Journal of Trace Elements in Medicine and Biology 58 (2020) 126445

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Serum zinc, copper, zinc-to-copper ratio, and other essential elements and minerals in children with attention deficit/hyperactivity disorder (ADHD)

Anatoly V. Skalny[a,b,c,e], Anna L. Mazaletskaya[a], Olga P. Ajsuvakova[a,b], Geir Bjørklund[d], Margarita G. Skalnaya[b,c], Jane C.-J. Chao[e], Lyubov N. Chernova[c], Roza A. Shakieva[f], Philippe Yu. Kopylov[b], Andrey A. Skalny[a,c], Alexey A. Tinkov[a,b,*]

[a] *Yaroslavl State University, 150003, Yaroslavl, Russia*
[b] *IM Sechenov First Moscow State Medical University (Sechenov University), 119146, Moscow, Russia*
[c] *RUDN University, Moscow, Russia*
[d] *Council for Nutritional and Environmental Medicine, Mo I Rana, Norway*
[e] *Taipei Medical University, Taipei, Taiwan*
[f] *Kazakh Academy of Nutrition, Almaty, Kazakhstan*

A R T I C L E I N F O

Keywords:
Neurodevelopment
Magnesium
Zinc
Chromium
Copper

A B S T R A C T

Background: Essential trace elements and minerals play a significant role in neurodevelopment. Although certain studies demonstrated impaired essential trace element and mineral status in children with ADHD, the existing data are insufficient. The objective of the present study was to assess serum trace element and mineral levels in children with ADHD.

Methods: Serum trace element and mineral levels in 68 children with ADHD and 68 neurotypical controls were assessed using ICP-MS at NexION 300D (PerkinElmer Inc., USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., USA).

Results: Serum Cr, Mg, and Zn levels in children with ADHD were 21 % ($p = 0.010$), 4 % ($p = 0.005$), and 7 % ($p = 0.001$) lower as compared to the healthy controls, respectively. In turn, serum Cu/Zn values were 11 % higher than those in the control group. Age and gender had a significant impact on serum element levels in ADHD. Particularly, preschool children were characterized by significantly increased Cu (+8 %; $p = 0.034$), and Cu/Zn (+19 %; $p < 0.001$) values, whereas serum Zn (-9 %; $p = 0.004$) level was decreased. In primary school-aged children only 6 % ($p = 0.007$) lower Mg levels were observed. Both boys and girls with ADHD were characterized by 8 % ($p = 0.016$) lower serum Zn levels and 10 % ($p = 0.049$) higher Cu/Zn values when compared to neurotypical girls. Boys with ADHD also had significantly higher Cu/Zn, exceeding the respective control values by 12 % ($p = 0.021$), predominantly due to a 7 % ($p = 0.035$) decrease in serum Zn. Serum Mg levels were also found to be significantly lower than those in neurotypical children by 5 % ($p = 0.007$). In adjusted regression models serum Cr ($\beta = -0.234$; $p = 0.009$) and Cu/Zn ($\beta = 0.245$; $p = 0.029$) values were significantly associated with ADHD, respectively. Two-way ANOVA revealed a significant impact of ADHD on Cr, Mg, Zn, and Cu/Zn, whereas age was associated with Cu, I, Mg, Mo, and Cu/Zn, whereas gender accounted only for variability in serum Mn levels. Principal component analysis (PCA) also revealed significant contributions of Mg, Zn, and Cu/Zn values to ADHD variability.

Conclusions: Hypothetically, the observed decrease of essential trace elements, namely Mg and Zn, and elevation of Cu/Zn may significantly contribute to the risk of ADHD or its severity and/or comorbidity.

1. Introduction

ADHD is a neurodevelopmental disorder characterized by hyperactive behavior, inattention, impulsivity, and problems in social interaction manifesting predominantly in childhood [1]. The prevalence of ADHD is recently estimated as 5–7 % [2,3], although previous studies reported a wider range from 2 % to 18 % [4]. Gender also has a significant impact on ADHD, with the latter being more prevalent in boys at a male:female ratio of 2:1 to 9:1 [5].

ADHD is also characterized by the presence of psychiatric

* Corresponding author at: Yaroslavl State University, 150003, Yaroslavl, Russia.
E-mail addresses: tinkov.a.a@gmail.com, tinkov.a.a@microelements.ru (A.A. Tinkov).

https://doi.org/10.1016/j.jtemb.2019.126445
Received 16 September 2019; Received in revised form 7 November 2019; Accepted 5 December 2019
0946-672X/

comorbidities (anxiety/depression, conduct disorder, etc.) that have a significant impact on functional outcome [6]. Particularly, 66 % of the children with ADHD in the present study were characterized by at least one comorbid psychiatric disorder [7]. Correspondingly, ADHD significantly affects the quality of life of both children [8] and adults [9]. ADHD is also associated with high societal and economic costs, although the values vary significantly in different countries [10,11].

Although genetic factors were shown to determine up to 60–80 % of ADHD cases, gene-environment interaction was shown to play an important role in ADHD [12]. Particularly, environmental influences mediate 10 %–40 % of ADHD variance [13]. Premature birth, maternal smoking [13], pollutant exposure [14] play a significant role in ADHD. Nutrition was also shown to have a significant impact on ADHD development. Particularly, even certain dietary patterns like low adherence to the Mediterranean diet were associated with increased risk of ADHD [15]. Micronutrient density of foods was also significantly inversely associated with ADHD risk [16] due to their role in brain development and functioning.

The existing data on micronutrient, and particularly essential trace element and mineral status of children with ADHD are insufficient. It has been demonstrated that children with ADHD are characterized by low Zn intake and status that may be involved in ADHD pathogenesis [17]. The results of meta-analyses demonstrated impaired iron [18] and magnesium [19] levels in ADHD, although certain contradictions persist. Data on other trace elements and minerals are less consistent. The existing indications of efficiency of nutritional therapy with zinc, magnesium, and iron [20] also support the role of low serum trace elements in ADHD.

Moreover, micronutrient levels were shown to have certain effects on treatment outcome in ADHD [21]. Particularly, zinc, iron, and magnesium supplementation may significantly improve ADHD symptoms in children with a deficiency of these trace elements [22]. Further investigations are required to reveal potential targets for nutritional therapy in ADHD.

Therefore, the objective of the present study was to assess serum trace element and mineral levels in children with ASD.

2. Materials and methods

The present study was performed in agreement with the principles of the Declaration of Helsinki (1964) and its later amendments (2013). The study protocol was approved by the Local Institutional Ethics Committee at Yaroslavl State University. All children and their parents participated in the study voluntarily and were informed about the contents of the study. Parents also signed informed consent (parents). All procedures involving children, including anthropometric examination and blood sampling, were performed in the presence of one of the legal representatives.

Sixty-eight children with diagnosed ADHD aged 4–9 years old and 68 gender- and age-matched neurotypical controls. Both control and ADHD groups consisted of 35 boys (51 %) and 33 girls (49 %). Cases and controls were also matched according to anthropometric parameters, including height, weight, and BMI to avoid any potential effect of obesity or growth delay on the outcome of the study. Therefore, no significant difference in age (6.4 ± 2.1 vs 6.4 ± 2.1, $p = 0.999$), weight (24.4 ± 7.7 vs 23.9 ± 7.9, $p = 0.709$), height (123.9 ± 14.1 vs 121.0 ± 20.2, $p = 0.342$), and BMI (15.6 ± 2.3 vs 15.8 ± 2.5, $p = 0.597$) was observed between the control and ADHD groups.

The examinees were contacted during annual medical examination. The diagnosis of ADHD (ICD-10: F90.0) was extracted from the clinical record of the outpatient department. ADHD was diagnosed using ICD-10 criteria including inattention; hyperactivity; impulsivity (not less than 3 symptoms of each). Only children not administering any specific treatment for ADHD were included in the study.

Exclusion criteria were used in order to alleviate the impact of side factors on serum trace element and mineral levels: a) the presence of other neuropsychiatric disorders; b) endocrine disorders (obesity, diabetes); c) dental amalgam fillings (or any other metallic implants); d) environmental exposure to metals due to habitation near sources of industrial metal exposure; e) exposure to passive smoking; f) acute infectious, surgical and traumatic diseases. Also, only children permanently (> 5 years) living in the selected location were included in the study in order to prevent the influence of the difference in geochemical and anthropogenic factors.

Venous blood samples were collected after overnight fasting via venepuncture of the cubital vein using 9-ml "Vacuette" tubes (Greiner Bio-One International AG, Austria) with subsequent centrifugation at 1600 g for 10 min to obtain serum. Serum samples with visual signs of hemolysis were excluded from the analysis. The obtained serum samples were diluted (1:15; v/v) with an acidified (pH = 2.0) diluent consisting of 1 % 1-Butanol (Merck KGaA, Darmstadt, Germany), 0.1 % Triton X-100 (Sigma-Aldrich, Co., St. Louis, USA), and 0.07 % HNO_3 (Sigma-Aldrich, Co., St. Louis, USA) in distilled deionized water (18 MΩ cm).

The obtained samples were analyzed for essential trace element (Co, Cr, Cu, Fe, I, Mn, Mo, Se, V, Zn) and mineral (Ca, Mg) levels using inductively-coupled plasma mass spectrometry at NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA). System calibration was performed trace element solutions prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT, USA). 10 μg/l solutions of yttrium and rhodium prepared from Yttrium (Y) and Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA) were used for internal online calibration. Laboratory quality control was performed daily using the certified reference materials (CRM) of human plasma ClinChek® Plasma Control, Levels I, II. The recovery rates for Level I and II during the whole period of study were 86 %–108 % and 88 %–113 %, respectively.

Statistical treatment of the obtained data was performed using Statistica 10.0 (Statsoft, OK, USA). Shapiro-Wilk test was used for data normality assessment. Due to Gaussian distribution data on serum trace elements and minerals were expressed as Mean ± SD. Paired group analysis was performed using parametric t-test. Two-way ANOVA was used for factorial analysis with subsequent assessment of the difference between gender- (male, female) or age-specific (preschool, primary school age) groups using Fischer-LSD test. Multiple regression analysis was used to assess the relative association between serum trace element levels and ADHD in a crude model containing serum levels of all elements studied (Model 1), serum levels of elements characterized by significant group difference (Model 2), and adjusted for anthropometric parameters (Model 3). The key patterns of serum trace element and mineral levels in children with ADHD were also investigated using PCA. All tests were considered as significant at $p < 0.05$.

3. Results

The obtained data demonstrate that children with ADHD are characterized by significant group difference in serum trace element and mineral levels in comparison to neurotypical controls (Table 1). Particularly, serum Cr, Mg, and Zn levels in children with ADHD were 21 %, 4 %, and 7 % lower as compared to the healthy controls, respectively. Serum Co levels also tended to decrease, being lower than the respective control values by 8 %. Oppositely, a nearly significant 5 % decrease in serum iodine concentration was detected in ADHD. In a general cohort of children with ADHD, the Cu/Zn ratio significantly exceeded the control values by 11 % (Fig. 1A).

Stratification by age demonstrated an age-specific difference in serum trace element and mineral levels in ADHD (Table 2). Particularly, preschool children with ADHD were characterized by a significant 8 % increase in serum Cu levels ($p = 0.034$), whereas serum Zn concentrations were 9 % ($p = 0.004$) lower in comparison to the respective control values. Moreover, the Cu/Zn ratio (Fig. 1B) was 16 % higher in