The Intercontinental Journal of Emergency Medicine

Original Article

DOI: 10.51271/ICJEM-0001

Investigation of changes in MDH enzyme activity in epilepsy

Bahadır Taşlıdere[1], Emre Can Güven[2], Beyza Oflaz Güven[2], Bedia Gülen[3]

[1]Department of Emergency Medicine, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey
[2]Basaksehir Public Hospital, Istanbul, Turkey
[3]Department of Emergency Medicine, Istanbul Medipole University, Istanbul, Turkey

Cite this article: Taşlıdere B, Güven EC, Oflaz Güven B, Gülen B. Investigation of changes in MDH enzyme activity in epilepsy. *Intercont J Emerg Med.* 2023;1(1):1-3.

Corresponding Author: Bahadır Taşlıdere, drbahadir@yahoo.com

Submit Date: 15/03/2023 **Accept Date:** 30/03/2023

ABSTRACT

Aims: Epilepsy is one of the most common neurological diseases. It is classified into three groups: focal onset, generalized onset and unclassifiable. A seizure type is difficult to determine. The malate dehydrogenase (MDH) enzyme has a critical role in the excitability of the brain, and it has been reported that recurrent seizures are seen when its regulation is disturbed. We investigated the contribution of MDH enzyme levels as a biomarker to seizure classification.

Methods: Our study was conducted prospectively by patients who were admitted to the emergency department of our hospital within a six-month period. Included in the study were 65 patients diagnosed with epilepsy, according to the International League Against Epilepsy (ILAE) classification. The patients were divided into two groups according to seizure type: focal onset and generalized onset. They were compared in terms of enzyme activity.

Results: The MDH values of the patients with primary epilepsy were found to be 105.94±111.43 and those with secondary epilepsy were 81.03±121.59 (p=0.141). The same test was used to compare the distribution of MDH values between seizure type groups. The MDH value was 47.56±38.65 in patients with focal-onset epilepsy and 109.76±128.44 in patients with generalized-onset epilepsy. A statistically significant difference was observed in the comparisons between the groups (p= 0.031)

Conclusion: Our results reveal the potential of MDH as a biomarker that can be used in epilepsy. In addition, the statistically significant difference between focal-onset and generalized-onset epilepsy indicates that it can be a usable biomarker in seizure classification. MDH enzyme level has a cut-off value of 109.76±128.44 IU/L strongly underlines that it should be used in the diagnosis.

Keywords: Epilepsy, malate dehydrogenase, generalized-onset

INTRODUCTION

Epilepsy is one of the most common neurological diseases and affects 1–3% of the general population.[1] Epilepsies with known etiology are called secondary epilepsy, while epilepsies whose causes cannot be determined are called idiopathic or primary epilepsy.[2] According to the new classifications made by the ILAE in 2017, epilepsies are categorized into three groups: focal onset (focal clonic, focal myoclonic, and focal tonic), generalized onset (tonic-colonic, absence), and unclassifiable.[3,4] Determining the seizure type is important for the treatment, follow-up, and prognosis of the disease. There are many mechanisms responsible for the pathogenesis of epilepsy, which result in different clinical manifestations.[5,6] One of them is oxidative stress and neurodegeneration.[7]

MDH is an enzyme involved in the mitochondrial tricarboxylic acid cycle, and oxidative stress can cause the enzyme activity to change. MDH has a critical role in brain excitability (encoded by the voltage-gated sodium channel α-subunit type I -SCN1A gene). When this gene regulation is disrupted, recurrent seizures may be observed. It has been shown that this enzyme level is increased in epilepsy patients.[8,9]

Appropriate biomarkers should be used to prevent progression and predict the prognosis of epilepsy, which has a very complex pathophysiology and classification. In particular, the distinction between focal and generalized seizures is important in the treatment and follow-up of patients. In our study, we first investigated the level of MDH in focal-onset and generalized-onset epilepsies. Then, we examined the change in MDH enzyme activity in primary and secondary epilepsy. Finally, we investigated whether changes in MDH enzyme activity could be used as a biomarker in the classification of epilepsy.

METHODS

The study was carried out with the permission of Bezialem Vakıf University Non-invasive Researches Ethics Committee (Date: 19.05.2021, Decision No: 2021/177). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted prospectively by analyzing patients who were admitted to the emergency department of

our hospital during seven-month period between 12/1/2019 and 06/30/2020. Included in the study were 65 patients diagnosed with epilepsy as a result of neuroradiological and electroencephalographic examinations. The patients were informed about their participation in the study, and their written consent was obtained. Excluded from the study were patients who did not give consent (and for whom there was a lack of data) and patients with seizures whose onset could not be determined and were therefore unclassified.

The diagnosis of epilepsy was made by the presence of typical interictal electroencephalogram (EEG) findings and clinical presentations, in accordance with ILAE epilepsy classifications. Excluded from the study were patients with a history of any traumatic brain injury, cerebral ischemia, transient ischemic attack, stroke, or neuroendocrinal tumors; patients with a history of neuropsychiatric procedures; patients with a history of psychoactive or central nervous system depressant drug use; patients with a history of drug or alcohol abuse; pregnant and lactating women; and patients with any hepatic dysfunction. Routine laboratory tests and malate dehydrogenase levels were determined from blood samples taken from the patients. Patients were divided into primary and secondary epilepsy groups. According to the seizure types, they were collected in two groups as focal-onset and generalized-onset seizures. They were then compared in terms of enzyme activity. Before administering any medication, at least 2 cc of study blood was taken into a gel-free biochemistry tube from patients with informed consent. The tubes were centrifuged at 3000/min for 10 minutes and the serums were separated and stored in eppendorf tubes at -80 degrees Celsius until the operating time.

Statistics

Behaviors of quantitative variables were expressed using centralization and measures of variance: Mean±SD. To show the behavioral differences of the group averages; Kruskal-Wallis H Test (number of groups>2) and Mann-Whitney U Test (number of groups=2) were used in cases where the assumptions of normality and homogeneity were not met. Statistical significance was determined as p = 0.05 for all cases. Statistical analyzes were provided with the IBM SPSS (Statistics Package for Social Sciences for Windows, Version 21, Armonk, NY, IBM Corp.) package program.

RESULTS

The mean age of the 65 patients participating in the study was 43.75±19.49 years. Of all the patients, 29 were female (44.6%) and 36 were male (55.4%). The mean age of the women was 46.3 ±19.4 years and the men 41.2±19.4 years. Thirty-five (53.8%) of the patients had primary epilepsy and 30 (46.2%) had secondary epilepsy. There were 16 patients (24.6%) with focal-onset epilepsy and 49 patients (75.4%) with generalized-onset seizures (Table 1).

The Mann–Whitney U test was used to compare the distribution of MDH values between epilepsy type groups. The MDH values of the patients with primary epilepsy were found to be 105.94±111.43 and those with secondary epilepsy were 81.03±121.59. No statistically significant difference was observed in the comparisons between groups (p=0.141).

The same test was used to compare the distribution of MDH values between seizure type groups. The MDH value was 47.56±38.65 in patients with focal-onset epilepsy and 109.76±128.44 in patients with generalized-onset epilepsy. A statistically significant difference was observed in the comparisons between the groups (p= 0.031) (Table 2). The results of routine blood tests taken from the patients are given in Table 3.

Table 2. The relationship between epilepsy and MDH

	MDH		P (m)
	Mean ± SD/	Median (Min–Max)	
Type of epilepsy			0.142
Primary	105.9 ± 101.4	71 (13 - 512)	
Secondary	81.0 ± 91.5	44 (5 - 658)	
Seizure types			0.031
Generalized-onset	109.76 ± 118.4	71 (13 - 658)	
Focal-onset	47.56 ± 38.65	34 (5 - 141)	

MDH: Malate dehydrogenase m=Mann Whitney U Test

Table 3. Laboratory results

Parameters	Unit	Values
MDH	U/L	95.06±116.7
WBC	10^9/L	10.9±4.7
Hemoglobin	gr/dl	13.1 ±2.8
Platelet	10^9/L	260±89.4
BUN	mg/dL	14.8±7.5
Creatinine	µmol/L	0.88±0.45
AST	IU/L	27.2±22.3
ALT	IU/L	26.5±18.2
Sodium	mEq/L	137±3.8
Potassium	mmol/l	4.6±4.3
Calcium	mmol/L	10.6±10.8
Neutrophil	10^9/L	7.6±7.9
Lymphocyte	10^9/L	2.9±1.9

MDH: Malate dehydrogenase, WBC: White blood cell, BUN: Blood Urea Nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

DISCUSSION

It has been shown that the incidence of epilepsy is higher in men than in women, and the male to female ratio varies between 1.42 and 2.12.[10-12] In our study, we found this ratio to be 1.24. The mean age of our patients was 43.75±19.49 years. In other studies, 47% of the patients were found to be 40 years or younger.[13] Epilepsies in which there is no underlying cause that triggered the seizures are called idiopathic or primary epilepsy. Most of the time, this condition is caused by genetic predispositions. According to studies, the most common cause of seizures in adults is stroke (23%). In our study, patients were grouped according to the etiological cause. Thirty-five (53.8%) were classified as primary epilepsy and 30 (46.2%) as secondary epilepsy.

Table 1. Age, gender, epilepsy

Female	Male	Type of epilepsy n (%)		Seizure types n (%)		Age
		Primary epilepsy	Secondary epilepsy	Generalized-onset seizures	Focal-onset epilepsy	Mean ± SD
29 (44.6)	36 (55.4)	35 (53.8)	30 (46.2)	49 (75.4)	16 (24.6)	43.7 ± 19.4

n: number, %: percent

In the latest current classification made by the ILAE, the terms "focal onset," "generalized onset," and "unclassifiable" epilepsy are used. Generalized epilepsies constitute approximately 40% of all epilepsies, and there is no obvious etiology other than genetic predisposition.[14,15] Focal seizures are more common in adults than are generalized seizures. Many studies have reported the frequency of generalized seizures to be 23–35% among all epilepsy syndromes.[16-18] In some studies, this rate goes up to 59%.[19] In our study, generalized-onset seizures were more common than focal seizures. There were 16 patients (24.6%) with focal-onset epilepsy and 49 (75.4%) with generalized-onset seizures. Because it causes unconsciousness more frequently, emergency admission rates are higher in epilepsies with generalized onset.[20]

The relationship between the MDH enzyme and epilepsy has been demonstrated in experimental epilepsy studies.[21,22] In addition, severe neurological disorders have been observed in MDH enzyme mutations in the Krebs cycle.[23] Studies have found 46.6±4.9 international units per liter (IU/L) in MDH control groups. According to the receiver operating characteristic (ROC) analysis, the cut-off value of MDH is 51.2 IU/L.[24] In our study, the MDH value was 47.56±38.65 IU/L in patients with focal-onset epilepsy, while the MDH value was 109.76±128.44 IU/L in patients with generalized-onset epilepsy. A statistically significant difference was observed in the comparison between groups (p= 0.031).

CONCLUSION

The result we obtained showed that MDH—one of the oxidative markers—can be used in epilepsy. Diagnostic difficulties in distinguishing between focal seizures and generalized seizures may ultimately cause serious difficulties in the follow-up and treatment of patients. A misdiagnosis as focal epilepsy may lead to inappropriate antiepileptic use and even unnecessary epilepsy surgery. The MDH enzyme level can be used as a biomarker instead of separating focal epilepsy from generalized epilepsy based only on semiology.[25] Moreover, the fact that the MDH enzyme level has a cut-off value of 109.76±128.44 IU/L strongly underlines that it should be used in the diagnosis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bezialem Vakıf University Non-invasive Researches Ethics Committee (Date: 19.05.2021, Decision No: 2021/177).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: This study was supported by the Scientific Research Project Department of Bezmialem Vakif University (TTU-2020-0302).

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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