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Assessment of Serum GLO1 Levels In Relation to Epilepsy

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Abstract

Background: Epilepsy is the most common neurological disease of childhood and adolescence. Methylglyoxal, an endogenous byproduct of glucose metabolism and a reactive carbonyl species, is a novel inhibitor of epileptic seizures. Methylglyoxal is metabolized by the enzyme glyoxalase 1. We aimed to investigate serum glyoxalase 1 levels between a group of newly diagnosed pediatric epilepsy patients and a healthy control group.

Material and Method: A total of 34 children from both sexes, aged between 1 and 17 years with newly diagnosed epilepsy and 21 healthy controls were included in the study irrespective of the epilepsy subtype. Informed consent was obtained from parents or guardians of all patients before initiation of the study. 5 cc venous blood sample was collected from each patient into plain biochemistry tubes under sterile conditions. Glyoxalase 1 levels were measured using ELISA methodology.

Results: Comparison of the gender distribution of 34 pediatric patients and 21 healthy controls showed that 61.8% of the epileptic patients were boys and 38.2% were girls. Control group included 66.7% males and 33.3% females. Body mass index (BMI) comparison of both groups showed a BMI of 23.38±9.66kg/m² (mean± SD) among epileptic patients and 20.31±3.94kg/m² (mean± SD) in healthy controls, with no statistically significant difference between groups (p=0.10). Comparison of serum glyoxalase 1 levels between groups showed a mean glyoxalase 1 level of 31.51ng/ml (2.08-132.68, min-max) in epileptic patients and 19.25ng/ml (11.74-87.97 ng/ml, min-max) in healthy controls. There was a statistically significant difference between glyoxalase 1levels of the study groups (p= 0.02).

Conclusion: The study groups did not show any significant difference in gender, BMI and age. However, serum glyoxalase 1levels were statistically significantly different between the groups. Based on our findings, we concluded that there is a positive correlation between serum glyoxalase 1 levels and epilepsy. We humbly hope that our results might contribute to current literature for future development of new treatment strategies for epilepsy patients using glyoxalase 1 inhibitors.

Keywords: Epilepsy; Pediatric Patients; Glyoxalase 1; Methylglyoxal.

Introduction

Epilepsy is the most common neurological disease of childhood and adolescence (1-4). The prevalence of childhood epilepsy is approximately 0.5 percent. About 50 percent of epileptic seizuresstart before the age of 5 years and 75% before the age of 20 years (5-7).

Serdaroglu et al., reported an epilepsy prevalence of 0.8% among Turkish children between the ages of 0 and 16 years. In their study, 55.2% of the subjects with epilepsy were grouped as generalized, 39% as partial, and 5.8% as unidentified (8-9). Epilepsy affects 3-5% of children and adolescents at some developmental stages of their lives (7).Two out of every three epilepsy cases start during the first two decades of life when development takes place and epilepsy can adversely affect children's development and cause distress in their families' lives. At the time when epilepsy diagnosis is established in a child, he/she faces with several psychological stressors in addition to challenges associated with their health condition.

Epilepsy is a neurological disease characterized by seizures (9). An epileptic seizure is a transient occurrence of signs and symptoms due to abnormal excessive or synchronous neural activity in the brain (9). A multitude of causes including genetic factors, environmental factors and illnesses have been implicated in the etiology of epilepsy (10). Antiepileptic agents may not be effective in all types of epilepsy or for all patients and may

produce adverse effects (11-13).

Investigating biological pathways and novel genes underlying epilepsy may enable the development of new therapeutic targets and provide valuable insight to the disease pathogenesis. Methylglyoxal (MG) is a novel inhibitor for suppression of epileptic seizures (14-15). Methylglyoxal is an endogenous byproduct of glucose metabolism and a reactive carbonyl species that is generated by the non-enzymatic fragmentation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate(14-15). Studies have shown that MG activates GABAA receptors at physiological concentrations (16-17). GABAA receptors are the major regulators of fast inhibitory synaptic transmission in the central nervous system(18).MG is metabolized bythe enzyme glyoxalase 1 (GLO1).GLO1expression and activity regulate endogenous MG concentrations in the brain(16). Few studies in animals demonstrated that increased GLO1 levels result in increased severity of epileptic seizures by reducing MG concentration in the brain (19-21).

These findings suggest that inhibiting the enzymatic activity of GLO1 and reducing the clearance of GABAergic MG may be a novel approach for prevention of epileptic seizures. Simonato et al., stated that identification of epileptogenic mechanisms may both contribute to prevention of epilepsy and development of new treatment strategies (23-25).

With these data in mind, we aimed to investigate serum GLO1 levels in pediatric epilepsy patients, which have not been formerly studied in this population by comparing the levels between a group of newly diagnosed epilepsy patients and a healthy control group.

Material Method

The study included a total of 34 children from both sexes, aged between 1 and 17 years who presented to SANKO University Sani Konukoglu Research and Practice Hospital and had newly diagnosed epilepsy(confirmed through routine workup including EEG and blood biochemistry) and 21 sex- and –age-matched healthy controls.

Epilepsy grading of the study patients was performed based on the International League Against Epilepsy (ILAE) 1981 classification (26).This study was approved by the Ethics Committee of SANKO University.

Exclusion criteria of the study were as follows:

- Patients diagnosed with diabetes and patients taking antidiabetic drugs for any reason,
- Patients with abnormal MRI results,
- Patients receiving multiple therapies for epilepsy,
- Patients on regular medications for a chronic disease,
- Patients diagnosed with depression or anxiety.

Consent was obtained from parents or guardians of all patients before initiation of the study who were informed about the nature of the study.All procedures were conducted in accordance with the ethical standards of the relevant committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Routine blood investigations and EEG were performed for all study patients. 5 cc venous blood sample was collected from each patient into plain biochemistry tubes under sterile conditions. Taken into anticoagulant-free, non-gel strait tubes, blood samples were centrifuged at 4000 rpm for 10 minutes and then transferred to Eppendorf tubes and stored at -80°C. These samples were kept overnight at 4°C in a refrigerator before the measurements and brought to room temperature 2 hours before being analyzed using the ELISA method.

Human GLO1 levels were measured quantitatively according to the manufacturer's instructions of kits with the Catalog Number 201-12-9603 (Shanghai Shanghong (SRB) Biotechnology, Shanghai /China). The analysis was performed using the sandwich enzyme immunoassay technique. The test hasa sensitivity of 0.438 ng/mL and a lower detection limit of 0.5 ng/mL.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS). Kolmogorov-Smirnov test showed a normal distribution of BMI and abnormal distribution of age and plasma GLO1 levelsamong the study and control groups.

Mann-Whitney U test was used for variables with a non-normal distribution and Student's t test was used for variables with a normal distribution. The $\chi 2$ test was used to analyze gender difference between groups. A p value less than 0.05was considered statistically significant.

Results

Gender distribution of 34 pediatric patients and 21 healthy controls was compared using the chi-square test. Test results showed that 61.8% of the epileptic patients were boys and 38.2% were girls. Gender distribution of the control group included 66.7% males and 33.3% females. No statistically significant difference was found between both groups in gender distribution (p=0.71;Table1).

BMI (body mass index) comparison of both groups showed a BMI of 23.38±9.66kg/m² (mean± standard deviation) among epileptic patients and 20.31±3.94kg/m² (mean± SD) in healthy controls, with no statistically significant difference between groups (p=0.10; Table1). Age comparison of the groups revealed that epileptic patients had a median age of 5 years (min:1-max:17) and control group had a median age of 6 years (min:2-max:17). There was no statistically significant age difference between the groups (p=0.18; Table1).

Comparison of serum GLO1 levels between groups showed that mean GLO1 level was 31.51ng/ml (2.08-132.68, min-max) in epileptic patients and19.25ng/ml (11.74-87.97ng/ml, min-max)in healthy controls (Table1). There was a statistically significant difference between GLO1 levels of the study groups (p= 0.02).

As can be seen from Table 2, there was no gender-related difference between serum GLO1 levels of epileptic patients and healthy controls (Epileptic patients, p=0.18;controls, p=0.8).

Table 1. Comparison of study variables between epileptic patients and controls.

	Epilepsy (n=34)	Control (21)	p
Gender Male/Female	21/13 61.8% / 38.2%	14/7 66,7% / 33,3%	*0.71
BMI (kg/m²) mean± standard deviation	23.38±9.66	20.31 ± 3.94	**0.10
Age (years) Median (min-max)	5(1-17)	6(2-17)	0.18
GLO 1 (ng/ml) Median (min-max)	31.51 (2.08-132.68)	19.25 (11.74 - 87.97)	0.02

n = number of individuals. *The Chi-square test was used for statistical analysis. ** Based on Student's t test.

Table 2. Association between GLO1 levels and gender among epileptic patients and controls.

GLO 1 (ng/ml)			
	Boys (n=21)	Girls (n=13)	p value
Epilepsy group (n=34)	40.17 (15.05-121.63)	18.62 (2.08-132.68)	0.18
Control group (n=21)	19.1 (11.74-84.27)	19.25 (16.09-87.97)	0.8

n = number of individuals. Mann-Whitney U test was used for statistical analysis.

Discussion

Epilepsy is a complex disease with a predisposition towards seizures. There are numerous barriers to the successful treatment of epilepsy.Moreover, the pathophysiological basis of epilepsy remains elusive. Thus, research efforts have focused on identification of novel genes and biological processes underlying epilepsy with the ultimate aim to develop new therapeutic agents (9,11,13).

GLO1is an enzyme that plays a role in the metabolism of MG(16).

Recently, findings have linked increased GLO1 levels to several behavioral patterns including psychiatric diseases (anxiety, depression) and neuropathic pain (17,27).

In 2009, Williams et al. found a positive association between increased GLO1 levels and anxiety in murine models, showing that mice with increased GLO1 expression displayed anxiety-like behaviours.They also demonstrated a relation between GLO1 levels and locomotor activity, motor coordination, learning and memory(28).

Apart from anxiety, studies in murine models of several diseases including neuropathic pain, depression and epilepsy found an association between elevated GLO1 levels and disease progression (21,27). For example, increased GLO1 expression showed a significant association with increased susceptibility to epileptic seizures in studies on recombinant inbred strains of mice as well as transgenic mice (21).

A positive correlation was observed between depressive behaviours and GLO1 levels in a study using tail suspension test in mice (21).

In an in vivo animal study in 2013, Distler et al. found that endogenous MG reduced the seizure susceptibility and severity and suggested that inhibition of GLO1 might be a novel therapeutic approach for epilepsy through increased endogenous MG action (21).

Similarly, Palmer et al. found a positive correlation between increased GLO1 release and susceptibility to epileptic seizures(17,29). Moreover, they observed increased MG concentration and reduced seizure frequency by GLO1 inhibition in mice with pilocarpine-induced convulsions (17,29).Here, one important consideration is that MG concentration should be in the physiological range to activate GABAA receptors (30).

In the present study, we first compared demographic characteristics of patient and control groups. BMI comparison between the groups did not reveal any statistically significant difference (p=0.10). Mean BMI (± SD) values were not significantly different in both groups (23.38 ± 9.66 for epilepsy group versus 20.31±3.94 for controls). In one study, increased dicarbonyl stress was associated with reduced GLO1 levels and decreased MG metabolism in obesity(31). In our study, mean BMI values of both groups were close to normal.

In this study, we compared serum GLO1 levels between 34 newly diagnosed, treatment-naïve epilepsy patients and 21 healthy controls. Epileptic patients had higher GLO1 levels (31.51 ng/ml) versus control group (19.25 ng/ml; p=0.02).
Some recent studies have found that gender differences exist in the epidemiology and pathophysiology of epilepsy (32-33). However, we did not observe any difference in gender distribution between the groups (p=0.71).

When we looked at whether there was a gender-related difference in GLO1 levels between groups, it was interesting to see that epileptic female patients(18.62 ng/ml) showed lower GLO1 levels compared to their male counterparts (40.17 ng/ml). However, no significant difference was found between their values (p=0.18). We did the same comparison for control subjects and found similar values for boys (19.1ng/ml) and girls (19.25ng/ml; p=0.8). This finding demonstrates the positive correlation between GLO1 and epilepsy.

Our study has a number of limitations. In particular, small

sample size is an important limitation and may have precluded demonstration of less clear associations between studied variables. Therefore, we believe that data with no proven associations between variables should not be generalized. In addition, we were not able to measure serum MG levels. If we had the chance to correlate GLO levels with serum MG levels, this could possibly add value to our findings.Also, we could not perform stratification of GLO1 levels by epilepsy subtypes. In the future, we wish to design a more detailed study involving such stratification.

A major aspect of our study is that for the first time, serum GLO1 levels were investigated in a sample of pediatric epilepsy patients in comparison to controls and we observed a significant difference between groups. We humbly hope that our results might contribute to current literature for future development of new treatment strategies for epilepsy patients using GLO1 inhibitors.

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