Original Article

The Serum Prolidase Enzyme Activity as a Biomarker for Evaluation of the Subclinical Vascular Damage in Children with Epilepsy

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Abstract

Backgroud: Epilepsy is a chronic medical condition requiring long term or even lifelong therapy. Various researches have shown that epilepsy patients have vascular risk factors such as abnormal lipids, insulin, elevated oxidative stress, chronic inflammation, and subclinical atherosclerosis. **Objectives:** The purpose of the present study was to determine serum prolidase enzyme activity as a biomarker in children taking antiepileptic drug treatment through comparison with control cases. **Materials and Methods:** The present study group consists of 61 children (20 females, 41 males) with epilepsy and a control group was formed of 32 healthy individuals (14 females, 18 males). Aspectrophotometric method was used to measure serum prolidase enzyme activity. **Results:** The epilepsy group demonstrated statistically significantly higher prolidase enzyme activity values when compared with the control group ($P = 0.003$). It was measured that the serum TOS and OSI values were significantly elevated in patients with epilepsy compared to controls ($P < 0.001$). However, serum TAS values were significantly lower in the epilepsy group than in the control group ($P = 0.032$). **Conclusions:** These results supported that epileptic patients taking the antiepileptic treatment had increased serum prolidase enzyme activity, suggesting that it may show an increased risk of subclinical vascular damage related to both chronic inflammation and fibrotic process associated with degenerated collagen turnover. Therefore, serum prolidase enzyme activity could be considered a useful biomarker for evaluation of the subclinical vascular damage in children with epilepsy on some antiepileptic drugs.

Keywords: Biomarkers, children, epilepsy, oxidative stress, serum prolidase enzyme activity, vascular damage

Introduction

Epilepsy is defined as a chronic condition characterized by recurrent unprovoked seizures. It is a common and serious neurological condition that occurs globally and can be a cause of disability in developing countries.[1,2] In most studies, epilepsy prevalence has been reported to be approximately 10.2–57/1000 in developing countries and 6–8/1000 in developed countries including Turkey.[3-8] The goal of antiepileptic treatment in childhood is to make the patient completely seizure-free without negatively affecting the brain functions. Therefore, treatment of the patient can be started with the drug known to be the most effective and appropriate to epileptic syndrome or the type of seizure determined from the history of a video recording. By taking age and gender into consideration while making this choice, drugs are selected which can be easily used and which have the fewest side effects.[1,9] When these different antiepileptic drugs are used over a prolonged period for patients with epilepsy, there may be some adverse effects containing metabolic and endocrine disorders, behavioral or psychiatric illness, idiosyncratic reactions, negative cognitive influences, and medication interactions.[10,11]

Furthermore, various researches have shown that epilepsy patients have vascular risk factors such as abnormal lipids, insulin, elevated oxidative stress, and elevated total plasma homocysteine.[12-15] Recent studies have also reported that long-term some antiepileptic treatment may increase the risk of vascular damage in patients with epilepsy.[16,17] It has been suggested that through the mechanism of endothelial dysfunction resulting from vascular inflammation, antiepileptic treatment in cases of intractable epilepsy could be a role in the pathogenesis of vascular damage.[18]

Prolidase enzyme activity is required for collagen biosynthesis and plays an important role in the breakdown of collagen and the intracellular proteins, especially in the final stage when imidodipeptides containing C-terminal proline or hydroxyproline are cleaved. Collagen is essential for the maintenance of connective tissue, and increased rates of collagen synthesis may lead to a change in the quality of collagen fibres.

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This enzyme is known to be active in human plasma and different organs.[19,20] The activity of prolidase enzyme is site-dependent with highest activities in the jejunum. Moreover, the studies indicated that fasting had different effects on the duodenal, jejunal, and colonic prolidase activities with the jejunal prolidase as the one most prone to dietary regulation.[21]

Prolidase enzyme activity and the pathophysiological role of prolidase have investigated some diseases such as lung cancer, hypertension, acute hemorrhagic stroke, and epilepsy.[22-25]

To our knowledge, there is no study regarding the serum prolidase enzyme activity in childhood epileptic patients. The goal of the present study is to evaluate the level of oxidative stress and serum prolidase enzyme activity in patients taking some antiepileptic treatment and control cases.

Methods

The present prospective investigation was supplied by February 2014 to April 2016 in the division of pediatric neurology of a tertiary university hospital, in Turkey. The acceptance of the present study was granted by the University Ethics Committee with respect to the Second Declaration of Helsinki (Date: 12.12.2014, No: 12). Written informed consent was obtained from the parents or legal guardians of these children. Evaluations were made of children aged 1–16 years with epilepsy, who were presented at the pediatric neurology outpatient clinic. The clinical, biochemical, electroencephalography, and brain magnetic resonance imaging (MRI) findings of epilepsy patients were evaluated and recorded in the follow-up.

The study group patients were those with at least 6 months of epilepsy with medical treatment. Blood samples were taken for examination in a period when the patient had no complaints, no acute infection table and were not using any drugs other than antiepileptic drugs. The control group comprised apparently healthy age and gender-matched children who were not using any medical drugs and had no chronic systemic or cardiovascular disease.

The serum samples were collected from these subjects within biochemical tubes. These samples were immediately centrifuged to separate plasma and accumulated at -20°C for analysis. Serum total antioxidant status (TAS) and total oxidant status (TOS) were measured using the automated evaluation method advanced by Erel.[26,27] The oxidative stress index (OSI) was described as the percentage rate of TAS values to TOS values. It is formulated as: OSI (Arbitrary Unit) = TOS (mmol H_2O_2 Equivalent/L)/TAS (mmol Trolox Equivalent/L).

A spectrophotometric method measuring proline levels was used to evaluate serum prolidase enzyme activity.

All statistical analyses in the study were performed using SPSS® for Windows version 18.0 software (Chicago, IL, USA). *The Kolmogorov-Smirnov test* was applied to quantitative data to determine conformity to normal distribution and values were stated as arithmetic means and standard deviations. The Chi-square test was applied to compare the demographic characteristics of groups. The comparison of continuous variables between two groups was performed with the student's *t*-test. The results were accepted as statistically significant if a value of P was <0.05.

Results

The patient's group composed of 61 children (41 males, 20 females) with epilepsy and the control group comprised 32 healthy children (18 males, 14 females). The mean age of the epilepsy group was 8.07 ± 5.05 years, while that of the control group was 7.74 ± 5.63 years. There were no statistically significant differences about the age and gender between two groups (P = 0.947) [Table 1]. The epilepsy type was found to be focal epilepsy in 14 cases, generalized epilepsy in 39 cases, and unclassified epilepsy in 8 cases. Of the 61 epilepsy patients, 27 were taking monotherapy and 34 were taking polytherapy [Table 1].

In the patients using polytherapy, 15 cases (44, 12%) were taking two antiepileptic drugs, 13 cases (38, 23%) were taking three antiepileptic drugs, and six cases (17, 65%) were taking four antiepileptic drugs.

The brain MRI examinations demonstrated abnormality in 27 of 61 patients. These abnormalities include cortical dysplasia (3), porencephalic cyst (4), periventricular leukomalacia (8), multicystic encephalomalacia (3), hydrocephaly (2), mesial temporal sclerosis (1), and subependymal nodules (1) and nonspecific gliosis (5).

We measured that the serum TOS and OSI values were significantly elevated in patients with epilepsy compared to controls (P < 0.001). However, serum TAS values were

Table 1: Demographic and clinical parameters of the epilepsy patient and control groups

	Patients (n=61)	Controls (n=32)	P*
Age, (years)	8.077±5.05	7.74±5.63	0.947
Gender, Female/Male, (n)	20/41	14/18	
BMI (kg/m^2)	16.93±4.42	17.44±4.25	0.887
Number of AED, (n)	1.96±1.02	-	
Duration (years)	5.14±3.25	-	
Epilepsy type, (n)			
Focal Epilepsy	14	-	
Generalized Epilepsy	39	-	
Unclassified Epilepsy	8	-	
Brain MRI Finding, (n)			
Normal	34	-	
Abnormal	27	-	
AED Therapy, (n)			
Monotherapy	27	-	
Polytherapy	34	-	

*Student's *t*-test, BMI: Body mass index, AED: Antiepileptic drug, MRI: Magnetic resonance imaging, values are mean±SD, significance was defined as P<0.05

significantly lower in the epilepsy group than in the control group ($P = 0.032$) [Table 2 and Figure 1].

The mean serum prolidase enzyme activity was 444.70±22.23 U/L in the epileptic patients and 366.44 ± 17.43 U/L in the controls. Thus, epilepsy group demonstrated statistically significantly higher prolidase activity values when compared with the control group ($P = 0.003$) [Table 2 and Figure 2].

Discussion

Oxidative stress has been defined as a disturbance of the equilibrium between the prooxidant and antioxidant systems in favor of prooxidation. The term oxidative stress is used to describe a number of chemical reactions involved in the production of free radicals and other reactive molecules that could potentially cause cellular injury.[28,29] Moreover, increased oxidative stress has been shown to play an important role in the pathogenesis of acute and chronic cerebral diseases.[30,31]

The findings obtained in this study demonstrated that there may be impaired oxidative status and increased serum prolidase enzyme activity in children using some antiepileptic medication.

Recent studies have shown increases in several oxidative stress indicators in epilepsy cases and this has strengthened the opinion that increased oxidative stress may contribute to some complications of epilepsy.[12-14] There are also various current studies that have speculated that long-term some antiepileptic drug use may alter serum lipids and may predispose the patient to vascular damage associated with chronic inflammation later in life. To investigate the development of vascular damage in epileptic children, several methods have been used containing different techniques.[17,32-39]

In a study of Egyptian patients with epilepsy, El-Farahaty *et al*. evaluated the atherosclerotic side effects of long-term antiepileptic treatment.[36] The serum fasting lipid profile was studied and biochemical, hormonal, and radiological imaging techniques were used in their study. The study concluded that following long-term monotherapy treatment using valproate, carbamazepine, lamotrigine, and topiramate. There were alterations in the biomarkers of vascular inflammation which could exacerbate atherosclerosis whereas the effect of levetiracetam was seen to be minimal. Similarly, in a study of 64 children with epilepsy by Sonmez *et al*. the effects of phenobarbital, carbamazepine, and valproate were evaluated on serum lipid profiles and lipoprotein (a).[37] It was concluded that treatment with phenobarbital, carbamazepine, and sodium valproate led to a considerable increase in serum lipoprotein values. In another cross-sectional study, intima-media thickness was determined to be significantly elevated in pediatric epileptic cases following monotherapy with either carbamazepine or phenytoin monotherapy for more than 18 months, compared to children in the controls.[35] However, Tokgoz *et al*. found no abnormalities in intima-media thickness evaluations in epilepsy patients compared to a control group.[38]

Similar to those results, it was observed that total oxidative values in the epileptic children were higher than those of the controls whereas the total antioxidative values were lower compared to the controls.[36,37,39] Increased oxidative stress index values were also determined in the current study patients with epilepsy [Figure 1]. These findings suggest that some antiepileptic treatment may be related to changes in the

Table 2: Mean serum TAS, TOS, and OSI values and prolidase activities in the epilepsy patients and control group

Parameters	Patients (*n* : 61)	Controls (*n* : 32)	*P**
TAS, (µmol Trolox equiv./L)	1.13±0.33	2.09±1.04	0.032
TOS, (µmol H_2O_2 Equivalent/L)	52.07±13.21	42.15±8.17	<0.001
OSI, (Arbitrary Unit)	4.89±2.03	2.53±1.17	<0.001
Prolidase Activity, (U/L)	444.70±22.23	366.44±17.43	0.003

*: Student's *t*-test, OSI: Oxidative stress index, TAS: Total antioxidant status, TOS: Total oxidant status

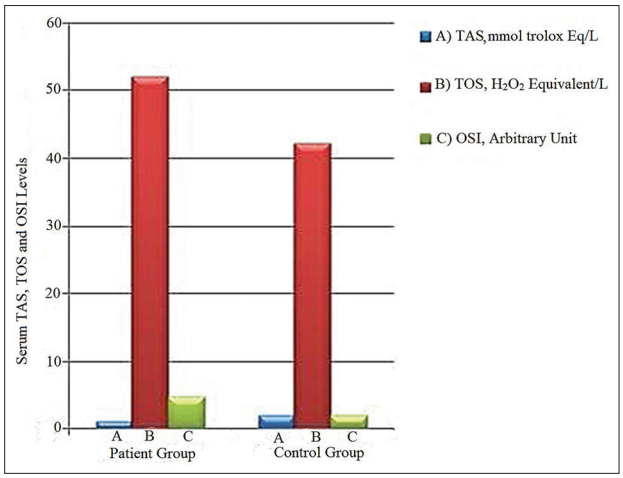

Figure 1: Oxidative and antioxidative parameters in the patient and control groups

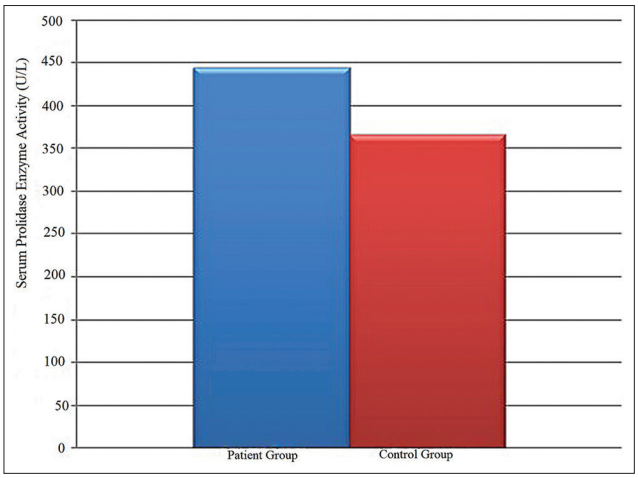

Figure 2: Comparison of serum prolidase activities in the patient and control groups

circulatory biomarkers of vascular inflammation which may then contribute to the acceleration of preclinical atherosclerosis and increased vascular damage in epileptic patients.

Prolidase, an exopeptidase with a significant role in collagen metabolism, is the main regulating enzyme in the metabolism of proline and hydroxyproline containing about 20% of total human collagen.[20] In previous studies, elevated serum prolidase activity has been associated with various diseases including diabetic neuropathy, liver fibrosis, bipolar disorder, anxiety disorder, and many other brain disorders.[40-43] Recently, Ozozen *et al*. have evaluated oxidative stress, inflammation, and the fibrotic process by measuring serum prolidase activity of adult with primary generalized epilepsy and they not found statistically significant differences in serum prolidase activity between patients with epilepsy and members of the healthy control group.[25]

In our study, we investigated serum prolidase activity (to evaluate collagen metabolism) and oxidative stress markers. This study showed that children with epilepsy have significantly increased serum prolidase activity and oxidative stress levels. Moreover, we found that there is an association between the oxidative–antioxidative status and prolidase activity in children with epilepsy.

The findings of the current study were not consistent with those of the study of Ozozen *et al*. with respect to serum prolidase activity in adult epileptic patients. The difference between the present study and other study is most likely related to the difference in the age ranges of the subjects. Epileptic children also may be more sensitive to external factors such as antiepileptic drug treatment than adults.

Toy *et al*. showed that compared with a control group, babies with intrauterine growth retardation had elevated serum prolidase enzyme activity.[44] In addition, Sezen *et al*. demonstrated significantly decreased serum prolidase enzyme activity in adult patients with cardiomyopathy while Yildiz *et al*. investigated the relationship both serum prolidase enzyme activity and the presence of coronary artery disease in adult patients.[45,46] In their study, serum prolidase enzyme activity was found to be higher in patients with coronary artery disease than in controls. In addition, a study by Demirbag *et al*. reported increased serum prolidase enzyme activity in hypertensive patients compared to a control group.[23]

In the current study, an important increase was seen in serum prolidase enzyme activity and oxidative stress index values in the epileptic children compared with the control group [Figure 2]. This finding is in accordance with the findings of similar studies that have investigated serum prolidase enzyme activity in patients with various disorders. The elevated serum prolidase enzyme activity and oxidative stress levels in the epileptic children in the current study suggest that both chronic inflammation and fibrotic process related to degenerated collagen turnover. Thus, these results may demonstrate the development of endothelial cell dysfunction and subclinical vascular damage.

The main limitation of this study was exclusively the use of biochemical methods. In addition, we did not measure the levels of tissue prolidase activity present study. However, we have published a new study showing similar results using radiological and cardiologic imaging methods in our clinic.[47]

Conclusion

In conclusion, the findings of this study supported that epileptic patients taking some antiepileptic drug treatment had elevated serum prolidase enzyme activity which could suggest an increased risk of subclinical vascular damage. Hence, it was hypothesized that elevated serum prolidase enzyme activity may be a useful biomarker for the evaluation of the subclinical vascular damage in epileptic children. Further clinical studies are needed on this subject.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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