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Quantification of chemical elements in blood of patients affected by multiple sclerosis

Giovanni FORTE *(a)*, Andrea VISCONTI *(b)*, Simone SANTUCCI *(b)*, Anna GHAZARYAN *(b)*, Lorenzo FIGÀ-TALAMANCA *(b)*, Stefania CANNONI *(b)*, Beatrice BOCCA *(a)*, Anna PINO *(a)*, Nicola VIOLANTE *(a)*, Alessandro ALIMONTI *(a)*, Marco SALVETTI *(b)* and Giovanni RISTORI *(b)*

(a) Dipartimento di Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy
(b) Dipartimento di Neurologia, Ospedale S. Andrea, Università degli Studi "La Sapienza", Rome, Italy,

Summary. - Although some studies suggested a link between exposure to trace elements and development of multiple sclerosis (MS), clear information on their role in the aetiology of MS is still lacking. In this study the concentrations of 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 and Zr were determined in the blood of 60 patients with MS and 60 controls. Quantifications were performed by inductively coupled plasma (ICP) atomic emission spectrometry and sector field ICP mass spectrometry. When the two groups were compared, an increased level of Co, Cu and Ni and a decrement of Be, Fe, Hg, Mg, Mo, Pb and Zn in blood of patients were observed. In addition, the discriminant analysis pointed out that Cu, Be, Hg, Co and Mo were able to discriminate between MS patients and controls (92.5% of cases correctly classified).
Key words: multiple sclerosis, elements, blood.

Riassunto *(Quantificazione dei livelli di elementi chimici nel sangue di malati di sclerosi multipla)*. - Gli studi finora effettuati hanno evidenziato un legame tra esposizione ad elementi in tracce e lo sviluppo della sclerosi multipla, senza fornire una esatta informazione sul ruolo eziologico di questi elementi. In questo studio i livelli di 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 e Zr sono stati determinati nel sangue di 60 pazienti affetti da sclerosi multipla e di altrettanti controlli. Le misurazioni sono state effettuate per mezzo delle tecniche di spettrometria di emissione atomica e di massa a plasma induttivamente accoppiato. Dal confronto con i valori dei controlli è stato osservato nei pazienti un incremento significativo nelle concentrazioni di Co, Cu e Ni ed un decremento in quelle di Be, Fe, Hg, Mg, Mo, Pb e Zn. Inoltre, l'analisi discriminante ha evidenziato che Cu, Be, Hg, Co e Mo sono in grado di discriminare tra i pazienti ed i controlli classificando correttamente il 92.5% dei casi.
Parole chiave: sclerosi multipla, elementi, sangue.

Introduction

Multiple sclerosis (MS) is the most frequent inflammatory disease of the central nervous system in young adult. Our knowledge about its pathogenesis is still incomplete and its aetiology remains unknown. The disease is considered to be an autoimmune disorder induced when myelin-specific CD4 T cells recognize components of the myelin sheath [1, 2]. Probably, the immune process is a consequence of the connections between environment and susceptibility genes. Dietary, habits and life-styles, infective disease, urban and industrial exposure could be considered additional factors. Available literature provides evidences of the involvement of environmental and occupational exposures, such as to organic solvents, to Zn used in manufacturing plant, to Ba contaminant in the ecosystems or to Co, Mn, Mo and Zn in arable soils in MS disease [3-7].

On the other hand, several trace or major elements have been investigated as possible causes of the disease. In fact, increased level of Cd, Cu, Fe and Pb, and a decrement of Mg and Zn in the aethiology of MS has been suggested [8]. Besides, Pb, as responsible of the

Indirizzo per la corrispondenza (Address for correspondence): Giovanni Ristori, Dipartimento di Neurologia, Ospedale S. Andrea, Università degli Studi "La Sapienza", Via di Grottarossa 1035-1039, 00189 Roma. E-mail:giovanni.ristori@uniroma1.it.

formation of antibodies against myelin proteins, is suspected to play a role in the pathogenesis of nervous system diseases such as MS [9]. As regards Hg, it has been suggested to be involved in factors causing the MS, i.e., destruction of the myelin sheath and reduction of the nerve conduction velocity [10, 11]. Moreover, the concentration of Mg and Zn has been shown to decrease in the central nervous system of MS patients, especially where pathological changes have been observed [12-14]. Additional studies have shown variations in Cu, Mg and Zn concentrations in serum and cerebrospinal fluid from patients with MS [15, 16]. Brain is able to concentrate physiological metals, i.e., Cu, Fe, Mn and Zn, up to toxic levels determining a possible production of oxidative species [17]. Thus, it is still unclear if aberrant metal interactions are a primary or secondary factor, or a consequence of the neurodegeneration.

Finally, studies performed up till now highlighted the possible role of elements into the MS pathogenesis without allowing definitive conclusions about the exact relationship between the metals and the pathology. To verify and support these assumptions a study to quantify the concentration of twenty-six elements (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 and Zr) in blood of MS patients and healthy donors has been undertaken.

Materials and methods

Subjects

Sixty patients (40 females ad 20 males) affected by definite MS were enrolled on the present study. Disease was defined according to the criteria of McDonald *et al*. [18]. Patients mean age was 38.7 ± 9.9 yrs and expanded disability status scale (EDSS) range was 0-7.5. Sixty age- and sex-matched controls (mean age of 38.4 ± 9.7 yrs) were recruited from healthy blood donors living in the same geographic area. Written consent from each participant was obtained. Subjects were interviewed in order to obtain detailed information on family, lifestyle, dietary and personal medical history. Exclusion criteria for both groups were: cardiological, respiratory, kidney or liver disease; intestinal absorption abnormalities, infections, assumption of thyroid hormones, lithium therapy, intake of vitamin or mineral supplements, vegetarian dietary, artificial metallic bodies.

Samples pre-treatment and analysis

After fasting overnight and drug abstention, 1 ml of blood was collected in plastic tubes in absence of anticoagulant to prevent a possible exogenous source of metals. Blood samples pre-treatment consisted in a microwave acid-assisted digestion. Calcium, Cu, Fe, Mg, Si and Zn were further quantified by inductively coupled plasma atomic emission spectrometry and Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr by the sector field inductively coupled plasma mass spectrometry. More details are reported elsewhere [19].

Statistical analysis

The levels of elements in blood of MS patients and controls are described in terms of mean and standard deviation (SD). The Mann-Whitney test was used for evaluating significance ($p \leq 0.05$) of differences between means. A stepwise discriminant analysis (SDA) to determine the set of variables that discriminated between MS and controls were carried out. A forward stepwise procedure guided by the statistical F values of each variable was applied. In order to validate the classification of the cases by the discriminant function a leave-one-out method was used. The SPSS 12.0.1 version statistical package was utilized (SPSS, Chicago, IL, USA).

Results and discussion

Table 1 reports the mean concentrations and the SD of the metals in blood of patients and controls. In particular, in blood of patients, Bi, V and W were under 0.1 ng ml^{-1}; Be, Co, Cr, Li, Sb, Tl and Zr were in the range of 0.1-1.0 ng ml^{-1}; while Ba, Cd, Hg, Mn, Mo, Ni and Sn were in the interval 1.0-10 ng ml^{-1}. The concentrations of Al, Pb and Sr were found to be in the order of tens of ng ml^{-1} and those of Si in the order of hundreds of ng ml^{-1}. In the case of the major elements, i.e., Ca, Cu, Fe, Mg and Zn, in this matrix the lowest and highest values were found for Cu and Fe, respectively.

Blood Cu levels in the patients were significantly higher than in the controls (1,445 ± 481 *vs* 926 ± 145 ng ml^{-1}). These findings were found in the cerebrospinal fluid (CSF) of patients, whilst Kapaki *et al*. observed Cu levels decreased in both CSF and serum of MS subjects [15, 16]. Anyway, Cu, a redox-active metal such as Zn, plays an important catalytic role in many antioxidant enzymes activities and in free radical formation. In addition, in an MS animal model Cu chelators have been seen to attenuate experimental autoimmune encephalomyelitis, this fact would suggest a crucial role of Cu and reactive oxygen species in initiation and progression of the disease [20]. As regards Co, mean levels in blood of patients were higher than in normal individuals (0.22 ± 0.10 *vs* 0.11 ± 0.06 ng ml^{-1}). This is an essential element for metabolism and cell surviving. In particular, Co as a component of cobalamin, takes

Table 1. - Element concentrations (ng ml^{-1}) in blood of multiple sclerosis (MS) patients and controls

	MS Mean ± SD	Controls Mean ± SD
Al	23.9 ± 21.3	15.9 ± 8.6
Ba	1.43 ± 0.11	1.17 ± 0.54
Be(*)	0.29 ± 0.11	0.41± 0.20
Bi	0.04 ± 0.02	0.03 ± 0.02
Ca	65,410 ± 11,264	68,006 ± 10,517
Cd	1.11 ± 0.56	0.95 ± 0.48
Co(*)	0.22 ± 0.10	0.11 ± 0.06
Cr	0.36 ± 0.18	0.41 ± 0.25
Cu(*)	1,445 ± 481	926 ± 144
Fe(*)	482,748 ± 83,763	546,285 ± 69,705
Hg(*)	3.91 ± 2.35	6.43 ± 4.44
Li	0.86 ± 0.36	0.87 ± 0.52
Mg	36,784 ± 5,804	40,392 ± 5,657
Mn	8.39 ± 3.93	7.80 ± 3.00
Mo(*)	2.11 ± 0.83	2.94 ± 1.65
Ni	1.17 ± 0.63	0.87 ± 0.66
Pb(*)	24.3 ± 12.7	35.6 ± 20.1
Sb	0.67 ± 0.47	0.46 ± 0.24
Si	220 ± 142	168 ± 72
Sn	1.87 ± 1.08	1.53 ± 0.62
Sr	23.9 ± 7.78	26.5 ± 12.5
Tl	0.14 ± 0.22	0.07 ± 0.04
V	0.09 ± 0.03	0.08 ± 0.05
W	0.07 ± 0.04	0.07 ± 0.04
Zn(*)	5,998 ± 1,492	6,579 ± 1,015
Zr	0.69 ± 0.47	0.50 ± 0.25

(*) $p \leq 0.05$

part in myelin formation, red blood cells production, metabolism of fats, carbohydrates, synthesis of proteins and conversion of folate to its active form. Considering the Co biological functions, an unbalance of this microelement cannot exclude its possible role in the disease. Iron levels in blood of MS subjects were significantly lower than in controls (482,748 ± 83,763 *vs* 546,285 ± 69,705 ng ml^{-1}). Several studies investigated Fe metabolism during inflammation, a process in which Fe would induce oxidative injury, while, at the same time, ferritin taking away Fe is able to protect against the oxidative damage [21-23]. Recent studies showed a gradual accumulation of Fe in MS brain suggesting a probable explanation for its peripheral decrement in our patients [8, 24]. Notwithstanding, the CSF did not seem to mirror the documented high Fe level in MS brain [25].

Although neurotoxicity appears to be associated with Hg and Pb contamination, a significant reduction in blood mean concentration of these elements in our patients has been observed. Mercury and Pb, considered environmental contaminants without a biological role, have been intensively investigated for several decades. Relatively to Hg, its involvement in the disease was related to the supposed massive release from dental amalgams, but the controversial results did not allow to confirm this issue [10, 26, 27]. Recently, some evidences suggests that Pb strongly binds to the Ca-regulated chaperone proteins and interacts with several others Ca-regulated enzymes, including protein kinase C and calmodulin [28-30]. As regards Mg, a significant decrement in the blood of MS patients was observed. In another study, the level of this element in serum and CSF of MS patients was found practically coincident with those of controls [16]. This essential element may affect the maintenance and function of nerve cells as well as the proliferation and synthesis of lymphocytes [13]. Moreover, Mg interacts with other minerals such as Al, Ca and Zn in biological systems, supporting the assumption that modification of this element may be a co-factor in the development of the disease. A significant decrement of Zn in blood of patients was observed, confirming its involvement in MS.

On the contrary, other studies found no difference in the Zn levels in serum and CSF of MS patients. [15, 16]. The role of this redox-active metal in immune response and during inflammation has been extensively investigated. Zinc could modify cytokine production by mean of matrix metallo-proteinases, it could stabilize the association of the myelin basic protein with brain myelin membranes and also initiate the autoimmunity response [31]. As regards Be and Mo, their mean blood levels resulted to be significantly lower in MS. This result needs of further studies to be clarified.

When the SDA was applied on the variables found different between the two groups, only a small groups of them seemed to have a role in adequately discriminating between MS and healthy individuals. In particular, the elements showing the best discriminating power (standardized coefficient > 0.3) were in the order Cu, Be, Hg, Co and Mo. Thus, these elements, three of them surely essential for a lot of cellular activities, contributed in the prediction of the group membership. The model was able to correctly classify the 92.5% of cases and the 91.7% of cases after cross-validation. Thus, these findings suggest that the SDA could be correctly applied to the MS as a diagnostic statistical test.

In conclusion, these results support the hypothesis that the picture of chemical elements in blood may be a potential discriminating factor for MS. However, further investigations are necessary also combining

this sight of the elements concentration with clinical information, demographic data, socio-economic status, occupation, nutritional factors and family history of the patients.

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