

Relation between Melatonin and Other Markers of Oxidant/Antioxidant Status in Epileptic Children: Effect of Valproate Therapy

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ABSTRACT

The aim of the present work was to investigate the relationship between serum melatonin levels and other markers of oxidant/antioxidant balance in epileptic children before and after treatment with the antiepileptic drug valproic acid (VPA). The study was conducted on twenty epileptic children prior to starting therapy, as well as on another twenty age and sex matched epileptic children receiving treatment with the antiepileptic drug VPA, for a minimum duration of one year. Fifteen age and sex matched healthy children were included as a control group. Serum melatonin, zinc, copper, and malondialdehyde (MDA) concentrations and erythrocyte superoxide dismutase (SOD) activity were measured in all subjects. Mean levels of melatonin and MDA were significantly increased while SOD activity was significantly decreased in both untreated and treated epileptics versus control. However, the melatonin and SOD were significantly lower in treated versus untreated epileptics. The serum zinc levels were significantly lower while the serum copper levels were significantly higher in treated versus untreated epileptics. Melatonin was negatively correlated to MDA and copper and positively correlated to SOD. It thus seems possible that oxidant stress is associated with epilepsy and is aggravated with VPA therapy leading to relative reduction in melatonin (in treated versus untreated epileptics) and absolute reduction in erythrocytic SOD and serum zinc concentrations.

Key Words: Epilepsy, serum melatonin, zinc, copper, and malondialdehyde (MDA,) erythrocyte superoxide dismutase (SOD), antiepileptic drug valproic acid (VPA).

INTRODUCTION

Epilepsy is a chronic disorder characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction. It affects all age groups. The incidence is highest in children with another but lesser peak occurring after the age of 60

years^(1,2). Approximately 10-30% of patients with epilepsy continue to have seizures despite medical treatment. Seizures are thought to be the result of an imbalance of neurochemicals and are associated with the production of free radicals which can damage brain cells^(3,4). The brain is especially vulnerable to oxy-radical injury due to its high oxygen consumption rate, its abundant lipid

content, and the relative paucity of antioxidant enzymes compared with other tissues⁽⁵⁾. The antioxidant enzymes CuZn-superoxide dismutase (SOD), Mn-SOD and glutathione peroxidase are present in intermediate amounts, and the catalase activity is very low. Thus, the glial and neuronal cells may be prone to oxidative stress which has been implicated as a contributory factor in various neurological disorders⁽⁶⁾.

It is widely accepted that trace elements play an important role in various metabolic processes, and zinc (Zn), copper (Cu) and selenium (Se) are known to be necessary for the activity of some enzymes. Although many authors have demonstrated an association between some trace elements and epilepsy, the relation has never been convincingly documented, because the data in the literature are conflicting. Moreover, little is known about the changes in trace elements induced by antiepileptic drugs (AEDs)⁽⁷⁾.

Melatonin, a neuromodulator produced by the pineal gland, has been shown to have antiepileptic activities in animal studies using several seizure models⁽⁸⁾ as well as in cases of childhood epilepsy⁽⁹⁾. A few mechanisms for anticonvulsant activity of melatonin have been suggested. It exerts neuroprotection due to its antioxidant, anti-excitotoxic and free radical scavenging properties within the central nervous system^(10,11,12). The function of melatonin as an antioxidant and free radical scavenger is facilitated by the ease with which it crosses morphophysiological barriers, as the

blood-brain barrier and intracellular and subcellular barriers^(13,14).

Carbamazepine (CBZ) and valproic acid (VPA) are widely used as antiepileptic drugs (AEDs) that are effective in treating different forms of epilepsy. Recent studies have indicated that AEDs, especially VPA, may trigger oxygen dependant tissue injury by several mechanisms. It is suggested that VPA impairs the liver function resulting in free radicals production. The latter seems to produce DNA oxidative damage in cells, including neuronal cells⁽¹⁵⁾. VPA may, also, compromise the antioxidant system.⁽⁴⁾

The aim of the present study was to evaluate the oxidant/antioxidant status in patients with epilepsy as represented by the levels of malondialdehyde (MDA: as a marker of lipid peroxidation), the antioxidants melatonin and superoxide dismutase, and the trace elements copper and zinc in erythrocyte and serum samples of patients with epilepsy. In addition, the effect of therapy with the antiepileptic drug valproic acid on the oxidant/antioxidant status was, also, evaluated. Melatonin (as a neuromodulator, not only an antioxidant) will be correlated to the other parameters studied.

SUBJECTS & METHODS

The study included 40 epileptic patients with generalized tonic-clonic seizures, chosen from the Neurology Clinic in Abu El Reish Pediatric Hospital, Cairo University. Their ages ranged between 3 and 12 years, and there were 18 males and 22 females. The 40 patients included: (I) 20 newly

diagnosed epileptic children prior to the administration of any form of antiepileptic drug (AED), their mean age \pm S.D. was 6.6 ± 3.9 years, and (II) 20 epileptic children on treatment with the AED Valproic acid for a minimum period of one year. Their mean age \pm S.D. was 7.4 ± 3.2 years. All patients were in the interictal period, or at least 12 hrs. seizure free from sampling time. Excluded from the study were children suffering from progressive brain disease or chronic medical or surgical illness or those receiving chronic medication other than the AED valproic acid. Fifteen normal healthy children (age and sex matched) served as a control group. Their mean age \pm S.D. was 7.6 ± 3.1 years. Detailed information on the study was given to each patient or subject and their accompanying relative, and a written consent to participate in the study was obtained.

All patients were subjected to full clinical examination and electroencephalographic study. The children were under normal daily light-dark cycles. From each case 10 ml venous blood was collected at about 10 p.m. Light remained dim during that nocturnal sampling. 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 that serum were kept frozen at -70°C . The second part of the blood sample was collected in a tube containing heparin, centrifuged, and the retrieved erythrocytes were washed thrice with cold physiological saline and lysed with double distilled water prior to

analysis for hemoglobin and SOD estimation.

All patients and controls were subjected to the following biochemical investigations:

1. The quantitative estimation of some serum trace elements (Zn^{2+} and Cu^{2+}) were measured by the atomic absorption spectrophotometer (UNICAM, 939/959). Sera were diluted 10 times with distilled water. The analysis wavelengths were 213.9 and 324.7 nm, respectively.
2. Serum malondialdehyde (MDA) (the most abundant degradation product of lipid peroxidation as a marker of lipid peroxidation). The principle of the method was based on the spectrophotometric measurement of the color adduct occurring during the reaction to thiobarbituric acid with malondialdehyde whose absorbance is measured at 530 nm⁽¹⁶⁾.
3. Hemoglobin concentration was assessed by conventional methods.
4. Superoxide dismutase (SOD) was assessed using the Ransod kit (Antrim, U.K.)⁽¹⁷⁾.
5. Serum melatonin level was quantitatively determined by an enzyme immunoassay method using the kit supplied by IBL Hamburg. Extraction was first determined in C18 reversed phase extraction column by applying the serum samples with 10% methanol in bidistilled water (V/V) and eluting with undiluted methanol followed by evaporation of methanol to dryness using liquid nitrogen then reconstitution of the samples with distilled water.⁽¹⁸⁾

Statistical analyses:

Data are expressed as means \pm S.D. Statistical comparison among different groups was performed using the one way analysis of variance (ANOVA) test followed by post-hoc test. To assess the relationship between the different studied biochemical parameters Pearson's correlation was used. Calculations were done using the statistical package SPSS for Windows, version 10.0. Statistical significance was defined as $p < 0.05$.

RESULTS

The results of the present study are shown in table (1) and figures (1-5).

The mean levels \pm S.D. of both serum melatonin and MDA showed a significant increase while that of erythrocytic SOD showed a significant decrease in untreated epileptics versus controls. The melatonin mean level then showed a significant decrease in the epileptic patients receiving treatment with VPA as compared to the untreated epileptic

group. However, that decreased melatonin level was still significantly higher than that of the control group. Meanwhile, the mean level of MDA further significantly increased while the SOD mean levels further significantly decreased in the epileptic patients treated with VPA compared to both controls and untreated epileptics.

The mean serum zinc level was significantly lower while the mean serum copper was significantly higher in the treated epileptics versus both controls and untreated epileptics.

Significant negative correlation was detected between melatonin and MDA ($r = -0.65$), while a significant positive correlation was detected between melatonin and SOD ($r = 0.52$). Negative correlations were detected between MDA and each of SOD ($r = -0.59$) and zinc ($r = -0.53$). Meanwhile, MDA was positively correlated with copper ($r = 0.61$). Also, other negative correlations were detected between serum zinc and copper levels ($r = -0.58$) and between melatonin and copper ($r = -0.43$).

Table (1): Serum melatonin, malondialdehyde (MDA), zinc and copper and erythrocytic superoxide dismutase (SOD) in various study groups.

	Control (n = 15)	Untreated Epileptics (n = 20)	Treated Epileptics (n = 20)
Melatonin (pg/mL)	32.7 \pm 8.5	165.2 \pm 102.4 (a)	83.8 \pm 40 (a) (b)
MDA (nmol/mL)	3 \pm 0.7	5.5 \pm 0.9 (a)	6.5 \pm 1.1 (a) (b)
SOD (U/gHb)	965.3 \pm 120.4	853.5 \pm 116.7 (a)	733.5 \pm 139.7 (a) (b)
Zinc (μ g/dL)	94.4 \pm 10.6	88.8 \pm 9.5	71.6 \pm 12 (a) (b)
Copper (μ g/dL)	93.6 \pm 9.9	97.5 \pm 14.9	126.1 \pm 19.4 (a) (b)

- Values are expressed as mean \pm S.D.

- (a): denotes significant difference between patient group and the control ($p < 0.05$)

- (b): denotes significant difference between treated and untreated epileptic patients ($p < 0.05$).

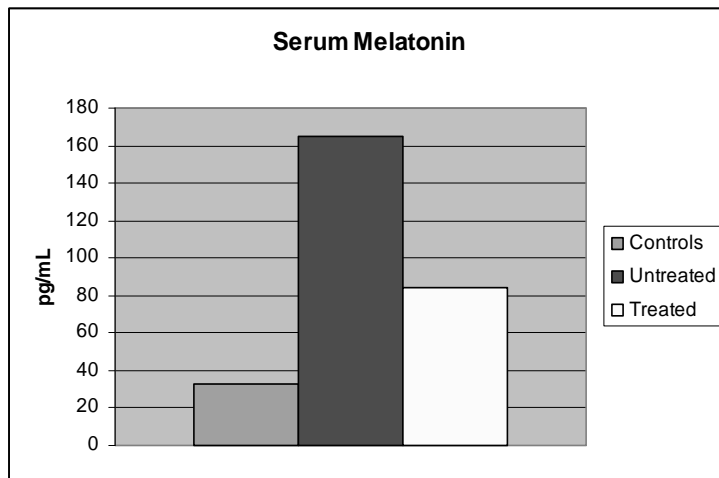


Figure (1): Serum melatonin levels in the different study groups.

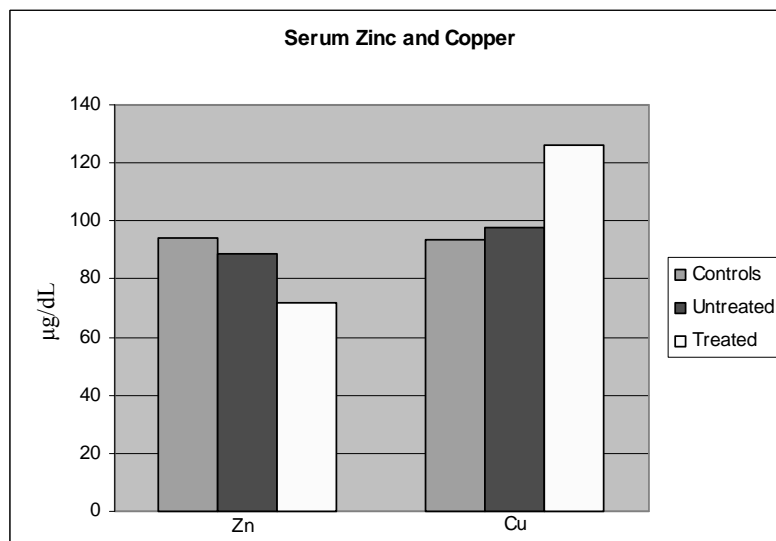


Figure (2): Serum Zinc and Copper levels in the different study groups.

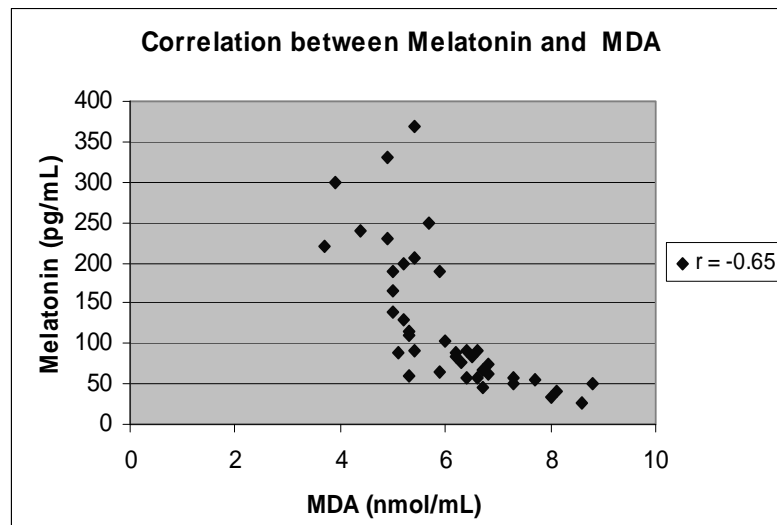


Figure (3): Scatter plot showing the correlation between serum melatonin and malodialdehyde (MDA) levels.

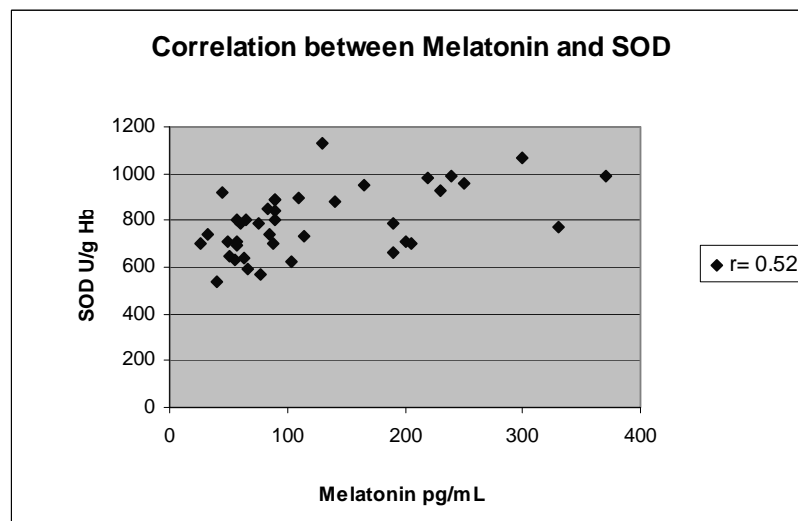


Figure (4): Scatter plot showing the correlation between serum melatonin and superoxide dismutase (SOD) levels.

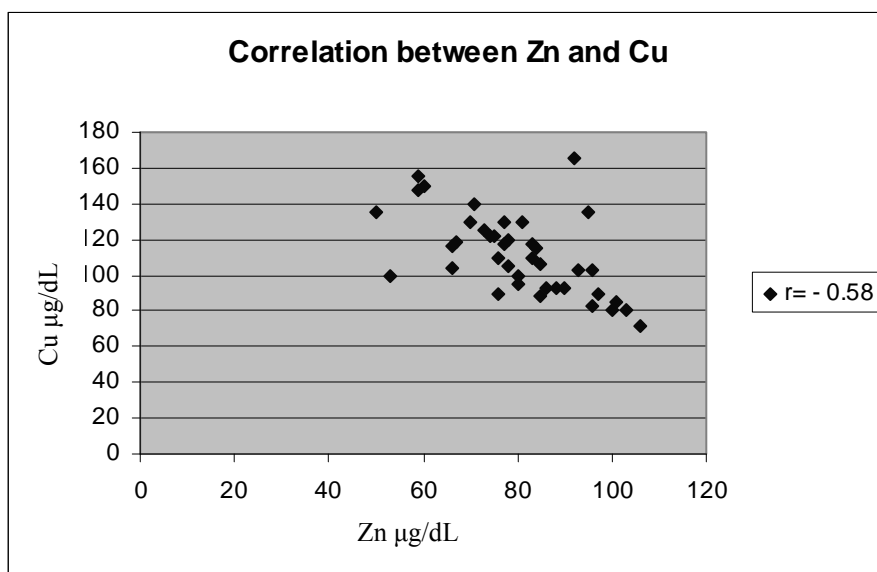


Figure (5): Scatter plot showing the correlation between serum zinc (Zn) and Copper (Cu) levels.

DISCUSSION

Evidence has accumulated about the involvement of reactive oxygen species (ROS) in epilepsy⁽¹⁹⁾. MDA levels showed significant increase in the untreated children compared to the MDA levels in the controls. MDA levels showed further significant increase in the treated patients receiving the antiepileptic drug (AED) valproic acid (VPA) indicating that the metabolism of VPA may trigger oxygen dependent tissue injury and elevate free radicals in the body^(4, 20). These free radicals cause a cascade of neurochemical events leading to neurodegeneration and cell death⁽²¹⁾. Long-term use of AEDs has been shown to increase free radical

formation and cause oxidative damage within neuronal cells⁽²⁰⁾. It was previously reported that VPA may have different effects on the antioxidant system^(4, 20, 22). Moreover, VPA enhances the clearance of selenium and zinc, subsequently leading to decreased synthesis of free radical scavenging enzymes^(23, 12).

The present results showed a significant increase in the mean serum levels of melatonin in both untreated and treated epileptic children compared to controls; however, the levels in the treated children were significantly lower than the levels in the untreated children. In addition, melatonin was negatively correlated to MDA and Cu^{2+} .

The current findings are in accordance with those of **Graham et al.**, who demonstrated that melatonin levels were high in unmedicated patients with complex partial epilepsy⁽²⁴⁾. Overproduction of melatonin may possibly be an attempt by the brain cells to produce a natural downregulator of cerebral epileptiform activity. Melatonin appears to enhance the major neurochemical GABA, whose function is to stop or inhibit seizures. In addition, melatonin blocks the neurochemical glutamate which promotes seizures⁽²⁵⁾. Lastly, melatonin is an anti-oxidant which means that it blocks the bad effects of free radicals which may cause brain damage. Melatonin scavenges oxygen free radicals like superoxide radical, hydroxyl radical, peroxy radical and peroxynitrite anion. Melatonin can, also, enhance the antioxidative potential of the cell by stimulating the synthesis of antioxidative enzymes like superoxide dismutase (SOD), glutathione peroxidase, and, also, the enzymes that are involved in the synthesis of glutathione⁽²⁶⁾. In many instances, melatonin increases the expression of mRNA's of the antioxidative enzymes⁽²⁷⁾. Such finding is confirmed, in the present study, by the decrease in the erythrocytic SOD in the treated epileptics more than the untreated children where the melatonin levels were lower. It is also confirmed by the positive correlation between melatonin and SOD. However, **Sandyk et al.**⁽²⁸⁾ demonstrated that high doses of melatonin may exert proconvulsive activity by decreasing

hypothalamic and cortical GABA levels⁽²⁵⁾.

The results of melatonin levels in the epileptic group receiving VPA are in accordance with the findings of **Bazil et al.**⁽²⁹⁾. Lower levels of melatonin, could possibly be attributed to the oxidant status induced by the anticonvulsant drug VPA⁽¹⁵⁾. This could have contributed to the decrease in melatonin levels found in medicated epileptics compared to untreated ones. Melatonin may thus be consumed by the oxidative process induced by VPA. **Gupta et al.**⁽³⁰⁾ also, detected a decrease in melatonin levels in patients treated with another AED, carbamazepine (CBZ). They attributed that to the additive increase in ROS due to the disease combined with CBZ, or possibly to a difference in melatonin kinetics in conditions of oxidative stress. **Li et al.**⁽³¹⁾ reported that melatonin directly interacts with MDA to form a new product. The findings suggest that melatonin may detoxify unsaturated carbonyls and protect against cellular damage induced by oxidative stress. This is confirmed by the negative correlation between melatonin and MDA, and the positive correlation between melatonin and SOD. **Gupta et al.**⁽¹²⁾ found that administration of melatonin to AEDs, increased the antioxidant level in epileptics receiving VPA. Melatonin, thus, as an adjunct, can be a putative neuroprotector in conditions involving oxidative stress like epilepsy^(10, 11, 12).

Altered Zinc (Zn^{2+}) metabolism has been implicated to play a role in the development of epilepsy. Intracellular Zn^{2+} homeostasis is

sensitive to patho-physiological environmental changes, such as acidosis, inflammation and oxidative stress. The mechanisms by which Zn^{2+} exerts its neurotoxicity include inhibition of glutamic acid decarboxylase activity, thereby resulting in decreased levels of GABA⁽³²⁾, and also, production of ROS and disruption of metabolic enzymatic activity, ultimately leading to activation of apoptotic and/or necrotic processes leading to acute neuronal injury⁽³³⁾. In the present study, serum Zn^{2+} levels showed a non-significant decrease in the untreated epileptic group, while there was a significant decrease in the serum Zn^{2+} level in the epileptic children receiving the VPA therapy. This could be attributed to the fact that VPA binds Zn^{2+} , thus protecting glutamic acid decarboxylase from the inhibitory effect of Zn^{2+} and hence increasing the level of GABA⁽³⁴⁾. In accordance with the present results, **Lerman-Sagie et al.**,⁽³⁵⁾ reported reduced erythrocyte Zn^{2+} content in their epileptics. **Kuzuya et al.**⁽³⁶⁾ reported lower levels of Zn^{2+} in patients who were treated with the AED VPA. In the prospective study done by **Altunbasak et al.**⁽³⁷⁾, the serum and hair levels of Zn^{2+} were found to be higher in untreated epileptic patients than those treated with VPA and controls. In accordance with the current results, is the work done by **Steidl et al.**, and **Armutcu et al.**^(38,39). Controversy exists, as in contrast to the current findings, **Yuen et al.**⁽⁴⁰⁾ reported normal levels of Zn^{2+} in white blood cells of epileptic patients on VPA or CBZ. They

suggested that AEDs might affect the intracellular Zn^{2+} level concentrations.

In the current study non-significant higher serum levels of Cu^{2+} in the untreated epileptic patients as compared to the control group were reported. However, there were significantly higher levels of Cu^{2+} in the epileptic patients receiving VPA therapy. This is in accordance with previous studies^(39,41). The significantly increased level of serum Cu^{2+} in epileptic patients receiving VPA therapy could be attributed to the influence of AEDs on the serum Cu^{2+} and ceruloplasmin concentrations by hepatic enzymes induction⁽⁴²⁾. **Tutor-Crespo et al.**⁽⁴¹⁾ also, reported that VPA may increase the hepatic synthesis of ceruloplasmin. Also, in some cases, a drug induced cholestasis may contribute to the increase in serum Cu^{2+} and ceruloplasmin. Another explanation was given by **Pippenger et al.**,⁽²³⁾ who attributed the high levels of serum Cu^{2+} in untreated epileptics to an inverse relationship between Cu^{2+} and Zn^{2+} . Thus the current study indicates that Cu^{2+} and Zn^{2+} homeostasis might be affected by VPA therapy, but not by the convulsive disorder itself.

However, controversy exists regarding serum Cu^{2+} in either untreated or treated epileptics. **Hurd et al.**, and **Kaji et al.**^(43,44) reported decreased levels of serum Cu^{2+} in epileptics receiving VPA therapy⁽⁴⁵⁾. On the other hand, **Verotti et al.**⁽⁷⁾ reported that VPA did not affect Cu^{2+} or Zn^{2+} serum concentrations.

In conclusion, it seems possible that oxidant stress is associated with epilepsy and is aggravated with VPA

therapy leading to relative reduction in melatonin and absolute reduction in erythrocytic SOD and serum zinc concentration respectively. Thus, using melatonin and zinc (in view of their antioxidant activity) as an adjuvant therapy with VPA seems beneficial and needs thorough investigation.

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الملخص العربي

العلاقة بين مستوى الميلاتونين في مصل الدم وحالة التأكسد و مضادات الأكسدة في الأطفال المصابين بالصرع و تأثير العقار المعالج (فالبروات)

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أستهدف هذا البحث دراسة دلالات حالة الأكسدة و مضادات التأكسد في الأطفال المصابين بالصرع، وأيضاً أستهدف تحديد مستوى الميلاتونين في مصل الدم ودراسة تأثير العقار المعالج فالبروات (VPA) على حالة الأكسدة في الأطفال المصابين بالصرع. و قد شمل هذا البحث ٤٠ طفلاً مصابين بالصرع تتراوح أعمارهم بين ٣ سنوات و ١٢ سنة و تم تقسيمهم الى مجموعتين:

١- مجموعة لم تبدأ أى علاج للصرع (٢٠ طفلاً).

٢- مجموعة تحت العلاج بالعقار (VPA) لمدة لا تقل عن سنة (٢٠ طفلاً).

و كذلك شمل البحث ١٥ طفلاً صحيحاً كمجموعة ضابطة.

وقد تم قياس الآتى في جميع المجموعات:

مستوى الميلاتونين والزنك والنحاس والمالون الثنائى الألدهيد (MDA) كمقياس لعملية الأكسدة، والسوبر أوكسيد ديسميوتيز (SOD).

و قد أظهرت النتائج ارتفاعاً ذو دلالة إحصائية في مستوى الميلاتونين و MDA بينما انخفض مستوى SOD انخفاضاً ذو دلالة إحصائية في كل من مرضى الصرع الذين يتلقون العلاج بعقار (VPA) والذين لا يتلقون علاجاً، مقارنة بالمجموعة الضابطة. بينما وجد انخفاضاً ذو دلالة إحصائية في مستوى الميلاتونين و MDA و SOD في مرضى الصرع الذين يتلقون العلاج بعقار (VPA) عن المرضى الذين لم يبدأوا أى علاج للصرع. و كذلك وجد انخفاضاً في مستوى الزنك بينما وجد ارتفاعاً في مستوى النحاس في المرضى الذين يتلقون VPA مقارنة بالمرضى الذين لم يبدأوا العلاج بالإضافة الى وجود علاقة عكسية بين الميلاتونين و MDA ، و بين الميلاتونين و النحاس، ووجدت علاقة طردية موجبة بين الميلاتونين و SOD .
وبذلك يتضح أن حالات الصرع مقرونة بزيادة في نسبة الأكسدة وأن العلاج ب VPA يزيد عنها سوءاً.