

Evaluation of whole blood zinc and copper levels in children with autism spectrum disorder

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Abstract Zinc (Zn) and copper (Cu) are important trace elements for cognitive development and normal neurological functioning. Autism spectrum disorder (ASD) is a common neurological disorder, which has previously been associated with the levels of some trace elements in the blood. However, clinical data regarding the potential implication of Zn and Cu in patients with ASD are still insufficient. Therefore, the aim of the present study was to investigate the whole blood levels of Zn and Cu in a cohort of 28 children with ASD and 28 age- and gender-matched healthy controls. Whole blood Zn and Cu levels were assessed using inductively-coupled plasma-sector

field mass spectrometry. Both in the control and in the ASD group, the values of whole blood Cu and Zn were characterized by a Gaussian distribution. The results indicate that the ASD children were characterized by ~10 % ($p = 0.005$) and ~12 % ($p = 0.015$) lower levels of whole blood Zn and Zn/Cu ratio, respectively, in comparison to controls. No significant difference in whole blood Cu was observed. However, Cu/Zn ratio was ~15 % ($p = 0.008$) higher in ASD children than that in the control ones. The results of the present study may be indicative of Zn deficiency in ASD children. Taking into account Zn-mediated up-regulation of metallothionein (MT) gene expression, these findings suggest a possible alteration in the functioning of the neuroprotective MT system. However, further investigations are required to test this hypothesis.

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Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by persistent deficits in social communication and social interaction, and restricted, repetitive patterns of behavior, interests or activities (APA 2013). ASD can be present from birth or can appear very early in the development. In the past decades, the number of children with ASD has increased at an alarming rate (ADDM Network 2014; Zablotsky et al. 2015). Current estimations in the US report that 1 in 45 children are diagnosed with ASD (Zablotsky et al. 2015) with a higher prevalence in boys (1 in 42) than in girls (1 in 189) (ADDM Network 2014). In part, this increase could be driven by improvements in diagnosis and increased awareness (Macedoni-Lukšič et al. 2015).

ASD is influenced by a variety of genetic, environmental, immunological, metabolic and nutritional factors (Essa et al. 2013; Saad et al. 2015a; Endreffy et al. 2016; Matelski and Van de Water 2016). Since both genetic and non-genetic factors are suspected to cause ASD, considerable efforts have focused on identifying non-genetic factors that might be involved in the etiopathogenesis of this disease, for early detection and treatment of individuals at risk. It has been proposed that certain heavy metals, especially mercury (Hg), may play a significant role in the pathogenesis of ASD (Kern et al. 2012, 2015; Mostafa et al. 2016).

Trace elements such as zinc (Zn) and copper (Cu) have been shown to be crucial for cognitive development, normal neurological functioning, and heavy metal detoxification (Bjørklund 2013; Böckerman et al. 2015). Previous studies also show that Zn deficiency, elevated Cu levels, and, therefore, low Zn/Cu ratio, are common in ASD children (Faber et al. 2009; Bjørklund 2013; Li et al. 2014; Macedoni-Lukšič et al. 2015). However, clinical data regarding the relationship between these two trace elements in ASD patients are still insufficient. Therefore, the aim of the present study was to test whether whole blood levels of Zn and Cu are altered in children with ASD compared to healthy control subjects. We hypothesize that whole blood levels of Zn and Cu will be altered in ASD children.

Material and methods

Subjects

28 children (5.83 ± 3.10 years) with ASD registered at the Pediatric Psychiatry Clinic were diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria (APA 2000). Another group of 28 healthy, age- (5.95 ± 2.92 years) and gender-matched subjects, free from any neuropsychiatric disorders was also recruited. Informed consent from the parents of each subject was obtained, and the Ethical Committee at Iuliu Hațieganu University of Medicine and Pharmacy approved all research protocols.

Blood sampling

Blood for determination of Zn and Cu were collected in 2 ml tubes containing lithium heparin (Greiner-Bio-One GmbH, Austria). The samples were taken from the cubital vein in the morning, after an overnight fast, and stored at -80 °C until analysis.

Zinc and copper assay

Whole blood Zn and Cu levels were assayed using inductively coupled plasma-sector field mass spectrometry (ICP-SFMS) on a Perkin-Elmer SCIEX ICP-MS (model ELAN DRC II, Toronto, Canada). Samples were previously digested with nitric acid (microwave assisted), following the method described by Rodushkin et al. (2000).

Statistical analysis

Data are presented as mean and their respective standard deviation (mean \pm SD) and coefficient of variation (CV, %). Data analysis was performed in Statistica 10.0 (Statsoft, Tulsa, OK, USA). One-way ANOVA was used to test potential differences between groups, previous normality and homogeneity of variance being assessed using Shapiro-Wilk and Levene's test, respectively. Correlation analysis for evaluation of a possible association between whole blood Cu and Zn levels and Childhood Autism Rating Scale (CARS) values in ASD children was performed using Pearson's correlation coefficient. Differences were considered significant at α level < 0.05 .

Results

The present study found that both the control and the ASD group had values of whole blood Zn, Cu, and Zn/Cu ratio characterized by a Gaussian distribution. It was also found that ASD children had whole blood Zn levels ~ 10 % lower than controls (5.54 ± 0.78 $\mu\text{g/ml}$ vs. 6.14 ± 0.76 $\mu\text{g/ml}$; ANOVA $p = 0.005$, Table 1). No significant difference in whole blood Cu was observed (1.22 ± 0.18 $\mu\text{g/ml}$ vs. 1.26 ± 0.16 $\mu\text{g/ml}$; ANOVA $p = 0.460$). However, the whole blood Zn/Cu ratio was also ~ 12 % lower in the ASD group as compared to the control values (4.49 ± 0.95 vs. 5.09 ± 0.86 ; ANOVA $p = 0.015$). Oppositely, ASD children were characterized by 15 % higher whole Cu/Zn values than the control ones (0.23 ± 0.05 vs. 0.20 ± 0.03 ; ANOVA $p = 0.008$). Correlation analysis indicated no correlation between the whole blood levels of Zn and Cu in a whole study group (autistic and healthy children) ($r = -0.04$; $p = 0.771$), ASD

Table 1 Whole blood levels of Zn and Cu ($\mu\text{g/ml}$), and Zn/Cu ratio in children with ASD and healthy controls

Parameter	Healthy controls	ASD children	<i>p</i> -value
Zn	6.14 ± 0.76 (12 %)	5.54 ± 0.78 (14 %)	0.005
Cu	1.22 ± 0.18 (15 %)	1.26 ± 0.16 (13 %)	0.460
Zn/Cu ratio	5.09 ± 0.86 (17 %)	4.49 ± 0.95 (21 %)	0.015
Cu/Zn ratio	0.20 ± 0.03 (16 %)	0.23 ± 0.05 (23 %)	0.008

Data presented as mean \pm SD (CV, %); significant difference at $p < 0.05$

patients ($r = -0.270$; $p = 0.165$), or in healthy controls ($r = 0.236$; $p = 0.226$). No significant correlation was observed between the studied metals and CARS values in children with ASD (CARS vs. Cu: $r = -0.244$, $p = 0.210$; CARS vs. Zn: $r = 0.160$, $p = 0.416$; CARS vs. Zn/Cu ratio $r = 0.205$, $p = 0.295$; CARS vs. Cu/Zn ratio $r = -0.272$, $p = 0.161$).

Discussion

The present study found that children diagnosed with ASD had lower whole blood Zn levels and Zn/Cu ratios than healthy controls, in agreement with data obtained analyzing plasma or serum samples (Faber et al. 2009; Li et al. 2014). An earlier study found elevated Zn levels in the plasma of ASD children (Vergani et al. 2011), a finding that is not supported by the results of the present study. The whole blood Zn and Cu levels, both in ASD and control children, reported here are well in agreement with the levels in healthy children in other studies (Smith et al. 1976; Kumar et al. 2004).

Changes in whole blood Zn levels may be the first to signal the development of a Zn-deficient state as erythrocytes contain significantly higher levels of Zn than plasma (Iyengar and Woittiez 1988). Moreover, erythrocyte and consequently whole blood Zn content may reflect a more long-term Zn status, than serum Zn level (Stewart-Knox et al. 2005). Earlier data indicate that brain structures are susceptible to Zn deficiency (Takeda and Tamano 2009), and this sensitivity may be increased during oxidative stress and associated with the development of certain neurological diseases (Cuajungco and Lees 1997). Taking into account the capacity of Cu ions to take part in Fenton reactions (Valiko et al. 2005), the interaction between Zn and Cu may have a significant impact on oxidative stress and associated diseases. One study found a significantly lower Zn/Cu ratio in ASD children compared with children with other neurological disorders (Macedoni-Lukšič et al. 2015). However, alteration of Cu and Zn status has been detected in numerous brain diseases including Alzheimer's, Parkinson's and prion diseases (Kozłowski et al. 2012; Stelmashook et al. 2014). Therefore, lower Zn/Cu ratio in children with ASD may reflect a generalized Zn deficiency, potentially also decreasing the efficiency of the anti-oxidant system.

Research suggests that Hg accumulation may occur as a consequence of metallothionein (MT) dysfunction in ASD children, which may be one of the effects of Zn deficiency (Bjørklund 2013). Zinc and Cu bind to and participate in the control of the synthesis of MT proteins, crucial in metal metabolism and protection (Sakulsak 2012; Bjørklund 2013).

Zinc ability to up-regulate the MT gene expression and to reduce the toxicity of heavy metals, may suggest that the administration of Zn to ASD children (with diagnosed Zn deficiency) may offer some improvements in a treatment protocol

along with other potential dietary interventions (Srinivasan 2009; Bjørklund 2013; Saad et al. 2015a, 2015b; Endreffy et al. 2016). It is important to monitor and follow the values for both Zn and Cu during Zn therapy, because these two trace elements are antagonists in function, but at the same time essential for living cells (Bjørklund 2013).

Conclusion

The present study shows a lower Zn level and Zn/Cu ratio in ASD children compared to healthy controls. Taking into account Zn-mediated up-regulation of MT gene expression, these results suggest a possible alteration in the functioning of the neuroprotective MT system. However, further investigations are required to test this hypothesis and to estimate the detailed mechanisms of the association between Zn deficiency and ASD.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest with respect to the authorship, and/or publication of this paper.

Ethical approval All procedures were in accordance with the ethical standards of the institutional and/or national research committee/s and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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