

# Investigation of PON1 activity and MDA levels in patients with epilepsy not receiving antiepileptic treatment

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**Purpose:** There are many studies dedicated to researching the etiopathogenesis of epilepsy. In such research, oxidative and antioxidant indicators of etiopathogenesis have also been examined under the scope. Drawing on a group of patients with epilepsy who were receiving no treatment, we have tried to evaluate whether or not an increase in oxidative indicators is linked directly with the disorder, independent of epileptic medicaments.

**Methods:** Thirty people in good health and 30 newly diagnosed with epilepsy and who received ambulatory treatment in the polyclinic of the Neurology Department took part in the study. The tests relating to serum malondialdehyde (MDA) levels and paraoxonase 1 (PON1) activity were carried out in the biochemistry laboratory.

**Results:** Even though the levels of MDA in the patient group ( $14.34 \pm 3.59$  nmol/mL) were found to be high compared to those of the control group, which consisted of people in good health ( $13.53 \pm 3.56$  nmol/mL), there was no statistically significant difference. PON1 activity in the serum taken from people in the patient group ( $0.65 \pm 0.17$ ) was lower in comparison to that observed in the serum of the control group ( $0.71 \pm 0.17$  U/L). Nonetheless, it was not so low as to have significance from a statistical point of view.

**Conclusion:** We conclude that such a high level of oxidative parameters should have been related to the disease and that statistically significant findings that emerged in some other studies could have been related to an antiepileptic treatment.

**Keywords:** epilepsy, paraoxonase 1, malondialdehyde, oxidative stress, epilepsy, biochemical marker

## Introduction

Epilepsy is one of the most common chronic diseases in developed and developing countries, affecting ~1% of the population.<sup>1</sup> Many genetic, radiological, and biochemical researches have been performed to determine the etiology of this disease. Impaired gamma-amino butyric acid (GABA) inhibition, impaired activation of neurons, and an increase in excitatory neurotransmitters are responsible for the pathophysiology of epilepsy.<sup>2-6</sup> Production of free radicals has a role in the regulation of biological function and damage to cell structures, as well as in the pathogenesis of neurodegenerative diseases, such as Parkinson's disease, stroke, epilepsy, and dementias, in the central nervous system.<sup>2,3</sup> Oxidative and nitrosative stress are regarded as possible mechanisms in the pathogenesis of epilepsy.<sup>4</sup> Oxidative markers have therefore become popular in recent years, and many of them have been studied.<sup>5</sup>

The paraoxonase (PON) gene family is localized in the long arm of chromosome 7, and includes *PON1*, *PON2*, and *PON3* genes, which are adjacent to each other. PON is

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a 43–45 kDa glycoprotein which is synthesized mainly by the liver. PON1 is a  $\text{Ca}^{2+}$ -dependent enzyme that is transported with high-density lipoproteins (HDLs) in circulation.<sup>6</sup> It is reported that the antioxidant effect of PON1 and PON3 protects HDLs and low-density lipoproteins (LDLs) from oxidation.<sup>7,8</sup> The antioxidant effect is probably due to the hydrolysis of activated phospholipids and/or lipid peroxide products. It is known that diet, pregnancy, and hormones can affect serum PON1 levels.<sup>9,10</sup>

Malondialdehyde (MDA) is a mutagenic, carcinogenic, and genotoxic compound that is the end product of lipid peroxidation and is the main secondary oxidation product of polyunsaturated fatty acids. It is known that the levels of this compound increase in several diseases that are related to free-radical damage. MDA is the most commonly used marker of oxidative damage.<sup>11,12</sup> Brain tissue is particularly susceptible to free-radical damage for several reasons (eg, high oxygen consumption or high concentrations of phospholipids can increase peroxidation and prevent regeneration of neurons), and the extent of damage is proportional to the amount of free radicals formed.

The results of some studies show that oxidative stress can play a role in the etiopathogenesis of epilepsy, and there are some studies which also report that this stress is generated by antiepileptic drugs (AEDs).<sup>13,14</sup> Our main aim in this study was to investigate the relationship of oxidative stress to PON1 activity and MDA levels by measuring these parameters in epileptic patients who were not using AEDs and have had nonprovoked seizures.

## Materials and methods

This study was started on June 10, 2013, and this study was approved by the Dicle University Faculty of Medicine Ethics Committee (approval number 293). Patients aged between 16 and 65 years who applied to the neurology clinic of the Dicle University Faculty of Medicine (DUTF) hospital with a prediagnosis of epilepsy were evaluated. Classification of epileptic seizures was made according to the epilepsy classification of the International League against Epilepsy (ILAE), which was published in 1981. The results of routine biochemistry, electroencephalogram, and cranial magnetic resonance imaging (MRI) were requested from the patients who were selected for this study, in order to exclude patients with provoked epileptic seizures and select patients with only nonprovoked epileptic seizure diagnosis. Patients with significant pathology in cranial MRIs (sinus vein thrombosis, encephalitis, intracranial tumors) were excluded to prevent erroneous assessment of oxidative markers. In addition, patients with known presence of neurological disease, hypertension, diabetes mellitus,

mental retardation, pregnancy, cardiogenic syncope, and psychogenic nonepileptic seizures; use of antioxidant drugs and agents; and those with a diagnosis of provoked epilepsy were also excluded from this study. As a result, 30 newly diagnosed patients were included in the study.

A total of 30 volunteers who did not have any other neurological or chronic diseases according to the anamnesis and who were similar in age and sex to the members of the patient group were included in this study as a control group. Informed consent forms were signed by the individuals in both the patient and control groups.

From the control group and patients in the interictal period, 5 cc of blood was drawn from the antecubital vein into the biochemical tubes. This blood sample was centrifuged for 5 minutes at 5,000 rpm with a Rotafix 32-A brand device, and serum was obtained and stored at  $-80^{\circ}\text{C}$  in a refrigerator at the Department of Biochemistry, DUTF. After the stored blood was defrosted, it was homogenized by vortexing using a M16 Vortex mixer.

## Paraoxonase enzyme activity measurement

The activity of PON1, which is a lipophilic, hydrophobic, antioxidant enzyme connected to HDL-cholesterol, was detected by using a commercial Reel Assay brand kit according to manufacturer's instructions. In this method, hydrolysis of the paraoxon (*O,O*-diethyl-*O-p*-nitrophenyl phosphate) substrate by PON1 enzyme generates a colored *p*-nitrophenol product. PON1 enzyme activity was then measured photometrically with an Architect C16000 autoanalyzer. The rate of paraoxon hydrolysis was measured by monitoring the increase of absorbance at 412 nm, and the enzyme activity was expressed as U/L.<sup>15</sup>

## Malondialdehyde level measurement

MDA levels were determined using a Northwest MDA assay kit according to manufacturer's instructions. MDA forms a pink complex in aerobic conditions as a result of incubation with thiobarbituric acid (TBA) at pH 3.4 and  $95^{\circ}\text{C}$ . This colored complex was read at 532 nm by spectrophotometry, and the absorbance was measured. The MDA concentration in the sample was determined by comparing the measured absorbance value to a MDA standard curve.<sup>16</sup>

## Statistical analysis

All the data were analyzed using the Statistical Package for Social Sciences (SPSS) program for Windows 16.0 (SPSS Inc., Chicago, IL, USA). For comparing the results of the control and patient groups, independent *t*-tests and

**Table 1** Demographic characteristics of control group and patients

Characteristics of patients	Total (n=60), n (%)	Control (n=30), n (%)	Patient (n=30), n (%)	P-value	$\chi^2$
<b>Sex</b>				0.795	0.067
Female	27 (45.0)	13 (43.3)	14 (46.7)		
Male	33 (55.0)	17 (56.7)	16 (53.3)		
<b>Education</b>				0.010	13.262
Uneducated	2 (3.3)	0 (0)	2 (6.7)		
Primary school	6 (10.0)	1 (3.3)	5 (16.7)		
Middle school	13 (21.7)	5 (16.7)	8 (26.6)		
High school	25 (41.7)	19 (63.3)	6 (20.0)		
University	14 (23.3)	5 (16.7)	9 (30.0)		
<b>Marital status</b>				0.008	7.051
Single	23 (38.3)	6 (20.0)	17 (56.7)		
Married	37 (61.7)	24 (80.0)	13 (43.3)		
<b>Living place</b>				0.098	4.643
Village	4 (6.7)	0 (0)	4 (13.3)		
District	9 (15.0)	4 (13.3)	5 (16.7)		
City	47 (78.3)	26 (86.7)	21 (70.0)		
<b>Lifestyle</b>				0.117	4.286
Alone	3 (5.0)	0 (0)	3 (10.0)		
With family	56 (93.3)	30 (100)	26 (86.7)		
Other	1 (1.7)	0 (0)	1 (3.3)		
<b>Disease history</b>				0.237	3.158
No	57 (95.0)	30 (100)	27 (90.0)		
Yes	3 (5.0)	0 (0)	3 (10.0)		
<b>Epilepsy history in family</b>				0.112	4.286
No	56 (93.3)	30 (100)	26 (86.7)		
Yes	4 (6.7)	0 (0)	4 (13.3)		

chi-square tests were performed. To find independent associations of age, variables with a  $P$ -value  $\leq 0.05$  from the bivariate correlation analysis were selected for multiple linear regression analysis. Results with  $P$ -values  $< 0.05$  were considered statistically significant.

## Results

The average age of our patients was  $24.6 \pm 8.15$  years, and that of the control group was  $23 \pm 4.83$  years. There was no statistically significant difference between the two groups with respect to age. The patient group consisted of 14 females (46.7%) and 16 males (53.7%) patients, and the control group consisted of 13 females (43.3%) and 17 males (56.7%) individuals. There was no statistically significant difference between the two groups in terms of sex.

There was a statistically significant difference between the patient and control groups with respect to education level and marital status. There was no statistically significant difference between the two groups with respect to living place, living status, disease history, and disease history in the family ( $P > 0.05$ ) (Table 1).

Epilepsy classification was made in accordance with the Classification of Epileptic Seizures of the ILAE, which was published in 1981. In 43.3% ( $n=13$ ) and 56.7% of patients

( $n=17$ ), partial and generalized seizures were detected, respectively. The distribution of partial seizures was found to be simple partial in 30.8% ( $n=4$ ) and complex partial in 69.2% ( $n=9$ ) of the patients. The distribution of generalized seizures was found to be tonic in 26.5% ( $n=4$ ), atonic in 5.9% ( $n=1$ ), and tonic-clonic in 70.6% ( $n=12$ ) of the patients. In the patients included in our study, absence, myoclonic and clonic seizures were not observed.

PON1 activity and MDA levels, both of which were investigated in our study, were statistically insignificant between the patient and control groups ( $P > 0.05$ ) (Table 2).

In the patient group, no statistically significant difference was noted for the relationship of PON1 activity to MDA levels and seizure types ( $P > 0.05$ ) (Table 3). There was no correlation between MDA levels and PON1 activity and age.

**Table 2** Relationship between PON1 activity and MDA levels in the patient and control groups

MDA levels and PON1 activity	Control (n=30), mean $\pm$ SD	Patient (n=30), mean $\pm$ SD	P-value
MDA (nmol/mL)	13.53 $\pm$ 3.56	14.34 $\pm$ 3.59	$> 0.05$
PON1 (U/L)	0.71 $\pm$ 0.17	0.65 $\pm$ 0.17	$> 0.05$

**Abbreviations:** MDA, malondialdehyde; PON1, paraoxonase 1; SD, standard deviation.

**Table 3** Relationship of MDA level and PON1 activity to seizure types

MDA levels and PON1 activity	Total (n=30), mean $\pm$ SD	Partial (n=13), mean $\pm$ SD	Generalized (n=17), mean $\pm$ SD	P-value
MDA (nmol/mL)	14.34 $\pm$ 3.59	14.41 $\pm$ 3.82	14.28 $\pm$ 3.53	>0.05 (0.928)
PON1 (U/L)	0.65 $\pm$ 0.17	0.69 $\pm$ 0.13	0.63 $\pm$ 0.20	>0.05 (0.345)

**Abbreviations:** MDA, malondialdehyde; PON1, paraoxonase 1; SD, standard deviation.

## Discussion

When studies related to epilepsy and oxidative stress were analyzed, it was found in some cases that oxidative stress parameters were high in epileptic patients, and on the other hand, in some studies, it was considered that the use of AEDs increased the levels of these parameters.<sup>17,18</sup>

In epilepsy patients, various oxidative markers and antioxidant defense mechanisms were studied, such as PON1, MDA, arylesterase, oxidative stress, and antioxidant capacity.<sup>18</sup> Varoğlu et al conducted a study on 61 epilepsy patients who were taking antiepileptic treatment.<sup>13</sup> When they compared PON, arylesterase, 8-hydroxyl guanine, and oxidized LDL levels in the patient and control groups, the levels of oxidative stress markers were high in all the antiepileptic treatment groups, whereas the PON levels were found to be significantly low. But as there was no patient group that had not undergone treatment for epilepsy in this study, it cannot be known whether this change in the levels of oxidative markers was due to disease or drugs.<sup>13</sup> The results of a study by Çevik et al showed that MDA levels in an epileptic patient group were significantly higher than those of the control group, and PON1 activity was found to be significantly low in the patient group. These results were thought to be related to AED use.<sup>19</sup>

In a study conducted by Tong et al on rats,<sup>20</sup> the animals were divided into two groups. Saline injection was given to the control group and 500 mg/kg of valproic acid injections (once a day) were administered to the other group. For observing the changes in rats, oxidative stress markers, such as TBA and hydroxyl peroxidase levels, were measured at 2, 4, 7, 10, and 14 days. To measure the levels of TBA, a MDA assay kit was used and the levels of MDA and TBA were increased. As a result of this study, on the 14th day of valproic acid injections, MDA levels were found to be significantly higher in the test group than in the saline-injected group.<sup>20</sup>

AEDs, at least in part, impair antioxidant systems. Some of them, especially from the older generation of AEDs (such as carbamazepine and valproic acid), may trigger oxygen-dependent tissue injury by several mechanisms.<sup>21</sup> Increased activities of superoxide dismutase and catalase and decreased

activities of glutathione peroxidase and glutathione reductase were observed in numerous studies of drug-naïve patients with epilepsy.<sup>22</sup> Also, it has been proposed that the long-term use of certain AEDs increases free-radical formation and causes oxidative damage in neurons.<sup>23,24</sup>

## Limitations

Our study has several limitations. One of them is that the number of patients was insufficient; therefore, the statistical analysis may not be satisfactory. On the other hand, we did not evaluate the relationship of MDA levels and PON1 activity with the frequency and duration of seizures.

## Conclusion

In conclusion, no statistically significant difference in the MDA levels between the patient and control groups was observed in our study. There were no differences in MDA levels and PON1 activity when comparing between the control group and the patient group that did not have antiepileptic treatment. Also, there was no correlation between MDA levels and PON1 activity and age in the patient group. Some articles in the literature reported that the levels of oxidative markers increased in epileptic patients. In the majority of studies, the impact of antiepileptic treatment on oxidative markers was ignored because the increase in oxidative markers might be related to the damage done to the liver by the antiepileptic treatment. We think that it might be helpful to include nonmedicated epilepsy patients in our future studies, because adding these patients can make the understanding of the effects of changes in oxidative markers on the pathophysiology of epilepsy easy.

## Disclosure

The authors report no conflicts of interest in this work.

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