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Concentration of elements in serum of patients affected by multiple sclerosis with first demyelinating episode: a six-month longitudinal follow-up study

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Summary. - Twenty-six chemical elements and oxidative status were determined in serum of 12 patients with first demyelinating episode and brain magnetic resonance imaging compatible with the disease at different time points. Quantifications of Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, V, Tl, W, Zn and Zr, as well as of serum oxidative status and antioxidant capacity were carried out. The results were compared with values obtained from healthy subjects living in the same geographic area. Concentration variability, expressed as coefficient of variation (CV), was evaluated over a six months longitudinal follow-up. The CV was higher for Li and Pb, while showed minimal variation for Ca, Cu, Mg and Zn - elements strictly body regulated. Significant difference ($p \leq 0.05$) in mean concentrations of Ba, Ca, Cd, Cr, Li, Mn, Mo, Ni, Sb, Si, Sn and Zr between patients at time 0 and controls was also found.

Key words: multiple sclerosis, chemical elements, serum, oxidative stress, first demyelinating episode.

Riassunto *(Livelli degli elementi nel siero di pazienti affetti da sclerosi multipla con primo episodio demielinizzante: uno studio longitudinale seguito per sei mesi)*. - Nel presente studio ventisei elementi chimici e lo stato ossidativo sono stati determinati a differenti punti di tempo nel siero di 12 pazienti con un primo episodio demielinizzante (FDE) ed una risonanza magnetica cerebrale compatibile con la malattia. I dosaggi hanno riguardato: Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn, Zr, stato ossidativo (SOS) e capacità antiossidante (SAC). I risultati sono stati comparati con valori ottenuti da soggetti sani accoppiati per età e sesso, residenti nella stessa area geografica. La variabilità degli elementi chimici, espressa come coefficiente di variazione (CV), è stata valutata in sei mesi di follow-up. Il CV era più alto per Li e Pb - elementi derivanti da probabile contaminazione esogena - mentre mostrava una minima variazione per Ca, Cu, Mg e Zn - elementi strettamente regolati dall'organismo. Non è stata trovata alcuna alterazione significativa della concentrazione media di ciascuna variabile nei pazienti con FDE durante tutto il periodo di follow-up. Al contrario, il confronto tra le concentrazioni medie in pazienti con FDE al tempo 0 e nei controlli mostrava una significativa differenza ($p \leq 0,05$) per Ba, Ca, Cd, Cr, Li, Mn, Mo, Ni, Sb, Si, Sn and Zr.

Parole chiave: sclerosi multipla, elementi chimici, siero, stress ossidativo, primo episodio demielinizzante.

Introduction

Several studies have described the importance of abnormal protein-metal interactions and oxidative stress in neurodegenerative diseases such as Parkinson's disease, familial amyotrophic lateral sclerosis, Wilson disease, Alzheimer's disease and multiple sclerosis (MS) [1]. Oxidative stress is implicated in the inflammatory process characterized by overproduction of reactive oxygen species and reactive nitrogen species. Free radicals can cause cellular damage and subsequent cell death because

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reactive oxygen species oxidize cardinal cellular components, such as lipids, proteins and DNA [2]. The toxic effects of metals are mediated through free radicals formation, cell membrane disturbance or enzyme inhibition but it is still unclear if aberrant metal interactions is a primary or secondary casual factor, or a consequence of the neurodegeneration. On the other hand, many trace elements like Cu, Fe and Zn are essential for human life, being components of enzymatic systems involved in principal cellular functions. In recent years, many studies have highlighted the relationship between the dietary intake of trace elements and the efficiency of the immune system. Frequently, deficiency of trace elements and vitamin, even if sub-clinical, has been associated with the impairment of host defence mechanisms. The role of Fe and Zn in immune response has been widely investigated, and recently Cu, Mn and Se have been object of attention [3-5]. These data suggest that metals may alter immune response.

Several epidemiological studies and animal models [6] indicate exposure to metals like a potential risk for induction of autoimmunity. Considering the complexity of their interaction with immune system, it seems possible an etiological role of metals in the pathogenesis of autoimmune disease. Several mechanisms are proposed for how metals act within the immune system and induce autoimmunity. Metal-binding to -SH or to other groups in protein, the interaction with MHC II, the capacity to modify cytokine production are just a part of the mechanisms in metal-induced autoimmunity. In MS a link between development of disease and metal exposure has been proposed suggesting a possible role of some metals in its pathogenesis [7-11]. Moreover accumulating data have indicated that oxidative stress is implicated as mediator of demyelization and axonal damage [12]. In this study, the global profile of trace elements and the oxidative status in patients with first demyelinating episode (FDE) and brain magnetic resonance imaging (MRI) compatible with MS have been evaluated by means of serial measures over a six-month period [13]. The data in MS patients were also compared with those in a healthy population.

Materials and methods

Subjects

Twelve patients (8 females and 4 males) with FDE and MRI compatible with MS were enrolled for the present study. The most common types of clinically isolated syndromes were optic neuritis, brainstem and spinal cord syndromes. Patients mean age was 28.2 ± 7.9 yrs and Expanded Disability Status Scale (EDSS) range was 0-2.5. Twelve age- and sex-matched controls (mean age, 28.3 ± 8.8 yrs; females/males, 7/5) were recruited from healthy blood donors of the same urban area. Exclusion criteria for both groups were: cardiological, respiratory, kidney or liver disease, intestinal absorption abnormalities, infections, assumption of thyroid hormones, intake of vitamin or mineral supplements, vegetarian dietary, artificial metallic bodies. Subjects were followed-up for 6 months with a blood test and clinical examination each month.

Metal analysis

After an overnight fasting and drug abstention, whole blood of patients was drawn with polyethylene syringes and transferred into a 15 ml polystyrene tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA). Blood was allowed to stand for ca. 30 min at room temperature and further centrifuged at 1500 rpm for 15 min to obtain serum. For the analysis of Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr, serum samples were 1+4 (v+v) water-diluted and the sector field inductively coupled plasma mass spectrometry was used. Instead, for the quantification of Ca, Cu, Fe, Mg, Si and Zn, serum was water-diluted 1+29 (v+v) and the inductively coupled plasma atomic emission spectrometry was used [14].

Oxidative status measurements

Both oxidative damage (SOS) and total antioxidant capacity (SAC) were determined in serum by means of a photometric system (FREE, Diacron, Grosseto, Italy). In particular, the SOS, as the sum of the content of hydro- and lipo-peroxides, was assessed by the d-ROMs test (Diacron, Italy) and expressed in arbitrary units, i.e., U.CARR, where 1 U.CARR corresponded to 0.08 mg of peroxyl and alcoxyl radicals for 100 ml of hydrogen peroxide. The range for normal condition was in the interval 250-300 U.CARR, while lower values indicated oxidative damage. The SAC, as the serum capability to resist to the oxidative action of a HClO solution, was expressed as μmol ml^{-1} of HClO. Normal values were over 350 μmol ml^{-1} of HClO.

Statistical analysis

Variability of chemical elements levels and oxidant status during the six-month follow-up was expressed as coefficient of variation (CV). In order to evaluate differences among mean values of the variables over time an analysis of variance (ANOVA) was performed. Student's *t* test was used for evaluating significance of differences between means of the patients at time 0 and controls.

Results and discussion

Table 1 reports the monthly measurements (from time 0 to time 5) of the serum variables as mean and standard deviation (SD), and the variability of the follow-up study assessed as CV (column 9). Monthly measurements in serum showed the high variability of the majority of metals, with a CV ranged > 0.4 - 1.0. In particular, the highest variability was for Li and Pb (CV, 1.04 and 1.05, respectively), whose presence in serum may be presumably related to exogenous exposure. Instead, other elements such as Ca, Cu, Fe, Mg, Mn, Mo, Sr and Zn displayed lower CV values (≤ 0.4), with the lowest variability in time-replicated measurements for Ca and Mg (CV, 0.17). For Ca, Cu, Fe, Mg and Zn this pattern could be a probable effect of the physiological homeostasis. They are, in fact, crucial for many biological activities and their levels are strictly regulated. In the last column of Table 1 the results of the ANOVA are also reported; it is evident that none of the elements showed a significant alteration in mean values across the six-month period. Nevertheless, Ba has shown a gradual slight increment of its concentration, whilst Zr has shown a decrement during the six months follow-up. Table 2 reports, in turn, the comparison between values obtained in patients at time 0 and controls. Serum analysis showed dissimilar mean levels of Ba, Ca, Cd, Cr, Fe, Li, Mg, Mn, Mo, Ni, Sb, Si, Sn and Zr in FDE patients at time 0 when compared with those in controls. In particular, all these elements were higher in their mean values in patients, with the exception for Fe and Li that were higher in the healthy group.

About Ba, its level increased in patients over the time and also respect to normal serum levels. This element is considered a contaminant without biological function. Chronic Ba exposures in the ecosystem and workplace, in fact, have been associated with high incidence clustering of MS [15]. Zirconium is the other metal displaying minor variations over time and, more crucially, important increases in patients in comparison to controls. The potential pathogenetic and predictive value of these changes in Zr concentration are currently under scrutiny. A relative insufficiency of Mg has been demonstrated in the brain of patients affected by MS, as well as by Parkinson's and Alzheimer's diseases [16]. Recently, Mg has been also tentatively used to reduce spasticity in MS patients [17]. These attempts should be treated with caution on the basis of the higher Mg content in serum of the FDE patients. The fact that Ca levels in patients were significantly higher than those in controls might suggest a pathogenic impact on T-cell function and demyelination. An increase in cytosolic Ca is, in fact, a critical second messenger controlling T-cell activation and proliferation through gene transcription [18]. The T-lymphocyte activation leads to a bifasic Ca flux. A prolonged Ca elevation is required for activation of transcription factors and for production of interleukin-2 [19]. Furthermore, several studies in rat suggest that extracellular Ca influx, *via* voltage-gated Ca channels, contributes to white matter damage in acute spinal cord injury and stroke. Iron is known to accumulate in MS brain and in myelin and oligodendrocites, two crucial protagonists in MS [7, 20]. Instead, in our patients a peripheral depletion of this element was found. The higher levels of serum Mo in patients compared to controls, seemed to substantiate the relationship between Mo and MS reported in literature [21]. Chronic environmental exposure through water and soils, in fact, was accountable for an increased risk of this pathology. With reference to Cd, in animal experiments Patra *et al.* evidenced that the neurotoxicity of this element occurred *via* the production of lipid peroxidation and inhibition of superoxide dismutase [22]. Manganese, in its turn, is a recognized neurotoxin which can act, like Cd, by the production of hydroxyl radicals and reduction of cellular antioxidants [23]. The evidences in the literature and the increased serum Cd and Mn levels found in this study could support the implication of these two elements in the MS.

Surprisingly, elements actually not recognized implicated in MS, such as Cr, Li, Sb, Si and Sn were different between patients and controls, but these findings cannot be simply explained or sustained by previous observations. On the contrary, no significant difference in serum has been revealed for those elements, such as Hg, Pb and Zn, for which effects on nervous system have been rather documented. The link between MS and Hg it is often connected to chronic intoxications, e.g. from Hg liberated from dental fillings, whilst Pb is recognized to interact with some Ca-regulated enzymes, include protein kinase C, causing oxidative damage and contributing to MS degeneration [24, 25]. The relationship between Zn and the immuno-pathogenesis of MS is rather complex. It has been shown that Zn stabilizes the association of myelin brain protein with brain myelin membranes, facilitates T-cell proliferation and chemotaxis, and influences T-cell responses to certain interleukins [26, 27].

As concern the oxidative metabolism, our patients did not appear to be injured. In fact, the reactive oxygen species were slightly overproduced in patients but, in the same time, the defence mechanisms were reasonably sufficient to resist to that overproduction. Also during the follow-up study no alteration in SOS and SAC levels was found in FDE patients.

Table 1. - Chemical elements (in ng ml^{-1}) and oxidative status (SOS in U.CARR, SAC in µmol ml^{-1} HClO) in 12 patients with FDE over six-month follow-up

	Time 0	Time 1	Time 2	Time 3	Time 4	Time 5	Time 0 - 5		
	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	CV	*p*
Al	2.93 (1.39)	2.68 (1.73)	2.79 (1.13)	2.39 (1.09)	2.52 (1.31)	2.74 (1.11)	2.67 (1.28)	0.48	0.94
Ba	1.32 (0.54)	1.19 (0.52)	1.36 (0.86)	1.39 (0.66)	1.51 (0.84)	1.8 (0.74)	1.43 (0.71)	0.51	0.38
Be	0.23 (0.06)	0.24 (0.09)	0.3 (0.15)	0.27 (0.12)	0.22 (0.11)	0.28 (0.12)	0.26 (0.11)	0.43	0.54
Bi	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.02 (0.01)	0.48	0.51
Ca	86,017 (11,115)	91,231 (19,887)	91,113 (14,485)	91,326 (16,157)	95,970 (14,203)	89,361 (15,476)	90,836 (15,184)	0.17	0.76
Cd	0.19 (0.11)	0.16 (0.09)	0.12 (0.08)	0.19 (0.11)	0.13 (0.09)	0.16 (0.10)	0.16 (0.1)	0.61	0.34
Co	0.21 (0.11)	0.14 (0.08)	0.20 (0.09)	0.18 (0.08)	0.16 (0.12)	0.17 (0.12)	0.18 (0.1)	0.58	0.56
Cr	0.36 (0.22)	0.38 (0.22)	0.40 (0.15)	0.46 (0.45)	0.35 (0.19)	0.47 (0.31)	0.40 (0.27)	0.67	0.85
Cu	1,034 (228)	1,084 (388)	1,037 (211)	1,045 (188)	1,076 (290)	977 (247)	1,042 (260)	0.25	0.94
Fe	1,318 (527)	1,344 (525)	1,364 (416)	1,669 (797)	1,604 (566)	1,569 (729)	1,478 (602)	0.41	0.58
Hg	1.50 (1.27)	1.30 (0.98)	1.48 (1.12)	1.73 (1.73)	1.57 (1.41)	1.58 (1.51)	1.53 (1.32)	0.86	0.98
Li	0.63 (0.37)	0.63 (0.49)	0.81 (0.53)	1.25 (1.34)	0.51 (0.32)	1.16 (1.33)	0.83 (0.86)	1.04	0.18
Mg	19,558 (3,127)	20,655 (3,162)	20,415 (3,524)	20,792 (4,001)	20,328 (3,519)	19,468 (3,832)	20,203 (3,453)	0.17	0.91
Mn	1.13 (0.33)	1.20 (0.66)	1.26 (0.4)	1.20 (0.35)	0.94 (0.28)	1.24 (0.57)	1.16 (0.45)	0.39	0.52
Mo	1.45 (0.49)	1.52 (0.62)	1.44 (0.36)	1.23 (0.34)	1.37 (0.38)	1.78 (0.96)	1.46 (0.57)	0.39	0.32
Ni	1.62 (1.24)	1.13 (0.54)	1.35 (0.48)	1.88 (1.53)	1.12 (0.39)	1.59 (1.13)	1.44 (0.99)	0.69	0.33
Pb	0.53 (0.45)	0.69 (0.65)	0.61 (0.32)	0.67 (0.40)	0.81 (1.19)	0.73 (0.94)	0.67 (0.71)	1.05	0.96
Sb	0.30 (0.16)	0.22 (0.13)	0.13 (0.12)	0.22 (0.15)	0.21 (0.17)	0.20 (0.14)	0.21 (0.15)	0.71	0.16
Si	627 (238)	730 (340)	738 (316)	782 (476)	718 (314)	845 (500)	740 (368)	0.50	0.81
Sn	1.36 (0.74)	1.13 (0.36)	0.93 (0.41)	1.12 (0.62)	1.01 (0.46)	0.95 (0.39)	1.08 (0.52)	0.48	0.34
Sr	34.9 (6.12)	36.1 (8.65)	37.3 (11.7)	37.8 (11.7)	34.5 (6.72)	40.8 (11.8)	36.9 (9.64)	0.26	0.65
Tl	0.06 (0.06)	0.07 (0.06)	0.06 (0.05)	0.06 (0.05)	0.06 (0.04)	0.06 (0.05)	0.06 (0.05)	0.82	1.00
V	0.07 (0.05)	0.07 (0.05)	0.07 (0.04)	0.08 (0.04)	0.07 (0.05)	0.06 (0.04)	0.07 (0.04)	0.63	0.95
W	0.04 (0.02)	0.07 (0.05)	0.05 (0.02)	0.05 (0.01)	0.05 (0.02)	0.04 (0.03)	0.05 (0.03)	0.59	0.28
Zn	864 (160)	943 (213)	951 (197)	983 (185)	1,045 (268)	1,012 (161)	966 (202)	0.21	0.33
Zr	0.97 (0.48)	1.22 (0.51)	1.12 (0.57)	0.79 (0.62)	0.67 (0.54)	0.73 (0.46)	0.92 (0.55)	0.63	0.08
SOS	326 (15.6)	333 (14.1)	324 (13.1)	328 (12.3)	322 (17.6)	329 (20.8)	327 (15.6)	0.05	0.67
SAC	338 (18.4)	333 (22.3)	318 (25.4)	330 (24.2)	329 (35.2)	328 (27.4)	329 (25.8)	0.08	0.56

SOS: serum oxidative status; SAC: serum antioxidant capacity; FDE: first demyelinating episode.

Table 2. - Comparison between multiple sclerosis (MS) patients at time 0 and controls. Element concentration in ng ml^{-1}; SOS in U.CARR; SAC in µmol ml^{-1} HClO.

	Patients mean (SD)	Controls mean (SD)		Patients mean (SD)	Controls mean (SD)
Al	2.93	1.99	**Mo*(*)***	1.45	0.86
	(1.39)	(1.10)		(0.49)	(0.35)
Ba*(*)*	1.32	0.69	**Ni*(*)***	1.62	0.44
	(0.54)	(0.42)		(1.24)	(0.35)
Be	0.23	0.26	**Pb**	0.53	0.65
	(0.06)	(0.11)		(0.45)	(0.34)
Bi	0.02	0.02	**Sb*(*)***	0.30	0.10
	(0.01)	(0.01)		(0.16)	(0.06)
Ca*(*)*	86,017	65,310	**Si*(*)***	627	141
	(11,115)	(5,620)		(238)	(66.3)
Cd*(*)*	0.19	0.10	**Sn*(*)***	1.36	0.71
	(0.11)	(0.07)		(0.74)	(0.45)
Co	0.21	0.16	**Sr**	34.9	35.7
	(0.11)	(0.09)		(6.1)	(8.95)
Cr*(*)*	0.36	0.14	**Tl**	0.06	0.04
	(0.22)	(0.05)		(0.06)	(0.02)
Cu	1,034	953	**V**	0.07	0.06
	(228)	(75.2)		(0.05)	(0.03)
Fe*(**)*	1,318	1,686	**W**	0.04	0.03
	(527)	(547)		(0.02)	(0.01)
Hg	1.50	1.08	**Zn**	864	781
	(1.27)	(0.47)		(160)	(120)
Li*(*)*	0.63	1.36	**Zr*(*)***	0.97	0.11
	(0.37)	(0.87)		(0.48)	(0.04)
Mg*(**)*	19,558	17,600	**SOS**	326	299
	(3,127)	(1,831)		(15.6)	(61.5)
Mn*(*)*	1.13	0.63	**SAC**	338	345
	(0.33)	(0.16)		(18.4)	(43.9)

(*): $p = 0.05$;*(**)*: $p = 0.10$; SOS: serum oxidative status; SAC: serum antioxidant capacity.

Conclusions

Significant longitudinal alterations did not seem to emerge in our patients with the adopted experimental scheme, although some trends might be identified. On the contrary, there were several differences in the elements content when compared to the control group. Some of these discrepancies can be tentatively explained, whilst, at present, others are far from a possible biochemical or clinical interpretation. A larger number of patients as well as a longer time of follow-up can probably help to clarify the clinical relevance of these preliminary results, also considering the clinical and demographic characteristics of the patients.

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