

Magnesium, Zinc and Copper Contents in Hair and Their Serum Concentrations in Patients with Epilepsy

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Objective: Although trace elements are found a little amount in body they have obvious structural functional importance and have an important role on a variety of biological processes. Since the abnormal metabolism of metal ions plays an important role in health and disease conditions, studies about them have been attracting great interest. The aim of our study was to evaluate magnesium, zinc and copper contents in hair and serum concentrations in patients with epilepsy.

Method: Twenty-seven patients (12 male, 15 female; mean age $42,73 \pm 16,23$ years) with epilepsy and 29 age-matched healthy subjects (12 male, 17 female; mean age $49,76 \pm 13,11$ years) were enrolled in this study. We assessed Mg, Zn and Cu contents in hair and their serum levels in all subjects with an atomic absorption spectrophotometer. Results were calculated in ppm for hair and in $\mu\text{g/ml}$ for serum. For comparison of means, Student's t test was used.

Results: The mean values of trace element concentrations of hair in patients were significantly reduced compared with the controls (mean magnesium concentration in patients, $111,33 \pm 37,33$ vs. control, $133,57 \pm 22,91$ [$p < 0,01$], mean zinc concentration in patients, $121,40 \pm 45,40$ vs. control, $176,96 \pm 43,10$ [$p < 0,001$], mean copper concentration in patients, $42,74 \pm 20,36$ vs. control, $60,22 \pm 22,32$ [$p < 0,05$]). Although the serum levels of magnesium and zinc are lower in patients with epilepsy than the levels in controls, these differences were not found to be significant (mean magnesium level in patients, $19,4 \pm 2,7$ vs. control, $23,3 \pm 12,3$ [$p > 0,05$], mean zinc level in patients, $1,54 \pm 0,79$ vs. control, $1,86 \pm 0,83$ [$p > 0,05$]). Furthermore, lower magnesium levels in patients with epilepsy were closely related to pharmacoresistant epilepsy.

Conclusion: These data indicate that concentrations of Mg, Zn and Cu are altered trace element concentrations become change in patients with epilepsy during interictal periods and these changes may be important, especially low magnesium levels, in anticonvulsant drugs designed for the clinical problem of pharmacoresistant epilepsy.

Key words: Epilepsy, hair, serum, trace element

Trace elements exist in very low concentrations in the body and their concentrations in the cerebral tissue is not equal in all parts of brain (1). Although they are minor building components in tissues, they play important

functional roles in peripheral and central nervous systems (CNS) (2-5). They play an important role in neuronal excitability (3-10).

It was shown previously that low magnesium level - induced epileptiform activity in rat entorhinal cortex slices changes with time from a pattern of serial seizure -like events (SLEs) to a state of continuously recurring epileptiform activity. It was proposed that the late activity is a model for pharmacoresistant SLEs since it was also refractory to phenytoin, carbamazepine, phenobarbital, and midazolam. It was demonstrated that low magnesium level -induced epileptiform activity in rat entorhinal cortex is an in vitro model for the transition from pharmacosensitive to pharmacoresistant SLEs (11).

Recently, hair analysis has been used to evaluate the trace element status in the body (11-13). Unlike blood, serum and urine, the hair provides historical information on concentrations of trace elements in the body as well as the nutritional condition over a long period of time (12,14). Furthermore, trace elements are often more concentrated in the hair than in bodily fluids. In addition, hair analysis provides information about intracellular accumulations of trace elements. The technique is more invasive for the detection of trace elements, although hair samples can be collected painlessly and stored easily (13,15,16). There are many investigations on the serum trace element levels of epileptic patients. We preferred to examine hair from the epileptic patients, since serum does not have stable balance of trace elements. Serum levels may show variability during the day. For more reliable measurements, a stable tissue should be used. In the light of these facts, we studied Mg, Zn, and Cu levels in hair and serum and compared with controls.

Material and Method

Twenty-seven patients (12 male, 15 female; mean age $42,73 \pm 16,23$ years) with epilepsy and 29 age-matched healthy subjects (12 male, 17 female; mean age $49,76 \pm 13,11$ years) were enrolled in this study. The demographic characteristics of patients and control subjects are presented in Table I. The clinical diagnosis was made according to the criteria of the International League Against Epilepsy (17) and electroencephalographic records. Since the geographic and environmental

Table I. The demographic characteristics of patients and control subjects.

Sex	Patients		Control Group	
	Number (%)	Age (Yrs)	Number (%)	Age (Yrs)
Female	12 (44.44)	39.26	17 (58.62)	47.29
Male	15 (55.56)	43.92	12 (41.37)	51.36
Total	27 (100)	42.73	29 (100)	49.76

conditions may affect the blood and hair levels of trace elements, all subjects had to be selected among the people who had been staying in Elazığ for at least 1 year. These subjects did not suffer from diabetes, kidney failure, or other disease nor had they been treated with drugs, which can interfere with the nutritional status of the elements (diuretics, antihypertensive drugs, etc). Hematocrit, blood urea nitrogen, creatinine, glucose, liver enzymes, total protein, sodium, potassium, and calcium were analysed in all subjects. We assessed trace element concentrations of hair and trace element levels of serum in all subject with atomic absorption spectrophotometer (Shimadzu AA-6701F) using a standard additional technique of Kirgbright (18).

Blood was drawn from the antecubital vein of each subject using a plastic syringe fitted with stainless steel needle. Ten ml blood was taken in the morning from each subject after overnight fasting period and before taking the morning dose of anticonvulsants. The blood was collected into a metal-free plastic tube, allowed to clot, and then centrifuged at 3000 g for 15 minutes. Centrifuged material was put into deionized tubes. Blood was burned over the beck fire at 250 °C. In order to perform a proper and complete burning, we put a certain amount of nitric acid/perchloric acid mixture (5/1, v/v) to the tubes. After burning organic matrix completely, inorganic remnants were taken to plastic tubes with certain dilutions. They were kept at +40 °C until assay.

All the subjects had black hair. There were not subjects with dyed hair or who have had any cosmetic hair treatment. They were allowed free access to shampoos. However, no subject used dandruff shampoos containing zinc pyrithione. Before the start of this study, all the patients were verbally informed of the aim of the study, and all agreed to participate. We obtained the hair samples from the patients when they had their hair cut by barbers on the same day with blood samples. The patients received ordinary routine haircut service to trim their hair. Hair samples were obtained by cutting the hair from the nape or occipital region with a stainless steel scissors. We used the portion of the hair sample that was originally located within 5 cm from the scalp. The distal ends of the hair

were discarded. Then they were washed several times with demineralised water and dried in an incubator. After weighing, hair samples were digested in a solution comprising concentrated nitric acid/perchloric acid mixture (5/1, v/v) for 24 hours and burned by heating mildly until all perchloric acid fumes disappeared. They were diluted by demineralised water to a defined volume and kept at +40 °C until the analyses. Results were calculated in ppm for hair and in µg/ml for serum. For comparison of means, Student's t test was used.

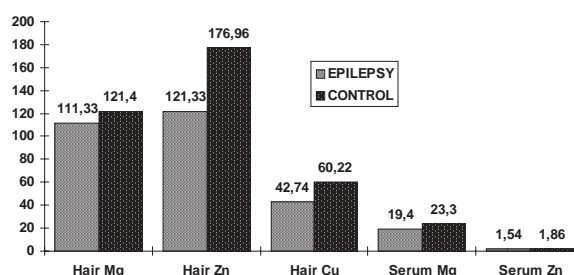


Figure 1. The mean values in ppm for hair and in µg/ml for serum trace element levels in epileptic patients and control subjects.

Results

Mean hair Cu, Mg, and Zn levels and mean serum Mg, and Zn levels are shown in Figure 1. The mean values of element concentrations of hair in patients [mean magnesium concentration in patients, 111.33 ± 37.33 vs. control, 133.57 ± 22.91 (p<0.01), mean zinc concentration in patients, 121.40 ± 45.40 vs. control, 176.96 ± 43.10 (p<0.001), mean copper concentration in patients, 42.74 ± 20.36 vs. control, 60.22 ± 22.32 (p<0.05)] were significantly reduced compared with the controls. Although the serum levels of magnesium and zinc were lower in patients with epilepsy than the levels in controls, these differences were not found to be significant [mean magnesium level in patients, 19.4 ± 2.7 vs. control, 23.3 ±

12.3 ($p > 0.05$), mean zinc level in patients, 1.54 ± 0.79 vs. control, 1.86 ± 0.83 ($p > 0.05$)]. Furthermore, lower magnesium levels in patients with epilepsy were closely related to pharmacoresistant epilepsy.

Discussion

There are a few studies of trace elements in hair for the pathogenesis of epileptic seizures; generally studies are restricted to serum, saliva or mononuclear cells. Advantages of the study reported here are high trace element levels in hair (19), which makes analysis easy; slow metabolic turnover rate of hair (20); and its being a reliable specimen for retrospective search of trace element status of body without daily variations. Also, collection of hair samples from patients is relatively simple and non-traumatic. It is important to emphasize that the serum is not suitable to determine the levels of some trace elements. Using tissues that have a stable element turnover is especially necessary for retrospective studies, because serum element levels are widely variable in a day, even in an hour depending on food intake. For that reason, hair is widely used for determination of trace elements in body and toxic substances absorbed from industrial plants (21-23).

Experimental studies intended to show their relationships with the epilepsy have been conducted since the early 1970s. Epileptic seizures were obtained in rats by intraventricular zinc injections in 1971, 1973, and 1982, and intracortical in 1983 (3-6). Other experimental studies were directed to the determination of the pathophysiological mechanisms yielding this epileptogenic effect, and it was shown that the zinc ions inhibited the activity of the Na-K-ATPase enzyme (4), which is known to concentrate in the hippocampus (24). It was claimed that this situation increased the neuronal excitability and led to seizure (25). It was suggested at the same time that a relationship might well exist between the zinc level in the hippocampus and gamma-aminobutyric acid (GABA) concentration (26). It is also known that it has an antiepileptic effect in humans. Zinc causes an increase in the taurin secretion and may well raise the seizure activity of zinc (25). Suh et al. (27) reported that the hippocampus showed significant vesicular zinc ion depletion following adrenalectomy. After the kainite injection, adrenalectomized rats showed proconvulsive seizure behavior, i.e. shortened latency to seizure onset time and increased seizure score.

A low Zn concentration was demonstrated in the hair from epileptics on prolonged anticonvulsant therapy (28). It was also reported that hair concentrations of Cu in both male and female epileptics, Zn in male epileptics, and Mg in female epileptics were significantly decreased when compared with those of age-matched and gender-matched controls. Hair Cu concentrations were significantly decreased in male epileptics; a significant decrease in hair Mg concentration was observed in female epileptics when compared with schizophrenics. An increased serum Cu

concentration was found in female epileptics and a decreased Zn concentration was found in male epileptics (29). However, we did not observe any difference for trace element levels in hair and serum between male and female epileptics. It was reported that the hair copper levels in 55 patients with epilepsy were significantly lower than those in the control group. The hair zinc levels in female patients were significantly lower than those in the control group (30). It was also demonstrated that long-term anticonvulsant therapy with valproic acid and carbamazepine could induce alteration in both the metabolism and distribution of Cu, Zn, and Mg (25). It was also concluded that both serum and hair zinc levels in epileptic patients deviated from normal before the beginning of valproic acid monotherapy, but returned to normal during valproic acid treatment (31). On the other hand, some studies (32,33) demonstrated that prolonged anticonvulsant therapy with valproic acid and carbamazepine did not affect levels of Cu, Zn, and Mg. We confirmed the findings of previous studies that some trace element levels in serum and hair are significantly lower in epileptics than controls. Reason of these alterations however, still sounds unclear.

It was shown previously that low magnesium level -induced epileptiform activity in rat entorhinal cortex slices changes with time from a pattern of SLEs to a state of continuously recurring epileptiform activity. It was proposed that the late activity is a model for pharmacoresistant SLEs since it was also refractory to phenytoin, carbamazepine, phenobarbital, and midazolam. It was demonstrated that low magnesium level -induced epileptiform activity in rat entorhinal cortex is an in vitro model for the transition from pharmacosensitive to pharmacoresistant SLEs (11). The reduction of hair and serum Mg levels in the epileptic patients is easier to explain. Mg is an essential element with a role in neuronal excitability. It inhibits the facilitating effect of calcium on synaptic transmission and also exerts a voltage dependent blockage of the NMDA receptor-channel (7). Lowering the extracellular Mg-concentration induces various patterns of epileptiform activity in combined rat entorhinal cortex-hippocampal brain slices (34). In addition, the efficacy of magnesium sulfate in the prevention and control of eclamptic convulsion has been validated in randomized controlled trials performed worldwide (35).

It is known that metals are indispensable components for certain enzymes in the brain, for example, dopamine- β -monooxygenase, tyrosine-3-monooxygenase, cytochrome c-oxidase (36) and GABA (25). They are also important for the development of the central nervous system (36,37), and play an important role in neuronal excitability (9,10). The abnormal metabolism of these metals involves severe neurological and mental disturbances (38,39). These results, together with previous studies, demonstrate that trace element levels in human body play an important functional role in the pathophysiology of epilepsy.

In conclusion, these data indicate that concentrations of Mg, Zn and Cu are altered trace element concentrations become change in patients with epilepsy during interictal periods and these changes may be important, especially low magnesium levels, in anticonvulsant drugs designed for the clinical problem of pharmacoresistant epilepsy. Forthcoming clinical trials will provide further information from understanding standpoint the exact roles of trace element levels in the central nervous system.

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