ISSN: 2717-8161 RESEARCH ARTICLE

New Trends in Medicine Sciences

New Trend Med Sci 2022; 3(2): 98-104. https://dergipark.org.tr/tr/pub/ntms

Assessment of Serum Cystatin C Level Under Levetiracetam Monotherapy in Patients with Epilepsy

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Article History
Received 25 Apr 2022
Accepted 30 May 2022
Published Online 16 Sep 2022

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DOI: 10.56766/ntms.1108819

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Abstract: Cystatin C is a cysteine protease inhibitor that has been shown to have antiviral, antibacterial and neuroprotective efficacy. The aim of this study is to assess the effect of levetiracetam monotherapy on serum cystatin C levels in patients with epilepsy. 30 patients, who were diagnosed with epilepsy for the first time and subsequently started on levetiracetam monotherapy, were included in the study as the study group, whereas 30 healthy volunteers were included in the study as the control group. Serum cystatin C and creatinine levels of patients were measured twice, once before they were started on the levetiracetam treatment and once after the completion of six months of treatment levetiracetam, whereas the serum cystatin C and creatinine levels of the healthy control subjects were measured once. Both the pre-treatment and post-treatment creatinine levels of epilepsy patients were found to be statistically significantly higher compared to the creatinine levels of the healthy control subjects. The pre-treatment and post-treatment serum cystatin C levels of epilepsy patients were found to be lower compared to the serum cystatin C levels of the healthy control subjects, albeit not statistically significantly. Additionally, serum cystatin C levels of epilepsy patients were found to have increased after the completion of the levetiracetam treatment, even though not statistically significantly. The observed increase in the levels of cystatin C, a neuroprotective agent, following completion of levetiracetam treatment in epileptic patients may suggest a neuroprotective effect of levetiracetam.© 2022 NTMS.
Keywords: Levetiracetam; Epilepsy; Cystatin C.

1. Introduction

The majority of seizures suffered by adults are focal seizures. The first choice of preference in the treatment of epilepsy is medical therapy, and levetiracetam is one of the most frequently used types of antiepileptic drugs in the treatment of focal seizures. It is usually possible to control the seizures in a vast majority of patients with the administration of right medication. The mechanisms of action of antiepileptics are different, and levetiracetam binds to synaptic vesicular protein 2A and acts by inhibiting Ca^{2+} release (1, 2). Levetiracetam easily crosses the blood-brain barrier, and its cerebrospinal fluid (CSF) half-life is 3 times longer than its plasma half-life (3). Most of the levetiracetam is metabolized through non-hepatic hydrolysis and the rest is excreted by the kidneys without any change.

Cite this article as: Şimşek F and Aşkın S. Assessment of Serum Cystatin C Level under Levetiracetam Monotherapy in Patients with Epilepsy. *New Trend Med Sci* **2022**; 3(2): 98-104. Doi: 10.56766/ntms.1108819

Cystatins are proteinase inhibitors that are expressed in many regions in the mammalian body. Cystatins protect the organism against endogenous proteases released from lysosomes. Cystatin C (Cys C) is widely distributed in body fluids such as CSF, saliva, blood plasma, and urine (4). Cys C has been identified as an alternative endogenous marker indicating the renal functions and is more sensitive than creatinine (5, 6). It has been shown that the Cys C level in serum is strongly dependent on the glomerular filtration rate (7). Cys C has many biological functions and has growth-supporting, inflammation-reducing, antiviral and antibacterial properties (8). It has been shown that serum CysC level is decreased in Alzheimer's disease, and CysC expression is found in experimental animal models of induction of transient forebrain ischemia and epilepsy (9-11). In an experimental study conducted with animals, in which an electrically induced status epilepticus model was created, Cys C protein levels were shown to have elevated and remain elevated for a few months (12). In another study, it was shown that exogenously applied Cys C protects neuronal cells from death in a concentration-dependent manner, i.e., that it has a neuroprotective effect (13).

Due to its neuroprotective effect, Cys C may be beneficial in terms of controlling neurodegenerative diseases, such as epilepsy, and the development of new treatment strategies to that effect. Accordingly, in this study, it is aimed to assess the effect of levetiracetam on Cys C.

2. Material and Methods

This study was carried out as a prospective clinical study. The ethics committee approval required to conduct the study was obtained from the local ethics committee of Atatürk University Faculty of Medicine (04/19/ 30.05.2019). Informed consents were obtained from all patients and healthy control subjects who participated in the study.

2.1. Patient Selection

The study was conducted with 30 patients who were diagnosed with epilepsy for the first time, at Atatürk University neurology outpatient clinic, between June 2019 and May 2020. The 1981 International Classification of Epileptic Seizures of the International League Against Epilepsy (ILAE) was used for diagnosis. Patients with focal epilepsy or secondary generalized tonic-clonic seizures were included in the study. Hypertension, diabetes mellitus, liver and kidney dysfunction, history of stroke, mental retardation, central nervous system disease such as multiple sclerosis, history of malignancy, history of depression, organic brain lesion on cranial magnetic resonance imaging, who presented with status epilepticus in the last two weeks patients with a history of infection and under 18 years of age were excluded. The demographic characteristics of the patients, types of the seizures the patients suffered, as well as the frequencies in which they suffered these seizures were recorded. Patients were started on levetiracetam (500 to 2000 mg/day) monotherapy following their diagnosis with epilepsy and were followed up for a minimum of 6 months thereafter.

2.2. Control Subjects

Control subjects were selected from among healthy volunteers of matching gender and of an an age group comparable to the patients.

2.3. Collection of serum samples

Venous blood samples were collected from the patient and control groups following a 12-hour fasting period. Blood samples were taken from the patients twice, that is once before they were started on the levetiracetam treatment and once after the completion of six months of treatment levetiracetam, provided that they were seizure-free for a period of at least 2 weeks before blood-sampling. On the other hand, blood samples were taken from the control group once. Blood samples taken were let rest at room temperature for half an hour, and were then centrifuged. Subsequently, centrifuged serum samples were placed in epandorf tubes, and preserved at -80 °C until they were studied.

2.4. Cystatin C test

Cystatin C levels were tested via the Enzyme-Linked Immunosorbent Assay (ELISA) method using a respective ELISA kit (SunRed, Lot: 201-12-1105, China). Test results were calculated in ng/ml in line with the manufacturer's instructions.

2.5. Statistical Analysis

All statistical tests were performed using the SPSS (IBM Statistical Package for the Social Sciences version 20) Software. Kolmogorov-Smirnov test was used to analyzed whether the research data conformed to the normal distribution. Categorical data were expressed as n (%), whereas numerical data were expressed as Mean±Standard Deviation (SD) in case of data that conformed to the normal distribution, and as median (minimum-maximum) in case of data that did not conform to the normal distribution. Student’s t-test was used for the analysis of numerical data that conformed to the normal distribution, whereas Mann-Whitney test was used for numerical data that did not conform to the normal distribution. Additionally, Wilcoxon test was used to analyze the data that did not conform to normal distribution in paired groups. Furthermore, Spearman's correlation analysis was used to determine the relationship between two numerical values that do not conform to the normal distribution. It has been accepted that the probability (p) values of ≤ 0.05 indicate statistical significance.

3. Results

There were 16 females and 14 males in both patient and control groups. The mean age of the patients group was calculated as 31.87±5.69 years, and the mean age of the control group was calculated as 31.47±5.78 years. The

mean age of the gender-matched groups was similar (p=0.790). The creatinine levels were found to be between 0.54 and 0.95 mg/dL, thus normal, in both the patient and control groups. The creatinine levels of the patient group both before and after the levetiracetam monotherapy were found to be statistically significantly higher than the creatinine levels of the control group (p<0.001, p=0.001, respectively). The post-treatment creatinine levels of the patient group were found to be lower than the pre-treatment creatinine levels of the patient group, albeit not statistically significantly (p=0.288). The pre-treatment and post-treatment serum Cys C levels of the patient group were found to be lower compared to the serum Cys C levels of the control group, albeit not statistically significantly (p=0.141, p=0.631, respectively). Additionally, serum Cys C levels of the patient group were found to have increased after the completion of the levetiracetam treatment, even though not statistically significantly (p=0.147) (Figure 1). The demographic characteristics and laboratory test results of the patient and control groups are given in Table 1.

Table 1: Clinical, demographic and laboratory data of the patient and control group.

	Patient	Control	p
Age±SD	31.87±5.69	31.47±5.79	0.79
Sex n(%)			
Female	16 (53.3)	16 (53.3)	1
Male	14 (46.7)	14 (46.7)	
Seizure type n(%)			
Focal Seizure	16 (53.3)	-	
Secondary Generalized Seizure	14 (46.7)	-	
Seizure frequency/6 month, med (min-max)	2 (1-6)	-	
LEV Drug Dose (mg/day), Med (min-max)	1000 (500-2000)	-	
Cystatin C (ng/mL), Med (min-max)			
Before LEV	3.281 (0.21-122.6)	6.38 (1.10-148.6)	0.141
After LEV	5.277 (0.25-155.51)		0.631
p	0.147		
Creatinine (mg/dL), Med (min-max)			
Before LEV	0.7 (0.5-0.9)	0.55 (0.4-0.8)	**<0.001***
After LEV	0.65 (0.4-0.9)		**0.001****
p	0.288		

LEV: Levetiracetam, SD: standard deviation, min: minimum, max: maximum, med: median, *It shows a significant difference in creatinine levels between the patient and control group before levetiracetam treatment, **It shows a significant difference in creatinine levels between the patient and control group after levetiracetam treatment.

Cystatin and creatinine levels before and after treatment according to seizure type are given in Table 2. There was no significant difference between the pre-treatment and post-treatment Cys C levels of the patient group with focal seizures and the patient group with secondary generalized seizures (p=0.983, p=0.114, respectively). Cys C levels of patients with focal seizures increased after treatment compared to pre-treatment, and there was a significant difference (p=0.049). There was no significant difference in Cys C levels before and after treatment in patients with secondary generalized seizures (p=0.975). Additionally, no significant relationship was found between the frequency of seizures and the serum creatinine and Cys C levels (r=-0.004, p=0.982; r=0.016, p=0.931, respectively). There was no significant correlation between the pre-treatment and post-treatment serum Cys C and creatinine levels (r=-0.066, p= 0.618; r= 0.009, p= 0.945, respectively). Dosage of the drug used in the patient group following the levetiracetam treatment was found to be correlated with the serum Cys C and creatinine levels in the negative direction, albeit not statistically significantly (r=-0.349, p=0.059; r=-0.199, p=0.292, respectively) (Figure 2). A statistically significantly positive correlation was found between the drug dosage and the frequency of seizures (r=0.541, p=0.002).

Table 2: Cystatin and creatinine levels before and after treatment by seizure type.

	Focal Seizure (n=16)	Secondary Generalized Seizure (n=14)	p
Before LEV, med (min-max)			
Cystatin (ng/mL)	2.83 (0.35-51.47)	3.72 (0.21-122.60)	0.983
Creatinine (mg/dL)	0.70 (0.50-0.90)	0.71 (0.50-0.90)	0.253
After LEV, med (min-max)			
Cystatin (ng/mL)	9.52 (0.25-155.51)	3.56 (0.44-39.83)	0.114
Creatinine (mg/dL)	0.67 (0.40-0.90)	0.65 (0.60-0.90	1

LEV: Levetiracetam, min: minimum, max: maximum, med: median.

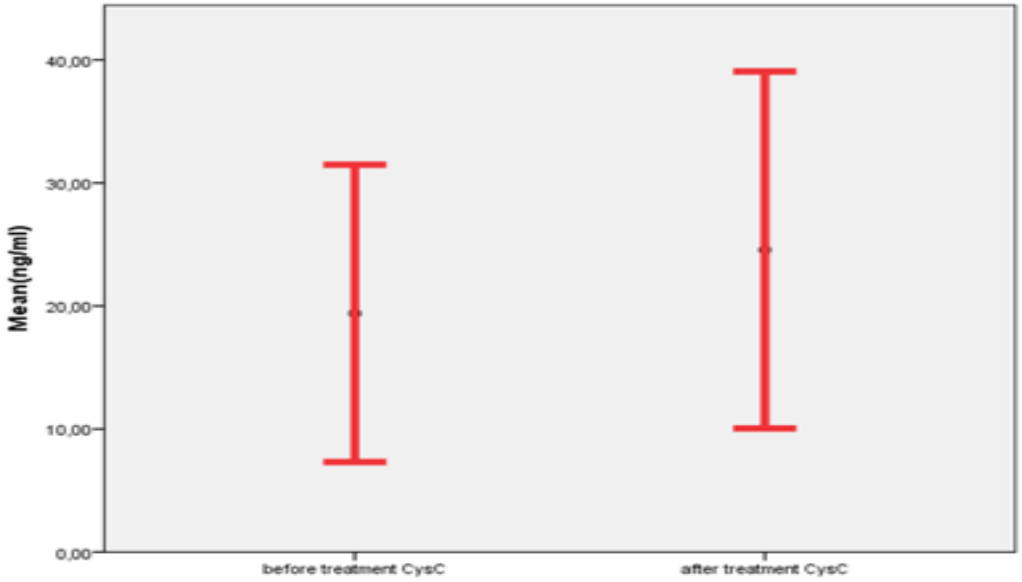

Figure 1. Mean serum cystatin C levels of patients before and after levetiracetam treatment.

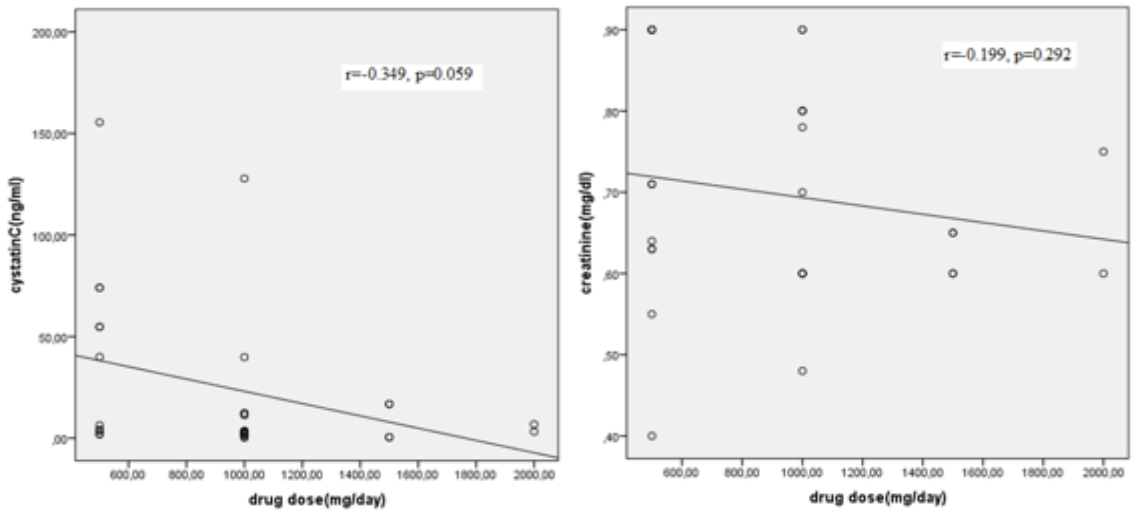

Figure 2. Levetiracetam drug dose correlation with serum cystatin C and creatinine levels. X-axis represents the levetiracetam drug dose in mg/day, whereas y-axis represents the serum cystatin C levels in ng/ml (left picture) and creatinine levels in mg/dl (right picture). Each point on the graph represents one patient. Some dots are darker because some patient values are the same. Dark dots represent more than one patient. There was a negative but insignificant relationship between the drug dosages and serum cystatin C and creatinine levels of the patients treated with levetiracetam (r=-0.349, p=0.059; r=-0.199, p=0.292, respectively).

4. Discussion

The creatinine levels of epilepsy patients both before and after the levetiracetam monotherapy were found to be statistically significantly higher than the creatinine levels of the healthy control subjects. Even though not statistically significant, a decrease was observed in the creatinine levels and an increase was observed in the Cys C levels, following the completion of the levetiracetam monotherapy. Levetiracetam was used at higher doses in patients with higher seizure frequency, and it was observed that as the dosage of the levetiracetam was increased, the Cys C levels decreased, however no statistically significant relationship was found between the two.

The seizures increase the consumption of adenosine triphosphate (ATP), since the energy need of the metabolism increases during seizures. Creatine is an important source of energy in that it accelerates ATP energy production by increasing the creatine phosphate pool to meet the high energy demand of the brain tissue during seizures (14, 15). Creatine phosphate contributes to the energy production by providing high energy phosphate molecules to adenosine diphosphate (ADP) during the production of ATP (Creatine phosphate+ ADP↔Creatinine+ATP). Thus, it is an expected outcome that the use of creatine phosphate during the above described ATP production would lead to a decrease in the creatine phosphate levels and an increase in the creatinine levels. Lee et al. reported to have observed a decrease in the creatine phosphate levels and an increase in the creatinine levels in their study conducted in the form of an experimental epilepsy model (16). In comparison, similar results were found in this study, since both the pre-treatment and post-treatment creatinine levels of the epilepsy patients were found to be statistically significantly higher than the creatinine levels of the healthy control subjects.

It was reported in various studies conducted in the form of experimental animal models available in the literature that the Cys C levels increased following status epilepticus and then decreased over time (11, 12). Such results suggest that the increases in Cys C levels occur not due to the neurodegenerative process, but as part of a cellular repair response during the acute period. In comparison, in this study, both the pre-treatment and post-treatment Cys C levels of the patients were found to be lower than the Cys C levels of the healthy control subjects. Additionally, Cys C levels of the patients were observed to have increased after treatment with levetiracetam, which were observed to have decreased however with increasing doses of levetiracetam. High doses of levetiracetam have been used in patients whose seizures cannot be controlled and who have more frequent seizures. The fact that a decrease was observed in the Cys C levels with higher doses of levetiracetam seems to be due to the increase in the frequency of seizures, and not to the increase in the drug dosage. In other words, the decrease in the Cys C levels suggests the neurodegenerative process. Along these lines, it has been shown in an experimental study that the exogenous Cys C protects neuronal cells under neurotoxic stimuli from death in a concentration-dependent manner (13). Accordingly, the increase in Cys C levels following levetiracetam treatment may suggest that levetiracetam has a neuroprotective effect. Cys C is the predominant cysteine protease inhibitor in the cerebrospinal fluid (CSF) and its levels in CSF are 5.5 times higher than in plasma (17). Accordingly, more meaningful results could have been obtained as a result of this study, were the Cys C levels to be checked in CSF instead of venous blood sample.

It has been demonstrated in various studies available in the literature that levetiracetam has less side effects on cognitive functions than the other antiepileptic drugs. In a study of healthy volunteers, carbamazepine had the greatest effect on neuropsychological tests and levetiracetam the least effect when compared to levetiracetam, carbamazepine or oxcarbazepine. In another study with healthy volunteers, levetiracetam was found to cause less cognitive deficits compared to carbamazepine (18, 19). The reason for levetiracetam having less side effects on cognitive functions compared to other antiepileptics may be attributed to the increase it causes in the level of Cys C, a neuroprotective agent. As a matter of fact, there are studies, in which Cys C levels were found to be decreased in patients with Alzheimer's disease (20). Findings indicating a decrease in Cys C levels in case of patients with diseases affecting cognitive functions, such as Alzheimer's disease, suggests that Cys C has a significant effect on cognitive functions.

It was reported in various studies that the risk of depressive symptoms were three times higher in individuals with renal impairment than in individuals without renal impairment (21). There are other studies, in which high serum Cys C levels and impaired kidney functions were found to be associated with depressive symptoms in older adults (22-24). In comparison, in this study, an increase was observed in Cys C levels following the levetiracetam treatment, which may explain the emergence of depressive symptoms observed in other studies available in the literature in relation to the use of levetiracetam following the treatment. Nonetheless, in this study, there were no patients with depressive symptoms both before and after the treatment.

It has been shown that creatinine levels vary depending on age, gender and body mass, unlike Cys C levels, which were not found to have been affected by these parameters (25). This result suggests that Cys C can be used with more ease and as a more reliable parameter in the follow-up of kidney functions of patients. The finding of lack of a significant difference between the post-treatment Cys C levels of the patients and the Cys

C levels of the healthy control subjects in this study supports the hypothesis that levetiracetam has no serious side effects on renal functions.

This study had some limitations, such as the relatively small number of the patients included in the study, the fact that it was based on a relatively shorter follow-up period and that different doses of levetiracetam were administered to each patient.

5. Conclusions

The fact that Cys C levels increased after treatment compared to pretreatment in epilepsy patients suggests that levetiracetam may have a neuroprotective effect. Levetiracetam, which increases Cys C levels, may have positive effects such as protecting cognitive functions and slowing down the neurodegenerative process, as well as negative effects such as increasing depressive symptoms. In order to provide more meaningful data on the relationship between changes in Cys C levels and drug doses, studies with larger numbers of patients and including higher drug doses are needed.

Limitations of the Study

The limited number of cases the lack of seizure control in all patients and the different seizures frequencies are the limitations of the study.

Acknowledgement

None

Conflict of Interests

The authors declare no conflict of interest.

Financial Support

This study recieved no financial support.

Author Contributions

Conceived and designed the analysis: F, SA. Contributed data or analysis tools: F, SA. Performed the analysis: F, SA. Wrote the paper: F, SA.

Ethical Approval

Ethics committee aproval was received fort his study from the study from the ethics committee of Atatürk University.

Data sharing statement

All data relevant to the study are included in the article.

Informed Consent

Written informed consent was obtained from all patients at the of the study.

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