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DETERMINATION OF TRACE ELEMENTS IN HAIR ANALYSIS USING ICP-MASS SPECTROMETRY

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ABSTRACT

Determining the trace element levels in the human hair is a significant analytical technique, a screening element in the evaluation of possible deficiencies, excesses, and/or biochemical imbalances in all bodies of these microelements. In this work of research, by an inductively coupled plasma– mass spectrometry (ICP-MS) analyzer, the authors have proposed the discovery of toxic trace elements (Al, Pb, Hg) and levels principal mineral elements (Ca, Mg, Cu, Zn) from the human body on healthy individuals with a good nutritional status. The study was performed on a sample of 75 adult women (30-35 years old) from different regions of the country, by taking 100 mg of hair from the base of the scalp with a length of 3 cm, for analysis and evaluation of trace elements. Twelve patients (16%) had high mean values of intracellular Mg (1.2 mmol / L), high Ca values (0.72 mmol / L), but low mean Ca / Mg ratios, (0.58). In addition, six patients (8%) had low mean values of Mg (0.004 mmol / L) and Ca (0.04 mmol / L) but a high Ca / Mg ratio. At the moment of analysis, all individuals did not have acute or severe intoxication signs with heavy metals. The environmental lifestyle of analyzed individuals, from various areas of the country, was observed in their hair cells, by present levels of trace elements.

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Introduction

The mineral analysis of the hair traces is useful in assessing the general condition of nutrients and health [1-3]. Trace elements evaluation in the hair may be used in diagnosing illnesses like checking human poisoning, cancer, and examining malnutrition [4, 5], benefiting from the opportunity to conveniently collect and store samples.

Hair mineral analysis is a test to measure the level of nutritional and toxic mineral elements found in hair tissue. The essential feature is the determination of the level of minerals stored in the cells and the interstitial spaces of the hair over 2-3 months and allows us to deduce what happens in other tissues even if it does not provide an assessment of the mineral content of other tissues of the body [6]. The components that are absorbed and temporarily circulate in the body before excretion and/or storage are measured by the blood, while the toxic elements and nutrition found in the hair produce a permanent exposure record, the concentrations being 10-15 times higher in the hair than in blood or urine.

The analysis of hair is one of the important biological monitoring of environmental pollution [7], and can be used to sensitize the individual to maintain a healthy lifestyle in their environment following the United Nations Global Program for Environmental Monitoring (GEMS) for the Environment [8].

In the literature, a series of works can be found that describe the quantification of the inorganic components of human hair, where the relationships among the common diseases and mineral composition of human hair are recognized. In this context we find a series of correlations: blood pressure levels were evaluated in combination with the concentration of Fe, Ca, Mg, Zn, Cu, Na, K, [9]; diabetic patients showed significantly lower levels of manganese and zinc [10]; in patients with lung cancer at different stages, higher levels of cadmium in the scalp hair were found compared to the control group and very high levels of cadmium in smokers compared to non-smokers [11].

The morphological status of the hair of the persons suffering from diabetes and hypertension cholelithiasis and kidney stone was evaluated by scanning electron microscopy [12].

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The method inductively coupled plasma– mass spectrometry (ICP-MS) used for determinations of trace elements analysis of product bulk and materials from human tissues, can be effectively used for determination of mineral levels in human hair for nutritional, toxicological, and pharmaceutical drug trials purposes [13-15].

Materials and Methods

The main objective of the present study was to diagnose intracellular extents of main mineral nutritive elements (Ca, Mg. Cu, Zn) and trace elements (Al, Pb, Hg) in the human hair, measured by (ICP-MS) instrument and correlating the status of trace elements concerning of possible human health risk. Metal testing, as main mineral nutritive elements (Ca, Mg, Cu, Zn) and trace elements (Al, Pb, Hg), were performed via inductively coupled plasma mass spectroscopy conventional (IPC-MS; Perkin Elmer 900): From the analysis of residual samples of digested hair (100 mg), with results calculated after the equivalence, 1 part per million (ppm) =1000 parts per billion (ppb) = 1mg/L).

In 75 adult females, aged between 30 and 55, all from different regions of the country, 100 mg of hair in the special cuvettes IPC-MS were sampled, with a length of 3 cm near the scalp, and used to obtain information about the status of nutrients in the last 6 to 8 weeks.

The calibration of IPC-MS has been made with the 10 milliliters of azotic acid (HNO3), 60% concentrated, in the dilutions 1/10, 1/100, 1/100, to the pressure of 35 atm. (315 psi). The blank of sample was prepared: 200 milliliters, solution in concentration 2% from HNO3 60% concentrated, (200 x 2 / 60 = 6.6 ml; 6.6 ml HNO3 + 193.4).

The samples of 100 mg hair were collected by cutting the first 3 cm closest to the scalp. The samples were mineralized with 10 ml HNO3 60% concentrated, for 15 minutes, in cuvettes with opened lips, and after that dilution has been done 1/ 1 = V/V. The digest of samples continued in IPC by micro-sounds, to a power of 1600 W, heated for 15 minutes, at 800 psi pressure, and results were interpreted after chromatography of graphics IPS –MS.

Results and Discussion

The results are presented in **(Tables 1-3)**, and can be compared with the other international studies from the medical literature. All the individuals of this study have been registered and followed the results of levels of intracellular concentration of mineral elements.

Considering total participants in the present study, 55 (73%) showed normal intracellular extents: Mg (normal range = 4.1-10.5 ppb parts per billion (ppb), 0.03-0.09 mmol / L, mean value = 0.06 mmol / L; SD = 0.2 mmol / L; p = 0.02); normal intracellular extents for Ca (normal values = 20-40 ppb, normal values = 0.1-0.2 mmol /L, mean value = 0.15 mmol /L; SD = 2.5, p = 0.05) and normal report Ca/Mg (range 4.5-7.5, mean value = 6.5) **(Table 1)**.

Table 1. Database of ICP-MS; Perkin Elmer 900 [16].

No pacient	Concentrations (Mean Value)				Reports Mean Value
No. Cr	**Ca (ppb)**	**Ca (mmol/L)**	**Mg (ppb)**	**Mg (mmol/L)**	**Ca/Mg (ppb)**
55	397.50	0.15	81.48	0.06	**4.87 ppb**
12	558.45	0.29	335.8	0.4	**1.66 ppb**
8	149.50	0.07	200.1	0.02	0.74 **ppb**
Reference	22-97 ppm male 18-47 ppm female		2-11 ppm 2-18 ppm		6.77 ppb 6.80 ppb

Twelve patients (16%) indicated vast extents of intracellular Mg (mean value = 1.2 mmol /L); high levels of Ca, (mean value =0.72 mmol /L), but low extents of report Ca/Mg, (mean value = 0.58) and 8 patients (8%) showed low extents Mg (mean value = 0.004 mmol/L); low extents of Ca (0.04 mmol /L) but high report of Ca/Mg (10).

Distinctly, 9 patients, in measurements of mass spectrometry, were registered with normal values for Ca (0.18 mmol/L), very low extent for Mg (0.006 mmol/L), and reported Ca/ Mg very high (30). At the same time, to these special cases, in 9 people performed the blood tests on the level of Ca and Mg: serum blood values determined for total Ca of 10.7 mg/dL or 2.30 mmol/L, (normal value = 9.1 -10.8 mg/dL; 2.19-2.54 mmol/L and value for Ca^{2+} was registered 1.41 mmol/L, (normal value for Ca^{2+} = 1.02-1.42 mmol/L).

For Mg in blood, the normal value of 2.53 mg/dL or 1.04 mmol/L, (normal value 1.6-2.5 mg/dL, 0.73-1.06 mmol/L), was registered. All normal values were for Ca and Mg in blood, but high values of intracellular report Ca/Mg gave us the signs of answer regarding physiopathology of dynamic rate ion Ca/Mg in the functionality of human cells. The Analysis of Cu and Zn concentrations in the hair samples of the selected patients, microelements as important for the assessment of nutritional and health status are presented in **Table 2**.

Zn and Cu were registered in high values but normal report Zn/Cu, in conformity with chromatography ICP-MS. Patients with elevated levels have been accused of muscle spasm, especially at night, under conditions of high physical stress. Moreover, the results can be compared with normal results of another Licensed Clinical Laboratories example 14: i) Zn references range (10-21 ppm); Cu references range (0.9-3.9 ppm); Zn/Cu acceptable range is (4-12); ii) example value data for female 47 years: Zn (16 ppm), Cu (0.9 ppm); Zn/Cu 17.78 (ppm) **(Table 2)**.

Table 2. Level of Intracellular Concentration of Trace Elements

No pacient	**Concentrations (Mean Value)**				**Reports Mean Value**
	Zn (ppm)	Zn (μg/dL)	Cu (ppm)	Cu (μg/dL)	Zn/Cu (μg/dL)
8	13.52	42.3	5.83	1.83	23.6
7	10.13	32.1	4.78	1.5	21.4
10	10.48	3.2	13.4	4.2	2.57
15	28.71	8.7	11.69	3.67	7.8
17	8.47	19.1	18.96	5.95	13.8
18	3.21	10.7	3.46	1.1	32
Reference Range	10-21		0.9-3.9		4-12

In **Table 3** was presented the selection of data reflecting the high levels of metal concentrations with toxic potential detected in hair samples, (N= normal values; H= high values)

Table 3. Intracellular concentration of toxic elements

Hg		**Pb**		**Al**	
C (μg/dL)	**Bounding**	**C (μg/dL)**	**Bounding**	**C (μg/dL)**	**Bounding**
0.84	N	0	N	0.11	N
0.63	N	0.17	N	0.09	L
3.5	H	0	N	0.2	N
0	N	0.03	N	0.3	H
0	N	0	N	0.23	H
0.18	N	0	N	0.2	N
0.18	N	0.01	N	0.3	H
0.21	N	0	N	0.4	H
0.5	N	0.02	N	0.8	H
0.12	N	0	N	0.4	H
0.25	N	0	N	0.15	N
0.44	N	0	N	0.2	N
2.46	H	0	N	4.9	H
1.29	H	0	N	1.8	H
0.042	N	0	N	0.1	L
2.46	H	0	N	0.5	H
1.29	H	0	N	0.7	H
0.042	N	0	N	0.4	H
0.55	N	0.88	H	3.42	H
0.63	N	0	N	0.4	H

Taking into account normal values of prospect of method used by Mass Spectrometry, MS Agilent (Hg, normal value = 0.63-0.99 μg/dL; Pb normal value =0.3-0.45 μg/d L; Al, normal value = 0.15-0.21), the samples analyzed for the 75 persons fall into the following categories; Hg, (μg/dL) = 15 persons with normal values, 5 persons with high value; Pb, (μg/dL)=19 person with normal values, 1 person with high value; Al, (μg/dL = 4 persons with normal values, 14 persons with high values and 2 persons with low values).

The mineral elements Ca^{2+} and Mg^{2+} are very necessary enzymatic reactions into the cellular and extracellular processes, including the system of coagulation, aerobic metabolism cells, and including the harmonic performance of different organ systems and endocrine glands for the good health of the body.

Only 1% to 3% of the total intracellular Mg exists as the free ionized form of Mg, which has a closely regulated concentration of 0.05 to 0.1 mmol/ L, the situation in which the data from our research does not fit (except point 2) **(Table 1)**, finding a deficit of Mg in the other samples, especially subjects from point 4 in the Table (0.006 mmol/L). Total cellular

Mg condensation may differ from 0.55 to 2 mmol/dL, considering the type of tissue investigated, with the highest Mg concentrations being found in skeletal and cardiac muscle cells [17].

Magnesium is the second most plentiful intracellular cation after potassium. It has a vital task in regulating various cellular functions and enzymes, which include signaling pathways, metabolic cycles, and ion channels.

To maintain the positional entirety of closely clustered phosphate groups, an important ion that is considered is the magnesium ion (Mg^{2}+). These clusters emerge in various separate sections of the cytoplasm and cell nucleus. The integrity of proteins, ribosomes, and nucleic acids is maintained by Mg^{2}+. Furthermore, this ion acts as an oligo-element with a role in energy catalysis. Poly-anionic charges are exhibited on the surface by cell walls and biological cell membranes. Because various membranes preferentially bind different ions, this result has significant implications for the transport of ions.

To regularly stabilize both Ca^{2}+ and Mg^{2}+ membranes, the phosphorylated and carboxylated head groups of lipids are cross-linked. In metabolism, Malignant cells use Mg^{2}+ ions more often than normal cells and they increase the magnesium uptake from stores in normal tissues, including muscles and bones. The serum Mg level is enhanced through Mg^{2}+ release from malignant tissues in patients with the malignant disease before therapy with cytostatic medicines.

Copper and Zn have a significant role in a smooth full body function. Thus, the mean values of Zn and Cu in hair in patients with epilepsy were reduced [18]. The mean values of the condensations of Mg, Zn, Cu (ppm) determined in the hair in pat its with epilepsy were much lower in comparison to the control group: 111.33 ± 37.33 Vs. 133.57 ± 22.91 ($p < 0.01$); 121.40 ± 45.40 Vs. 176.96 ± 43.10 ($p <0.001$); 42.74 ± 20.36 Vs. 60.22 ± 22.32 ($p <0.05$).

Zinc is an important trace element for the immune system, neurogenesis, zinc deficiency can lead to poor cognitive performance, altered attention, and motor development [19]. Zinc concentrations in the hair samples of learners with lower IQ were found to be lower (141.70 ± 88.56 µg / g) compared to regular students (198 ± 90.90 µg / g) in P = 0.01, and the copper level for the samples of the IQ lower group (11.90 ± 3.97 µg / g) compared to 13.23 ± 3.97 µg/ g for the control group at P = 0.18 without significant difference between the two groups [20].

A recent study reported the level of concentrations of Zn, Ca, Mg in scalp hair at children with growth retardation compared to the control group (average values): Zn 157 ± 25 µg / mL and 1218 ± 42.29 (p = 0.001); 9.97 ± 3.99µg / mL and 11.83 ± 2.53 (p = 0.5); Ca 1168 ± 231 µg / mL and 1417 ± 245 (p = 0.001); Mg 168 ± 50 µg / mL and 206 ± 40 µg / mL (p = 0.005). It is found from these data that the levels of Zn, Ca and Mg in the scalp hair were significantly lower in the children with growth retardation in comparison to those of the controls (P <0.05) [21].

Children's hair is a commonly used biomarker for monitoring exposure to heavy metals. A total of 40 hair samples from Libyan children were analyzed using inductive coupled plasma mass spectrometry (ICP-MS) to determine the level of heavy metals (Cd, Pb, and Hg) in children with autism and normal children.

The findings indicated that the concentrations of these metals in children with autism are higher than in the group of normal children (µg / g) Pb 40.99 ± 32.02 vs. 37.00 ± 14.61: Cd 0.82 ± 0, 98 vs. 0.42 ± 0.27; Hg 7.84 ± 10.57 vs. 4.07 ± 2.54 [22].

Concentrations of trace metals were determined in the hair, children in the surrounding areas of Athens, Greece [23]. The values of the concentrations of heavy metals with toxic potential determined by the polluting factors of the environment (water, air) in the hair of the children are found as average values (µg/g) for Hg between 0.52 - 0.36 and Pb 3.31 - 0.80. The heavy metal content of the hair is the result of the exposure and the absorbed dose that is correctly interpreted offers the possibility of evaluating the potential of the exposure. The analysis of the heavy metal concentrations in the hair of the population tested for inhabitants of Krakow proves by the concentrations (mg kg-1 of dry matter): Zn (Female 260.86, Male 241.68) and Pb (Female 2.46, Male 3.04) a potential environmental hazard on near-professional danger hair of people [24].

Nearly, 60% of magnesium ions are reserved in tissues and about 40% of magnesium ions chip in intermediary metabolism. Approximately 70% of these ions exist in the Mg^{2+}free form. The other 30% of the ions are related to citrate, phosphate, proteins (particularly albumin), and other complexes. Within very narrow limits (0.65-1.05 mmol/dL; 1.58-2.25 mg/dL), the serum levels of magnesium are kept constant. There is a regulation that happens in the kidneys, particularly through the ascending loop of Henle. The complex 3-D structure, which is formed through the hydration of the Mg^{2+} ion in an aqueous environment, determines the transport mechanisms.

Cancer is known to be the main problem in the diagnostic process. In support of this aspect, the study of correlating the status of trace elements in the body, evaluated by the mineral analysis of the hair concerning the occurrence of cancerous diseases is approached by Czerny *et al.*, (2014). The values of the concentrations of metals determined by ICP-MS for the population from Poland of female origin cancer patients were divided into three groups, considering the cancer type: cancer with high glycolytic activity (D), alimentary tract cancer (HG), and hormone-dependent cancer (H) are presented as follows in, **(Table 4)** [25].

Table 4. Content of tested elements in hair of cancer patients

Metals	**Concentration Level (µg/g)**			
	Control	**H**	**HG**	**D**
Ca	425.25 ± 80.93	800.12 ± 403.05∗∗∗	275.86 ± 102.89∗∗∗,+++	689.43 ± 282.36eee ∗∗∗
Hg	27.16 ± 8.14	20.53 ± 14.06∗∗∗	9.58 ± 4.69∗∗∗,+++	eee 15.77 ± 7.61∗∗∗
Cu	13.15 ± 3.12	87 ±2.57∗∗∗,+++,†	7.99 ± 4.22∗∗∗	11.45 ± 5.67∗∗∗

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Zn	141.23 ± 32.11	130.58 ± 39.01*,+++	125.09 ± 47.28**	74.55 ± 27.53***,†††,eee
Hg	0.02 ± 0.02	0.04 ± 0.05***	0.04 ± 0.06***	0.06 ± 0.06***
Pb	0.85 ± 0.54	0.94 ± 0.53	1.18 ± 0.98*	1.19 ± 0.91**
Al	1.10 ± 1.01	2.23 ± 2.5***	3.37 ± 3.69***	3.97 ± 4.65***

Statistical difference versus control, $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively, +++, ++, +, Statistical difference versus D group, $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively, †††, ††, †, Statistical difference versus HG group, $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively, Statistical difference versus H group, $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively, Hormone-Dependent Cancer Group (H).

Conclusion

The present study confirms the usefulness of the analysis of hair elements in screening tests in different diseases in a female population of different ages. Life environment of analyzed individuals, from different regions of the country, styles of alimentation, quality of air or water drank can be reflected in hair cells by analysis of trace elements of all body.

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