Blood Levels of Trace Elements, Electrolytes, and Oxidative Stress/Antioxidant Systems in Epileptic Patients

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Abstract. Epileptic patients exhibited variably altered status of trace elements, electrolytes, and free radical scavenging enzyme activities. We investigated the effect of epilepsy and long-term antiepileptic drug therapy on the serum level of some trace elements (zinc, selenium, and copper), electrolytes (calcium, magnesium, sodium, and potassium), and antioxidants (glutathione peroxidase, and uric acid) and plasma levels of lipid peroxidation index (malondialdehyde), total antioxidant capacity, and ceruloplasmin. Seventy epileptic patients and fourteen controls were recruited in this study. In the treated group (particularly with valproate), we reported increases in the levels of zinc, calcium, sodium, malondialdehyde, and glutathione peroxidase and decreases in the levels of copper, total antioxidant capacity, and ceruloplasmin with no difference in selenium, magnesium, and potassium. However among untreated epileptics, uric acid level was increased and total antioxidant capacity was markedly lowered. We conclude that the above parameters balance differs in epileptics comparable to controls and hence their correlation to seizures pathophysiology and their degree of control or resistance to antiepileptic drug therapy. Better regulation of the lipid peroxidation and antioxidants and fewer disturbances in mineral metabolism were observed in monotherapy versus polytherapy and with carbamazepine versus valproate therapy.

Keywords: trace element, electrolyte, antiepileptic drug, oxidative stress, antioxidant

Introduction

Epilepsy is a chronic dynamic important medical problem with about one in eleven persons experiencing at least one seizure at some point. The tendency to have recurrent, unprovoked seizures occurs with a prevalence of about 0.5%, and a cumulative lifetime prevalence of 3%. It covers a range of different conditions with varying etiology (1). Certain minerals balance is crucial for a healthy nervous system and neuronal susceptibility to excitability. Several reports suggested that the body electrolytes (sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), and magnesium (Mg²⁺)) and the level of some trace elements play a vital role in seizure condition to develop (2, 3). It has been reported that increased generation of free radicals or reduced activity of antioxidative defense mechanisms can cause some forms of seizures and in addition, increases the risk of seizure recurrence (4, 5). Many antiepileptic drugs (AEDs) are metabolized to generate reactive metabolites with the capability of covalent binding to macromolecules as proteins or other vital biomolecules and hence eliciting systemic toxicity (5 – 7). Peroxidation of membrane lipids caused by an increase of generation of free radicals or decrease in the activities of antioxidant defense systems have been suggested to be critically involved in seizure control (4). Malondialdehyde (MDA) is one of the important aldehydes resulting from membrane lipid peroxidation products. It was found to be formed during the non-enzymatic auto-oxidation of polyunsaturated fatty acids (7). Free radical scavenging activity was indicated by the total antioxidant capacity (TAC). Antioxidant defense mechanisms was indicated to involve glutathione peroxidase (GSH-Px), which is one of the most important members of the body antioxidant defense systems (8), copper (Cu²⁺) (9, 10), zinc (Zn²⁺) (9, 11, 12), sele-
nium (Se^{2+}) (13), ceruloplasmin (Crl) (a copper-binding protein), and uric acid (6, 14, 15).

It has been suggested that AEDs have occasionally been associated with significant adverse effects on the antioxidant defense system (5, 8). The existing knowledge about the impact of epilepsy and AEDs on trace elements and free radical/antioxidant system is poor and controversial. Therefore, in this study, we purposed evaluation of the level of some trace elements (Zn^{2+}, Se^{2+}, and Cu^{2+}); electrolytes (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}); and lipid peroxidation MDA/antioxidant markers (TAC, GSH-Px, Crl, and uric acid) in a total of seventy epileptic patients.

Materials and Methods

Seventy chronic epileptic patients with idiopathic generalized tonic-clonic seizures (male = 33, female = 37) were included in this study. All patients were in the interictal period or at least 12 h seizure free from sampling time. All were randomly recruited from the outpatient epilepsy clinic, Assiut University Hospital, Assiut, Egypt. Excluded from this study were those with progressive brain disease or other chronic medical or surgical illnesses or chronic medication besides AEDs. Fourteen normal healthy (age- and sex-matched) volunteers served as a control group. This study was accepted by the regional Ethical committee. Detailed information on the study was given to each patient and all subjects gave their written consent to attend this study. For on the study was given to each patient and all subjects by the regional Ethical committee. Detailed information about sampling time. All were randomly recruited from the outpatient epilepsy clinic, Assiut University Hospital, Assiut, Egypt. Excluded from this study were those with progressive brain disease or other chronic medical or surgical illnesses or chronic medication besides AEDs. Fourteen normal healthy (age- and sex-matched) volunteers served as a control group. This study was accepted by the regional Ethical committee. Detailed information on the study was given to each patient and all subjects gave their written consent to attend this study. For each patient, the following information were obtained: complete medical history and clinical examination with special emphasis on age, sex, type and frequency of seizure, duration of illness, AED(s) used with their doses, and age of starting treatment and control of seizures. After the baseline evaluation, the patients were divided into two main groups: untreated epileptic patients (n = 15) and treated epileptic patients (n = 55). The treated epileptics were subdivided according to the type of the used antiepileptic drug therapy into three groups:

Group I: Patients on carbamazepine (CBZ) (n = 34) in a dose range of 400 – 1200 mg/day (741.0 ± 189.66).

Group II: Patients on sodium valproate (VPA) (n = 9) in a dose range of 349 – 1500 mg/day (761.0 ± 331.61).

Group III: Patients on polytherapy with CBZ and VPA (n = 16), patients on CBZ and phenytoin (PHT) (n = 4), and patients on CBZ, VPA, and PHT (n = 2).

The quantitative estimation of some serum trace elements and electrolytes were undertaken for all patients and controls. From each case, after an overnight fast, 10 ml venous blood was collected. The blood sample was divided into two parts: one part was allowed to clot at room temperature and centrifuged at 5000 rpm for 10 min and the serum was collected, and aliquots of this serum was kept frozen at −70°C until they were used to assay of serum trace elements (Zn^{2+}, Se^{2+}, and Cu^{2+}), electrolytes (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}), and antioxidant markers (GSH-Px and uric acid). The second part of the blood sample was collected in a tube containing heparin, centrifuged, and the separated plasma was kept frozen at −70°C in aliquots that were later used for assays to determine MDA, TAC, and Crl.

Serum Zn^{2+}, Cu^{2+}, Se^{2+}, Ca^{2+}, and Mg^{2+} were determined by Atomic absorption/Flame-Emission spectrophotometer (model A A-630-02; Shimadzu, Kyoto) using an acetylene flame and hollow cathode lamps. Sera were diluted 10 times with distilled water. The analysis wavelengths were 213.9, 324.7, 196.0, 422.7, and 285.2 nm, respectively. Serum Na^+ and K^+ were determined after a 1:200 dilution of the sample using flame photometer (model PEP7; Medica Scientific, England Genway).

Plasma TAC was determined spectrophotometrically according to the method described by Miller et al. (16), which use 6-hydroxy-2,5,7,8-tetramethylchroman, 2-carboxylic acid as a standard. Plasma MDA was determined spectrophotometrically according to the method described by Buege and Aust (17) in which MDA reacts with thiobarbituric acid (TBA) with the production of a pink pigment. Plasma Crl was determined spectrophotometrically according to the method of Houchin (18). Serum GSH-Px activity was determined by a spectrophotometer according to the method of Mills et al. (19), as modified by Hafeman et al. (20), using reduced glutathione and H_2O_2 as substrates, and the remaining reduced glutathione was determined using Ellman reagent. Serum uric acid was determined by a colorimetric US plus kit, distributed by Roche Diagnostics GmbH, Mannheim, Germany.

The serum level of AED(s) were determined in the therapeutic drug monitoring (TDM) lab, Assiut University Hospital, Assiut Egypt, using the fluorescence polarization immunoassay system of Abbott (EPIA) using TDXFLX apparatus (Abbott Lab., Wiesbaden, Germany). The approximated therapeutic serum level of CBZ is 4 – 10 µg/ml and that of VPA is 50 – 100 µg/ml.

Statistical analyses

Data are expressed as means ± S.D. Statistical comparison among different groups was performed by using ANOVA tests. Moreover, Pearson’s correlation was performed between different biochemical parameters. Calculations were done with the statistical package SPSS for Windows, version 10.0. Statistical significance
was defined as $P < 0.05$.

**Results**

This study included a total of seventy patients (male = 33, female = 37) (treated group = 55, untreated group = 15) recruited from the out-patient epilepsy clinic of the Department of Neurology, Assiut University Hospital, Assiut, Egypt. All were chronic epileptics with mean duration of illness of 16.47 and 4.53 years for the treated and the untreated group of epileptics, respectively. Fourteen age- and sex-matched healthy volunteers were chosen as controls for comparison. The demographic, clinical data of the epileptic patients are shown in Table 1. The level of different trace elements, electrolytes, and oxidative markers in controls and patients treated with different AEDs (CBZ, VPA, and polytherapy) are shown in Tables 3 and 5.

The untreated group of epileptics exhibited unaltered Zn$^{2+}$ levels compared to controls. In contrast the VPA-treated group exhibited significantly higher levels of Zn$^{2+}$ compared to controls ($P < 0.05$) and CBZ-treated group ($P < 0.001$) (Tables 2 and 4).

The untreated group of epileptics exhibited significantly higher levels of Cu$^{2+}$ compared to controls ($P < 0.05$), while among the treated group of epileptics, the CBZ-treated group exhibited significantly lower Cu$^{2+}$ levels in comparison to controls ($P < 0.05$) (Tables 2 and 4).

The Se$^{2-}$ levels were unaltered among the untreated

**Table 2.** Serum levels of zinc, copper, selenium, calcium, magnesium, sodium, and potassium in controls and untreated and treated epileptic patients

<table>
<thead>
<tr>
<th>Characteristic of patients</th>
<th>Mean ± S.D. (Range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 14</td>
<td>Untreated, n = 15</td>
<td>Treated, n = 55</td>
</tr>
<tr>
<td>Zn$^{2+}$ (µg/dL)</td>
<td>85.57 ± 19.95 (60–120)</td>
<td>74.53 ± 17.27 (55–100)</td>
</tr>
<tr>
<td>Cu$^{2+}$ (µg/dL)</td>
<td>96.43 ± 18.75 (75–125)</td>
<td>117.2 ± 26.87 (85–160)</td>
</tr>
<tr>
<td>Se$^{2-}$ (µg/dL)</td>
<td>8.95 ± 1.01 (7.5–10.5)</td>
<td>9.41 ± 1.05 (7.5–10.5)</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mg/dL)</td>
<td>10.04 ± 0.65 (9–11)</td>
<td>9.56 ± 0.5 (8.5–10)</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mg/dL)</td>
<td>1.91 ± 0.14 (1.70–2.15)</td>
<td>1.96 ± 0.17 (1.7–2.2)</td>
</tr>
<tr>
<td>Na$^{+}$ (mmol/L)</td>
<td>138 ± 3.8 (132–143)</td>
<td>152.67 ± 11.02 (135–170)</td>
</tr>
<tr>
<td>K$^{+}$ (mmol/L)</td>
<td>4.23 ± 0.59 (3.6–5.3)</td>
<td>3.65 ± 0.41 (3.1–4.1)</td>
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$P1$: untreated group versus controls, $P2$: treated group versus controls, $P3$: untreated group versus treated, NS: non significant.

**Table 3.** Levels of plasma malondialdehyde (MDA), plasma total antioxidant capacity (TAC), ceruloplasmin (Crl), serum glutathione peroxidase (GSH-Px), and serum uric acid in controls and untreated and treated epileptic patients

<table>
<thead>
<tr>
<th>Characteristic of patients</th>
<th>Mean ± S.D. (Range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 14</td>
<td>Untreated, n = 15</td>
<td>Treated, n = 55</td>
</tr>
<tr>
<td>MDA (mmol/mL)</td>
<td>1.34 ± 0.62 (0.67–2.44)</td>
<td>1.96 ± 0.95 (0.77–3.52)</td>
</tr>
<tr>
<td>TAC (mg/mL)</td>
<td>1.64 ± 0.14 (1.45–1.80)</td>
<td>0.89 ± 0.23 (0.57–1.20)</td>
</tr>
<tr>
<td>Crl (mg/dL)</td>
<td>31.14 ± 6.43 (22–40)</td>
<td>28.67 ± 4.15 (22–35)</td>
</tr>
<tr>
<td>GSH-Px (U/mL)</td>
<td>0.69 ± 0.00 (0.55–0.80)</td>
<td>0.46 ± 0.00 (0.38–0.52)</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.64 ± 0.53 (4.9–6.25)</td>
<td>7.27 ± 1.04 (5.8–8.6)</td>
</tr>
</tbody>
</table>

$P1$: untreated group versus controls, $P2$: treated group versus controls, $P3$: untreated group versus treated, NS: non significant.
group of epileptics in comparison to controls, while the VPA-treated group exhibited significantly elevated Se2+ levels (P<0.01) (Tables 2 and 4).

The levels Ca2+ were significantly higher among the CBZ- and VPA-treated group of epileptics in comparison to controls (P<0.05) and the untreated group of epileptics (P<0.001) (Tables 2 and 4).

No difference was observed in the levels Mg2+ in all studied groups of epileptics in comparison to the controls (Tables 2 and 4).

Na2+ levels were significantly higher among the untreated group (P<0.001) and CBZ- and polytherapy-treated groups of epileptics (P<0.01) in comparison to the controls (Tables 2 and 4).

K+ levels were significantly lower among the untreated group of epileptics in comparison to the controls (P<0.01) and the treated group of epileptics (P<0.001) (Tables 2 and 4).

The levels of MDA (an index of extracellular membrane lipid peroxidation) were significantly higher in both the untreated (P<0.05) and treated (P<0.001) groups of epileptics with marked elevation among
the VPA- and polytherapy-treated groups of epileptics (P<0.001) (Tables 3 and 5).
Significant reduction of TAC levels were observed among the untreated and treated groups of epileptics (P<0.001) in comparison to controls with marked reduction among the polytherapy- (P<0.001) and VPA-treated groups of epileptics (P<0.05) compared to the CBZ-treated group (P<0.01) (Tables 3 and 5).
No difference in the levels of Crl (a copper-binding protein) were observed in the untreated group of epileptics when compared to controls, while significant reduction of Crl levels were observed in CBZ- and VPA-treated group of epileptics (P<0.001) (Tables 3 and 5).
No difference in the levels of GSH-Px were observed in the untreated group of epileptics when compared to controls while significant elevation in the GSH-Px levels were observed in CBZ- and VPA-treated group of epileptics (P<0.001) (Tables 3 and 5).
Significantly higher levels of uric acid were observed among the untreated group compared to the control and the prescribed dose are important variables. Age of the patients, whether children or adults, is not a strong variable as it is believed that the plasma levels of these elements (e.g., Cu2+, Zn2+, Se2+, and Mg2+) and antioxidant enzymes (e.g., GSH-Px) do not change after the age of 3 years (30). Further prospective and experimental studies are necessary for confirmation.

Altered Zn2+ metabolism has been implicated to play a role in the development of epilepsy. Zn2+ inhibits glutamic acid decarboxylase activity, thereby resulting in decreased levels of GABA or an increase in carbonic anhydrase activity (11). In our study, the untreated epileptics exhibited unaltered Zn2+ levels and the VPA-treated epileptics showed significantly higher level of Zn2+ (P<0.05) (Tables 2 and 4). The studies of the relationship between Zn2+ levels and AED therapy have yielded contradictory results. In contrast to our study, Kuzuya et al. (31) reported lower levels of Zn2+ in patients who were treated with mono-AEDs (CBZ, PHT, and VPA) with no dose related changes. Yuen et al. (32) reported normal levels of Zn2+ in white blood cells of epileptic patients on VPA or CBZ. They suggested that AEDs might affect the intracellular Zn2+ level concentrations. Steidl et al. (33) reported significant lowering of serum Zn2+ in patients treated with AEDs for ≥5 years. Lerman-Sagie et al. (34) reported reduced erythrocyte Zn2+ content in their epileptics. In the prospective study done by Altunbasak et al. (35), the serum and hair levels of Zn2+ were found to be higher in untreated epileptic patients than those treated with VPA and controls and returned to normal level after VPA treatment. Consistent with our study, Kürekçi et al. (12) reported higher levels of Zn2+ in treated groups of patients that remained within the normal range and attributed this to the normal physiological variation in serum Zn2+ concentrations (circadian variation) and is unlikely related to the anticonvulsant drugs or epilepsy (36, 37). Others attributed the increased serum Zn2+ levels to measuring such levels at least 2 h later than VPA intake, that is, at the peak of VPA concentration (38). However, in our study and that of Kürekçi et al. (12), the Zn2+ levels were measured after an overnight fast before taking the next morning VPA dose. The real mechanism(s) of the possible effect of AEDs on Zn2+ concentrations is not completely understood, although some authors (9) have suggested that AEDs could decrease Zn2+ (with a concomitant Cu2+ increase) modifying the neurotransmitter regulation. VPA binds Zn2+, thus protects glutamic acid decarboxylase from the inhibitory effect of Zn2+ and hence increased level of GABA (21).

We reported higher levels of serum Cu2+ in untreated epileptics (P<0.05), which we attributed to inverse relationship between Zn2+ and Cu2+ concentration (9, 10). However, the results in the treated epileptics are contradictory. We reported significantly lower levels
among the CBZ-treated epileptics \((P<0.05)\) (Tables 2 and 4). However, this was different from the previously reported normal \((7, 8, 10, 12, 39–42)\) or decreased \(\text{Cu}^{2+}\) levels \((43, 44)\) in different groups of epileptics. Kaji et al. \((45)\) and Hurd et al. \((46)\) reported low serum \(\text{Cu}^{2+}\) levels in VPA-treated epileptics. In contrast, many studies reported normal \((7, 8, 10, 12, 39–42)\) or decreased \(\text{Cu}^{2+}\) levels \((43, 44)\) in different groups of epileptics. We reported unaltered \(\text{Cu}^{2+}\) levels in untreated epileptics and in CBZ-treated group of epileptics \((3, 42)\) which agrees with many studies \((42, 58, 61, 62)\). While we found no difference in the serum \(\text{K}^{+}\) levels in different treated groups (Tables 2 and 4), White et al. \((58)\) reported elevation of \(\text{K}^{+}\) levels during periods of intense seizure activity. Generally, the deficiency in \(\text{K}^{+}\) is correlated to hypomagnesaemia and hypocalcaemia \((42)\). We think that the \(\text{K}^{+}\) deficiency in our untreated epileptics may in the future, result into serious neurological symptoms \((42)\).

Many studies suggest that membrane lipid peroxidation may be causally involved in some forms of epilepsies, and the decrease in free radical scavenging enzyme activity is believed to cause the increased risk of seizure recurrence \((4, 12, 63)\) and idiosyncratic drug reaction encountered in the management of epilepsy \((55, 63, 64)\). In addition, Many conventional AEDs are metabolized to generate reactive metabolites with capability of covalent binding to macromolecules \((65)\). We reported elevated MDA in untreated \((P<0.05)\) and treated patients \((P<0.001)\) (Tables 3 and 5) which agrees with many studies \((4, 5, 7, 41, 66, 67)\). We attributed the marked elevation of MDA levels among our VPA-treated group, whether utilized as mono-therapy or in combination with other AEDs, to excess generation of VPA metabolites with increased body burden of free radicals, which is responsible for serious side effects such as acute pancreatitis and hepatotoxicity \((5, 6)\). Added to this burden is our reported decrease in the utilized antioxidant markers as TAC and Crl which was evident in all groups of our patients, particularly the polytherapy-\((P<0.001)\) and VPA-\((P<0.05)\) treated groups (Tables 3 and 5) \((43, 55, 68, 69)\). However, none of our patients reported side effects from VPA therapy. In contrast, Tutor-Crespo et al. \((70)\) who reported increased Crl attributed this to a drug-induced cholestasis.

\(\text{GSH-Px}\), which is considered as important antioxidant \((8)\), was also reported to be reduced in our untreated epileptics and increased in the treated epileptics, particularly in the VPA- and polytherapy-treated
groups (P<0.001 and P<0.01, respectively) (Tables 3 and 5). It is important to emphasize that in all our treated patients, the drugs were prescribed at the usual dosage and all serum levels of AEDs were within the therapeutic ranges and none of our patients reported significant side effects from the AEDs, which is consistent with many studies (5, 10, 12, 39, 71). GSH-Px deficiency is suggested to be a cause of childhood intractable epilepsy and those who suffered intolerance to AEDs improved after discontinuation of AEDs and selenium substitution (72). Graf et al. (6) have demonstrated that GSH-Px can be depressed in VPA-treated patients with clinically defined toxicity of the drug, while Yüskel et al. (5) believed that VPA affects the antioxidant system, but did not agree with the comment that a decrease level of GSH-Px is an indicator of the risk of toxicity in some clinical applications. In contrast to our study, Verrotti et al. (8) found unaltered GSH-Px levels among their epileptics. The significantly increased levels of GSH-Px in our VPA-treated group could be attributed to induction of hepatic synthesis of GSH-PX and transport to blood (12).

In this and many previous studies, differential effect on antioxidant bioindicies was observed as our CBZ-treated epileptics reported no significant differences in the levels of the antioxidant markers, which is consistent with many studies (5, 10, 12). Yüskel et al. (5) concluded that antioxidant systems in epileptic patients on CBZ therapy are better regulated in comparison with epileptic children on VPA therapy. CBZ may be a better anticonvulsant for the control of free radical-related seizures (post-traumatic epilepsy) and for clinical management and treatment of female epileptic patients during pregnancy (41). In contrast, the metabolic intermediates of CBZ (e.g., the 10,11-epoxide and trans-diphenyridiol derivative were found to be stable, and teratogenicity has rarely been reported in patients with CBZ monotherapy (73).

Uric acid is an effective antioxidant (74). The observed hyperuricaemia (P<0.001) (Tables 3 and 5) among untreated epileptics in this study could be a compensatory mechanism trying to counteract oxidative stress encountered in epilepsy (14). Also, we suggest that hyperuricaemia observed in our VPA- (P<0.001) and CBZ-treated epileptics (P<0.05) may be related to alteration of the renal excretion of the drug (75) or a cellular protective mechanism against peroxidative damage (14).

The results of this study suggested that the homeostasis of trace elements, electrolytes, membrane lipid peroxidation, and antioxidants is altered in epileptic patients. Levels of the studied indices found in our study were different from the results of some other studies. We suggested that the age of the patients; the type and duration of epilepsy; and the type, duration, and doses of drug treatment are important variables added to the difference in methodologies used. Further prospective studies are need for more information. Differential effects were detected among different AEDs treatments in which CBZ was found to be better anticonvulsant for the control of free radical related seizures and the level of trace elements were better regulated with CBZ than with VPA. We also concluded that adequate trace elements and antioxidants supply is important for brain functions and prevention of neurological diseases and further elucidiation of the pathological actions of such substances in the brain should result in new therapeutic approaches.

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