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The Effects Of Antiepileptic Drugs On Serum And Hair Trace Element Levels

Antiepileptik İlaçların Serum Ve Saçta Eser Element Düzeylerine Etkileri

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Aim: To assess whether antiepileptic drugs (AEDs) could affect serum and hair trace element levels, we studied 40 children with epilepsy.

Materials and Methods: Their serum zinc (Zn), copper (Cu), magnesium (Mg) and hair Zn levels were measured by atomic absorption spectrophotometer prior to and 3 months after therapy with VPA (sodium valproate) or PB (phenobarbital).

Results: In both treatments, epileptic children were found to have lower serum Zn concentrations. When serum Zn levels were compared after VPA and PB therapies, those who received PB therapy were observed to have a less significant decrease in serum Zn level.

Conclusion: Although none of our patients showed any symptoms of Zn deficiency, we should be cautious about this in patients who receive VPA and/or PB therapy.

Key Words: ***Antiepileptic drugs, epilepsy, trace element levels***

Amaç: Antiepileptik ilaçların serum ve saç eser element düzeylerini etkileyip etkilemediğini göstermek için epilepsi tanısı alan 40 çocuğu değerlendirdik.

Gereç ve Yöntem: Serum Zn, Cu, Mg ile saç Zn düzeyleri fenobarbital veya valproik asit tedavisi başlanmadan önce ve 3 ay sonra değerlendirildi.

Bulgular: Her iki tedavi ile de serum Zn düzeyleri düştü. Fenobarbital veya Valproik asit tedavisi alan epileptik hastalar karşılaştırıldığında, fenobarbital tedavisi alanlarda serum Zn düzeylerindeki düşme daha belirgindi.

Sonuç: Hastalarımızın hiçbirinde Zn eksikliğine bağlı bulgular görmesek de, Fenobarbital veya Valproik asit tedavisi alan çocuklarda bu açıdan dikkatli olunmalıdır.

Anahtar Kelimeler: ***Antiepileptik ilaçlar, epilepsi, eser element düzeyleri***

Trace elements, despite their low concentration in the body, play an important role in various metabolic events. They are also important for the development of the nervous system, myelination of nerve fibers, and neuronal excitability. Their depletion may cause serious health hazards, such as impaired central nervous system maturation in fetal and neonatal animals which emerges in the case of zinc (Zn) depletion (1). Possible mechanisms explaining the epileptogenicity of Zn in the brain could be the inhibition of glutamic acid decarboxylase (GAD) which is the rate-limiting enzyme for γ- aminobutyric acid (GABA) synthesis or an increase in carbonic anhydrase activity (2). Other trace elements which play an important role in neuronal excitability are copper (Cu), which may produce seizures due to possible inhibition of sodium-potassium ATP ase activity, and magnesium (Mn), which inhibits the facilitating effect of calcium on synaptic transmission and also exerts a voltage-dependent blockage of the N- methyl-D- aspartate receptor-channel (3,4).

Serum and/or hair trace metal homeostasis might be affected by antiepileptic therapy or by the convulsive

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disorder itself. Despite the obvious importance of trace elements, their relation to epilepsy has never been convincingly documented because research results cited in the literature are inconclusive. For one thing, it is not clear whether it is epilepsy itself or antiepileptic therapy which affects serum/hair trace element levels. To give another example, Zn levels were reported to be high, normal and even decreased in untreated and treated epileptics (5-7). In addition, the plasma Cu and Mg levels in epileptic patients treated with AEDs seem to be conflicting (8-12). Therefore this study was undertaken to evaluate whether AEDs affect serum and hair trace element levels.

Methods

We included in our study a total of 40 patients who were diagnosed with epilepsy according to the International League Against Epilepsy (Commission of the Classification and Terminology of the International Leaque Against Epilepsy, 1981) criteria. Following the baseline evaluation, patients were subdivided into two groups according to their therapy: group A, 20 patients (11 males, 9 females; average age, 2.24±0.35 years) treated with PB and group B, 20 patients (13 males, 7 females; average age, 7.69±1.12 years) treated with VPA. For both groups, serum Zn, Cu, Mg and hair Zn levels were identified. These measurements were made prior to and 3 months after therapy with AEDs. VPA and PB received by the patients was prescribed at the normal dosage and all plasma levels of AEDs were within the therapeutic range during the time of study. Patients were excluded from the study if they were receiving medical therapies that might interfere with the nutritional status of the trace elements or if they were receiving trace element supplements prior to the assessment. Similarly, children with other systemic illnesses other than epilepsy, such as diabetes, renal failure, malnutrition or infectious diseases were excluded from the study. Informed consent was obtained from the parents.

Samples

Blood samples were drawn from patients who were diagnosed with epilepsy before the beginning of the treatment with AEDs and three months later in the morning between 8.00-9.00. Venous blood specimens were drawn into trace element collection tubes containing EDTA as anticoagulant. They were centrifuged at 3000g for 15 min and serum was seperated and kept at -20 C until assay analysis. During analyses, samples of 0.5 ml serum were diluted with 2 ml deionized water for Zn and with 0.5 ml for Cu and assayed directly. In the blood samples drawn 3 months after the treatment, AED levels were also assessed. Hair specimens were also obtained from both groups prior to and three months after the treatment. Hair specimens were cut by stainless steel scissors 1 cm from the hairline in the neck and kept at room temperature until determination of Zn content. During analysis; in order to wipe out contaminating material such as hair dye and dirt, hair specimens were placed in an acetone/hexane mixture (3:5, v/v) for 24 hours. They were then washed several times with demineralized water and dried in an etuve. After weighing, hair samples were digested in a solution comprising concentrated nitric acid/ percloric acid (5:1, v/v) for 24 hours and burned by heating mildly until all perchloric acid fumes disappeared. The samples were then diluted with demineralized water in plastic tubes to a defined volume and assayed.

Equipment and Analyses

All the element analyses were performed with an atomic absorption spectrophotometer (Hitachi Zeeman-8000 Atomic Absorbtion Spectrophotometer). Each measurement was performed three times and averages were used. Result were expressed in μg/g for hair, in μg/dl for Zn and Cu, mg/L for Mg.

Statistical Analysis

Statistical analysis of the data were computed using SPSS for Windows. Results are given as mean±SD. Prior to and following the antiepileptic treatment, serum Zn, Cu, Mg and hair Zn levels were interpreted using Wilcoxon Paired Sample Test. $\alpha = 0.05$ was accepted as margin of error. Correlations were determined by Pearson's test and $p<0.05$ was considered statistically significant.

Results

The main results of the study are presented in Table 1. When the effects of anticonvulsant therapy on trace elements were analyzed in epileptics, there was only a significant decrease in serum concentrations of Zn; the differences between serum Cu, Mg and hair Zn levels of the two groups of patients were not statistically significant ($p>0.05$). Zn concentrations in the serum of epileptics treated with PB were found to be significantly lower when compared to those treated with VPA ($p<0.05$). When the serum concentrations of VPA and PB were evaluated in relation to the serum Zn, Cu, Mg and hair Zn, no significant relationship was found. Patients treated with PB monotherapy had significantly lower levels of serum Zn compared to patients treated with VPA. This parameter showed significant difference according to age ($p<0.05$).

Table 1. Relevant data of the two groups of patients traeated with PB or VP

	Group A		Group B	
	Baseline	After 3 months	Baseline	After 3 months
Sex (F/M)	9/11	9/11	7/13	7/13
Age (year)	2.24±0.35 (Range 0.3-4)		7.69±1.12 (Range 1.5-14)	
Zn (μg/ml)	99.0±8.0	56.65±4.62	85.90±2.79	69.75 ± 3.07
Cu (μg/ml)	111.22±13.06	114.55± 7.38	112.65±9.33	111.45±5.97
Mg (mg/L)	24.33±2.74	24.13±0.81	19.50±0.64	19.44±0.53
Hair Zn (μg/g)	134.0±9.41	130.5±7.9	136.59±8.56	134.0±7.1

Discussion

Epileptogenesis is a challenging disease because its mechanisms are not well established. Various experimental and human studies demonstrated that the abnormal metabolism of trace elements might be involved in the pathophysiology of epilepsy. In addition, AEDs can also alter the homeostasis of trace elements. However, the association between altered homeostasis of trace elements in epileptic patients to epilepsy *per se* and/or the effect of anticonvulsant drugs therapy has never been clearly understood (9,13,14). A possible explanation for conflicting reports on the effects of anticonvulsants on trace element levels might be the methods and biological material used in the measurements. Additionally, most studies have generally compared long-term AED recipients to healthy control patients (15,8,6).

On the other hand, the number of studies focusing on the trace element levels of epileptics prior to and during treatment is rather limited (14-17). We believe that the results of such studies may be more reliable in showing whether AEDs affect the kinetics of trace elements. Therefore, we compared serum concentrations for the trace elements in epileptic patients who had not undergone treatment with AEDs to the results of the mono-AED-treated patients in order to verify the factors affecting concentrations of trace elements in serum. We observed that, after three months of therapy with VPA or PB, serum Zn levels dropped whereas no meaningful difference occurred in Cu, Mg and hair Zn levels. Verrotti *et.al.* studied serum Cu and Zn levels of 36 epileptic children who were being treated with VPA or CBZ (carbamazepine) and, contrary to our study, found no meaninigful difference between their serum Cu and Zn levels prior to and 1 year after the treatment and those of healthy control children (14). On the other hand, Altunbasak *et.al.* observed 16 epileptic patients undergoing VPA treatment for 6 months and reported that their serum and hair Zn levels were higher than those of the control group prior to the study but that the levels were normalized with treatment, thus making Zn replacement therapy unnecessary (17). Another study with serum has demonstrated that serum Zn and Cu levels in epileptic patients under chronic antiepileptic therapy differed somewhat from the control level, but remained within the normal range (13) . Lerman *et.al.* observed that plasma zinc levels of 3-18 year-old patients receiving VA for petit mal epilepsy were not different from those of the control group, alhough the erythrocyte zinc levels were significantly lower than the control group. Lerman *et.al.* concluded that AEDs may affect the intracellular distribution of Zn (9). Steidl *et.al.* stated that PB results in Mg deficiency in red blood cells although serum Mg and serum Zn levels stay normal, and that therapy of magnesium lactate produces significantly higher RBC Mg and serum Zn with improved clinical EEG and biochemical findings (18). In another study conducted by Ilhan *et.al.*, average hair Cu, Mg and Zn levels were found to be significantly lower in epileptic patients than in the control group. However, they did not find a significant difference between the hair Cu, Zn, Mg and serum Mg and Zn levels of epileptic patients who received antiepileptic treatment and those who did not. On the contrary, Suzuki *et.al.* showed that the hair and serum Zn levels both decreased in 17 epileptic patients who were receiving combined VPA and other AED treatments (16). The results of this study are partially concordant with those of Altunbasak *et.al.* and tend to conflict with other data in the literature. In this study we were not able to verify whether the serum and hair trace element levels of epileptic and healthy children differed prior to the treatment. Our set was inconvenient for this purpose and constitutes one of our limitations. The treatment may not have led to a change in hair Zn levels because Zn turnover in this tissue is slower than plasma and red cells. Had we examined hair Zn levels much later, we may have observed a meaningful decrease in this compartment too. The duration of drug treatment and the prescribed dose are also important variables. It is important to underline that all patients treated in our study were precribed drugs at the normal dosage and all serum levels of AEDs were within the therapeutic range. Age may have been one of the factors why serum Zn levels were significantly lower in PB group. Regardless of whether the patient is a child or adult, age is not considered a strong variable because the plasma levels of these trace elements are not believed to change after the age of 3 (19). Studies examining the relationship between epilepsy and trace element levels have been conducted on adults and children over 3. This study brings to mind whether infants are particularly sensitive to the effects of AEDs on trace element homeostasis. To our knowledge, this study is the first to examine the effects of PB treatment on serum and/or trace element levels in infancy. More advanced studies of the effects of AEDs on the trace element homeostasis in infants may result in new therapeutic approaches.

Conclusion

Based on these observations, we can speculate that AEDs might upset the homeostatic balance of trace elements and cause abnormalities of serum or other tissue trace element concentration. Although none of our patients showed any symptoms of Zn deficiency, we should pay attention to potential Zn deficiency especially in infants treated with VPA or PB treatment.

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