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Regular paper

Bioelements in hair of children with selected neurological disorders

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We have analyzed concentrations of magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn) and iron (Fe) in hair of a group of 82 children with mental retardation, in which 9 patients suffered from epilepsy, 18 from the Down's syndrome and 55 from cerebral palsy. Girls comprised little over 50% of the patients. In the group of boys with epilepsy, we found Mg, Ca, Cu and Fe deficiency, and normal level of Zn. In the group of girls with epilepsy, apart from low Fe concentration, a high level of Ca, Mg, Zn, and Cu was noted. For girls with the Down's syndrome, a high or normal level of Ca, Mg, Zn and Cu was found, whereas the Fe concentration varied and presented itself in a non-characteristic way. Both groups of children with cerebral palsy, i.e. boys and girls, displayed low Fe concentration in their hair; low Cu level was found in older patients as well. In this group of patients, we also noted high concentrations of Ca, Mg and Zn in girls and normal in boys. A high concentration of Ca in girls with cerebral palsy requires separate analysis. The obtained results could be useful as guidance in the direction and determination of the amount of possible patient nutritional supplementation.

Key words: bioelements, epilepsy, Down's syndrome, cerebral palsy, hair, children

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Abbreviations: Mg, magnesium; Ca, calcium; Cu, copper; Fe, iron; Se, selenium, Zn, zinc

INTRODUCTION

The group of bioelements consists of macro and micronutrients.

Macronutrients are chemical compounds whose concentration in the environment (as well as in the human body) is relatively high. Because the level of macroelements among the various types of environments and groups of organisms differs, there are different reference values (Aggett, 1985; Macioszczyk, 1987; Maathuis, 2009; Rodwell *et al*., 2015). In regards to the human body, a macronutrient is a chemical element whose required daily intake is higher than 100 mg and which is necessary for the healthy development. Ca and Mg are in this group. Ca is an important constituent of bones, it plays a pivotal role in the muscle contraction and relaxation, and also, among other functions, it regulates the electrical conduction system of the heart. Mg in necessary for the formation of the bone, teeth and for normal nerve and muscle function, it regulates the function of enzymes and is related to the metabolism of Ca and potassium.

Micronutrients are chemical compounds that occur in very low (trace) amounts in humans and plants. The required daily intake of these elements for humans is less than 100 mg. Fe, Cu and Zn are among the group of micronutrients.

Fe plays a vital role in the catalysis of enzymatic reactions, it is also an essential part of cofactors such as the heme groups that make up hemoglobin, the protein which transfers oxygen. Cu is involved in the redox reactions of essential metabolic processes, such as mitochondrial respiration and synthesis of melanin. Zn, as part of many specific enzymes, plays a role in gene transcription, signal transduction and regulation of apoptosis.

A deficiency or excess of macro and micronutrients could lead to a homeostasis disorder, because these elements arenecessary not only for the developmental purposes (especially of the bone structures), but they are also part of the body fluids, enzymes, high energy compounds. Bioelements influence and regulate the activity of individual organs, as well as the whole organism.

Epilepsy is a group of neurological disorders that result from temporary incapacity of the brain, which leads to rapid electrical discharge within the brain cells. Loss of consciousness and seizures are characteristic symptoms of epilepsy. It is thought that the brain damage that occurred in the perinatal or a later period is the cause of this disease (Thurman *et al*., 2011).

The Down's syndrome is a congenital genetic disorder caused by the presence of additional chromosome 21. Patients with the Down's syndrome have lower cognitive abilities than among the average healthy population, and mental retardation varies from mild to moderate (Roizen & Patterson, 2003).

Cerebral palsy is a disease deriving from the damage to the central nervous system in the prenatal or perinatal periods, or within the next few years after birth (Gajewska, 2009).

The aim of our study was to measure the concentrations of Mg, Ca, Zn, Fe, in the hair of children with epilepsy, Down's syndrome and cerebral palsy and determine the basis for a possible bioelement supplementation in children with the mentioned disorders. It is important to conduct further studies, because publications on the subject of trace element concentration in patients with neurological disorders, published so far (Fung, 2002; Lech, 2002; Hartley, 2003; Kilpinen, 2009; Lima *et al*., 2010; Tórsdóttir *et al*., 2011; Amouian *et al*. 2013; Kheradmand *et al*. 2014; Prasad *et al*., 2014; Saad *et al*., 2015; Saghazadeh *et al*., 2015) show ambiguous results and no clear conclusions.

MATERIALS AND METHODS

We analyzed the concentrations of Mg, Ca, Cu, Zn and Fe in hair of a group of 82 children with mental retardation, in which 9 patients suffered from epilepsy (6 girls, 3 boys), 18 from the Down's syndrome (10 girls, 8 boys) and 55 from cerebral palsy (26 girls, 29 boys). All children within our study group suffered from moderate to profound mental retardation.

The collected test samples of hair were washed via shaking in a detergent solution (shampoo) with deionized water for 15 minutes. Afterwards, the detergent was carefully rinsed out with deionized water, and additionally, the samples were shaken in acetone for 5 minutes. Thus cleaned hair was then dried to dry matter in a dryer (in temperature of approx. 50°C). The samples were then put into a desiccator for 24 hours.

These samples were then mineralized with the mixture of 65% ultra-clean acids: HNO_3 and $HClO_4$ (in the proportion of 3:1 v/v), and then diluted with deionized water (0.06 μS · cm^{-1}) to a set volume (25 mL).Concentration of such elements as Ca, Mg, Zn, Cu and Fe was estimated by the means of atom absorption spectrophotometer using a flame technique called F-AAS, and a spectrophotometer manufactured by Perkin Elmer (model 2100 with flame atomizer and hollow cathode lamps). The measurement was carried out in the flame of C_2H_2 with the use of air as the oxidizing gas. The proportion of gases was 2.5/8, and excitation was performed at a resonant wave length of λ=422.7 nm for Ca, λ=285.2 nm for Mg, λ=213.9 nm for Zn, λ=324.8 nm for Cu and λ=248.3 nm for Fe. For each quantified element, the obtained results are presented as μg or ng per gram of dry matter of hair.

RESULTS AND DISCUSSION

The results obtained for each bioelement concentration (Ca, Mg, Zn, Cu, Fe) are shown in Table 1.

In the group of boys with epilepsy, apart from normal Zn concentration, a Ca, Mg, Fe deficiency was noted (Table 2). Results obtained for the group of girls with the same disorder indicate increased levels of Ca, Mg, Zn and Cu, while the Fe concentration presents itself in a non-characteristic way- ranging from high to low concentrations. (Table 3).

In the group of patients with the Down's syndrome, the hair of boys displayed a decreased level of Fe, but was characterized by normal levels of Ca, Mg, Zn and Cu (Table 2). The girls' hair had low or normal Mg concentration, and normal or high Zn levels. While the Cu and Fe concentrations varied, we have noticed the tendency for high concentrations of Ca (Table 3).

In the most numerous group – patients with cerebral palsy, both, the girls' and boys' hair had low Fe levels, and in the elder patients, regardless of sex, we also found decreased levels of Cu. Concentration of Ca was high in girls and normal in boys, whereas levels of Mg and Zn were slightly increased in girls and normal in boys. (Table 2, 3).

Neurological disorders, leading to disability and social exclusion, are caused by perinatal, genetic or environmental factors. Retardation and deviation of mental and physical development coexist with lower immunity, and higher frequency of infectious and somatic diseases. The severity of the disorders depends on nourishment and absorption of nutrients. Abnormal physical and mental development (Fung, 2002) and malnutrition lower the chances of full recovery of the central nervous system and affect the child's ability to adapt to the environment.

Optimization of nutritional support, based on previous assessment of the bioelement supply in children with neurological disorders, could lead to improvement of treatment and rehabilitation effects (Kilpinen-Loisa, 2009).

According to systematic review conducted by Hartley, most children with neurological disorders have a subclinical or severe deficiency of bioelements (Hartley, 2003).

Kheradmand and coworkers (2014) in their paper compared Zn and Cu concentrations in blood serum of 70 patients with epilepsy aged from 6 months to 15 years, of whom 35 children had intractable and 35 patients had controlled epilepsy. Statistically significant Cu insufficiency was found in a group of patients with drug-resistant epilepsy. In another study, similar group's deficiency was not significant.

Meta-analysis of 40 articles regarding the role of minerals in epilepsy and febrile seizures, brought by Saghazadeh and coworkers (2015), included the comparison of Zn, Mg, Cu and selenium (Se) concentrations in blood serum, hair, and spinal fluid in patients with epilepsy and febrile seizures. The concentration of Zn in untreated patients with epilepsy was significantly higher than in the control group, while concentration of Zn in patients with febrile seizures was lower. Concentrations of Cu, Mg and Zn were within normal range in patients treated with anti-epileptic drugs, whereas children with untreated epilepsy showed low Mg and high Zn and Cu levels.

In the Prasad and coworkers (2014) study, an association of serum trace elements and minerals with genetic generalized epilepsy (GGE) and idiopathic intractable epilepsy (IIE) was investigated. 200 patients with GGE and the same number of patients with IIE took part in this study. The authors had found that children with GGE had a relevantly low Ca, Mg and Zn levels. A significant decrease in Zn concentration was found in patients unresponsive to treatment, in comparison to patients with drug- controlled epilepsy, and also, no significant difference in Ca, Mg and Zn levels were found between these two groups. Low concentrations of Ca, Mg and Zn and high level of Cu was associated with GGE, while patients with IIE had a low Zn concentration. The study performed indicated a correlation of high levels of Ca, Mg, Zn and Cu in girls, in contrast to boys, and an increased Fe level in both groups. In the study performed by us, patients with epilepsy did not suffer from drug resistant epilepsy.

Zn plays an important role as a micro-element. Some research (Saad, 2015) suggests a moderately beneficial influence of Zn treatment in patients with IIE, therefore Zn supplementation could be used in the epilepsy treatment protocol, especially in patients with the drug resistant epilepsy.

In Amouian's and coworkers (2013) study, 150 children with epilepsy had total blood count, Fe, Zn, Mg and Ca determined.

The Mg concentration was significantly low, whereas Ca, Fe and Zn levels were increased.

In patients with the Down's syndrome, Tórsdóttir and coworkers (2001) assessed the Cu homeostasis, in-

Table 1. Bioelements' concentration in the hair of children with neurological disorders.

Epilepsy								Mean	S.D.	Min	Max
Age	Sex	Ca	Mg	Zn	Cu	Fe	Ca	725.9	411.8	110.0	1172.5
13.3	F	1147.9	41.6	385.6	54.2	11.3	Mg	51.3	31.4	15.7	98.2
14.6	F	1033.0	98.2	213.4	15.8	19.4	Zn	213.4	67.8	164.5	385.6
15.0	F	792.0	38.4	228.1	13.6	13.6	Cu	18.6	17.3	5.0	54.2
16.0	F	1172.5	86.8	203.5	42.2	18.0	Fe	16.2	6.3	7.1	28.0
16.0	F	1037.4	80.2	193.5	11.5	28.0	**Norm** (Dunicz-Sokołowska)				
18.5	F	649.6	60.4	179.8	9.2	22.3	Ca	Mg	Zn	Cu	Fe
9.0	M	413.1	23.9	164.5	7.5	12.6	412.1	28.1	172.4	12.6	15.8
16.0	M	110.0	15.7	185.0	8.6	7.1					
17.7	M	177.6	16.3	167.6	5.0	13.8					
Down's syndrome								Mean	S.D.	Min	Max
Age	Sex	Ca	Mg	Zn	Cu	Fe	Ca	446.4	386.9	103.8	1355.7
5.0	F	237.0	17.0	180.7	9.2	9.2	Mg	24.7	14.3	8.9	57.3
7.0	F	833.3	38.6	133.9	9.6	22.9	Zn	183.7	56.7	102.6	339.5
7.0	F	597.6	41.7	102.6	10.7	7.2	Cu	10.8	5.1	6.4	25.3
10.0	F	1054.6	26.2	241.3	7.9	9.2	Fe	13.4	7.5	7.2	30.6
11.0	F	495.2	22.6	339.5	25.3	30.6	**Norm** (Dunicz-Sokołowska)				
11.0	F	573.7	38.4	272.4	21.6	9.6	Ca	Mg	Zn	Cu	Fe
12.0	F	176.9	13.1	200.6	7.1	9.5	367.4	25.1	161.6	12.1	16.0
13.4	F	677.0	36.7	181.2	9.8	9.8					
16.2	F	923.0	57.3	184.4	11.2	10.0					
16.3	F	1355.7	43.3	152.7	12.7	28.0					
6.0	M	148.9	8.9	153.9	11.5	8.9					
7.0	M	119.7	10.0	157.1	7.5	12.5					
10.0	M	171.5	12.5	226.6	7.5	13.8					
15.4	M	123.0	12.4	176.4	13.7	8.7					
15.5	M	128.1	16.8	140.1	7.2	9.6					
17.2	M	168.7	20.4	136.8	6.4	8.9					
17.5	M	148.2	16.9	176.8	7.8	24.7					
19.5	M	103.8	11.9	149.1	7.2	8.3					
Cerebral palsy								Mean	S.D.	Min	Max
Age	Sex	Ca	Mg	Zn	Cu	Fe	Ca	793.0	627.8	34.4	2667.2
5.0	F	1201.4	148.9	155.1	13.5	7.4	Mg	49.2	46.2	2.5	164.3
6.0	F	996.2	90.8	248.4	10.8	6.0	Zn	210.3	96.0	93.8	594.1
9.0	F	2059.2	114.4	163.8	9.6	9.6	Cu	9.1	2.0	4.3	14.3
9.0	F	980.0	60.4	203.4	11.1	8.6	Fe	8.4	1.8	5.4	12.7
10.0	F	1831.2	141.7	156.6	8.7	6.2	**Norm** (Dunicz-Sokołowska)				
11.0	F	709.6	33.9	357.5	9.5	6.8	Ca	Mg	Zn	Cu	Fe
12.0	F	1124.5	61.9	197.7	9.7	12.1	367.4	25.1	161.6	12.1	16.0
12.0	F	905.1	48.8	224.5	7.1	8.0					
12.5	F	524.5	48.2	162.7	7.8	9.1					
12.6	F	1083.6	60.8	155.4	9.1	10.4					
14.0	F	2066.1	134.7	204.2	11.3	8.5					

14.2	F	1108.0	45.9	408.4	10.9	8.5
14.5	F	553.7	35.6	156.6	7.6	7.6
15.2	F	1403.2	114.0	140.6	8.9	8.9
15.5	F	696.5	37.8	419.8	8.3	8.3
16.0	F	2214.2	164.3	366.0	12.9	10.3
16.1	F	1046.0	21.3	305.3	11.3	11.3
16.4	F	925.9	33.5	167.3	8.7	7.4
17.0	F	419.8	24.2	290.1	11.5	12.7
17.1	F	2667.2	161.8	168.0	6.2	5.4
18.0	F	1677.0	83.8	172.9	9.0	6.4
19.0	F	1369.1	157.2	163.3	7.4	8.6
19.2	F	1151.4	82.2	181.7	4.3	6.8
19.3	F	795.5	65.0	263.9	10.2	10.2
19.5	F	1500.5	37.0	594.1	8.9	8.9
19.7	F	1807.3	133.7	227.1	7.9	6.9
6.0	M	414.4	19.8	152.2	9.9	9.9
7.0	M	325.3	11.9	93.8	7.1	9.5
9.0	M	283.1	21.1	132.2	8.2	10.5
9.0	M	455.5	21.1	186.2	9.4	8.2
11.0	M	877.6	11.7	125.0	14.3	9.1
11.0	M	185.5	15.6	187.9	9.6	8.4
12.0	M	622.0	26.3	197.4	8.4	9.6
12.0	M	628.6	17.8	177.0	7.1	7.1
12.5	M	221.9	15.1	168.1	5.7	6.6
13.3	M	145.2	13.2	161.8	9.9	7.7
13.7	M	365.5	12.4	135.0	11.1	9.9
13.8	M	222.1	24.7	167.5	9.1	9.1
13.9	M	142.9	8.6	149.0	8.6	9.9
13.9	M	34.4	2.5	150.0	9.8	6.1
14.1	M	170.2	15.1	157.5	6.4	5.6
14.2	M	186.2	19.5	199.2	9.1	6.5
14.7	M	166.2	13.7	193.7	12.4	12.4
15.0	M	996.1	19.5	511.7	7.8	6.5
15.0	M	192.9	12.2	145.1	5.7	7.3
15.1	M	507.7	29.9	207.1	9.6	7.2
15.7	M	308.0	29.0	187.7	10.4	8.3
15.7	M	278.5	27.0	187.4	10.3	10.3
15.9	M	293.5	10.9	158.2	10.9	10.9
16.2	M	1151.8	61.4	303.3	9.8	9.8
16.9	M	135.4	11.4	136.2	6.1	7.0
16.9	M	166.3	14.3	182.5	10.5	5.7
18.2	M	836.7	33.2	182.5	6.1	7.0
19.1	M	126.1	16.0	162.2	7.6	6.7
19.6	M	357.0	26.8	216.7	8.9	8.9

Concentration of each bioelement									
					Norm (Dunicz-Sokołowska)				
Ca	709.5	575.4	34.4	2667.2	Ca	Mg	Zn	Cu	Fe
Mg	44.0	40.8	2.5	164.3	367.4	25.1	161.6	12.1	16.0
Zn	204.8	86.0	93.8	594.1					
Cu	10.5	6.8	4.3	54.2					
Fe	10.4	5.1	5.4	30.6					

cluding the ceruloplasmin concentration and superoxide dismutase activity (SOD1). SOD1 activity was found to significantly decrease with age. The study we performed was a one-time only event, and therefore cannot be used for comparison.

Analysis of Zn resources in children with the Down's syndrome (Lima *et al.*, 2011) had shown low levels of Zn. The obtained results could lead to a conclusion that changed nutritional status in individual patients with the Down's syndrome may be the reason for clinical disorders that occur with age. In our own research for the group of children with the Down's syndrome, we obtained normal Zn hair concentration for boys and a decreased level for girls.

Another study (Lech, 2002) had analyzed the lead (Pb), Cu and Mg levels in the hair of 153 children and adolescents, aged 1–18, with selected neurological disorders. A significant decrease in the average hair Mg concentration was found and a slight increase in average Cu concentration. Both of the results obtained were statistically significant.

Table 2. Bioelements' concentration in the hair of boys with neurological disorders.

	Mean	S.D.	Min	Max					
Epilepsy					**Norm** (Dunicz-Sokołowska)				
Ca	233.6	159.1	110.0	413.1	Ca	Mg	Zn	Cu	Fe
Mg	18.6	4.6	15.7	23.9	296.2	21.3	159.5	12.4	15.5
Zn	172.4	11.1	164.5	185.0					
Cu	7.0	1.8	5.0	8.6					
Fe	11.2	3.6	7.1	13.8					
Down's syndrome					**Norm** (Dunicz-Sokołowska)				
Ca	139.0	24.2	0.0	0.0	Ca	Mg	Zn	Cu	Fe
Mg	13.7	3.9	8.9	20.4	292.5	20.8	154.0	12.0	15.8
Zn	164.6	29.1	136.8	226.6					
Cu	8.6	2.6	6.4	13.7					
Fe	11.9	5.5	8.3	24.7					
Cerebral palsy					**Norm** (Dunicz-Sokołowska)				
Ca	372.3	284.1	34.4	1151.8	Ca	Mg	Zn	Cu	Fe
Mg	19.4	10.8	2.5	61.4	292.5	20.8	154.0	12.0	15.8
Zn	183.2	73.3	93.8	511.7					
Cu	9.0	2.0	5.7	14.3					
Fe	8.3	1.7	5.6	12.4					
Sum					**Norm** (Dunicz-Sokołowska)				
Ca	315.2	262.0	34.4	1151.8	Ca	Mg	Zn	Cu	Fe
Mg	18.2	9.6	2.5	61.4	292.5	20.8	154.0	12.0	15.8
Zn	178.7	63.9	93.8	511.7					
Cu	8.7	2.1	5.0	14.3					
Fe	9.3	3.3	5.6	24.7					

Table 3. Bioelements' concentration in the hair of girls with neurological disorders.

	Mean	SD	Min	Max					
Epilepsy					**Norm** (Dunicz-Sokołowska)				
Ca	972.1	207.6	649.6	1172.5	Ca	Mg	Zn	Cu	Fe
Mg	67.6	24.7	38.4	98.2	594.3	39.2	193.7	12.9	16.1
Zn	234.0	76.1	179.8	385.6					
Cu	24.4	18.9	9.2	54.2					
Fe	18.8	6.0	11.3	28.0					
Down's syndrome					**Norm** (Dunicz-Sokołowska)				
Ca	692.4	362.0	0.0	0.0	Ca	Mg	Zn	Cu	Fe
Mg	33.5	13.5	13.1	57.3	450.7	30.1	171.8	12.3	16.3
Zn	198.9	69.5	102.6	339.5					
Cu	12.5	6.0	7.1	25.3					
Fe	14.6	8.9	7.2	30.6					
Cerebral palsy					**Norm** (Dunicz-Sokołowska)				
Ca	1262.2	571.7	419.8	2667.2	Ca	Mg	Zn	Cu	Fe
Mg	82.4	47.9	21.3	164.3	450.7	30.1	171.8	12.3	16.3
Zn	240.6	109.9	140.6	594.1					
Cu	9.3	2.0	4.3	13.5					
Fe	8.5	1.9	5.4	12.7					
Summary					**Norm** (Dunicz-Sokołowska)				
Ca	1085.1	541.0	176.9	2667.2	Ca	Mg	Zn	Cu	Fe
Mg	68.6	44.0	13.1	164.3	450.7	30.1	171.8	12.3	16.3
Zn	229.7	97.2	102.6	594.1					
Cu	12.2	9.0	4.3	54.2					
Fe	11.4	6.3	5.4	30.6					

The Fe, Cu and Mg concentrations in Kalra's *et al.* study were found to be relevantly low in children with cerebral palsy, which justified the need of supplementation (Kalra, 2015). In our research, in all groups we had found low concentrations of Fe, both, for the girls and the boys.

Multivariate analysis of the selected metals in the hair of cerebral palsy patients in comparison to healthy control group, performed by Khalique *et al.* (2006), had shown a significant influence in the affected patients' physiology.

In the study presented above, we have analyzed the concentrations of selected bioelements in the hair of children with certain neurological disorders. The analyses performed show an abnormal (high or low) bioelement concentrations in hair, however they are not comparable to the serum levels because the two- hair and serum make different compartments.

As a point of reference for our research, we have used a group of 7400 children and young adults who had 5 basic bioelements and oxic metals determined by hair analysis in a study that could be considered as a referential norm in the hair of Polish population (Dunicz-Sokolowska, 2006; Dunicz-Sokolowska, 2006).

CONCLUSIONS

1. Analysis of the selected bioelements in the hair of patients with epilepsy, Down's syndrome and cerebral palsy has shown differentiated results.
2. The assessment of concentrations of these bioelements only in the hair of the studied group, without simultaneous analysis of the blood serum levels, does not indicate the need for bioelement supplementation in the patients with selected neurological disorders.

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