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# Effects of levetiracetam and valproic acid treatment on liver function tests, plasma free carnitine and lipid peroxidation in childhood epilepsies

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ABSTRACT

*Background and aims:* The relationship between anti-epileptic usage and oxidative damage has not yet been clearly understood. In our study, we investigated oxidative stress parameters, carnitine levels, liver function tests (LFT) and their relationship in epileptic children treated with valproic acid or levetiracetam.

*Method:* LFTs, serum free carnitine and oxidative damage markers and their relations with each other were determined in patients who are on valproic acid or levetiracetam treatment at least for 6 months. 25 patients on therapeutic doses of valproic acid, 26 patients on therapeutic doses of levetiracetam and 26 healthy volunteers as controls were included. LFTs, ammonia, carnitine, lipid peroxidation biomarker malondialdehyde (MDA) and a sensitive marker of DNA damage, 8-hydroxy-2-deoxyguanosine (8-OHdG) levels were measured. Results of patients are compared to healthy controls. The data is evaluated with IBM SPSS Statistics 22.0.

*Results:* Ammonia and MDA levels were elevated in patients using levetiracetam; 8-OHdG levels were elevated in both patient groups. Carnitine levels were significantly low in patients under valproic acid therapy, however they were not found to be correlated with MDA, 8-OHdG or LFTs. MDA showed positive correlation with ammonia and 8-OHdG in the levetiracetam group.

*Conclusion:* We did not observe hepatotoxicity in patients under therapeutic doses of valproic acid. However, epileptic children under therapeutic doses of levetiracetam showed significantly elevated levels of MDA and 8-OHdG, which is supportive for oxidative damage under levetiracetam therapy. This result was observed for the first time in childhood epilepsies and further studies are needed to understand its mechanism.

## 1. Introduction

The prevalence of childhood epilepsies changes in different parts of the world. It is shown to be between 3,2–5,5/1000 children in developed countries, whereas a much wider range and higher prevalence is observed in developing countries as 3.6–44/1000 children affected (Camfield and Camfield, 2015). The use of drugs in the management of epilepsy is accompanied by adverse effects such as idiosyncratic reactions, dose-related neurocognitive effects and complications of long-term use such as organ insufficiency (Baker et al., 1997; French and Pedley, 2008). While selecting the appropriate treatment, adverse effect profile is solemnly considered as comparative studies have shown an equivalency among different drugs (Cramer et al., 2010; Mattson et al., 1985; Schmidt, 2011).

Valproic acid (VPA), which has been widely used since 1960's, is one of the most common agents to treat epilepsy (Tomson et al., 2016). Levetiracetam (LEV), on the other hand, was approved by FDA for children under 16 years of age in 2006. Shortly after approval, levetiracetam also became one of the most common used anti-epileptic drug (Weijenberg et al., 2015). Augmented oxidative stress and damage may occur in human body due to various circumstances. Not only epilepsy itself, but also anti-epileptic drug usage has been shown to cause oxidative damage (Yuksel et al., 2000; Patsoukis et al., 2004). Lipid peroxidation, which is determined by measuring malondialdehyde (MDA)

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levels, is one of the most frequently used method for determining oxidative damage in cells (Romero et al., 1998). Lately, 8-hydroxy-2'-deoxyguanosine (8-OHdG), was confirmed as a novel and a more sensitive marker of oxidative damage. 8-OHdG is produced due to hydroxylation of guanosine and can be measured in serum or urine samples (De Martinis and de Lourdes Pires Bianchi, 2002; Cooke et al., 2003). Antioxidants, such as L-carnitine have a protective role, in terms of acting as neuromodulatory, neuroprotective and cytoprotective and are both synthesized in *vivo* and/or taken by nutrition (Binienda and Ali, 2001). The intake of VPA was shown to gradually lower the level of L-carnitine (Zelnik et al., 1995).

Despite the efforts, the relationship between anti-epileptic medications, oxidants and anti-oxidant system has not yet been clearly understood (Abdel-Wahab et al., 2015; Schulpis et al., 2006; Natarajan et al., 2006). To address this issue, we aimed to investigate the outcome of VPA and LEV as two commonly used agents in childhood epilepsies, on oxidative stress parametres, carnitine levels and liver function tests.

## 2. Materials and methods

We recruited patients prospectively from the outpatient epilepsy clinic of the department of pediatric neurology, Ankara University Medical Faculty. Studies were performed with the approval of ethics committee of the medical faculty (decision number: 13-626-16). The study was also approved and supported by Scientific Research Projects Commission of Ankara University.

From October 2016 to April 2017; 51 pediatric patients who were diagnosed with generalized epileptic seizure, followed up at the Ankara University Medical Faculty Pediatric Neurology department who fulfilled the selected criteria are included in the study (Table 1). The diagnosis of epilepsy was made by the pediatric neurologists according to the criteria of International League Against Epilepsy (ILAE) (Fisher et al., 2014). None of the cases had any identifiable etiology. All selected patients were under therapeutic doses of VPA and LEV for a minimum of 6 months and underwent detailed clinical investigations to exclude the presence of any medical comorbidity. All patients underwent central nervous system magnetic resonance imaging (MRI) to exclude accompanying possible abnormalities.

A group of 26 healthy children served as controls. All control cases had normal anthropometric measurements including weight, height and head circumference, and all of them were in normal developmental status with respect to the peers of their age. All control cases underwent detailed clinical investigations to exclude the presence of any medical comorbidity. A detailed examination was performed in which demographic characteristics and medical history were questioned. Patients under any drugs, with recent or acute illnesses, chronic medical conditions or under any kind of stress and younger than 2 years were not included as control group.

Written informed parental consent was obtained for both research and publication of the results of each patient and control case who were included in the study.

### 2.1. Biochemical measurements

Venous blood sample and dry-blood sample were drawn from study participants in the morning, after a 12-h fasting period and before taking their medications (for therapy groups). All blood samples were delivered to the laboratory within an hour. The blood sample then divided into two groups: one group [a basic biochemistry tube for liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) and an EDTA tube for ammonia] was assayed within one hour at the central biochemistry laboratory. Second group was allowed to clot at room temperature and centrifuged at 3000 rpm for 10 min; serum was collected and stored at −80 °C until assay. Reagents for biochemical analysis (MDA and 8-OHdG) were purchased from Cayman Chemical (Ann Arbor, Michigan, USA) and were of analytical grade or the highest grade available. The commercial enzyme linked immunosorbent assay (ELISA) kits were used to determine the level of 8-OHdG in serum samples, according to the manufacturer's instructions. MDA level was measured using the thiobarbituric acid-reactive substances (TBARS) method according to manufacturer's instructions. Dry-blood samples retained on the Guthrie paper are dried for 24 h at room temperature without any contact with light and were refrigerated at 4 °C in sealed bags of low gas permeability until assayed. The samples were transferred via cold chain to Delta Laboratory (Ankara, Turkey) and assayed with immuchrom kits in Sciex 3200 device using mass spectrometry.

### 2.2. Data analysis

All statistical tests were performed using IBM SPSS software (version 22, SPSS, IBM Corp., Armonk, New York, USA). Quantitative variables are expressed as the mean ± standard deviation (SD), and qualitative variables as numbers and percentages. For categorical variables Pearson's Chi-squared test and Fisher's exact Chi-squared test were used to compare the patient and control groups. The Levene test was used to test the homogeneity in the distribution of study data. Comparison of homogenous data was performed by one-way analysis of variance (ANOVA) and non-homogenous data was analyzed via Welch test. When a significant difference was found between groups, Tukey's post hoc HSD test was applied. Pearson correlation analysis was used in the determination of the relationship between two numerical values with normal distribution. Values of $p < 0.05$ were accepted as indicative of significance.

## 3. Results

Samples are examined in three groups. Group 1; 26 patients under LEV therapy, group 2; 25 patients under VPA therapy and group 3; 26 children as control group. No significant difference of mean age or gender inequality is observed among groups ($p > 0,05$; $p = 0,148$) (Tables 2 and 3).

Liver function tests in both therapy groups and in control group

**Table 1**
Inclusion and exclusion criteria for patients under VPA and LEV therapy.

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Patients older than 2 years of age and diagnosed with generalized epileptic seizure according to ILAE | Patients with structural central nervous system abnormalities, intracranial infections, head trauma, birth trauma, neonatal insults or hypoxic ischemic encephalopathy. |
| Patients under a therapeutic dose of VPA (20-60 mg/kg/day) and LEV (20-60 mg/kg/day) at least for 6 months | Children with cerebral palsy, metabolic disorder affecting the CNS, neurobehavioral disorder, neurodegenerative disorder, and drug-induced neurological manifestation |
| Patients with normal anthropometric measurements (weight, height and head circumference) and in a good nutritional status, within normal developmental stage according to peers | Patients with poor compliance, low/high doses, or sub-therapeutic range of AEDs or under polytherapy |
| | Cases with abnormal anthropometric measurements, feeding difficulties or malnutrition, with developmental delay or taking antioxidant drugs/vitamins |

**Table 2**
Gender distribution among groups. No significant difference among groups was observed (p = 0,148).

| | | Gender | | Total |
|---|---|---|---|---|
| | | Girls | Boys | |
| Levetiracetam Group | Number | 11 | 15 | 26 |
| | Percentage | 42.3% | 57.7% | 100.0% |
| Valproic Acid Group | Number | 14 | 11 | 25 |
| | Percentage | 56.0% | 44.0% | 100.0% |
| Control | Number | 18 | 8 | 26 |
| | Percentage | 69.2% | 30.8% | 100.0% |

**Table 3**
Age distribution among groups.

| | | Number | Age, months (mean) | Variable | p value |
|---|---|---|---|---|---|
| Groups | Levetiracetam | 26 | 113.04 | Valproic acid | .946 |
| | | | | Control | .819 |
| | Valproic Acid | 25 | 117.84 | Levetiracetam | .946 |
| | | | | Control | .957 |
| | Control | 26 | 122.12 | | |

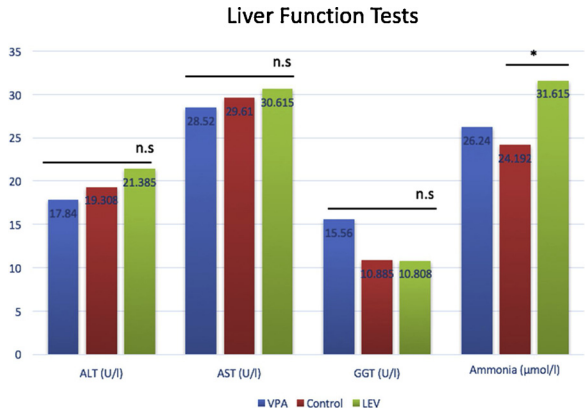


**Fig. 1.** Transaminase and ammonia levels of the therapy groups and control group. * Symbolizes statistically significant difference. ALT, AST and GGT levels were in normal range and showed no significant difference in both groups. (VPA; p = 0.832, p = 0.887, p = 0.295, respectively. LEV; p = 0.681, p = 0.903, p = 1.000 respectively.) Ammonia levels in LEV group was significantly higher compared to control group (p = 0.035) whereas VPA showed no significant difference (p = 0.768).

were in normal range (ALT < 35 U/l, AST < 35 U/l, GGT < 38 U/l, ammonia < 35 µmol/l) and mean value of therapy groups didn't show any significant difference from each other or control groups. However, mean ammonia level of LEV group is shown to be higher than control and is found statistically significant (p = 0,035) (Fig. 1).

There was no significant difference in the duration of drug usage among patient groups (p = 0,327).

The antioxidant molecule carnitine was found the highest in the control group and the lowest in VPA group. This difference between groups was statistically significant (p = 0,001) (Fig. 2). Although carnitine levels were lower in LEV group it was not statistically significant when compared with the control group.

Correlation between the duration of drug usage and carnitine levels are examined in therapy groups. No correlation between carnitine level and duration observed in LEV group, however in VPA group, the mean free carnitine value is found to be higher in patients who were on VPA for less than 10 months and carnitine levels showed a decay as the duration was increased and this difference was statistically significant (p = 0,001) (Fig. 3).

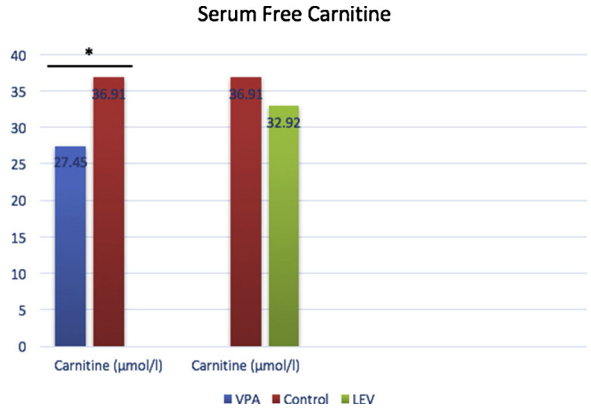


**Fig. 2.** Mean serum free carnitine levels of therapy groups and control group. * Symbolizes statistically significant difference. VPA group showed significantly low serum free carnitine levels (p = 0.001).

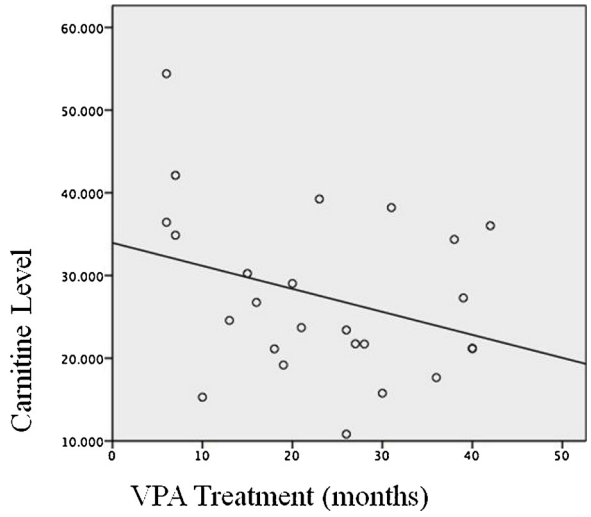


**Fig. 3.** Serum free carnitine level – VPA therapy duration correlation graphic. X-axis shows serum free carnitine levels in µmol/l and y-axis shows duration of VPA treatment of patients in months. Every single dot represents a patient in the graphic; specifically, after 10 months, the decrease in serum free carnitine becomes more apparent (p = 0.001).

8-OHdG, a marker of oxidative DNA damage is found high in both therapy groups compared to control group. The level of 8-OHdG was highest among patients under LEV therapy. The difference between therapy groups and controls were found to be statistically significant (p = 0,018; p = 0,030) (Fig. 4).

MDA, a marker of lipid peroxidation was found higher in LEV group when compared to VPA and control group; this value was found statistically significant respectively. (p = 0,002, p < 0,001) (Fig. 4). MDA levels in VPA group was also high, however was not found statistically significant compared to control group. Patients under LEV treatment showed the highest MDA values.

Correlation analyzes showed no significant results among liver function tests and carnitine level. The duration of the treatment had no statistically significant impact on oxidative damage markers MDA and 8-OhDG or on LFT's in both groups (p > 0,05 in all variables). In LEV group, in which ammonia was between normal ranges but the mean value was significantly higher than controls, showed positive correlation with MDA (r = 0,470, p = 0,015; low correlation) (Fig. 5). At the same time, in LEV group, both oxidative damage biomarkers (MDA and 8-OHdG) showed positive correlation with each other (r = 0,510 p = 0,008; medium correlation) (Fig. 5).

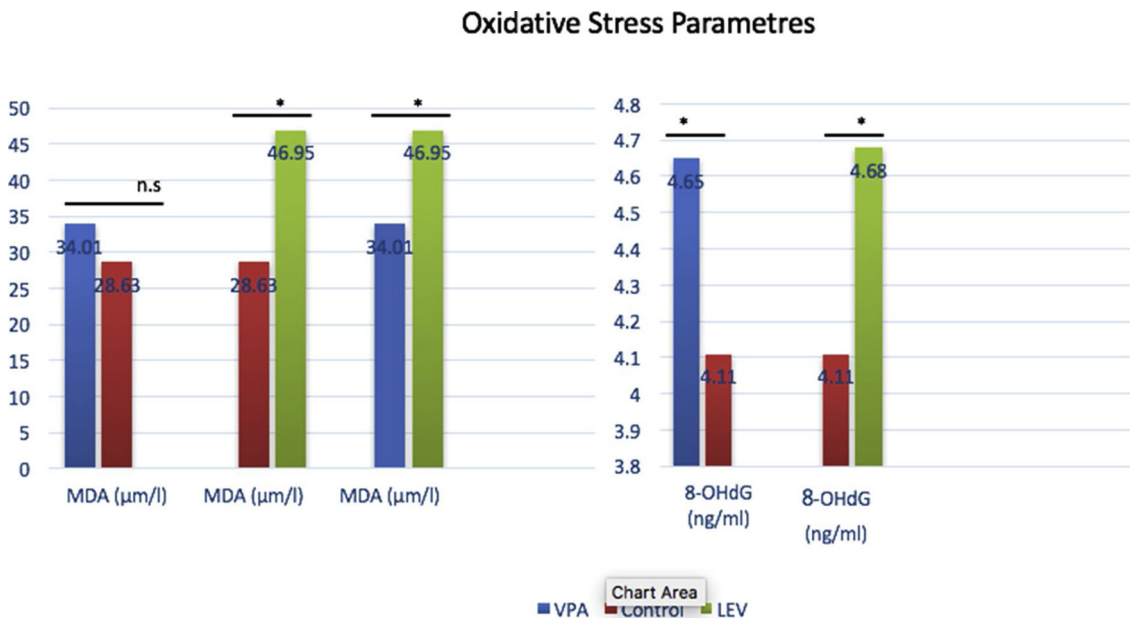


**Fig. 4.** Oxidative stress parametres for therapy groups and control group. * Symbolizes statistically significant difference. MDA (left-side graphic) was found the highest in LEV group (p = 0.000, p = 0.002 respectively) 8-OHdG was high both in VPA and LEV groups compared to controls (p = 0.030, p = 0.018 respectively) and showed no significant difference from each other (p = 0.985).

## 4. Discussion

Epilepsy, which is common in pediatric population, is an important health problem considering its impact on child health, behavior and development. In terms of safety and less adverse effects, one-drug therapy is the main goal in treatment strategy. For the first line therapy, the most common agent in treating generalized epilepsy is VPA. LEV, on the other hand, has become widely used after its approval from food drug administration (FDA) in 2006 and became applicable even in neonatal population (Hirtz et al., 2003; Wilmshurst et al., 2015).

Many researches on VPA, its side effects or effects on oxidative system have been done so far (Michoulas et al., 2006; Verrotti et al., 2008; Chang and Abbott, 2006), but effects of LEV on oxidative system still remain unclear. In our study, we compared effects of VPA and LEV therapy on liver functions, oxidative stress using 8-OHdG, a very sensitive and specific marker of DNA damage; MDA and antioxidant system via measuring free carnitine levels.

We showed that VPA therapy did not alter liver functions. Similarly, Hoshino et al. and Incecik et al. also suggested that the use of VPA did not have any effect on transaminase levels (Hoshino et al., 1995; İncecik et al., 2014). Hepatotoxicity and fatality due to VPA usage have been shown in many publications since 1977 (Scheffner et al., 1988; Anderson and Choonara, 2010). Elevated LFT's are the most common toxic effect of VPA usage, followed by hyperammonemia, hepatic encephalopathy and scarcely Reye-like syndrome (Nanau and Neuman, 2013). Although the data is controversial among many studies as most of them differ in terms of patient age, size of study group or dosage of VPA used; we conclude that in our study we have found the liver functions within normal limits since our VPA patients had no additional risk factors or comorbidities and were treated with appropriate doses of VPA. Considering that LEV is excreted from the kidneys, it does not have adverse effects on the liver, our data also support that patients under LEV therapy do not show altered transaminase levels.

We showed no increase in ammonia levels under VPA or LEV

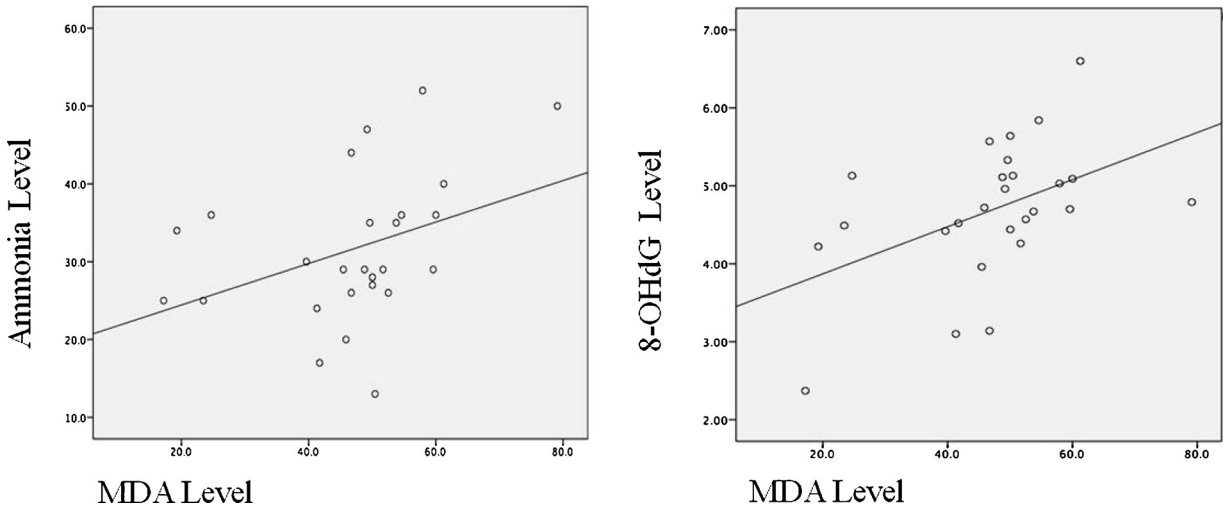


**Fig. 5.** Correlation graphics for LEV group. In both graphics, the dots represent one patient. On the left, low correlation between MDA and ammonia levels are demonstrated. X-axis shows ammonia levels in µmol/l and y axis shows MDA levels in µm/l. On the right side, correlation between MDA and 8-OHdG is shown. X-axis shows 8-OHdG levels in ng/ml and y-axis shows MDA levels in µm/l.

therapy. In VPA group mean ammonia level was slightly increased and was not statistically significant compared to controls. There was no connection between the duration of therapy and the levels of ammonia. Several studies (Tesen et al., 2017; Tseng et al., 2014) have suggested an increase in ammonia levels under VPA therapy such as Verotti et al. (Verrotti et al., 1999). However, our data does not support this outcome. Firstly, the elevation shown is similar to the one we showed in LEV group, it is within the normal ranges. Secondly, according to their data, there are some patients receiving VPA slightly beyond upper therapeutical limits. Lastly the epilepsy subtypes examined do not match in both studies. In LEV group, on the other hand, mean ammonia level was found higher and was statistically significant. Moreover, the elevated levels showed correlation with increased MDA levels which also showed significance. Literature shows only one case report by Roh et al., of which a patient under VPA therapy with uncontrolled seizures is treated with LEV as a second antiepileptic which generated hyperammonemic encephalopathy. The condition turned back to normal after LEV discontinuation (Roh et al., 2014). Therefore, we suggest monitoring ammonia and LFT's of patients under LEV therapy closer in order to get more idea about possible liver toxicity.

When the anti-oxidant molecule, carnitine was taken into consideration, we showed that VPA therapy decreases serum free carnitine level, which is concurrent with the literature (Zelnik et al., 1995; Verrotti et al., 1999; Van Wouwe, 1994). We also showed that there was positive correlation with the length of usage of VPA and the carnitine levels and it was statistically significant. Although there are studies which show correlation between low carnitine and high ammonia levels, the reason behind elevated LFT's and hepatotoxicity is not yet clear. We have hypothesized that augmented LFT's correlated with lowered carnitine; however, we found no such correlation as all LFT's were within normal range. Although mean serum free carnitine level was low, it was between normal ranges as Fung et al. also have shown (Fung et al., 2003). LEV group also had slightly low levels of serum free carnitine compared to control patients, but mean value remained in between normal range as in VPA patients. Our study is the first to examine the influence of LEV therapy on serum free carnitine levels among pediatric patients. We showed that serum free carnitine is not affected by adequately dosed LEV therapy.

Oxidative stress can be observed both in physiological aging and in diseases including epilepsy. The stress is due to imbalance between reactive oxygen species and antioxidants; resulting in augmented reactive oxygen species to attack lipid, protein or nucleotide structures. As our brain is rich in lipid in myelin sheath and uses a lot more oxygen than most tissues, it is more vulnerable to the attack of reactive oxygen species (Beal, 1998; Fridovich, 1970).

Our study demonstrated that appropriately-dosed VPA therapy did increase the levels of MDA and 8-OHdG. However, the rise in MDA level was not statistically significant. Menon et al. compared epileptic patients on VPA and on carbamazepine with patients who do not receive any therapy and found out no significant increase in MDA or nitric-oxide levels; suggesting epilepsy itself is a reason for oxidative damage (Menon et al., 2012). Moreover, two different studied from Turkey also showed VPA treatment did not increase MDA levels significantly (Yuksel et al., 2000; Cengiz et al., 2000). Verotti et al., also compared oxidative status of epileptic patients in one year period and found out that only the patients who formed obesity during time had significantly higher MDA levels (Verrotti et al., 2008). We conclude that, appropriate doses of VPA usage do not cause a pathologic lipid peroxidation. Patients with extra risk factors such as obesity might show higher levels as shown in the previous study. Our patients did not have any extra risk factors and all the patients had normal body mass index. VPA usage significantly altered the level of 8-OHdG, which is a sensitive and specific marker of DNA damage due to oxidative stress. We hypothesized that liver toxicity and oxidative markers and/or carnitine had a correlation; however, this pitch does not have any correlation with any of our data. Moreover, the duration of the treatment did not correlate with high levels of 8-OHdG. Relevant studies concerning 8-OHdG levels under VPA therapy shows that VPA has an effect on the elevation of 8-OHdG. Schulpis et al. showed high doses of VPA administration increased both LFT's and 8-OHdG levels, where higher doses of VPA administration correlated with higher levels of 8-OHdG. However, these results do not coincide with our findings as our patients received adequate doses of VPA and did not have elevated LFT's (Schulpis et al., 2006). Moreover, in vitro experiments on animals (Natarajan et al., 2006), show direct VPA administration on liver do increase oxidative damage in liver cells resulting in increased levels of transaminases, however more experiments are needed to back this hypothesis up.

Patients under LEV therapy showed a distinct increase in both MDA and 8-OHdG levels and they were both statistically significant compared to control patients. Moreover, these two parameters did show medium positive correlation which also was significant. Correlation analysis, however, showed no significance in terms of duration of the treatment. We conclude that levetiracetam's effects on oxidative system is independent of the timespan it is used. There are few studies concerning the effects of LEV on oxidative system. Several animal experiments showed that post seizure rat brains had decreased levels of MDA after levetiracetam administration (Marini et al., 2004; Roy et al., 2015) Two adult trials on stroke patients also showed that LEV had neuroprotective affects (Belcastro et al., 2008; Kutlu et al., 2008). Pearl et al. also supported this data by administrating prophylactic LEV to patients with head trauma and showed that only one patient developed epilepsy and thus LEV had protective effect on brain (Pearl et al., 2013). When 8-OHdG status is investigated, there is not any data about epileptic patients on LEV and 8-OHdG status. There is one recent study on rats' reproductive system. Baysal et al. administrated rats with high doses of LEV and investigated their effects on specimen (Baysal et al., 2017). They showed that rats who were administrated with higher doses of LEV had higher levels of 8-OHdG in their specimen. Most of the studies are done on animal models or on different patient population. Effects of LEV in epilepsy on oxidative system are mostly evaluated on animals and on liver tissues, thus can explain why it differs from what we have found.

As mentioned earlier; Menon et al. compared epileptic patients receiving different therapies with a control group of epileptic patients who do not receive any anti-epileptic drugs. They found out that seizures induce oxidative stress independent of the type of the treatment (Menon et al., 2012). The weak point of our study is that we don't know whether the disease, epilepsy itself, contributes to these high levels specifically in levetiracetam group. Since epilepsy itself also causes oxidative damage as previous animal experiments also have shown on tissue level and as the trial by Menon et al. suggests, we still do not have enough data collected about this subject in the literature. Therefore, with the data we have collected, we demonstrate that patients under LEV therapy, treated with adequate doses and who have no comorbidities or risk factors show altered oxidative status.

Because this is a pilot study to show whether LEV has any effect on oxidative system, we cannot strongly determine the consequences of LEV usage in routine epilepsy treatment among patients. According to our results we have shown that LEV has a negative impact on oxidative system. In humans, oxidative damage is thought to be involved in pathophysiological pathways of several diseases including ADHD (Joseph et al., 2015), depression (Jimenez-Fernandez et al., 2015) or autism (James et al., 2004). Clinically levetiracetam use is also linked to behavioral changes among children (Halma et al., 2014; Mbizvo et al., 2012). We believe this new finding may be a key to further investigations in this topic. However, yet it is early to state any practical consequence.

## 5. Conclusion

We presented the first results comparing the effects of oxidative stress caused by VPA and LEV on pediatric population. We have shown

that both drugs have impact on oxidative status. Patients under VPA showed lower levels of MDA and 8-OHdG compared to LEV and these levels did not have any correlation with either liver functions or low carnitine levels. Carnitine was lowered under VPA however it was within the normal range for children of this age. Within the LEV group, on the other hand, damage due to oxidative stress was much higher and also the two markers showed positive correlation. LEV also altered ammonia levels which barely correlated with MDA levels.

In conclusion, our study for the first time shows that LEV administration results in oxidative damage in children with epilepsy making a significant increase in MDA and 8-OHdG levels. Considering the effects of oxidative damage on various diseases, this finding may be a key point to explain adverse effects of levetiracetam via oxidative damage in pediatric population. Further studies recruiting more patients and measuring oxidative status before and after therapy will enlighten us more and help us to understand the effects of the drugs, mostly LEV, better.

## Author contributions

P.H., S.T., G.D. and T.E. conceived and designed research; P.H., P.A and G.Ö.T. recruited patıents; P.H. and Ö.D. performed experiments; P.H. and T.E. analyzed data; P.H., T.E., Ö.D. interpreted results of experiments; P.H. prepared figures; drafted manuscript; P.H., S.T., G.D., P.A., G.Ö.T and T.E. approved final version of manuscript.

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## Declaration of interests

Authors have nothing to declare.

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