Journal of the Neurological Sciences 359 (2015) 11–17

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Level of neurotoxic metals in amyotrophic lateral sclerosis: A population-based case–control study

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ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 18 September 2015
Accepted 12 October 2015
Available online xxxx

Keywords:
Amyotrophic lateral sclerosis
Blood
Hair
Metals
Motor neuron disease
Urine

ABSTRACT

The association between exposure to toxic metals and amyotrophic lateral sclerosis (ALS) was explored in a population-based case–control study in the Sardinia island (Italy), a region characterized by elevated rates of ALS cases. In 34 patients with ALS (mean age, 62 ± 10 years) and 30 controls (mean age, 65 ± 11 years), Al, Cd, Hg, Mn and Pb were determined in blood, hair and urine by sector field inductively coupled mass spectrometry. Results indicated that, in blood, concentrations of Al ($p = 0.045$) and Pb were higher ($p = 0.026$) in ALS patients than in control subjects. In hair, a depletion of Al ($p = 0.006$) and Mn ($p = 0.032$) concentrations in ALS subjects respect to controls was found. In urine, no significant differences between cases and controls were observed. Thus, some metals seemed to be associated with ALS degeneration, but a definitive conclusion is still far considering the multiple risk factors (genetic mutations, environmental toxicants and stressors) involved in the disease. Finally, the interpretation that deregulated metal concentrations can be a consequence of the degenerative process, rather than a cause, is also valid.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative syndrome characterized by adult-onset progressive loss of motor neurons in the motor cortex, brainstem and medulla spinalis. The disease causes muscular wasting, paresis and inevitable death usually within 2–5 years from the diagnosis. About 90–95% of ALS cases are considered as sporadic, without any documented family history while the remainder cases are most often dominantly inherited. The four most important genes causing ALS are: C9ORF72, responsible for ca. 40% of familial cases and 5–7% of sporadic cases, which gives rise to an expansion of an intronic hexanucleotide repeat; SOD1 gene on chromosome 21 (ca. 20% of familial cases and 2–7% of sporadic cases) which encodes Cu/Zn-SOD; FUS gene on chromosome 16 (ca. 5% of familial cases and <1% of sporadic cases), which encodes the protein fused in sarcoma; and TARDBP (ca. 3% of familial cases and 1.5% of sporadic cases), which encodes TAR DNA binding protein-43 (TDP-43). Many mechanisms as excitotoxicity, oxidative stress, aberrant protein aggregation, defective axonal transport, mitochondrial dysfunction, and altered RNA metabolism have been incriminated in the molecular and cellular pathways leading to ALS [71].

ALS has a worldwide incidence of about 2 cases per 100,000 subjects and a prevalence of 4–7 cases per 100,000 subjects [26]. Chiò et al. [22] reported a prevalence of 3.4 cases and incidence of 1.75 cases per 100,000 subjects in USA, while a prevalence of 5.4 cases and incidence of 2.1 cases per 100,000 subjects in Europe. Mean age at onset is ca. 60 years for sporadic ALS and ca. 50 years for familial ALS and sporadic ALS is more prevalent in men than in women [48]. Additionally to genetics, also viral, inflammatory or oxidative mechanisms as well as electromagnetic fields, smoking, diet, physical activity, trauma, and occupational and environmental exposure to toxic substances have been considered as risk factors in the development of ALS. In this context, it has been suggested that metals can contribute to ALS [39,80]. In particular, Al, Cd, Hg, Mn (at high doses) and Pb are known to be toxicants for cellular and biochemical activities at level of the central nervous system (CNS). Aluminum is a possible cause of neurodegeneration because of its pro-oxidant activity; it accelerates Fe-driven peroxidation of lipids and aggravates oxidative damage by enhancing the radical species

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http://dx.doi.org/10.1016/j.jns.2015.10.023
0022-510X/

formation [27]. An in-vivo and in-vitro study demonstrated that the Fe initiated lipid oxidation was the mechanism involved in Al-mediated oxidation of myelin [84], and in mice exposed to Al by diet a reduction of the myelin sheath was observed [34]. Moreover, mice injected with aluminum hydroxide showed a significant increase in cell death in the spinal cord and motor cortex [79]. An in-vitro study indicated that Cd decreases the content of Zn ions, changed the conformation of Cu/Zn-SOD protein to decrease its enzyme activity, and caused oxidative stress-induced neural cell apoptosis [38]. In humans, inorganic Hg can be taken up predominantly by corticomotor neurons according to the hypothesis that ALS originates in these upper motor neurons. In addition, several factors, as the nature of toxin, the exposure's duration and the amount of oxidative stress, are thought to affect the rate of Hg entry into motor neurons [65]. After respiratory exposure of rats and primates to Hg vapour, the metal accumulated in anterior horn cells of the spinal cord, causing axonal atrophy and distal weakness similar to the clinical picture in human ALS [74]. In addition, long-term exposure to low concentrations of methyl-Hg (MeHg) showed in mice overexpressing the mutant human SOD1 gene an anticipation of ALS onset in case of an underlying genetic predisposition for ALS [40]. Although the relationship between increased Mn levels and its disruptive effects on the neurochemistry of neurotransmitters has been debated, elevated Mn has been suggested to alter concentrations of γ-aminobutyric acid (GABA), dopamine, and glutamate neurotransmitters in brain. In this context, rat brain endothelial cells are an efficient carrier for Mn [3]. Spectroscopy analysis in rats has demonstrated that mitochondria in the basal ganglia accumulated the highest amount of Mn following exposure [60]. Elevated Mn concentrations were reported in spinal cord sections from 7 autopsied ALS patients [59]. The in-vivo study of Dang et al. [24] showed that expression of a disease-causing form of TDP-43 is sufficient to disrupt Mn homeostasis in the central nervous system. The disruption in the spinal cord but not in brain may explain why the TDP-43^{A315T} mice show symptoms of locomotive and not cognitive decline.

The neurotoxic effects of Pb are well known [4]. Lead mechanisms, such as oxidative damages to mitochondria and neural tissues or the amplification of the glutamate excitotoxicity, imply a plausible role of this metal in ALS pathophysiology. It is possible that exposure to occupational or environmental Pb can be the triggering pathogen in genetically susceptible individuals as described in a human case-report [64]. In a case–control study, results suggested the association between elevated blood Pb level and the higher risk of ALS in patients, by accounting for bone turnover [28]. Moreover, there is evidence that some polymorphisms in the genes for δ-aminolevulinic acid dehydratase (ALAD) resulted significantly associated with bone Pb levels in ALS patients compared with controls and may affect the ALS risk [42]. Paradoxically, a study concerning the Pb exposure in transgenic mice expressing the G93A SOD-1 mutation showed a delay in the progression of ALS [6]; this was supported by the observation that higher blood and tibia Pb levels increase survival of ALS patients [43].

In Italy, the prevalence of ALS was ca. 7 cases per 100,000 subjects and the incidence was ca. 2–3 cases per 100,000 subjects [52]. In particular, it has been reported that in Sardinia (an Italian island) the prevalence rate was high, with ca. 8 cases per 100,000 subjects [29]. Other sources reported that in 2012 ca. 170 subjects with ALS were living in Sardinia and ca. 50 new cases per year were estimated [2]. The higher rates of ALS cases in Sardinia probably reflects the conserved nature of that island population combined with a historical founder effect [19,20]. Recent studies conducted on Sardinian ALS patients have revealed two causative genes operating in that island population, namely the A382T-encoding allele of TARDBP and the C9ORF72 repeat expansion [21]. In this context, Borghero et al. [14] found that >40% of all ALS Sardinian cases carried a mutation of the aforementioned ALS-related gene, representing the highest percentage of cases that are genetically explained outside of Scandinavia and largely higher than in other Caucasian populations. Moreover, the island population has a uniquely high incidence of several autoimmune diseases such as type 1 diabetes and multiple sclerosis [32,54].

In this context, the study of populations, such as Sardinians, that have proven to display increased rates of ALS and to exhibit peculiar genetic patterns implicated in ALS, can add original information. No data are available in Sardinia to show the association of ALS with metals quantified directly in human blood, urine, and hair of ALS cases and controls. Analysis of blood samples supplies information of metals exposure at short or medium term; metals may be bound to red blood cells (e.g., Cd, Mn and Pb) or plasma components (Al), or occur unbound in blood. Urine analysis is useful to identify recent (Hg) and long-term (Cd) exposures. Because metals are excreted in hair, this matrix has been suggested for assessing exposure to metals (e.g., Pb, Hg, Mn) and it is recognized as a storage compartment. Considering that hair grow ca. 1 cm per month it would be possible to determine the metals load for a long period of time [63].

In search of putative association between metals and ALS outline, a small-scale survey was devoted to the determination of neurotoxic metals (Al, Cd, Hg, Mn and Pb) in blood, hair and urine of ALS cases compared to healthy controls. In addition, the association between metals and some individual factors as sex, age, duration and type of the disease, and body mass index (BMI), was explored to check if metals in concert with these variables can be implicated in ALS pathology.

2. Subjects and methods

2.1. Characteristics of cases and controls

Thirty-four subjects affected by definite ALS accordingly to the EI Escorial criteria and 30 controls enrolled in this study were natives of Sardinia and have the same ethnic origin. Three experienced ALS Health Units placed in different areas of Sardinia performed the recruitment of patients. Age and gender-matched controls were blood donors, with no history of neurological diseases, and resident in the same geographical areas of patients. The study started in the year 2013. The study protocol presenting the collecting procedure and the aim was approved by the Institutional Ethical Committee of the University of Sassari and an informed written consent was obtained from each subject. A questionnaire was also administered to subjects and personal data (as gender, age, BMI, disease type and duration, smoke and alcohol consumption, presence of metallic prosthesis, occupational exposure to metals) were noted down. Descriptive characteristics of patients and controls were reported in Table 1. All subjects were non-smokers and non-drinkers. Considering the job, none of the 64 subjects was occupationally exposed to metals and none of them had metallic prosthesis in the body.

Table 1
Descriptive characteristics of ALS subjects and controls.

	ALS	Controls
Subjects (no.)	34	30
Mean age (years)	61.9 ± 9.7	64.9 ± 11.0
Mean BMI (kg/m^2)	23.6 ± 3.3	25.3 ± 3.5
Mean duration of disease (years)	5.50 ± 2.65	
Sex (no.)		
Females	13	12
Males	21	18
ALS type (no.)		
Familial	1	
Sporadic	33	
Site of onset (no.)		
Bulbar	16	
Spinal	18	