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Plasma concentrations of the trace elements copper, zinc and selenium in Brazilian children with autism spectrum disorder

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ABSTRACT

The association between the plasma levels of trace elements, such as copper (Cu), zinc (Zn) and selenium (Se), in people with autism spectrum disorder (ASD), has attracted the interest of many physicians in the very recent years, because the impaired homeostatic regulation of trace elements, including their levels in the bloodstream and their potential neurotoxicity, contribute to the onset and exacerbation of ASD. In this study, we investigated 23 pediatric subjects ($\leq$ 18 yrs old, both sexes) with ASD, all residents in the city of Campo Grande in Brazil, by searching for their micronutrient levels in plasma in relation with metabolic and nutrition biomarkers. Aside for the few evidence reported, generally, the Brazilian cohort of ASD children here examined did not show a marked difference in micro-nutrient intake in relation with their resident geographical area and their dietary habit or metabolic state, although a slight difference in the levels of magnesium and phosphorus was retrieved due to sex difference.

1. Introduction

The relationship between plasma levels of trace elements such as copper (Cu), zinc (Zn), and selenium (Se) in subjects with autism spectrum disorder (ASD) has polarized the interest of many physiologists in recent years, due to the evidence that the impaired homeostatic regulation of trace elements, their levels in the bloodstream and their potential neurotoxicity, altogether contribute to the ASD etiology [1–5]. Furthermore, Cu, Zn, and Se are trace elements essential for many physiological functions, as they act as a cofactor in various enzymatic processes, and hence their biochemical regulation is of major importance [6]. The plasma concentration of these elements may be affected by different habits in the dietary intake, by environmental factors and other ones that can play a role in the pathophysiology of ASD.

In ASD subjects, the plasma levels of Zn have been reviewed elsewhere in the literature, where it has also been reported evidence about further trace elements [7,8]. The role of Zn is strategic in the function of the gut and the gastrointestinal system during the neural development, as Zn may affect the function of the brain-gut axis [9]. Zinc deficiency has been associated with an increased incidence of seizures, attention deficit, hypotonia and hyperactivity in subjects with haploinsufficiency of proteins from the ProSAP/Shank family, a pathology known as Phelan-McDermid syndrome. This would mean that Zn is a prerequisite to allow the assembly of the ProSAP/Shank scaffold and organize the post-synaptic density of excitatory synapses, i.e., a correct neuro-development [10,11]. This activity affects substantially also the levels of ProSAP1/Shank2 and ProSAP2/Shank3 and the impairment in social relationship and behavioral attitudes characterizing ASD [10]. Therefore, a correct level of Zn uptake is crucial in the elucidation of ASD etiopathogenesis and the related dietary supplementation [12–16].

Furthermore, the role of Cu in ASD etiopathogenesis seems to be closely linked to Zn, at least as the Zn/Cu ratio has been reported as a biomarker of ASD [17]. Cu is toxic for neurodevelopment [5]. Actually, Cu overload is a causative factor of the synaptic pathology occurring in ASD, as an excess plasmatic level of Cu can disrupt Zn homeostasis, particularly if in association with a genetic deletion or knockdown of the Cu-transported complex COMMD1, as Cu and Zn act as antagonist chemicals in neurobiology [18]. Finally, the relationship between Se and ASD has been particularly highlighted for selenoproteins [19,20]. As many further micronutrients, Se participates in the neurodevelopmental mechanisms leading to the correct synaptogenesis, particularly in its inhibition towards Cu-mediated neurotoxicity [21,22].

Brazilian children with ASD lack of an exhaustive survey in the

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literature about the levels of these micronutrients, if we except very few papers on children without ASD [23]. Knowing the plasmatic levels of these micronutrients in ASD is therefore of major importance.

The aim of the present study was to evaluate plasma Cu, Zn, and Se concentrations in children with ASD from the Center-West region of Brazil (Mato Grosso) and elucidate the relationship between ASD etiopathogenesis and micronutrient intake, by investigating further nutritional and metabolic parameters.

2. Materials and methods

2.1. Subjects

Twenty-three individuals of both gender, under 18 years of age, with ASD and all residents in the city of Campo Grande in Brazil, were enrolled in the study. The diagnostic criteria were in accordance with the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) published by the American Psychiatric Association (2013). The study protocol was approved by the Ethics Committee for Research in Human Beings of the Federal University of Mato Grosso do Sul. Written informed consent was obtained from the parents and/or from law tutors of each child. The patients were registered in the CAMS/APAE of Campo Grande/MS and residents of this city. A neurologist of our scientific team of experts confirmed the diagnosis.

2.2. Sample preparation

All plastic or glass materials used in the study were previously immersed for a minimum of 24 h in Extran (Merck, Darmstadt, Germany) solution at 5%, rinsed thoroughly in tap water and again immersed for a period of at least 24 h in nitric acid solution (Merck, Darmstadt, Germany) at 10% to decontaminate any residue of metal. They were then rinsed thoroughly with ultrapure Milli-Q water type (Millipore, Bedford, USA) and dried in an oven at 70 °C. Blood samples for analysis of laboratory data were collected by a nurse in the morning with fasting subjects. Each sample was immediately transferred to a polypropylene vacuum tube used to collect trace elements (BD Vacutainer Systems) and centrifuged for 15 min at 3000 *g*. The plasma was distributed in polypropylene Eppendorf tubes and immediately frozen at -18 °C. For the determination of trace elements, samples were previously thawed, and 0.5 ml of each was diluted with a solution of 4.5 ml ultrapure water Milli-Q type with nitric acid (HNO3) at 1% and 0.01% Triton X® (surfactant), reaching a final volume of 5 ml for the analysis.

2.3. Nutritional status

Subjects respective weight and height were recorded at the beginning of the evaluation. Nutritional status was verified through the weight-for-age (W/A), height for age (H/A) and weight for height (P/A) scores, taking as reference the WHO growth curves 2007 recommended by the Brazilian Society of Pediatrics. The weight value was obtained using a mechanical scale (Filizola®), which is commonly used in pediatrics. Children under two years of age were weighed on the same pediatric scale. From the age of 2 years, the weight was measured to balance the adult type, with 100 g graduation. In any case, the child or adolescent remained standing without support, with arms outstretched over the body, barefoot, wearing light clothing, positioned on the platform center and avoiding jogging. Stature in children under two years of age was measured with the same ruler anthropometric and supine. The child remained to lie flat on a horizontal surface, wearing only light clothing, with the fixed upper limit (zero) set the pole head, eyes turned upward, avoiding bending or neck extension and the lower limit, mobile, adjusted to the plane of the feet [24]. The height of older children was measured with a vertical stadiometer of the scale. The heel, the buttocks, the back and the occipital pole head, were positioned along the vertical stadiometer bulkhead, arms to the side, watching the body [24]. Furthermore, nutritional assessment of patients was accomplished by collecting measurements of arm circumference (AC) and triceps skinfold (TSF). To measure the circumference of the arm, the technique used is described in ref 24 and follows these steps. The subjects flexed the elbow at 90 degrees with the palm facing up, then the calculated distance between anatomical landmarks and the central point is marked with a demographic pencil, wrapping his arm with anthropometric tape, so that it is fixed on the marked point, and the assessment performed [24]. For the triceps, skinfold measurement technique was: assessed in the orthostatic position, arms extended and relaxed throughout the body The bend was measured /pinched with a compass skinfold Lange® vertically at the midpoint of the back of the arm, between the acromion process of the scapula and the olecranon process of the ulna [24].

2.4. Plasma evaluation of micronutrients

Plasma concentrations of Cu, Zn, and Se have been performed with an ICP-OES instrument (Optical Emission Spectrometer with Plasma Argon Inductively Coupled, Thermo-Fisher®). ICP-OES analysis is a technique based on observations of radiation emission elements, which are constituents of the sample, in a coupled plasma. If the solution of hydride generation (sodium borohydride and sodium hydroxide) is used, the approach potentiates the reading spectrum in order to trace an investigated element in the concentration of parts per billion (ppb). The wavelengths for reading Cu, Zn, and Se have been 324.75 nm, 213.86 nm, and 196.09 nm, respectively. For the determination of trace elements, samples were previously thawed, and 0.5 ml of each was diluted with a solution of 4.5 ml ultrapure water Milli-Q type with nitric acid (HNO3) at 1% and 0.01% Triton X® (surfactant), reaching a final volume of 5 ml. Finally, for the construction of Cu and Zn calibration curves were used solutions multielement standard at concentrations of 50, 100, 150 and 200 mg/l and Se concentration of 50, 100, 150 and 200 mg/L. All samples were read in triplicate.

2.5. Statistics

Data were expressed as mean ± standard deviation (SD). A Kolmogorov-Smirnov (KS) test was applied for comparisons between small samples. Where the Shapiro-Wilk test and the KS test assessed for a nonparametric feature of samples, a Wilcoxon Mann-Whitney test was applied, otherwise, a one-way ANOVA (Tukey's post hoc test) performed. Correlations were evaluated using a Pearson test, at a significant level of $p < 0.05$. The other results of the variables evaluated in this study were presented in the form of a descriptive statistics or tables. Statistical analysis was performed using the software SigmaStat, version 3.5 or InStat, version 3.0, considering a 5% significance level.

3. Results

The results showed that the prevalence of underweight (13.0%), overweight (8.7%) and obese subjects (30.4%) matched perfectly previously obtained data from ours for Brazilian children, which are still in the process of further investigation and reproducibility. The average concentration of Cu and Zn were within the range of the commonly accepted reference values, although the average plasma concentration of Se appeared lower than expected. However, plasma levels of Cu and Zn remain within normal limits. Plasma Se concentration appeared somehow below previous data retrieved by our studies with adult residents in the city of Campo Grande (data not shown). These results with ours suggest the need for evaluation of mineral metabolism in children with ASD seeking the benefits of supplementation listing.

Table 1 shows that the distribution of subjects was randomly assessed, as main shared parameters, i.e., ages and sex, were equally distributed. The subjects did not present any difference in further nutritional and metabolic pattern, to prevent any indirect statistical or