Serum Prolidase Enzyme Activity Level: Not a Predictive Biomarker for Epilepsy

Zeynep Ozozen Ayas\textsuperscript{1}, Dilcan Kotan\textsuperscript{2}, Mehmet Akdogan\textsuperscript{3}, Mustafa Ercan Gunel\textsuperscript{4}

\textbf{ABSTRACT}

\textbf{Objective}: Oxidative stress (OS) and inflammation are considered responsible for the pathogenesis of epilepsy. Prolidase has an extremely important role in proline recycling for collagen synthesis. Higher than normal proline levels have been shown to increase OS. Furthermore, prolidase activity is associated with inflammation during fibrotic process. No study has yet investigated the relationship between epilepsy and prolidase enzyme activity (PEA). In this study, we aimed to contribute to the existing literature by assessing postictal PEA levels, which are correlated with inflammation and OS, to determine whether PEA levels may be used as a biomarker for epilepsy.

\textbf{Materials and Methods}: This study included patients with epilepsy who presented to the emergency department within first 6 h of a seizure.

\textbf{Results}: The epileptic group included 27 patients (16 males, 11 females) and the control group included 31 healthy individuals (11 males, 20 females). The mean age of the epilepsy group (n=27) and healthy control group (n=31) was 43.1±20.2 and 51.9±21 years, respectively. Serum PEA levels were 1171.90±343.3 in the epileptic group and 1137.1±295.6 in the control group. There were no significant differences between two groups (p>0.05).

\textbf{Conclusion}: Our study results suggest that although PEA is an enzyme associated with OS and inflammation, it is still not an ideal biomarker for epileptic patients. This study is important because it investigated PEA in patients with idiopathic epilepsy for the first time.

\textbf{Keywords}: Prolidase, epilepsy, biomarker

\textbf{Introduction}

Epilepsy, a disorder with an overall population prevalence of 1%, is characterized by recurrent seizures resulting from the hyperexcitability of neurons throughout the cortex or in localized brain regions \citep{1}. A variety of pathophysiological mechanisms have been identified for epilepsy. Markers in physiological pathways have recently gained even more importance in the diagnosis and prognosis of the disease. Oxidative stress (OS) and inflammation caused by increased serum prolidase enzyme activity (PEA) play an important role in the pathogenesis of epilepsy. Studies have shown that PEA increases in conditions such as chronic liver disease, lung cancer, hypertension, and acute hemorrhagic stroke \citep{2-5}. This increase has been reported to be secondary to OS.

In an experimental study, increased proline in rat brain tissue was reported to reduce the total radical antioxidant potential and to cause OS. This result was related to an aspect of the neurological dysfunction seen in hyperprolinemia \citep{6}. A clinical study found that prolidase and total oxidant status levels were higher and total antioxidant status levels were lower in Alzheimer patients than in the control group. It has been reported that OS and collagen degradation play an important role in cognitive destruction \citep{7}.

Prolidase activity is also related to inflammation in the fibrotic process. Prolidase inhibitors have been reported to cause a reduction in immune mediator and receptor expression \citep{8}. Patients with chronic hepatitis infection have an increased prolidase activity with collagen turnover \citep{9}.

No study has yet investigated the relationship between epilepsy and PEA. In this study, we aimed to contribute to the existing literature by assessing postictal PEA levels, which are known to
be correlated with inflammation and OS, to determine whether PEA levels may be used as a biomarker for the disorder.

Materials and Methods
This study included patients with epilepsy who presented to the emergency department of the hospital within 6 h of an idiopathic generalized tonic-clonic seizure. There were 27 patients of the age range 18-88 years. The healthy control group comprised 31 healthy individuals of the same age range. Exclusion criteria included ischemic cerebrovascular accident, aneurysm, arteriovenous malformation, intracranial mass, alcohol use, antidepressant-antipsychotic use, metabolic disorders, prolonged postictal confusion, syndromic seizures, and hypersomnia or insomnia. Laboratory studies were conducted with the patients’ remaining serum samples taken for routine laboratory testing. The ethics committee of Sakarya University School of Medicine approved the study. Written informed consent was obtained from patients who participated in this study.

Data Collection
All patients underwent neurological examination, electrocardiography, complete blood count, and biochemical testing. After the completion of all procedures, the prolidase levels of the serum samples were measured using the ELISA technique.

Samples
The blood samples were taken into non-EDTA tubes, centrifuged at 4°C and at 3000 rpm for 10 min to obtain serum. Prolidase enzyme levels were maintained for analysis by storing blood serum at −80°C (Siemens). All measurements were performed in the same order after thawing the samples.

Prolidase Assay
Serum prolidase activity was quantified using the method described by Myara et al. [10] and optimized by Ozozen et al. [11]. Spectrophotometric method was used to measure proline level. A total of 100 μL serum and 500 μL preincubation solution (50 mmol/L tris-hydrochloride solution at pH 7.8, with 1 mmol/L endogenous antioxidant glutathione (GSH), 5 mmol/L manganese (II) chloride (MnCl2), and 0.1 Triton X-100) were mixed; this mixture was then incubated at 37°C for 3 h. A 100-μL volume of the preincubation serum was added to 100-μL 144 mmol/L Gly-Pro solution and was incubated at 37°C for 30 min. Then, following incubation, 1-mL 0.45 mol/L trichloroacetic acid solution was added to the incubation tube and the reaction was stopped. This mixture was centrifuged at 1500 rpm for 5 min, and 500-μL supernatant was removed. The supernatant was used for proline quantification with the method described by Myara et al. and modified by Chinard [10-12].

Statistical Analysis
The Kolmogorov-Smirnov test was used to test the normality of the distribution of variables. Continuous parametric variables were compared using the independent-samples t-test. Continuous nonparametric variables were compared using the Mann-Whitney U-test. The continuous variables were reported as mean ± standard deviation. Pearson’s chi-squared test was used to detect the relationship between the two categories of variables. Statistical significance was set at p<0.05. All analyses were performed using IBM Statistical Packages for the Social Sciences Statistics, Version 22.0 (Armonk, NY: IBM Corp.).

Results
The epileptic group comprised 27 patients (16 males, 11 females) and the control group comprised 31 healthy individuals (11 males, 20 females) (Table 1). The mean age of the epileptic patients (n=27) and the control group (n=31) was 43.1±20.2 and 51.9±21 years, respectively (Table 2). The demographic variables of both groups were similar. Serum PEA levels were 1171.9±343.3 in the epileptic group and 1137.1±295.6 in the control group (Table 3). There were no significant differences between patients with epilepsy and the members of the healthy control group (p>0.05).

Discussion
In our study, the serum level of PEA, an enzyme related to OS and inflammation, was evaluated as a biomarker of the pathogenesis of epilepsy among patients with primary generalized epilepsy. There were no statistically significant differences in serum PEA levels between patients with epilepsy and members of the healthy control group.

Prolidase, a protease that degrades imidopeptides into proline or hydroxyproline residues, plays an important role in the metabolism of collagen [13]. Proline and hydroxyproline form approximately 25% of collagen connective tissue and play an important role in maintaining its rigidity. The prolidase enzyme has been detected in erythrocytes, leukocytes, dermal fibroblasts, kidney, brain, heart, thymus, and uterus. A wide tissue distribution suggests that changes in PEA may play an important role in the pathogenesis of some diseases like as chronic liver disease, lung cancer, hypertension and result in many disorders.

Epilepsy is a disorder of the central nervous system, which is characterized by recurrent seizures as a result of neuronal hyperexcitability [1]. Various physiological mechanisms have been defined for epilepsy. The cellular mechanisms of epileptogenesis include cellular damage, gliosis, increased expression of intermediate-early genes (c-fos, c-jun), increased growth factors, neurogenesis, synaptogenesis, alterations of glutamate and GABA signaling, inflammatory mediators, alterations of voltage-gated ion channels, and excitotoxic antibodies [14]. Prolidase activity has been related to OS during the process of fibrosis and in various diseases [15]. Inflammation and OS play an important role in the pathogenesis of epilepsy.

Oxidative Stress resulting from excessive free radical release probably plays a role in the initiation and progression of epilepsy [16]. Maintaining low levels of reactive oxygen species (ROS) is critical to normal cellular functions, and thus prolonged increments of ROS constitute a risk for an increase in neurodegeneration observed in epilepsy. Brain is particularly sensitive to OS. Increased serum PEA increases proline and hydroxyproline levels. In a study on rat brains, both acutely and chronically increased proline.

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Table 1. Sex evaluations of epileptic and healthy control groups

<table>
<thead>
<tr>
<th></th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Total, n (%)</th>
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<tbody>
<tr>
<td>Epileptic group</td>
<td>16 (28)</td>
<td>11 (19)</td>
<td>27 (47)</td>
</tr>
<tr>
<td>Healthy group</td>
<td>11 (19)</td>
<td>20 (34)</td>
<td>31 (53)</td>
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Mann-Whitney U-test

Table 2. Mean age of epileptic and healthy control groups

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<th>Mean age±SD</th>
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<tr>
<td>Epileptic group</td>
<td>43.1±20.2</td>
</tr>
<tr>
<td>Healthy group</td>
<td>51.9±21</td>
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Mann-Whitney U-test. SD: standard deviation

Table 3. Serum PEA levels of epileptic and healthy control groups

<table>
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<tr>
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<th>PEA level Mean±SD</th>
<th>p</th>
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<tr>
<td>Epileptic group</td>
<td>1171.9±343.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Healthy group</td>
<td>1137.1±295.6</td>
<td>&gt;0.05</td>
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Pearson’s chi-squared test. SD: standard deviation, p<0.05: statistically significant.
affected some OS parameters and augmented in vivo and in vitro OS, suggesting this as the mechanism underlying brain dysfunction among patients with increased proline levels [17].

Recently, many clinical studies have been published supporting this relationship. Gonullu et al. [5] reported increased OS levels, reduced antioxidant levels, and increased PEA among patients with acute hemorrhagic stroke compared to the controls. Similarly, a study showed that serum PEA was increased and antioxidants were reduced among obese patients, suggesting OS to be the cause [18]. Patients with diffuse anxiety disorder were shown to have significantly increased total antioxidant levels, OS indexes, and serum PEA compared to those in the control group [19]. On the other hand, our study failed to demonstrate any significant difference between the serum PEA levels of the epileptic patients and healthy individuals. A study with results similar to that of our study demonstrated no significant difference between the serum PEA levels of patients with major depressive disorder and those of the controls [20].

Recent preclinical studies on acute seizures and chronic epilepsy models have revealed that the development of neuroinflammation in the brain contributes to underlying neuronal hyperexcitability in seizure onset and generalization [21]. Regarding the relationship of epileptogenesis with inflammation, it has been found in animal studies that the synthesis of cytokines and adhesion molecules increased due to inflammation [22, 23]. The levels of circulating inflammatory proteins are considered to be potential biomarkers for epileptogenesis [24]. Experimental studies have shown that immune mediators (IL-β, TNF-α, toll-like receptors, and TGF-β) have a prominent role in seizure onset [25]. IL-β is related to febrile seizure and status epilepticus, whereas TNF-α acts through glutamate receptors. Toll-like receptors are released by injured or dead neurons and play a role in seizure spread and recurrence. TGF-β is related to the opening of the brain-blood barrier. One study reported that proidase inhibitors caused a reduction in TGF-β1 and receptor expressions [8]. The proidase enzyme probably exerts an inflammatory effect through TGF-β1 and receptor expressions. Patients with chronic hepatitis exhibit increased collagen turnover and proidase activity [9]. Similarly, it was reported that serum PEA may be used for the diagnosis and management of idiopathic pulmonary fibrosis, a disease state created by a reaction to an injury [26]. However, a review reported that proidase activity per se may not reflect disease activity, and is clinically important only in combination with other biochemical markers [27].

The limitations of our study are the small sample size, the single measurement of the PEA level, and the lack of measurement of the levels of inflammatory mediators or OS levels. There is a need for larger and more detailed studies to elucidate the relationship between inflammation and OS, and the role of PEA in the epileptogenesis of the latter.

Our study aimed to investigate whether PEA, a marker related to inflammation, could be a novel biomarker for both diagnosis and treatment (for the management of future treatment models involving anti-inflammatory agents) in epileptic patients. This study is significant because of its pioneering role in the investigation of PEA among patients with idiopathic epilepsy. It is important to develop effective biological markers to diagnose epilepsy early in its course and to determine its prognosis. In this study, however, no significant difference was found between the epilepsy patients and the control group with respect to the serum levels of PEA. According to our results, although PEA has been reported to have a relationship with OS and inflammation, it is not an ideal biomarker for epileptic patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Sakarya University School of Medicine (Decision No:050.01.04/113).

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

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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References


