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Short Communication

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ABSTRACT

Huntington' disease (HD) is an autosomal dominant neurodegenerative disease characterized by progressive motor, psychiatric, and cognitive deterioration. HD is, together with spinocerebellar ataxias, spinobulbar muscular atrophy and dentatorubral-pallido- luysian atrophy, one of the nine disorders caused by an expansion of glutamine residues in the causative protein where the polyglutamine expansion cause aberrant protein folding. Since an excessive metal's accumulation in organs may induce protein misfolding and oxidative stress, we have studied the blood concentration of essential (Cr, Co, Cu, Fe, Mn, Mo, Ni, Se, Zn) and nonessential (As, Cd, Sb, Sn, V) trace elements in HD patients.

We found increased levels of the essential elements iron, chromium, selenium and zinc and of the nonessential element arsenic in the blood of HD patients.

Since alteration in metals homeostasis may contribute to the pathogenesis of neurodegenerative disease and could eventually constitute a target for therapy, we may suggest the utilize of the blood metal profile as a further *in vivo* tool to study and characterize Huntington disease.

Introduction

Huntington' disease is an autosomal dominant neurodegenerative disease characterized by progressive motor, psychiatric, and cognitive deterioration, such as loss of self and spatial awareness, depression, dementia, and weight loss [1].

The prevalence disorder in North America, North Western Europe and Australia ranged from 6-14 cases per 100000 individuals [2]. Treatments are focused to suppressing the "corea", the involuntary, irregular movements of the arms and legs and the mood-altering characteristics of the disease [3].

A trinucleotide CAG repeat expansion in exon 1 of the Huntingtin gene (HTT) causes the disorder; the number of CAG repeats expands from the normal range of 16-20 repeats to >35 repeats in patients [4]. The mutant huntingtin protein has an elongated polyglutamine tract at the amino terminus that cause protein aggregation and the subsequent toxicity [5].

The striatum and in cerebral cortex are the principal sites affected by neuronal death and glial activation, because the mutant huntingtin protein (mHTT) is expressed here and cause the disruption of several downstream pathways [3]. The Huntington protein in fact, is deputed in several key functions such as DNA transcription and maintenance, protein homeostasis and transport, cell cycle regulation and cell signalling. The presence of nuclear inclusions and cytoplasmic aggregates in the brain is one of the most striking hallmarks of HD.

HD is one of nine inherited neurodegenerative disorders caused by an expansion of glutamine residues in the causative protein [5]. The others eight are spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7, 17, spinobulbar muscular atrophy (SBMA), and dentatorubral-pallido- luysian atrophy (DRPLA). These nine disorders arise from aberrant protein folding as a result of polyglutamine expansion; AD, PD, and ALS are also characterized by the presence of misfolded and aggregated proteins and therefore all these disorders are known as protein conformational diseases [3]. Other neurodegenerative polyglutamine disorders, such as amyotrophic lateral sclerosis (ALS),

Alzheimer's disease (AD) and Parkinson's disease (PD) have characteristics in common with HD [6]. In fact, AD, PD, and ALS have in common with HD neuronal dysregulation, the late onset of the disorder, an altered energy metabolism and global changes in gene expression, finally suggesting that the chronic expression of misfolded proteins may cause progressive neuronal toxicity through common mechanisms [3].

Essential elements such as copper, zinc and manganese are essential for life but they are required in trace levels since excessive metal accumulation in brain is deleterious and may cause several detrimental effects that lead to neurodegeneration such as induce oxidative stress, mitochondrial dysfunction, and protein misfolding [7]. The neurotoxicity subsequent to excessive trace elements accumulation is associated with multiple neurological diseases such as AD, ALS, Parkinson disease (PD), Wilson's disease (WD) and abnormal Fe, Cu and Mn homeostasis has been observed both in the brain of HD patients and in animal models [8].

As alteration in metals homeostasis may contribute to the pathogenesis of neurodegenerative disease and could eventually constitute a target for therapy, we have studied the blood concentration of 15 essential and nonessential trace elements antimony (Sb), arsenic (^{75}As), cadmium (^{111}Cd), chromium (^{52}Cr), cobalt (^{59}Co), copper (^{63}Cu), iron (^{56}Fe), lead (^{208}Pb), manganese (^{55}Mn), molybdenum (^{98}Mo), nickel (^{60}Ni), selenium (^{78}Se), tin (^{118}Sn), vanadium (^{51}V) and zinc (^{66}Zn) in HD patients.

The aim of this investigation is to study the blood metal profile in order to a further characterization of the Huntington disease.

Patients and methods

The study enrolled 18 HD patients (10 males and 8 females) with genetic diagnosis of disease, and equal number of healthy controls. The ethical standards specified in the 1964 Declaration of

Helsinki was followed in this investigation; moreover, our study was approved by the internal review board of the Department of Medical Sciences (DSM-ChBU). Informed consent was obtained from patients or their legal representative. Venous blood was collected in heparinized vacutainer BD tubes (Becton Dickinson Labware, Franklin Lakes, USA) and stored at −20 °C until required for analysis. The quantification of 15 trace elements was performed by using a Thermo Xseries II ICP-MS instrument (Thermo Scientific, Germany), following the protocol already described in previous studies [9,10]. Calibration curves, with Rhodium and Germanium as internal, were prepared using multi-element standard solutions dissolved in acidified ultrapure water, at concentrations from 0.25 ng mL^{1} to 50 ng mL^{-1}. Certified Reference materials (Seronorm Whole Blood SWB-L2) and blank reagents were used to verify analytical performances. A Thermo Xseries II ICP-MS instrument (Thermo Scientific, Germany) equipped with a CETAC ASX 500 Model 520 (CETAC Technologies, USA) auto sampler and a peristaltic pump nebulizer was used for instrumental determinations. Instrumental parameters, such as torch position, ion lenses and gas output, were optimized daily, but general operating conditions were: forward power 1.40 kW, coolant gas flow rate 13.0 L min^{-1}, auxiliary gas flow rate 0.70 L min^{-1}, nebulizer gas flow rate 0.90 L min^{-1}, dwell time 75 ms, 3 replicates. The Collision Cell Technique (CCT), performed with a Helium/Hydrogen mixture (95/5) at a flow rate of 4.75 mL min^{-1}, and mathematical equations were used to overcome interferences.

For statistical analysis we utilized Graph Pad Statistics Software Version 6.0 (GraphPad Software, Inc., USA). Unpaired two tailed t-test was employed to evaluate the significance of difference between patients and controls (p values of < 0.05 was considered significant).

Results and discussion

We found increased levels of the essential elements chromium, iron, selenium and zinc and of the nonessential element arsenic in the blood of HD patients, as shown in Table 1 and Figure 1; values

were expressed as μg L^{-1} ± standard deviation (SD). We also registered lower concentrations of antimony, lead and vanadium in patients' blood compared to controls. No significant differences were found for the essential elements copper and manganese.

Cadmium, cobalt, molybdenum, nickel and tin were below the limit of quantitation (LOQ, 2.0 μg L^{-1}) in the analyzed samples.

Enzymes deputed in cellular activities regulation usually contain metals as cofactors [7]. Iron is an essential bioactive metal that participates in many biological functions; it is the cofactor of many proteins and enzymes, the most important is hemoglobin [11]. In biological systems iron has two oxidation states, ferrous (II) and ferric (III) that bind oxygen; proteins containing Fe are involved in key functions such as cellular respiration and regulation of cell survival [12]. In the brain Fe is involved in the biosynthesis of neurotransmitters, myelin formation and energy metabolism.

However, Fe(II) in excess in the brain can cause neuronal damage and cell death, by increasing oxidative stress generating highly cytotoxic free radicals. There is a group of neurodegenerative pathologies (Neurodegeneration of the Brain with Iron Accumulation, NBIA) characterized by the Fe accumulation in particular sites of the brain: the nuclei or basal ganglia located at the base of both cerebral both cerebral and densely interconnected hemispheres with the cerebral cortex and other structures such as the thalamus and the trunk of the brain. These sites are mainly involved in controlling the movement but also in the emotional and attention aspects that guide the finalized movement. Post-mortem studies have reported pathological changes in the nuclei of the base in HD. There are several neurological disorders in which Fe altered homeostasis has been observed such as PD, AD, HD, ALS; increased Fe is also seen in the brain of patients with HD, and the protein responsible of HD pathology (Htt) is supposed to be involved in the regulation of Fe homeostasis [13]. Moreover, Fe is essential for mitochondria functions and mitochondrial bioenergetic dysfunction was demonstrated in neurodegeneration in Huntington's disease [12].

We found that the altered homeostasis of iron is also reported by blood analysis that has shown higher values of Fe in HD patients compared to controls. In addition, higher values of other three essential elements, Cr, Se and Zn were recorded in patients.

The essentiality of chromium is still controversial, but since the 1950s it was suggested that it plays an important role in the metabolism of carbohydrates in humans. Cr is still utilized in nutritional supplementation but its beneficial effects are conflicting and an excessive intake of Cr(III) was suggested to be carcinogenic [14]. Cr contained in inorganic compounds is poorly absorbed while a larger amount is absorbed by organic compounds; metal is then linked to transferrin (Tf), the protein that transports iron mobilized from deposits into the blood and transferred to systemic circulation [15]. Chromium in fact, such as iron, is imported by the cells via a "transferrin-receptor complex for transferrin" and since both these elements compete for transferrin binding sites and both were found significantly higher in the blood of HD patients in comparison to healthy controls, the possible role of Cr in the pathology of HD disease surely deserves further investigations, as Tf binding was suggested to be a natural protective mechanism against the toxicity of chromium through blocking Cr(III) cellular accumulation [16].

Selenium and zinc are strictly involved and linked together in cytosolic defense against reactive oxidative stress [17], since they are cofactors of key enzymes of the cellular anti-oxidative systems. In fact, the Cu–Zn superoxide dismutase (SOD1) catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide that is subsequently reduced by the seleno-enzyme glutathione peroxidase (GPX).

Then, a possible hypothesis, that should be followed by further investigations, is that the excess of iron in the cell inducing oxidative stress via ROS production causes the upregulation of gene coding for the first line defense antioxidants enzymes SOD and GPX, revealed by the increase of their cofactors in the blood of HD patients,

We found significant differences in As, Pb, Sb and V levels between patients and controls that deserve additional investigation, even if the concentrations of these nonessential elements were in the range of reference values in blood [18,19].

Conclusions

Altered homeostasis of trace elements, such as iron, have been observed in patients and animal models of HD. Recent findings suggested that metal imbalance was related with HD pathogenic changes in enzymes sensitive to metals or depend on metals as cofactors, such as ATM. We found abnormal concentrations of several metals in the blood of HD patients and propose that the metal profile may represent a useful tool for further insights of the pathology. One therapeutic approach against HD or other neurodegenerative diseases could be targeting metals found in abnormal concentrations, since agents that target these metals may slow down or potentially reverse the course of the disease. The study of metals profile in the blood of HD patient could help in identify potential ions target for novel therapeutics approaches. Moreover, we may suggest that a future application could be the in deep study of a variety of trace elements to discriminate between neurological diseases.

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Conflicts of interest: the authors declare no conflict of interest.

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Table 1 Blood trace elements concentrations (µg/L ± S.D)

Metals	HD Patients	Controls	*p*
As	**2.4±0.18**	**1.2±0.11**	**<0.0001 (***)**
Cr	**26±0.12**	**19±0.17**	**0.0304 (*)**
Cu	913±65	963±84	0.5569 (NS)
Fe	**534616±8320**	**452556±8140**	**0.0072 (**)**
Mn	18±0.12	21±0.13	0.0811 (NS)
Pb	**43±0.33**	**58±0.39**	**0.0115 (*)**
Sb	**2.9±0.15**	**4.4±0.22**	**0.0138 (*)**
Se	**138±12**	**101±16**	**0.0057 (**)**
V	**2.8±0.26**	**5.1±0.47**	**0.0032 (**)**
Zn	**5668±870**	**4640±523**	**0.0096 (**)**

235 Note: In bold statistically significant metal ions. NS: not significant. * (< 0.05), ** (< 0.01),*** (<
236 0.001)
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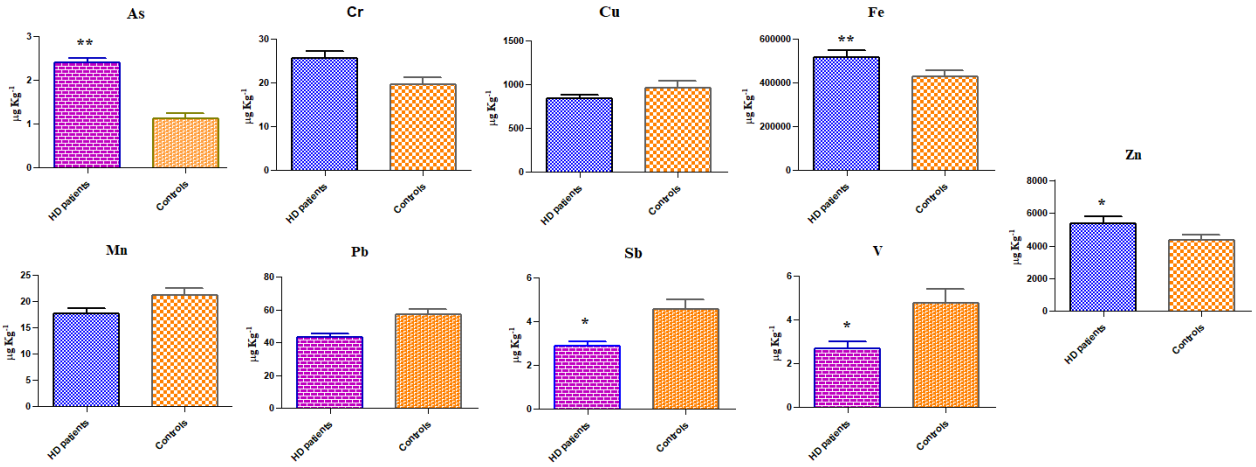

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