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ОРИГИНАЛЬНАЯ СТАТЬЯ

HAIR AND BLOOD MULTIELEMENT PROFILE FOR METABOLIC IMAGING OF THE MAJOR UNIPOLAR DEPRESSION. STUDY RATIONALE AND DESIGN

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SUMMARY: We analyzed 41 elements (Ag, Al, As, Au, B, Ba, Be, Bi, Cd, Ca, Co, Cr, Cu, Fe, Ga, Ge, Hg, I, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, Rb, Sb, Se, Si, Sn, Sr, Ti, Tl, V, W, Zn, and Zr) (multielement profile, MP) in the blood and hair of 48 depressed (15 men and 33 women) and 48 healthy control subjects (23 men and 25 women) in the randomized double-blind prospective clinical-epidemiological investigation. Depression was diagnosed by the psychiatrist following the DSM-IV clinical criterion and by the self-administration of the Beck Depression Inventory (BDI). The blood and hair samples were analyzed by the inductively coupled plasma mass spectrometry (ICP-MS) for all the elements, and by the differential pulsed anodic stripping voltammetry (DP-ASV) and electro thermal atomic absorption spectrometry (ET-AAS) for the selected elements, as a quality control. The statistical data analysis methods are enumerated. This paper is the general introduction to the series of papers where the individual fate of the enumerated elements would be analyzed with respect to the presence or absence of the human major depression. And what is to be followed thereafter with the more complex multifactoral methods of the data analyses for the study of the possible element clustering and interactions relative to the depression.

Introduction

Today, depression is the most prevalent human mental impairment in the world (Licino, Wong, 2005), and hence a great burden to the primary health care system of any country (US Department of Human Health and Health Services, 1993). Indeed, about fifty percent of the unnecessary medical diagnostic procedures can be ascribed to the patients with the unrecognized depression (Chwostick, Katon, 2003). Moreover, depression is the accompanier of the old age (Shammugham, Alexopoulosu, 2005), many hormonal (Whybrow, Bauer, 2005) and chronic diseases (Raison et al., 2005), the cardio- and/or cerebral-vascular insults (Frasure-Smith et al., 1993; Carney. Freedland, 2005), major surgery (Momčilović, 2000), and malignant diseases (Coups et al., 2005).

The clinical picture of depression is well documented and its major signs and symptoms are the basis of now widely adored DSM-IV classification system (APA, 1994). However, the underlying biochemical and metabolic changes in depression are poorly understood. One of the well documented causative and/or associative factors in human major depression are the metals of (in alphabetic order) cadmium, lead, mercury, and molybdenum (Momčilović, 1999). Since, until recently, it was not possible to perform the high accuracy multielement analysis on the same biological matrice sample, our knowledge of metal associated depression was limited to the data from the single element studies. Apparently, there are no data available to show how the group of several different elements would behave together to reflect the underlying disease, and what may be the respective complex element clustering and interaction; if any.

Over the last two decade the multielement profile (MP) analysis of hair confirmed itself as a reliable biopsy material indicator of the human trace element (TE) and mineral nutritional status (Passwater, 1983; Chatt and Katz, 1988). Some researchers still hold sharp criticism on the validity of the hair as a biopsy material for the assessment of the human TE and mineral nutrition status, because of the large within the subject TE variation in the longitudinal sampling, a danger of possible external environmental hair sample contamination, and large between the laboratory variability (Klevay et al., 2002, 2004). Such criticism seems to ignore the fact that deposition of TE in the hair is irreversible, unidirectional process, with hair acting as a biological "memory tissue", in contrast to the TE blood concentration, that is subjected to the immediate compartmental equilibration between the various tissues of the body, i.e., homeostatic regulation. It is evident that the blood TE homeostatic regulation came

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at a cost, and that such tough homeostatic regulation is not necessary to be maintained in the hair TE deposition. Meaning, that there may be no significant correlation between the blood and hair element content, and that element content of the hair may, indeed, vary highly in comparison to their value in the blood. The potential of modern MP analysis, as an overall indicator of metabolic activity of the body, is still very new in the clinical practice, and it will take some time for the MP analysis to get adequate professional appreciation and acceptance (Watts, 2005); it may remain to stay so for quite some time since the preventive medicine does not have all the lure and glamour of the curative medicine.

The aim of this paper is to outline in sufficient detail the design of a prospective randomized double blind clinical-epidemiological investigation where the concentration of 41 elements in the hair and blood was analyzed in human subjects suffering from the major unipolar depression. The presentation and visualization of such a so-called multielement profiles (MP), is a complex task that requires a separate analysis of the behavior of every studied element in the depression; first individually and later on there clustering and interactions. In order to avoid unnecessary duplication in the future manuscripts, we showed here the data on common background, i.e., data on subjects and methods and guiding principles of the entire study.

Subjects and methods

Randomized double blind prospective clinical epidemiological investigation. We followed the principles of the randomized double blind prospective clinical-epidemiological study (Whitehead, 1983,;Spilker, 1984, 1986, 1987). Over the period of three years a total of 96 subjects was involved; there were 48 subjects (15 men and 33 women) who were clinically diagnosed by the licensed board-certified psychiatrics to have unipolar major depression according to the DSM-IV diagnostic criteria (APA, 1994). All the examined depressed subjects were ambulatory, legally fully responsible (i.e. their life decision making capacity was not restricted by the law), and showed no life-threatening suicidal intentions. In addition to the clinical exam and clinical diagnosis of the major unipolar depression, their depressive symptoms were further scrutinized by the self administered Beck Depression Inventory (Beck, 1978). Controls were 48 apparently healthy individuals of similar age (23 men and 25 women) who volunteered for the study. The age distribution of both groups appeared to be very similar (Fig.1).

Ethical considerations. The study was planned and conducted in the full compliance with the ethical principles of the Helsinki Declaration and local Laws and regulations (Derenzo, Moss, 2006). Before giving their written informed consent, depressed subjects were informed orally and in writing about the aim of the study and the procedures involved by both their psychiatrist during the office clinical examination at the "Dom zdravlja Centar", Zagreb, Croatia, and later, at the time of the actual sample collection, by the specialist in internal medicine and occupational health at the Institute for Medical Research and Occupational Health, Zagreb, Croatia. All the control subjects were duly informed about the aim of the study and sampling procedure for blood and hair collection and analysis before they gave their informed consent. None of the depressed participants refused to participate in the study; the matter of fact is that they were pleased with that extra attention, i.e., that somebody appears to be really caring about their health condition. The benefit to the depressed and control participants was the expert professional interpretation of their hair MP and pertinent health counsel relative to their individual nutritional status and health condition.

Glassware and chemicals. All the glassware was Pyrex®, calibrated pipettes were Transferpipette® (Brand,

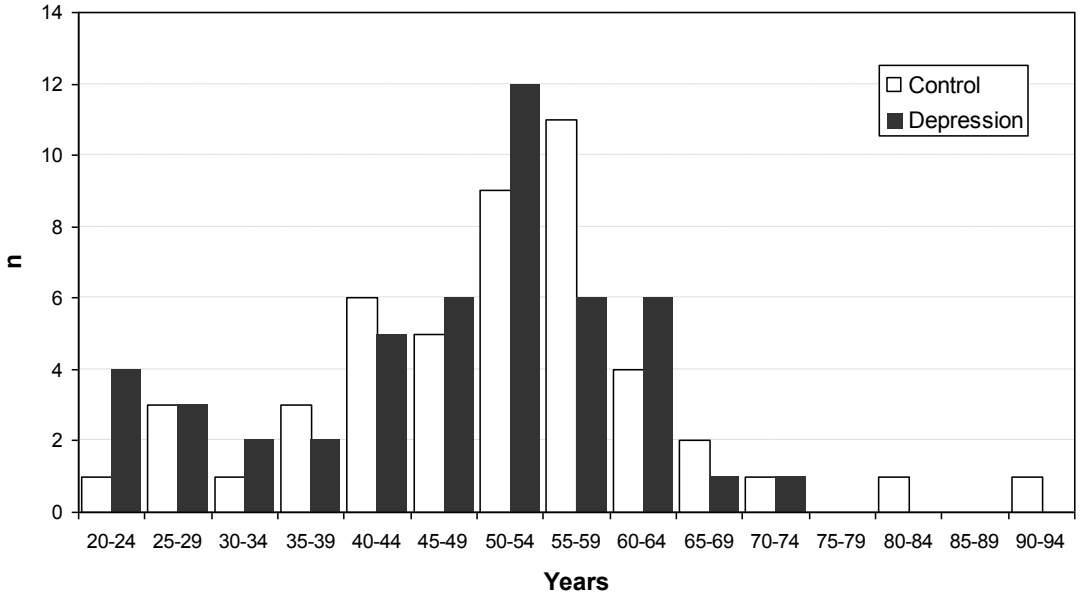

Fig.1. Age distribution of control and depressed subjects

Wertheim, Germany), and all the chemicals were Suprapur (Merck. Darmstadt, Germany), if not stated otherwise. Only redistilled H_2O was used for the analysis. Nitrogen (99.999% v/v) was supplied by Montkemija (Bakar, Croatia).

Hair collection and pre-treatment. Hair analysis was performed following the IAEA recommendations (IAEA 1980), and by following all the other valid analytical methods and procedures (Burgess, 2000). About 0.5-1.0 g of the hair was cut from the occipital head region of all the 96 subjects and stored in the prepared numbered envelope before they were randomly assigned for the analysis (Table 1). Individual hair samples were cut prior to the chemical analysis to be less than 1 cm long, stirred 10 min in an ethylether/acetone (3:1 ww), rinsed three times with the redistilled H_2O, dried at 85°C for one hour to the constant weight, immersed one hour in 5% EDTA, rinsed again in the redistilled H_2O, dried at 85°C for 12 hours, wet digested in HNO_3/H_2O_2. in a plastic tube and analyzed for the multielement profile (MP).

Hair standards. We used Human Hair Reference Material (Shangai Institute of Nuclear Research, Academia Sinica, Shanghai 201849, China).

Blood collection and pre-treatment. Venous blood was collected in the coded plastic vials (BD Vacutainer®, Beckton Dickinson, Plymouth, UK), which were randomly assigned for the analysis. Blood was not collected in 15 cases for various reasons (lost samples, L); sometimes the concentration of element under the consideration was below the detection limit of the analytical method (not detected, ND) (Table 1). Blood analysis was performed following the valid analytical methods and procedures (Burgess, 2000). Blood for DPASV and ET-AAS analysis was dried at 105°C, and ashed in the electrical furnace at 450°C for 24 hours. The cooled ash samples were dissolved in 5 ml of redistilled H_2O. Blood for the ICP-MS (0.5 ml) was digested in a microwave owen with 0.1 g of HNO_3 (Khimmed Sintez, Moscow, Russia) at 175°C.

Blood standards. We used liophylized SeronormTM Trace Elements Whole Blood Reference Standards Level 1 (OK0036), Level 2 (MR9067), and Level 3 (OK0337) for MP in blood (SERO AS, Billingstadt, Norway). Five ml of redistilled H_2O were added to every reference standard and stirred gently at room temperature for two hours to equilibrate. One ml of such equilibrated standard was pipetted in a 25 ml quartz glass vial, dried at 105°C, and ashed in the electrical furnace at 450°C for 24 hours. The cooled ash samples were dissolved in 5 ml of redistilled H_2O with 0.1 ml conc. HNO_3 added.

Coding. The collected samples of hair and blood were coded double blind and randomly sent for the analysis such that neither the acting MD nor the laboratory stuff knew who was the subject and what was his diagnosis (healthy or depressed). When the analytical results were provided, the code was broken and the results of the element analysis matched to the appropriate subjects. The entire code matrix data set for the collected hair and blood in control and depressed subjects of both sexes is shown in Fig.2. It should be noted that every subject represents almost 1% of the total population studied. Such a "topological" code data presentation would be of help to follow every individual subject case, element after element, throughout the entire study.

To get the multielement profile (MP), a total of 41 element was analyzed in every blood and hair biomatrice sample: Silver (Ag), Aluminum (Al), Arsenic (As), Gold (Au), Boron (B), Barium (Ba), Beryllium (Be), Bismuth (Bi), Cadmium (Cd), Calcium (Ca), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Gallium (Ga), Germanium (Ge), Mercury (Hg), Iodine (I), Potassium (K), Lanthan (La), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), Lead (Pb), Platinum (Pt), Rhubidium (Rb), Antimony (Sb), Selenium (Se), Silicon (Si), Tin (Sn), Strontium (Sr), Titanium (Ti), Thalium (Tl), Vanadium (V), Wolfram/Tungsten (W), Zinc (Zn), and Zirconium (Zr). The available reference values for the elements are shown in Table 2 (ANO Center for Biotic Medicine, Russia, 2004), and their actual position in the relevant portion of the periodic system is visualized in a novel way (Fig. 3) (Momčilović, 2006).

Instruments. The blood and hair samples were analyzed by the inductively coupled plasma mass spectrometry (ICP-MS) for all the elements, and by the differential pulsed anodic stripping voltammetry (DPASV) and electro thermal atomic absorption spectrometry (ET-AAS), as a quality control for the selected elements.

Differential pulsed anodic stripping voltammetry (DPASV). All DPASV analyses were performed with the Central unit μAutolab type II (Eco Chemie, Utrecht, Netherlands) and Electrode unit (663 VA Stand, Metrohm, Herisau, Switzerland). Static mercury drop electrode (SMDE), glass carbon auxiliary electrode, and Ag/AgCl reference electrode (saturated in 3M KCl/L), were also all from Metrohm. The software backup was provided by the General purpose electrochemical system (GPES) for Windows – version 4.9 and Frequency response analysis (FRA) for Windows – version 4.9 with USB support (Eco Chemie 2001).

Electro thermal atomic absorption spectrometry (ET-AAS). All the ET-AAS analyses were performed with the Aanalyst 600 (Perkin Elemer Instruments, Shelton, CT, USA).

Inductively coupled plasma mass spectrometry (ICP-

Table 1. Actually analyzed biomatrices (n = number of samples)

	Hair (n = 96)			Blood (n = 81)	
	Women	Men		Women	Men
Control	23	25	Control	17	22
Depression	15	33	Depression	12	30

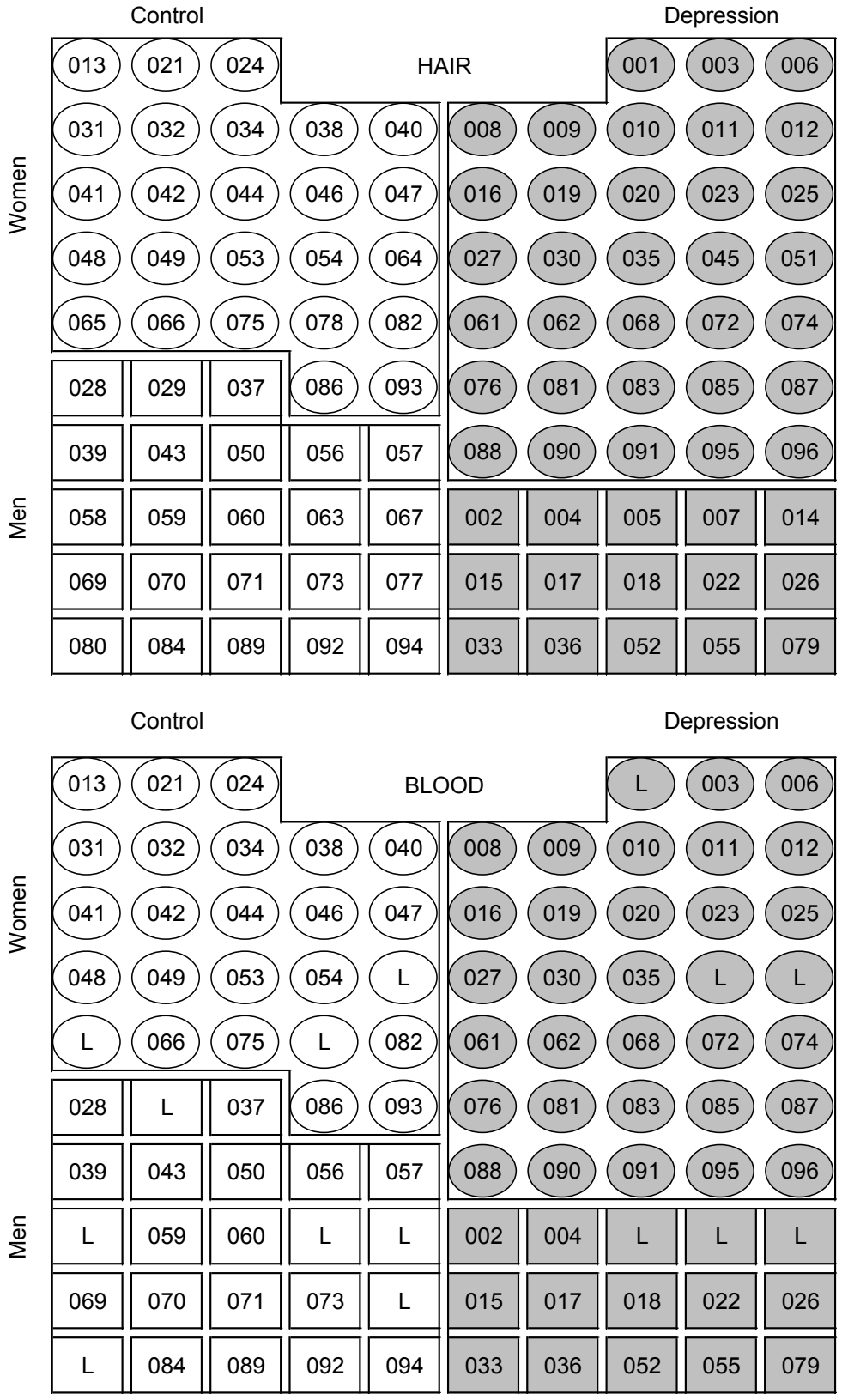

Fig.2. Code matrix sequence. □ Control men, ○ Control women, ■ Depressed men, ● Depressed women. The number within the symbol denotes the subject's sequential number in this randomized double blind prospective clinical-epidemiological investigation

Table 2. ANO Center for Biotic Medicine Reference values (μg/g)

Element	Hair		Blood
	Women	Men	
Silver (Ag)	0.00-0.80	0.00-0.80	0.00-0.005
Aluminum (Al)	0.00-15.00	0.00-40.00	0.00-0.040
Arsenic (As)	0.00-1.00	0.00-0.10	0.00-0.050
Gold (Au)	0.00-0.15	0.00-0.15	0.00-0.00005
Boron (B)	0.00-5.00	0.00-5.00	0.00-0.013
Barium (Ba)	0.00-5.00	0.00-5.00	0.00-0.07
Beryllium (Be)	0.00-0.10	0.00-0.10	0.00-0.00001
Bismuth (Bi)	0.00-1.00	0.00-1.00	0.00-0.016
Calcium (Ca)	600.0-3000.0	300.0-1000.0	32.00-85.00
Cadmium (Cd)	0.00-0.05	0.00-0.15	0.00-0.007
Cobalt (Co)	0.006-0.200	0.01-0.200	0.0002-0.04
Chromium (Cr)	0.15-1.00	0.15-1.50	0.02-0.300
Copper (Cu)	11.0-17.0	10.0-25.0	0.80-1.50
Iron (Fe)	10.0-50.0	10.0-65.0	300.0-600.0
Gallium (Ga)	0.00-0.200	0.00-0.200	not assessed
Germanium (Ge)	0.00-0.200	0.00-0.200	0.00-0.050
Mercury (Hg)	0.00-2.00	0.00-2.00	0.00-0.010
Iodine (I)	0.65-9.00	0.65-8.00	0.020-0.600
Potassium (K)	25.0-110.0	50.0-250.0	1000.0-1200.0
Lanthanum (La)	0.00-0.100	0.00-0.100	0.00-0.0005
Lithium (Li)	0.005-0.100	0.010-0.060	0.00-0.02
Magnesium (Mg)	60.0-200.0	30.0-70.0	25.0-45.0
Manganese (Mn)	0.25-1.80	0.15-1.00	0.010-0.400
Molybdenum (Mo)	0.00-0.150	0.00-0.150	0.0008-0.060
Sodium (Na)	50.0-250.0	80.0-850.0	500.0-5000.0
Nickel (Ni)	0.00-1.00	0.00-1.00	0.00-0.090
Phosphorus (P)	140.0-170.0	135.0-200.0	250.0-450.0
Lead (Pb)	0.00-1.00	0.00-1.50	0.00-0.20
Platinum (Pt)	0.00-1.00	0.00-1.00	0.00-0.002
Rubidium (Rb)	0.00-2.00	0.00-2.00	0.00-2.50
Stibium (Sb)	0.00-1.00	0.00-1.00	0.00-0.004
Selenium (Se)	0.20-1.80	0.15-2.00	0.06-0.30
Silicium (Si)	13.0-50.0	11.0-150.0	0.00-4.00
Tin (Sn)	0.00-1.00	0.00-0.50	0.00-0.002
Strontium (Sr)	0.00-6.00	0.00-6.00	0.00-0.07
Titanium (Ti)	0.00-3.00	0.00-3.00	0.00-0.005
Thelurium (Tl)	0.00-0.10	0.00-0.10	0.00-0.0005
Vanadium (V)	0.00-0.10	0.00-0.10	0.00-0.0002
Wolfram (W)	0.00-0.50	0.00-0.50	0.00-0.001
Zinc (Zn)	180.0-230.0	150.0-200.0	3.50-8.50
Zirconium (Zr)	0.00-0.80	0.00-0.80	not assessed

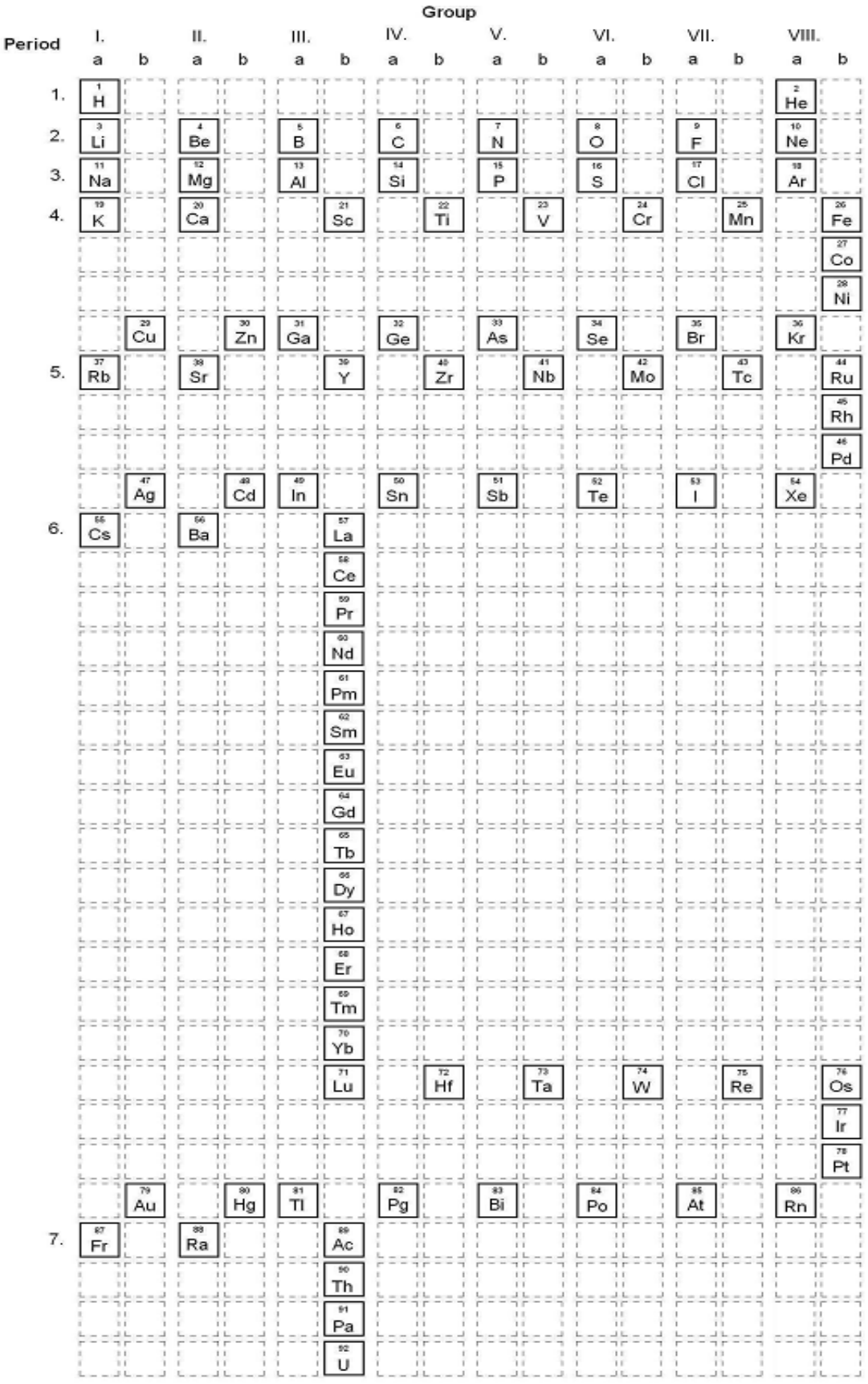

Fig.3. Novel visualization of the relevant part of the periodic system of elements

MS). All the ICP-MS analyses were performed with the Elan 9000 (Perkin-Elmer, USA). The chemicals were pro analysis grade (Khimmed Sintez, Moscow, Russia)

Statistical methods. The overall difference in MP between the respective control and depressed men and women was assessed by ANOVA, whereas the pair wise comparisons were made by using post-hoc Scheffe's test with the significance set at $p < 0.05$. The best-fit quadratic or other type polynomial curve of rank data for every element is provided where appropriate, and blood vs. hair data were plotted. After analyzing the relationship of every element under consideration towards human major depression, the entire MP data set will be analyzed by the multiple regressions, multiple correlation, and factor analysis to study if the intricate element grouping is associated with the human major depression. All analyses were run on SAS version 6.11 (SAS Institute Inc., Cary, NC, USA). Feinstein's (1977) caveats on the statistical inference trespasses in clinical and epidemiological trials were carefully considered.

Discussion

Depression is the multifactor disease that is governed by a complex system modulated by life style, dietary, psychological, spiritual, nutritional, and physiological aspects (Bongiorno, 2005). The focus of here presented outlay of the randomized double blind prospective clinical-epidemiological investigation is on the dietary and physiological aspects of the human major depression. Indeed, our preliminary data on MP showed the strong association of the human major depression with hair iodine deficiency, but apparently normal thyroid T3, T4, and TSH tests (Momčilović et al., 2005). The depression also appeared to be associated simultaneously with selenium and copper deficiency in this very same data set (to be published). Further the more, the multifactor nature of the depression in relation to the MP showed to be of considerable complexity since excess mercury appears to suppress the iodine metabolism in preference to the better known mercury suppression of selenium metabolism. The matter of fact is that, in the world of the multielement profiles, we are dealing with a system of many compounds (elements), all interacting with one another at once, and wherefrom a regular and predictable behavior emerges in statistical form from seeming chaos (Ball, 2004). Now and again we are confronted with the gestalt phenomenon, a distinctiveness of the life process where the characteristics of the whole system cannot be deduced by knowing the characteristics of the parts of such a whole system; doesn't matter how deep our knowledge of that part may be (Keller, 1985). How we may comprehensibly integrate the metabolism, energetics, and signal transduction (Ockner, 2004) of all these elements that appear in our food or as a food contaminants (Reilly, 2002, 2004), remains to be elucidated.

Today, there are about 6.5 billion of people living on the planet Earth; more than 850 million of them are hungry, and about 2 billion is undernourished and suffer from the lack of proteins, vitamins, and mineral elements (Difimije, 2006). It is well known since Pavlov (1927) that our brain is (still) an unsurpassed sensitive analytical instrument and what may transmute all these nutritional impact signals of deficiency and/or excess upon our physiology and metabolism, into a broad spectrum of mental disturbances. Alternatively, the relative abundance or deficiency of elements may change the characteristics of the intricate human metabolic network, i.e., change the expression of the genetic make up, so that the brain may differently receive and perceive the internal neuronal signals. Since every element of our body has its own, specific metabolic story of nutrition and toxicity in health and disease (Vohra, 1982; Skalny, 1999), the new insight we get from the individual element study within the frame of a complex multielement profile in the depressed people, may help us to draw our attention to the new possibilities underlying the biochemistry of this most common mental impairment of the human race.

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