)	9.50 (6.45 – 55.40)		(S)
SGOT (IU/L)				
Mean±SD	29.99±21.93	18.58±11.14	-2.842	0.004
Median (Range)	22 (9.10 – 96.70)	16.40 (5.60 – 45)		(S)
Creatinine (mg/dl)				
Mean±SD	0.30±0.07	0.30±0.09	-0.212	0.832
Median (Range)	0.31 (0.19 – 0.53)	0.34 (0.15 – 0.44)		(NS)
BUN (mg/dl)				
Mean±SD	11.65±5.83	11.52±4.66	-0.337	0.736
Median (Range)	10 (1.09 – 32.80)	10.80 (4 – 19.50)		(NS)
Ca (mg/dl)				
Mean±SD	10.02±0.71	10.06±0.70	-0.302	0.762
Median (Range)	9.91 (8.32 – 11.79)	10.12 (8.90 – 11.60)		(NS)
Na (mmol/L)				
Mean±SD	138.41±6.05	139.53±4.52	-1.459	0.145
Median (Range)	138 (128 – 155)	140 (132 – 150)		(NS)
K (mmol/L)				
Mean±SD	4.78±0.77	4.46±0.76	-1.844	0.065
Median (Range)	4.90 (2.71 – 6.60)	4.36 (3.15 – 6.01)		(NS)
Mg (mmol/L)				
Mean±SD	2.24±0.68	2.04±0.31	-1.335	0.182
Median (Range)	2.11 (1.04 – 4.50)	2.06 (1.35 – 2.80)		(NS)
CRP (mg/dl)				
Mean±SD	10.62±17.31	3.53±1.31	-2.977	0.003
Median (Range)	5.30 (0.50 – 85.44)	3.54 (1.35 – 5.40)		(S)
MMP-2 (ng/ml)				
Mean±SD	8.58±2.77	17.94±5.17	-6.979	<0.001
Median (Range)	9.10 (1.68 – 13.50)	18.60 (2.67 – 26.10)		(HS)

Continuous variables were expressed as mean ± SD & median (range); b: Mann Whitney U test; p-value<0.05 is significant; Sig.: Significance.

Table 5: Correlation between serum level of MMP-2 (ng/ml) and study variables among the studied subjects (N=86)

Variables	Serum MMP-2 (ng/mL)	
	R	p-value (Sig.)
Age (years)	+0.234	0.030 (S)
Weight (kg)	+0.258	0.016 (S)
Weight percentile	-0.132	0.225 (NS)
Duration of epilepsy (years)	+0.075	0.633 (NS)
Duration of hospitalization (days)	+0.148	0.343 (NS)
Hb (g/dl)	+0.468	<0.001 (HS)
RBC ($x10^6/mm^3$)	+0.031	0.779 (NS)
WBC ($x10^3/mm^3$)	+0.039	0.723 (NS)
Platelet count ($x10^3/mm^3$)	-0.017	0.874 (NS)
Ca (mg/dl)	-0.014	0.896 (NS)
Na (mmol/L)	+0.178	0.101 (NS)
K (mmol/L)	-0.181	0.096 (NS)
Mg (mmol/L)	-0.100	0.358 (NS)
PT (sec)	+0.171	0.116 (NS)
PTT (sec)	+0.272	0.011 (S)
PT concentration	-0.232	0.031 (S)
INR	-0.117	0.283 (NS)
TSB (mg/dl)	+0.266	0.013 (S)
DSB (mg/dl)	+0.247	0.022 (S)
Total serum protein (g/dl)	+0.359	0.001 (S)
Albumin (g/dl)	+0.100	0.360 (NS)
SGPT (IU/L)	-0.144	0.186 (NS)
SGOT (IU/L)	-0.027	0.802 (NS)
Creatinine (mg/dl)	+0.023	0.836 (NS)
BUN (mg/dl)	-0.131	0.229 (NS)
CRP (mg/dl)	-0.180	0.097 (NS)

r: Spearman's rank correleation coefficient; p-value<0.05 is significant; Sig.: Significance.

Table 6: Relationship between clinical data and serum MMP-2 among patient group (N=43).

Clinical data	N	Serum MMP-2 (ng/ml)				Test	p-value (Sig.)
		Mean	±SD	Median	(Range)		
Type of epilepsy							
Focal	13	8.59	±3.08	9.40	(3.36 – 12.36)	0.089[c]	0.956
Peti mal	4	9.09	±3.44	8.25	(6.35 – 13.50)		(NS)
GTC	26	8.50	±2.62	9.05	(1.68 – 12.10)		
Duration of epilepsy							
≤2.5 years	22	8.55	±2.99	9.05	(2.97 – 13.50)	-0.304[b]	0.761
>2.5 years	21	8.61	±2.59	9.20	(1.68 – 12.33)		(NS)
Seizure frequency							
Daily	18	8.15	±2.49	8.87	(2.97 – 11.36)	1.814[c]	0.612
1-5 times/week	10	8.78	±3.77	9.46	(1.68 – 13.50)		(NS)
1-5 times/month	11	9.23	±2.51	9.86	(4.60 – 12.36)		
≤Once a month	4	8.25	±2.33	7.50	(6.35 – 11.65)		
Lines of treatment							
Two/three Lines	23	7.92	±3.16	8.69	(1.68 – 13.50)	3.924[c]	0.141
Four Lines	10	10.17	±1.23	9.82	(8.80 – 12.33)		(NS)

Clinical data	N	Serum MMP-2 (ng/ml)				Test	p-value (Sig.)
		Mean	±SD	Median	(Range)		
Five Lines	10	8.52	±2.42	8.88	(4.23 – 11.65)		
Previous status epilepticus							
No	13	9.68	±2.38	9.94	(4.60 – 12.36)	4.294[c]	0.231
Once	11	7.49	±2.90	7.65	(1.68 – 11.36)		(NS)
Twice	8	7.88	±3.25	7.68	(3.36 – 13.50)		
3-5 times	11	8.89	±2.47	9.56	(2.97 – 11.65)		

Continuous variables were expressed as mean ± SD & median (range); b: Mann Whitney U test; c: Kruskal Wallis H test; p-value<0.05 is significant; Sig.: Significance.

Table 7: Serum Matrix Metalloproteinase-2 as a diagnostic marker for refractory epilepsy; ROC curve analysis.

Cut-off value	SN (95%CI)	SP (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)	AUROC (95%CI)	p-value (Sig.)
≤13.50 ng/ml	100% (91.8-100)	88.37% (74.9-96.1)	89.6% (79-95.1)	100%	94.19% (83.4-98.1)	0.937 (0.863-0.978)	<0.001 (HS)

ROC curve: Receiver Operating Characteristic curve; SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUROC: Area Under Receiver Operating Characteristic curve; 95%CI: 95%Confidnce Interval; Sig.: Significance; p-value<0.05 is significant.

DISCUSSION

Epilepsy is one of the most common and important neurological conditions in the world, affecting over 50 million people each year. There are five to ten cases for every 1,000 people [5].

An EEG is used in conjunction with a comprehensive and trustworthy medical history of the patient to make the diagnosis of epilepsy. Despite these precautions, medical histories by themselves could not always be enough, which could make diagnosis difficult in certain situations. Thus, it is essential to investigate novel strategies to improve the accuracy of epilepsy diagnosis [6].

The zinc-dependent proteases known as matrix metalloproteinases (MMPs) have a variety of substrates, such as receptors, extracellular matrix elements, cytokines, and cell motility factors [7]. They are the main class of proteolytic enzymes in charge of maintaining the integrity of the cell membrane and reorganizing the extracellular matrix. MMPs are essential for maintaining normal physiological processes in adulthood, controlling the growth of organs, including muscles and nerves, and aiding in the healing process following injuries to the nervous system. MMP-2 and MMP-9 are mostly expressed in the brain [8].

According to our findings, there were no statistically significant variations between the patient and control groups in terms of age, weight, gender distribution, or weight percentile. The study attempted to reduce potential confounding variables that could otherwise affect the results by ensuring comparability in these important demographic characteristics.

Similar to our results, a study by Soliman et al. [9] comprised 34 people over the age of two who had been diagnosed with epilepsy; their ages and sexes were matched to a group of 34 healthy controls.

In our study, 60.5% of the patient group disclosed being consanguineous. These elevated proportions draw attention to possible hereditary elements that may be causing epilepsy in the affected population. Consistent with our findings, Yasir et al.'s study [10] discovered a substantial correlation between paternal consanguinity and the incidence of epilepsy. In particular, cousins accounted for 59.1% of the parents of epileptic children, non-relatives within the same family for 13.6%, and no parental relationship for just 22.7% of cases.

In Chentouf et al. [11] the greater number of consanguineous marriages between cases and controls, which facilitates intra-family connections. There appears to be a 2.15-fold increase in the probability of both monogenic and polygenic epilepsies in first-degree paternal consanguinity.

When Asadi-Pooya [12] evaluated Iranian children who had epilepsy, he discovered that their parents' consanguinity was much greater in these patients than in the general population. This led him to

believe that parental consanguinity could be a risk factor for epilepsy.

However, our research revealed that 39.5% of participants had a family history of epilepsy, whereas 60.5% had not.

Yasir et al. [10] found that 68.2% of children had a positive family history of epilepsy, whereas 31.8% had no family history of the condition, which is in contrast to our findings.

There could be a number of reasons for the discrepancy in our study's results from those of previous studies. The first factor that could affect the prevalence of a family history of epilepsy is diversity in the research population, either in terms of demographics or genetic background. The mismatch may also be caused by methodological variations, such as the standards employed to identify a family history of epilepsy or the procedures followed in the data collection. Additionally, the results may differ depending on the sample size and location of the research, since genetic or environmental factors may cause a higher or lower prevalence of epilepsy in a given population [13].

The distribution of epilepsy types in our study, which showed 30.2% with focal, 9.3% with petit mal, and 60.5% with GTCs, demonstrates the variety in epilepsy types among the population under study. These findings were based on the EEG results. Compared to other studies, Keränen et al. [14] found that 26.5% of patients had generalized seizures and 56% of patients had focal seizures. Another study by Ahmed and Said revealed that 27.6% of the children reported experiencing focal seizures, and 60.9% of the included children had GTCs. It highlights the variety of clinical presentations that we found.

Seizure frequency varied, with 41.9% reporting daily seizures, 23.3% reporting 1–5 times per week, 25.6% reporting 1–5 times per month, and 9.3% reporting once a month or less. According to research by Ahmed and Said, the majority of seizures happened three to five times a month (80.8%), lasted three to five minutes (57.1%), and were preceded by observable triggering variables (69.2%) [15].

The MRI results revealed that 34.9% of the patients had normal results, and 65.1% had abnormalities such as hypoxic damage (4.7%), micro-lizenchephally (9.3%), corpus callosum agenesis (4.7%), cerebral atrophy (14%), and mid-brain atrophy (7%). In 7% of patients, there was cerebral palsy (CP) that is a clinical diagnosis based on history and examination plus imaging studies. CP caused by Oxygen deprivation near childbirth which cause brain damage in the form of hypoxic ischemic lesion which can be observed in MRI. In agreement with our study, a study by Khosronejad et al. [16] revealed that 29.16% of individuals had normal brain MRI findings. 9.72% of patients had neural migration abnormality, and 13.88% of patients had focal lesions (mass, dysplasia, etc.) and hippocampal abnormalities.

Hemoglobin levels were significantly different between the patient and control groups in terms of complete blood count, with a mean of 11.07±1.22 g/dl in the former group and 13.10±1.23 g/dl in the latter. Contrary to our research, Suo et al. [17] examined a number of laboratory indicators in individuals suffering from refractory epilepsy and found no discernible variations in hemoglobin levels. According to our investigation, there were no appreciable variations in the two groups' platelet counts, white blood cells, or red blood cell counts. Suo et al. [17] found that there were no appreciable changes in the hematocrit, leukocyte, erythrocyte, and platelet levels of 21 individuals with refractory epilepsy, which is consistent with our findings. Eroglu et al. [18] demonstrated, in contrast to our findings, that WBC, neutrophil, and lymphocyte counts were significantly higher in epilepsy patients during seizures and significantly lower in these values during the seizure-free time compared with controls.

There were no discernible variations in the two groups' serum electrolyte values for calcium, sodium, potassium, and magnesium. There were no statistically significant differences in the serum electrolyte concentrations between the two groups, as shown by the same mean and median values for each electrolyte. In contrast to our results, Kose et al. [19] showed that patients with refractory epilipsy had elevated Na and K levels.

Tests for kidney and liver function revealed notable variations in a number of parameters. With p-values of 0.002 and 0.002, respectively, total serum bilirubin and direct serum bilirubin levels were significantly lower in the patient group as compared to the control group. Amer et al. [20] reported that there was significant difference in total bilirubin levels between children with refractory epilepsy and healthy controls. Additionally, the patient group's total serum protein levels were significantly lower, with a p-value of less than 0.001.

Our analysis revealed that the patient group had greater levels of aspartate and alanine aminotransferase, with p-values of 0.004 and 0.003, respectively. In line with our findings, a research by Amer et al. [20] found that children with refractory

epilepsy had greater levels of aspartate and alanine aminotransferase than healthy controls.

According to our research, there were no significant variations in the two groups' blood urea nitrogen, albumin, or creatinine levels (p-values > 0.05). Based on our findings, a research by Amer et al. [20] found no discernible differences in blood urea nitrogen and creatinine levels between children with refractory epilepsy and healthy controls.

With a p-value of 0.003, the mean CRP level in the sick group was significantly higher than in the control group. Zhong et al. [21] demonstrated, in accordance with our investigation, that the CRP level was considerably higher in epilepsy patients as compared to healthy controls. Our findings are at odds with a research by Kopczynska et al., which found no discernible variation in CRP between individuals with epilepsy and a healthy control group [22].

A substantial rise in CRP levels in the blood in epileptic patients, possibly associated with long-term use of antiepileptic drugs (AEDs). Research has demonstrated that individuals receiving enzyme-inducing AEDs, such as phenytoin and carbamazepine, exhibited elevated CRP levels in contrast to those receiving lamotrigine or valproate. Long-term use of AEDs may cause inflammation, with variations in CRP indicating the degree of inflammation [21]. This could lead to atherosclerosis.

According to our research, there was a significant drop in the mean MMP-2 concentration between the patient and control groups, with a highly significant p-value of less than 0.001. Soliman et al. [9] and Wang et al. [23] also showed lower serum MMP-2 levels in epileptics, which is consistent with our findings. Further research is necessary to address the link between MMP-2 and epileptogenesis that this event raises.

Previous research on animal brain tissue with epilepsy revealed no changes in MMP-2 levels, which contradicts our findings. Increased MMP-9 activity in the rat brain induced by pilocarpine was shown in a study by Hoehna et al. [24] using rat models; no statistically significant significance for MMP-2 was established.

Elevated MMP-9 protein and activity levels were seen in the CSF of adult epilepsy patients in another investigation by Li et al. [25], suggesting a possible correlation with generalized tonic-clonic seizures. In their investigation on the hippocampus of mice, Mizoguchi et al. [26] highlighted the importance of MMP-9 in the development of seizures, with no statistically significant effect for MMP-2. MMP-2's significance to structural remodeling in epileptogenesis, with increased mRNA, protein, and activity following convulsions, is proposed, despite the fact that its role in neuronal cell death in epilepsy is denied. Reduced serum MMP-2 may have resulted from elevated enzymatic activity in brain tissue, which consumed peripheral MMP-2 and moved into the brain to take part in the epileptogenesis process. Future research combining serum and animal brain tissue collection is warranted by this notion [27].

Many noteworthy relationships were found in the correlation analysis between the research variables and the serum level of MMP-2. Among the individuals under study, there was a positive link found between MMP-2 levels and the following parameters: total serum protein levels, direct and total serum bilirubin levels, hemoglobin (Hb) levels, weight (kg), partial thromboplastin time, and PT concentration. On the other hand, age at onset and MMP-2 levels showed a substantial negative link, as reported by Soliman et al. [9], suggesting that patient prognostic category has a major influence on serum MMP-2 levels.

According to Wang et al. [23], MMP-2 levels decline with aging. Additionally, they revealed no discernible variation in MMP-2 levels between males and females. Inconsistencies in these results could be ascribed to variations in sample sizes, age ranges, and measurement techniques [28].

According to our findings, there were no appreciable variations in MMP-2 levels according to the kind of epilepsy, how long it had been present, how frequently seizures occurred, past status epilepticus, various treatment modalities, or the history of previous status epilepticus. Wang et al.'s [23] findings, which were consistent with our own, revealed a non-statistically significant difference (p-value surpassing 0.05) between serum MMP-2 and seizure types, seizure history, seizure frequency, time since the last seizure, and history of antiepileptic drug usage. Contrary to what we found, Soliman et al. [9] found that patients with focal epilepsy had statistically significantly lower serum MMP-2 levels than those with generalized epilepsy.

According to our research, MMP-2 is a useful tool for diagnosing refractory epilepsy, especially when using a cut-off value of ≤13.50 ng/ml. While the specificity of 88.37% (95% CI: 74.9-96.1) highlights its capacity to appropriately eliminate those without the disease, the high sensitivity of 100% (95% CI: 91.8-100) demonstrates its usefulness in correctly identifying children with refractory epilepsy. The

probability of a true positive result is indicated by the positive predictive value of 89.6% (95% CI: 79-95.1), whereas the reliability of a negative test result in ruling out refractory epilepsy is highlighted by the 100% negative predictive value. The test's overall performance is deemed credible due to its impressive 94.19% (95% CI: 83.4-98.1) accuracy.

Furthermore, the robustness of MMP-2 as a diagnostic marker for refractory epilepsy is further supported by the area under the ROC curve of 0.937 (95% CI: 0.863-0.978). The aforementioned findings indicate the possible clinical usefulness of MMP-2 in precisely and promptly identifying patients with refractory epilepsy.

With an MMP-2 concentration cut-off value of 111.5 ng/ml, Soliman et al. [9] were able to achieve an AUC of 0.922, 85.29% sensitivity, and 97.06% specificity. Additionally, Wang et al. [23] employed a cut-off value of 175.40 ng/ml, which produced an AUC of 0.697, 71.13% sensitivity, and 62.66% specificity.

Although MMP-2 may play a part in neurodevelopmental processes and neurological illnesses, its exact significance in the pathophysiology of epilepsy is yet unknown. Studies suggest that MMP-2 downregulation may prevent inflammatory cells from migrating into the central nervous system and from breaking down the blood-brain barrier. According to our research, drug-resistant epileptic subjects had the lowest levels of MMP-2, which may indicate a neuroprotective function. Nevertheless, more research is needed to determine MMP-2's precise function in the etiology of epilepsy [4, 29].

CONCLUSIONS

This study lends credence to the idea that MMP-2 may be essential for facilitating prompt and accurate treatment interventions for kids with refractory epilepsy.

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