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Serum and Cerebrospinal fluid levels of cooper, iron and zinc in patients with Ataxia type SCA-2 from the province of Holguín in Cuba.

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ABSTRACT
SCA-2 Ataxia constitutes a health problem for the province of Holguín and potentially for all Cuba. 757 persons in Cuba suffer this disease and almost 8000 descendents are at risk. The present work investigated serum and cerebrospinal fluid (CSF) copper (Cu), iron (Fe) and zinc (Zn) levels in 25 patients with SCA-2 Ataxia (14 men and 11 women) and in 30 neurologically normal persons (14 men and 16 women), employing atomic absorption techniques. A significant decrease of serum Zn concentration and CSF Cu, Fe and Zn levels were detected in patients with respect to controls. These results indicate an impairment of homeostasis for these important microelements in type SCA-2 Ataxia. These evidences support the application of new strategies for treating this disease.

Key words: SCA-2 Ataxia, copper, iron, zinc, cerebrospinal fluid, serum.

RESUMEN
La Ataxia de SCA-2 constituye un problema de salud para la provincia de Holguín y potencialmente para toda Cuba. 757 personas en Cuba sufren esta enfermedad y casi 8000 descendientes están en riesgo. El presente trabajo investigó en suero y en el fluido cerebroespinales (LCR) los niveles cobre (Cu), hierro (Fe) y cinc (Zn) en 25 pacientes con Ataxia tipo SCA-2 (14 hombres y 11 mujeres) y en 30 personas neurológicamente normales (14 hombres y 16 mujeres), empleando las técnicas de absorción atómicas. Una disminución significante de Zn en suero y de Zn en los pacientes con respecto a los controles. Estos resultados indican un deterioro de homeostasis para estos importantes microelementos en la Ataxia tipo SCA-2. Estas evidencias apoyan la aplicación de nuevas estrategias para tratar esta enfermedad.

Palabras clave: Ataxia tipo SCA-2, vobre, hierro, zinc, fluido cerebroespinal, suero.

INTRODUCTION
The genetic disease known as type SCA-2 Ataxia is characterized by progressive cerebellar ataxia, including nystagmus, slow ocular movements and in some patient's ophthalmoparalysis. Pyramidal refluxes are present, and the deep reflexes of the tendons are active at the onset and absent as the illness progresses. There are no other phenotypes associated with the SCA-2 gene. SCA-2 Ataxia is a genetic health problem for the country and specifically for Holguín, due to the existence of 168 affected families in all the national territory with 757 patients and almost 8000 descendents in risk of suffering from this illness during the next years.1

The existence of the genetic anticipation phenomenon in 77% of the cases is an alert for the possibility of the appearance of the disease in childhood, where the course is more severe and dramatic. The existence of a Presymptomatic and Prenatal Diagnostic Program poses the necessity of developing some therapeutic
procedures to modify the course and severity of the disease during the presymptomatic stages. From an ethical and humanitarian point of view, this justifies carrying out predictive diagnostic studies, which are included in the main research strategies lined out by the Cuban Health System for patients with ataxia. SCA2 is a molecular based disease, which has been associated with neurotoxicity. The identification of factors that contribute to explain the phenotypic variability of patients and carriers and that are in some way related with a delay in the onset of the disease, are basic points in the studies we have been conducting. Our efforts are directed towards clarifying the etiology (genetic and non genetic factors), as well as towards obtaining a therapy for SCA2 aimed at delaying the onset of the disease and improving the patient’s quality of life. Microelements play a crucial role in the human nervous system. Zinc (Zn) and copper (Cu) are antagonistic, and they have a clear relationship, not only in the context of pathology, but also in normal physiology. After iron (Fe), Zn is the most abundant microelement in nervous tissue. Decreased Zn concentrations have been implicated in the pathophysiology of some neurodegenerative diseases (Parkinson’s disease, Alzheimer’s disease and Friedrich’s Ataxia). To our knowledge there is no information concerning CSF and serum concentrations of microelements in patients with hereditary cerebellar ataxias. The present study was conducted to investigate the levels of Fe, Cu, and Zn in patients with SCA2 type Ataxia compared with a control population, with the aim of determining possible future “therapeutic targets”.

MATERIALS AND METHODS
The study was coordinated and ethically approved by the Ataxia Clinic of Holguín (CIRAH), where the patients were attended. Twenty five patients from the province of Holguín were clinically and genetically diagnosed as Type SCA-2 Ataxia by a Polymerase chain reaction (PCR) for the detection of the SCA2 mutation, as described by John, Weitzner and Rosen (1991). The average age of men was 39.9 ± 3.4 years (n = 14) and of women, 41.0 ± 2.6 years (n = 11). Thirty control subjects were selected from the same geographical region: 16 men (age 40.2 ± 2.7 years) and 14 women (age 39.0 ± 3.1 years). After informed consent, lumbar CSF and venous blood samples were taken from each fasted patient or control between 8.00 and 10.00 a.m. Lumbar puncture was performed (interspaces L3-L4 or L4-L5) in patients and in control subjects who underwent surgery with spinal anesthesia, without any known neurological or psychiatric disease. Blood samples were collected by venipuncture and centrifuged to obtain serum. For analysis, 2 ml CSF and 3 ml serum were employed. Serum and CSF samples were frozen at - 60 °C and protected from light exposure with aluminum foil until analysis. Control serum (Serenorm®) from NYCOMED (England) was used as reference material.

An atomic absorption spectrophotometer (AAS, SP9) equipped with a graphite oven (all from Pye Unicam) was employed for the analysis of CSF Fe, Cu and Zn. CSF samples were centrifuged 5 minutes at 3500 rpm and 500 µl of the supernatant fluid were appropriately diluted with nitric acid. The triplicates aspirations of the samples were averaged for calculations. The materials employed were previously cleansed overnight with 10 % nitric acid (Merck AnalaR, Germany) and rinsed more than 4 times with deionized distilled water.

Serum Zn, Fe and Cu were determined by AAS after a 1:9 dilution. Fe analysis included serum deproteinization to eliminate interference from proteins and as Zn is read in the UV region (213.9 nm) a 140 M NaCl solution was added to the standards to simulate the matrix and the scatter effect. The lamps employed were of hollow cathode type. The standard curve was prepared employing standard solutions of each metal (SIGMA) under the same instrument conditions as the samples. All sample analysis was triplicates. The control serum displayed similar values as those proposed by the commercial producers. Results were processed with the statistical program SPSS 10.0.5 for Windows. All the data were introduced in the corresponding forms and later in the data bases. The mean and standard deviations (SD) of each group were calculated. Normality was tested by the Kolmogorov-Smirnov test and the comparison of means between patients and controls was carried out employing the t-Student’s test. Statistical significance was considered if p < 0.05.

RESULTS AND DISCUSSION
Serum microelements
Serum copper, iron and zinc concentrations in controls and SCA-2 patients are shown in Table 1. Serum Zn levels were significantly decreased in patients of both sexes with respect to controls (p=0.000 and p=0.012), while Fe concentration was significantly decreased only in male patients (p=0.046). Serum Cu levels showed no variation in patients and controls.
Table 1. Serum copper, iron and zinc levels in patients with SCA-2 type ataxia from Holguin, Cuba.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cu (µmol/l)</th>
<th>Fe (µmol/l)</th>
<th>Zn (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 30</td>
<td>14.97 ± 4.63</td>
<td>14.81 ± 5.70</td>
<td>9.80 ± 2.78</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 25</td>
<td>14.24 ± 3.76</td>
<td>10.89 ± 1.74</td>
<td>7.35 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>p = 0.548</td>
<td>p = 0.07</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>Male patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 15</td>
<td>13.79 ± 4.05</td>
<td>10.45 ± 1.56</td>
<td>7.30 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>p = 0.331</td>
<td>p = 0.046</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>Female patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 10</td>
<td>14.92 ± 3.37</td>
<td>11.55 ± 1.87</td>
<td>7.43 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>p = 0.816</td>
<td>p = 0.121</td>
<td>p = 0.012</td>
</tr>
<tr>
<td>Male controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 14</td>
<td>15.14 ± 5.18</td>
<td>14.81 ± 6.57</td>
<td>10.28 ± 3.02</td>
</tr>
<tr>
<td>Female controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 16</td>
<td>14.82 ± 4.25</td>
<td>14.81 ± 5.03</td>
<td>9.38 ± 2.57</td>
</tr>
</tbody>
</table>

A: Comparing Control vs Patients;  
B: Comparing Male Controls vs Male Patients  
C: Comparing Female Controls vs Female Patients.

Values expressed as µmoles/ l.

The significant decrease of serum Zn levels in the group of SCA-2 patients and of Fe levels in male patients with respect to controls constitutes an indirect evidence of their possible association with the physiopathology of SCA-2.

The World Health Organization has defined that people with Zn values below 10.7 µmol/l are considered at risk of deficiency for this metal.9 48.9 % of the world population and 45.8% of Latin America and the Caribbean are in this category.10 Our results show that in all the patients studied, serum Zn concentrations were below this value. In the control group, 21 subjects (72.4 %) were beneath this level. Taken as a whole, these results could point to a systemic deficiency for these metals in the population and more so in patients with SCA-2, which can alter central nervous system function. Increasing evidence suggests that zinc appears to protect against oxidative stress and zinc deficiency has been reported to result in oxidative damage to various macromolecules and enhanced lipid peroxidation.11 Further investigations are required to clarify the causes and magnitude of Zn deficiency in this province.

CSF microelements

The levels of CSF copper, iron and zinc are shown in Table 2. All three microelements are significantly decreased in patients with SCA-2 (p=0.000), independent of gender. The decreased Zn and Fe concentrations in the CSF could be an expression in the CNS of a systemic deficiency or of altered transport mechanisms for the microelements at the blood CSF barrier due to the disease. However, dietary deprivation hardly causes any decrease in Zn concentration in the brain, unlike peripheral tissues.12

The negative impact of low Zn levels has been recently reported in neurodegenerative diseases, such as Parkinson’s disease, Alzheimer disease and Friedreich’s Ataxia.5,7 Exogenous zinc exerts neuroprotective actions through antagonism of the activation of NMDA receptors in the cerebellum, the brain area most affected in type SCA-2 Ataxia, and by being an antioxidant.12,13

The evidence available does not permit definition of the cause of these abnormalities, whether zinc deficiency or abnormal zinc transport is the primary factor. The pathological implications of disturbed metal ion homeostasis confirm the vital roles these metal ions play in the catalytic function of many enzymes, in gene regulation (zinc-finger proteins), and in free radical homeostasis.14

These results strongly suggest that these metals could be directly or indirectly related with the onset and/or clinical outcome of type SCA-2 Ataxia and they could be possible therapeutic targets for the symptomatic treatment of this illness.

This work is the first report of decreased serum and cerebrospinal fluid concentration of microelements in patients with type SCA-2 Ataxia, and the main results suggest that low serum and CSF zinc concentrations might be related with the risk for Ataxia type SCA-2, although they could also be associated with the...
pathophysiology of this disease. These findings could open a new door towards delaying the progression of SCA-2 Ataxia through a supplemental treatment with these microelements.

Table 2. CSF copper, iron and zinc levels in patients with SCA-2 type ataxia from Holguín, Cuba.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control N = 30</td>
<td>90,23 ± 14,22</td>
<td>0,17 ± 0,03</td>
<td>0,18 ± 0,06</td>
</tr>
<tr>
<td>Patients N = 25</td>
<td>40,32 ± 14,56</td>
<td>0,11 ± 0,02</td>
<td>0,072 ± 0,02</td>
</tr>
<tr>
<td>Male patients N = 15</td>
<td>38,65±12,67</td>
<td>0,11±0,02</td>
<td>0,07±0,02</td>
</tr>
<tr>
<td>Female patients N = 10</td>
<td>42,81±17,44</td>
<td>0,10±0,02</td>
<td>0,07±0,02</td>
</tr>
<tr>
<td>Male controls N = 14</td>
<td>91,70±15,35</td>
<td>0,17±0,03</td>
<td>0,18±0,07</td>
</tr>
<tr>
<td>Female controls N = 16</td>
<td>88,96±13,57</td>
<td>0,18±0,03</td>
<td>0,18±0,05</td>
</tr>
</tbody>
</table>

A: Comparing Control vs Patients; B: Comparing Male Controls vs Male Patients C: Comparing Female Controls vs Female Patients.

Values expressed as mg/l for Zn and Fe and µg/l for Cu.

CONCLUSIONS
The important underlying assumption in these studies is that many of the individuals in the selected group of Ataxic patients have less-than-optimal zinc status that has resulted in a zinc-dependent change in the level of the biomarker or the adequacy of the functional response. Thus, the provision of increased dietary zinc alone as a zinc supplement should be able to restore optimal physiological function or normal levels of the biomarker. Indeed, trace elements might be related with the pathogenesis of Ataxia type SCA-2.

REFERENCES