Szabat Przemysław, Poleszak Julita, Szabat Marta, Boreński Grzegorz, Wójcik Magdalena, Milanowska Joanna. The concentration of trace and toxic elements in the hair and in plasma of children with the autism spectrum disorder. Journal of Education, Health and Sport. 2019;9(3):559-572. eISNN 2391-8306. DOI http://dx.doi.org/zenodo.2619965
http://ojs.ukw.edu.pl/index.php/johs/article/view/6748

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

© The Authors 2019;
This article is published with open access at Licensee Open Journal Systems of Kazimierz Wielki University in Bydgoszcz, Poland
Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author (s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non commercial license Share alike. (http://creativecommons.org/licenses/by-nc-sa/4.0/) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 18.02.2019. Revised: 18.02.2019. Accepted: 31.03.2019.

The concentration of trace and toxic elements in the hair and in plasma of children with the autism spectrum disorder

Przemysław Szabat[1], Julita Poleszak[1*], Marta Szabat[1], Grzegorz Boreński[1], Magdalena Wójcik[1], Joanna Milanowska[2]

[1]*Student Science Club at the Department of Applied Psychology, Medical University of Lublin*
[2]*Department of Applied Psychology, Medical University of Lublin*

* E-mail adres: julita.poleszak@wp.pl

ORCID ID:

Przemysław Szabat https://orcid.org/0000-0001-5796-1900
Julita Poleszak https://orcid.org/0000-0002-5166-6262
Marta Szabat https://orcid.org/0000-0001-6309-2027
Grzegorz Boreński https://orcid.org/0000-0002-5359-7555
Magdalena Wójcik https://orcid.org/0000-0002-0999-6284
Joanna Milanowska https://orcid.org/0000-0001-9741-1583

Abstract

Introduction: Explanation of pathogenesis and selection of an effective therapeutic form to treat children with autism spectrum disorders (ASD) is still one of today's challenges.

The aim of the study: Review the research regarding the content of individual trace elements and toxic elements in the hair and in the serum of autistic children.

Material and method: Standard criteria were used to review the literature data. The search of articles in the PubMed database was carried out using the following keywords: *autism, ASD, trace elements, toxic elements.*

Description: There are significant differences in the concentrations of essential trace elements and levels of toxic metals in hair and serum in children with autism spectrum disorders compared to healthy children. Many children with autism spectrum disorders suffer from a deficiency of essential trace elements (including Zn, Cu, Mn, Mo, J, Ni, Cr and others) and high toxic metal loads (Pb, Hg, Cd, Al.).

Summary: The deficiency of essential trace elements and the presence of toxic heavy metals seem to play an important role in the pathogenesis and severity of autistic disorders.

***Keywords*:** autism, elements, ASD

INTRODUCTION

The Autism Spectrum Disorder (ASD) includes a series of neurodevelopmental disorders which are characterized by serious problems in communication and social interactions and the occurrence of restrictive and repetitive types of behavior [1]. In most cases ASD is diagnosed during the first 5 years of the child's life. The incidence of autism spectrum disorder is constantly increasing. Currently, the diagnosis of autism is made in 1 of every 160 children [1]. The explanation of pathogenesis and selection of an effective therapeutic form for treatment of autism spectrum disorders remains one of today's challenges. It is known that the causes of autism are multifactorial [2]. They include mainly genetic factors, but attention is also paid to the impact of epigenetic and environmental factors.

Some studies indicate the relationship between autism and epigenetic changes in gene expression caused by environmental factors [3]. Children, unlike adults, are more exposed to the effects of substances present in the environment. This is due to the immaturity of metabolic pathways and poorly developed mechanisms of renal excretion in young children, as a result of which their detoxification abilities are limited [4]. Additionally, in early development stages, exposure to even a small amount of toxic environmental factors can cause impairment of cognitive functions.

The relationship between levels of toxic elements in plasma, including mercury, lead and cadmium in people with autism, has attracted the attention of many scientists in recent years because impaired homeostatic regulation of toxic elements and their potential neurotoxicity contribute to occurrence and exacerbation of ASD.

The aim of this paper was to review the research regarding the content of individual trace elements and toxic elements in the hair and in the serum of autistic children. Standard criteria were used to review the literature data. The search for articles in PubMed database was carried out using the following keywords: *autism, ASD, trace elements, toxic elements.*

THE ROLE OF INDIVIDUAL ELEMENTS IN A HUMAN ORGANISM

Micronutrients are important components of the human diet [5]. These elements in physiological concentration are necessary for the proper functioning of the body. However, their shortages and excess can lead to unwanted effects [6, 7].

Micronutrients

The daily demand for zinc is 10-15mg. This element plays an important role as a component of many enzymes: it participates in the synthesis of proteins and participates in DNA replication. It is also a component of many proteinases and insulin, and is an element of carbonic anhydrase and respiratory enzymes. Its deficiency leads to inhibition of cell division. However the excess zinc in the body is harmful and may cause vomiting, weakness, insomnia, impaired memory, and dysfunction of T lymphocytes [8, 9].

Chromium is an element necessary to maintain homeostasis. It is found in the active centers of many enzymes and responsible for the proper function of insulin [10, 11]. Its physiological concentration suppresses appetite and helps maintain physiological cholesterol levels [10]. The chromium present in the human body remains on the third oxidation stage. However, small amounts of chromium in the VI oxidation state are harmful to humans. They damage the digestive system, cause skin changes, they also are mutagenic, embryotoxic, teratogenic and carcinogenic [12]. Chromium is the most common reason for professional contact eczema.

Selenium is another trace element necessary for the proper functioning of the human body. It interacts with vitamin E, enhancing the antioxidant effect, inhibiting the aging process and protecting the cell membranes. It is a component of glutathione peroxidase, which protects against hemoglobin. Selenium has a beneficial effect on rheumatic and cardiovascular diseases [13, 14]. Acute selenium poisoning is manifested by dyspnea, tachycardia, diarrhea and even death [15].

Copper is a component of cytochrome oxidase respiratory enzymes and ascorbic oxidase without which it is not possible to breathe at the cellular level [16]. It takes part in the synthesis of hormones and substances important for organism function: adrenaline, melanin, collagen and elastin. It is also a part of ceruloplasmin. Its excess can lead to food disorders, hyperthyroidism, cirrhosis of the liver, allergic reactions. A specific variation of the pathological accumulation of copper in the body is genetically determined Wilson's disease.

Manganese is a microelement which as a component of many bioactive substances and is involved in important biological processes such as: protein and carbohydrate metabolism, synthesis of mucopolysaccharides and thyroid hormones. It supports the functioning of the central nervous system, interacting with group B vitamins. It is also an activator of arginase - one of the urea cycle enzymes. Symptoms of acute intoxication may include throat burning, nausea and vomiting, gastrointestinal disorders. Chronic poisoning damages the nervous, respiratory and sexual systems [17].

Molybdenum is one of the elements forming metalloflavoproteins such as xanthine oxidase or aldehyde dehydrogenase [18]. The case of acute molybdenum poisoning with a cumulative dose of 13.5 mg in 18 days is described. The patient had acute psychosis with visual and auditory hallucinations, epileptic seizures. This led to cerebral damage in the frontal area and after a year, toxic encephalopathy with cognitive deficits, depression and PTSD were diagnosed [19].

Toxic elements

Mercury in the form of vapor, compounds or liquid is a poisonous element. The accumulation of this substance occurs in the liver and kidneys and the methylmercury forms are deposited in the nervous system [20]. Severe vapor poisoning leads to inflammation of the bronchi and lungs. Inhaled vapors enter the bloodstream, where after being absorbed by erythrocytes mercury goes to the oxidation process. It disturbs many biochemical processes by destroying biological membranes and connecting to body proteins. Other symptoms include: hemorrhagic enteritis, circulatory insufficiency, salivation, vomiting, diarrhea with admixture of blood, burning in the esophagus, kidney damage. Chronic poisoning gives non-specific symptoms: weakness, pain in the extremities, headaches, progressive CNS damage, hand tremors, and lack of gait.

Lead is a metal toxic to humans. None of the scientific studies confirmed that lead fulfills any function in the human body, despite it occurs in it in small amounts of 120 mg [22]. Inhaled or consumed, it passes into the bloodstream where it disturbs the function of many enzymes by binding to the -SH group contained in many of them [23]. Nerve-destructive acts destroying myelin sheaths, reducing the number of neurons and impairing their growth and disrupting neurotransmission [23]. It also damages the kidneys causing nephropathy. Symptoms of lead poisoning are: abdominal pain, feeling of weakness in the hands, wrists, ankles, increase in blood pressure, anemia. High levels of calcium and iron in the body act to a certain degree of protection, while low concentrations sensitize to lead poisoning.

Cadmium, another heavy metal, is also toxic to humans. Acute, fatal poisoning gives ingestion of 350 to 3500 mg. It accumulates in the kidneys and liver and acts similarly to lead by nephrotoxicity. It damages the glomeruli and renal tubules. It may interfere with the mineral metabolism of the body, affecting the distribution of iron, magnesium, copper, calcium or zinc. Cadmium disturbs the process of oxidative phosphorylation, sodium-potassium transport, sugar metabolism [24]. It also causes anemia, osteoporosis, proteinuria, disrupts insulin secretion, increases lipid oxidation, and at the same time increases the production of free radicals. In the brain, it induces apoptosis of neurons.

CONCENTRATION OF ELEMENTS IN CHILDREN WITH AUTISM - A LITERATURE REVIEW

Knowledge of the concentration of individual elements in autistic children can help in determining deficits or excess micronutrients, the normal level of which affects the health condition of people with ASD.

Table 1. Characteristics of the reviewed research showing the concentration of elements in children.

References	Study group	Control group	Material	Results
Zhai Q, *et al.* 2019 [25]	78	58	hair	the levels of Pb, As, Cu, Zn, Hg in hair samples were higher in the ASD group than in control group
Wu LL, *et al.* 2019 [26]	113	141	plasma	children with ASD had lower levels of Zn in plasma (by 6%) compared to the control group no significant differences was detected in blood Cu, Fe, Mg levels between groups
Li H, *et al.* 2018 [27]	180	184	plasma	children with ASD had much higher levels of Hg and As($p < 0,001$) and

				lower level of Cd in plasma compared to the control group; no significant differences was detected in levels of Pb between groups
Skalny AV, *et al.* 2017 [28]	48	0	plasma	the levels of Ni, Cr, Se Al, As, Mn in plasma were lower in ASD group than in control group
Skalny AV, *et al.* 2017 [29]	74	74	hair	children with ASD had lower levels of Cr (by 29%),J (by 41%), V (by 24%), Be (by 50%), Sn (by 34%) in hair samples compared to the control group; no significant differences was detected in levels of Hg, Zn, Cu between groups
Fiłon J, *et al.* 2017 [30]	30	30	hair	children with ASD had lower levels of Zn in hair samples compared to the control group; children with ASD had higher levels of Cd and Mn in hair samples compared to the control group
Crăciun EC, *et al.* 2016 [31]	28	28	plasma	children with ASD had lower levels of Zn (by 10%) and Zn/Cu (by 12%) in plasma compared to the control group; no significant differences was detected in blood Cu levels between groups
Tabatadze T, *et al.* 2015 [32]	30	30	hair	children with ASD had lower levels of Zn, Mn, Se, Mo in hair samplescompared to the control group; children with ASD had higher levels of Pb, Hg, Cd in hair samplescompared to the control group;

Mohamed FB,*et al.* 2015 [33]	100	100	hair	the levels of Hg, Pb, Al in hair samples were higher in the ASD group than in control group
Li SO, *et al.* 2014 [34]	60	60	plasma	the levels of Zn in plasma and Zn/Cu were lower in the ASD group than in control group(*p*<0,001); the levels of Cu in plasma were higher in the ASD group than in control group(*p*<0,001);
Yasuda H, *et al.* 2013 [35]	1967	0	hair	Zn deficiency was found in 584 (29.7%) children; Mg deficiency in 347 (17.6%) children; Ca deficiency in 114 (5.8%) children the presence of Al was found in 339 (17.2%) children; Cd in 168 (8.5%) children; Pb in 94 (4.8%) children; Hg in 55 (2.8%) children
Blaurock B, *et al.* 2012 [36]	44	0	hair	the levels of Al, As, Cd, Hg, Ni, Pb, V in hair samples were higher in the ASD group than in control group; the levels of Fe, J, Se, Zn, Mn, Mo in hair samples were lower in the ASD group than in control group;
Palma de G, *et al.* 2012 [37]	44	61	hair	the levels of Mo, Li, Se in hair samples were higher in the ASD group than in control group;
Lakshmi P, *et al.* 2011 [38]	45	50	hair and nails	children with ASD had higher levels of Cu in hair and nails compared to the control group(*p*<0,001); the study showed higher Pb and Hg levels in the hair and nails of children with ASD compared to the control group (*p*<0,001);

				Se levels were lower in the ASD group than in control group (*p*<0,001)
Blaurock B,*et al.* 2011 [39]	25	25	hair	children with ASD had higher levels of As (*p*=0,01), Cd (*p*=0,03), Ba (*p*=0,003), Pb (*p*=0,03), Ce (*p*=0,003) in hair compared to the control group(*p*<0,001);
Majewska M,*et al.* 2010 [40]	91	75	hair	the younger children (3-4 years old) with autism had lower Hg levels in hair samples compared to the control group the older children (7-9 years old) with autism had higher Hg levels in hair samples compared to the control group
Fido A, *et al.* 2005 [41]	40	40	hair	children with ASD had much higher levels of Pb, Hg, U in hair samples compared to the control group (*p*<0,001); no significant differences was detected in levels of Sb, As, Be, Cd, Al between groups
Al-Ayadhi LY, *et al.* 2005 [42]	77	0	hair	the levels of Hg, Pb, As, Cd in hair samples were higher in the ASD group than in control group; the levels of Cu, Cr, Mn, Fe, Co in hair samples were lower in the ASD group than in control group;

The results of the presented studies indicate that there are differences in the concentrations of essential trace elements in hair and serum in children with autism spectrum disorders compared to healthy children (table 1). Although the level of concentration of microelements in some of the studies was increased in the group of children with ASD compared to control group, in most studies a marked reduction in concentrations and even deficiency of trace elements in autistic children were found.

Most studies draw attention to reduced levels of zinc in the hair and serum of children with ASD [26, 30, 31, 34, 36]. H. Yasuda et al. observed a significant correlation of zinc concentrations with age in children with ASD ($r = 0.377$, $p<0.0001$) indicating that children under 3 years old are more susceptible to zinc deficiency than the older ones [35]. Due to the fact that Zn has an important role in the synthesis of nucleic acids and proteins as well as in the growth and repair of tissues, its normal concentration is extremely important in pregnant women and infants. H. Kurita et al. in mouse studies reported that zinc deficiency in the uterus induces epigenetic fetal changes in the modification of genes encoding the metallothionein promoters [43].

Other frequent deficiencies in children with ASD, in addition to insufficient levels of zinc, are magnesium deficiencies. This condition was found by H. Yasuda in 17.6% (347) of children diagnosed with autism [35]. As in the case of zinc, examination of the hair samples of children with ASD diagnosis allowed to observe a significant correlation of magnesium concentration with age ($r = 0.362$, $p <0.0001$) suggesting that the youngest children are also at higher risk of magnesium deficiency than older children [35].

In addition to deficiencies in the essential micronutrients, recent studies show that children with ASD compared to healthy children have high levels of toxic metal pollution, such as Pb, Hg, Cd and Al [25, 27, 30, 32, 33, 36, 38 , 39, 40, 41, 42]. This suggests a possible pathophysiological role of heavy metals in the genesis of autism spectrum disorder symptoms. An American study evaluating the prevalence of autism in areas with varying levels of lead, mercury and arsenic pollutions has shown that in areas with higher concentrations of lead in the air occurs a higher incidence of ASD than elsewhere. Similar results were obtained for environmental contamination with mercury and arsenic [44].

Japanese scientists conducting research on a group of 1 967 children with ASD showed that among the excess toxic metals, the most common is excess aluminum ($n = 339$, 17.2%), followed by cadmium ($n = 168$, 8.5%), then lead ($n = 94$, 4.8%), and on the fourth place of mercury ($n = 55$, 2.8%) [35]. Concentrations of toxic metals in patients with autism are well correlated with the intensification of the disease. Increasing the concentration of heavy metals in the hair significantly correlates with the increased severity of ASD [45]. Differences in toxic metal concentrations are also observed depending on the age of children with ASD. In a study conducted by M. Majewska et al. younger autistic patients had lower Hg levels in the hair samples, while older ones had higher levels than their corresponding controls [40]. This suggests that autistic children differ from healthy children by mercury metabolism, which seems to change with age.

Calcium deficiency is likely to further enhance the toxic effect of lead on cognitive and behavioral development in children [46]. A significant relationship between calcium deficiencies in the diet and the concentration of lead in the blood was found in 3 000 American children [46]. Therefore, one of the activities aimed at protection against the accumulation of toxic metals is to maintain normal levels of other essential elements.

CONCLUSION

This review shows that many children with autism spectrum disorders suffer from a shortage of essential trace elements and high toxic metal loads. This suggests that these phenomena may play an important part in the pathogenesis and intensification of autistic disorders. Deepening, long-term mineral deficiencies may lead to health and developmental problems in a child.

It appears that patients with autism may respond well to an appropriate nutritional approach that replenishes nutrient deficiencies. A proper diet will not only provide the necessary level of essential nutrients, but also may improve the detoxification ability by removing toxic metals from the body.

The dietary approach in such patients may be helpful in treatment of children with autism spectrum disorders.

References

1. Weintraub K. Autism counts. *Nature*. 2011; 479: 22–24.
2. Bailey A, le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, et al. Autism as a strongly genetic disorder: Evidence from a British twin study. *Psychol. Med*. 1995; 25: 63–77.
3. Murdoch JD, State MW. Recent developments in the genetics of autism spectrum disorders. *Curr. Opin. Genet. Dev*. 2013; 23: 310–315.
4. Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: A pilot study. *Neuroendocrinology*. 2002; 23: 303-8.
5. Murray R, Granner D, Mayes P, Rodwell V. Biochemia Harpera. Wydawnictwo Lekarskie PZWL, Warszawa, 1995 (wydanie III). ISBN 83-200-1798-X.
6. Lityński T, Jurkowska H. Żyzność gleby i odżywianie się roślin. Warszawa: Państwowe Wydawnictwo Naukowe. 1982; 146, 279. ISBN 83-01-02887-4.
7. Farago ME. Plants and the Chemical Elements Biochemistry: Uptake, Tolerance and Toxicity. *Weinheim, Nowy Jork: VCH*. 1994; 4. ISBN 3-527-28269-6.
8. Gapys B, Raszeja-Specht A, Bielarczyk H. Rola cynku w procesach fizjologicznych i patofizjologicznych organizmu. *Diagn. Lab*. 2014; 50: 45-52.
9. Puzanowska-Tarasiewicz H, Kuźmicka L, Tarasiewicz M. Funkcje biologiczne wybranych pierwiastków. III. Cynk – składnik i aktywator enzymów. *Pol. Merk. Lek*. 2009: 27: 419-422
10. Roczniak W. Analysis of the Content of Chromium in Certain Parts of the Human Knee Joint. *International Journal of Environmental Research and Public Health*. 2018.doi: 10.3390/ijerph15051013.
11. Scharf B. *Molecular analysis of chromium and cobalt-related toxicity*. *Scientific Reports*. 2014. doi: 10.1038/srep05729.
12. Kołacz R., Bodak E., Toksyczność metali ciężkich. Ek*otoksykologiczne problemy chowu zwierząt w rejonach skażeń metalami ciężkimi.* ELMA, Wrocław--Rudna 1997, 43-54.
13. Aaseth J, Thomassen Y, Alexander J, Munthe E. Selenium in Biology and Medicine. *Westport. C.T*. 1981, 418-421.
14. Levander OA. Biochemical and Nutritional Aspects of Trace Elements. *New York*.1982; 345.
15. Van Vleet J, Ferrans VJ. Nutritional Diseases: Research Directions in Comparative Pathobiology. *Alan r. Liss, Inc*. 1986; 359-396.

16. Mizerski W. Tablice biologiczne. Wyd. IV. Warszawa. Wydawnictwo Adamantan. 2004. ISBN 83-7350-059-6.

17. Treble RG, Thompson TS, Lynch HR. Determination of copper, manganese and zinc in human liver. *Biometals*. 1998; 11: 49-53.

18. Murray RK, Granner DK, Rodwell VW. Biochemia Harpera ilustrowana. Wyd. VI uaktualnione. Warszawa: Wydawnictwo Lekarskie PZWL. 2008; 119. ISBN 978-83-200-3573-5

19. Momcilovic B. A Case Report of Acute Human Molybdenum Toxicity from a Dietary Molybdenum Supplement. *Arhiv za higijenu rada i toksikologiju.* 1999; 50 (3): 289-297.

20. Albińska J, Góralski J, Szynkowska M, Leśniewska E,Paryjczak T. Rtęć w tłuszczach zwierząt łownych pochodzących z terenu województwa łódzkiego. *Rocznik Ochrona Środow.* 2013; 13: 525-539.

21. Seńczuk W. Toksykologia. Podręcznik dla studentów, lekarzy i farmaceutów. Wydanie IV. Warszawa: Wydawnictwo Lekarskie PZWL. 2002. ISBN 83-200-2648-2.

22. Emsley J. Nature's Building Blocks: An A-Z Guide to the Elements. *Oxford University Press*. 2011. ISBN 978-0-19-960563-7.

23. Rudolph AM, Rudolph CD, Hostetter MK, Lister GE, Siegel NJ. Rudolph's Pediatrics. Wyd. 21st. *McGraw-Hill Professional*. 2003; 369. ISBN 978-0-8385-8285-5.

24. Ejnik J, Robinson J, Zhu J, Försterling H. Folding pathway of apo-metallothionein induced by Zn2+, Cd2+ and Co2+. *J Inorg Biochem*. 2002; 88 (2): 144–152.

25. Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W.Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. *Environ Res.* 2019;171:501-509.

26. Wu LL, Mao SS, Lin X, Yang RW, Zhu ZW. Evaluation of Whole Blood Trace Element Levels in Chinese Children with Autism Spectrum Disorder. *Biol Trace Elem Res.* 2019; doi: 10.1007/s12011-018-1615-4.

27. Li H, Li H, Li Y, Liu Y, Zhao Z. Blood Mercury, Arsenic, Cadmium, and Lead in Children with Autism Spectrum Disorder. *Biol Trace Elem Res.* 2018;181(1):31-37.

28. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Radysh IV, Skalnaya MG, et al. Assessment of serum trace elements and electrolytes in children with childhood and atypical autism. *J Trace Elem Med Biol.* 2017;43:9-14.

29. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Bjørklund G, Skalnaya MG, et al. Hair toxic and essential trace elements in children with autism spectrum disorder. *Metab Brain Dis.* 2017;32(1):195-20.

30. Fiłon J, Ustymowicz- Farbiszewska J, Karczewski J, Żendzian-Piotrowska M. Analysis of trace element content in hair of autistic children. *J Elem*. 2017; 22(4): 1285 - 1293

31. Crăciun EC, Bjørklund G, Tinkov AA, Urbina MA, Skalny AV, Rad F, et al. Evaluation of whole blood zinc and copper levels in children with autism spectrum disorder. *Metab Brain Dis*. 2016;31(4):887-90.

32. Tabatadze T, Zhorzholiani L, Kherkheulidze M, Kandelaki E, Ivanashvili T. Hair Heavy Metal And Essential Trace Element Concentration In Children With Autism Spectrum Disorder.*Georgian Med News.* 2015; 248:77-82.

33. Mohamed Fel B, Zaky EA, El-Sayed AB, Elhossieny RM, Zahra SS, Salah Eldin W, et al. Asessment of Hair Aluminum, Lead, and Mercury in a Sample of Autistic Egyptian Children: Environmental Risk Factors of Heavy Metals in Autism. *Behav Neurol*. 2015;doi: 10.1155/2015/545674.

34. Li SO, Wang JL, Bjørklund G, Zhao WN, Yin CH. Serum copper and zinc levels in individuals with autism spectrum disorders. *Neuroreport*. 2014; 25(15):1216-20.

35. Hiroshi Y, Yuichi Y, Toyoharu T.Estimation of autistic children by metallomics analysis. *Scientific Reports.* 2013; doi: 10.11299/brte.19.57

36. Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T. Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism. *Maedica (Buchar).* 2012; 7(1):38-48.

37. De Palma G, Catalani S, Franco A, Brighenti M, Apostoli P. Lack of correlation between metallic elements analyzed in hair by ICP-MS and autism. *J Autism Dev Disord.* 2012 Mar;42(3):342-53.

38. Lakshmi P, Geetha A. Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. *Biol Trace Elem Res*. 2011; 142(2):148-58.

39. Blaurock-Busch E, Amin OR, Rabah T. Heavy metals and trace elements in hair and urine of a sample of arab children with autistic spectrum disorder. *Maedica (Buchar).* 2011; 6(4):247-57.

40. Majewska MD, Urbanowicz E, Rok-Bujko P, Namyslowska I, Mierzejewski P. Age-dependent lower or higher levels of hair mercury in autistic children than in healthy controls. *Acta Neurobiol Exp (Wars).* 2010;70(2):196-208.

41. Fido A, Al-Saad S. Toxic trace elements in the hair of children with autism. *Autism*. 2005;9(3):290-8.

42. Al-Ayadhi LY. Heavy metals and trace elements in hair samples of autistic children in central Saudi Arabia. *Neurosciences (Riyadh).* 2005; 10(3):213-8.

43. Kurita H, Ohsako S, Hashimoto S, Yoshinaga J, Tohyama C. Prenatal zinc deficiency-dependent epigenetic alterations of mouse metallothionein-2 gene. *J. Nutr. Biochem.* 2013;24:256–266.

44. Dickerson AS, Rahbar MH, Bakian AV, Bilder DA, Harrington RA, Pettygrove S, et al.Autism spectrum disorder prevalence and associations with air concentrations of lead, mercury, and arsenic. *Environ Monit Assess.* 2016; 188(7):407.

45. Geier DA, Kern JK, King PG, Sykes LK, Geier MR. Hair toxic metal concentrations and autism spectrum disorder severity in young children. *Int J Environ Res Public Health.* 2012; 9(12): 4486-97.

46. Mahaffey KR, Gartside PS, Glueck CJ. Blood lead levels and dietary calcium intake in 1 to 11 year-old children: The second national health and nutrition examination survey, 1976 to 1980. *Pediatrics*. 1986;78:257–262.