Autism spectrum disorder: Trace elements imbalances and the pathogenesis and severity of autistic symptoms

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ABSTRACT

The identification of biomarkers as diagnostic tools and predictors of response to treatment of neurological developmental disorders (NDD) such as schizophrenia (SZ), attention deficit hyperactivity disorder (ADHD), or autism spectrum disorder (ASD), still remains an important challenge for clinical medicine. Metallomic profiles of ASD patients cover, besides essential elements such as cobalt, chromium, copper, iron, manganese, molybdenum, zinc, selenium, also toxic metals burden of: aluminum, arsenic, mercury, lead, beryllium, nickel, cadmium. Performed studies indicate that children with ASD present a reduced ability of eliminating toxic metals, which leads to these metals’ accumulation and aggravation of autistic symptoms. Extensive metallomic studies allow a better understanding of the importance of trace elements as environmental factors in the pathogenesis of ASD. Even though a mineral imbalance is a fact in ASD, we are still expecting relevant tests and the elaboration of reference levels of trace elements as potential biomarkers useful in diagnosis, prevention, and treatment of ASD.

1. Introduction

Autism Spectrum Disorder (ASD) refers to a neurodevelopmental disorder that is characterized by persistent deficits in verbal and non-verbal social communication, social interactions, and limited, repetitive patterns of behavior, interests, activities (Fig. 1) (American Psychiatric Association, 2013). ASD was first described in 1943 and since then, we have seen a large increase in the incidence of ASD worldwide (Kanner, 1943).

Epidemiological data reveal that it is boys who are four times more likely to suffer from ASD than girls. In addition, in 3/4 patients with ASD, the disorder is accompanied by mental retardation. Landrigan et al. (2012) reported that in the case of neurodevelopmental disability, a dramatic increase might be observed, which currently affects more than 10 % of children born in the US each year. To date, the etiology of the disease has not been explained, although it is believed that it is most likely the result of gene and environmental factors interactions (Steyaert and De la Marche, 2008).

The participation of ‘ASD fraction’, which is associated with complex inheritance and genetic heterogeneity, was confirmed at only 30–40 % (AlSagob et al., 2015). It turned out that no single anomaly pre-dominates, and nearly 80 % of children with ASD possess a normal genome, whereas the remaining 20 % present different polymorphisms of unknown significance, or de novo mutations (Landrigan et al., 2012; AlSagob et al., 2015). Thus, it is clear that non-genetic factors can play a significant role in the etiology of ASD.

Numerous studies confirm increased neuroinflammatory activity and neuronal damage in the brain of ASD patients (Chez et al., 2007; Enstrom et al., 2005; Fatemi et al., 2008; Laurence and Fatemi, 2005; Morgan et al., 2012, 2010; Pardo et al., 2005; Tetreault et al., 2012; Vargas et al., 2005; Zimmerman et al., 2005). Evidence of brain inflammation in ASD entails the reactivity of microglial cells and

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astocytes, activation of inducible nitric oxide (NO)-synthase (i-NOS), and increased pro-inflammatory cytokines and chemokines and a loss of neurons (Eissa et al., 2020; Matta et al., 2019; Siniscalco et al., 2018; Tabatadze et al., 2015).

Most epidemiological studies confirm the increase in the number of ASD cases related to the increase in environmental pollution, mostly by heavy metals, trichloroethylene, or vinyl chloride (Kinney et al., 2010; Rossignol et al., 2014). The accumulation of toxic metals in the organism might significantly impair the homeostasis and proper functioning of the vital organs (Rehman et al., 2017; Pratush et al., 2018; Wu et al., 2016; Gobbina et al., 2015; Grochowski et al., 2019a; Baj et al., 2020; Grochowski et al., 2019b). Autistic children are found to present elevated levels of heavy metals such as: lead, mercury, and cadmium in their hair. Tabatadze et al. (2015) confirmed the existence of high levels of lead (78 % and 16 %), mercury (43 % and 10 %), cadmium (38 % and 8 %) in children with ASD as compared to healthy children, respectively. At the same time, the statistical results of the research indicate a deficiency of trace elements such as zinc, manganese, molybdenum, and selenium in the hair, which is clearly associated with the occurrence of ASD (Tabatadze et al., 2015). Based on epidemiological studies, numerous neurotoxic environmental factors have been identified that might contribute to neurodevelopmental disability and ASD. Grandjean et al. (Grandjean and Landrigan, 2006), as well as Landrigan et al. (2012), list the following xenobiotics as causative agents in ASD etiology: lead, methylmercury, polychlorinated biphenyls, organophosphorus pesticides, organochlorine pesticides, asphalts, car exhaust fumes, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, and perfluorinated compounds. What seems to be crucial here, is a synergistic action and adverse effects of the exposure to the several xenobiotics at the same time (Dorea, 2019). The following review is primarily based on the studies on the children who are already diagnosed with ASD, whilst, the most probable episode of the highest toxicity concerns pre- and postnatal periods and further persistent high concentrations of toxic metals might contribute to the maintenance of ASD symptoms.

2. Toxic metals burden in autistic children

Even relatively low concentrations of toxic metals are dangerous for young children (Osman et al., 2019; Hsueh et al., 2017; Choi et al., 2017). The danger increases as a result of the additive effect. Since heavy metals disrupt the function of numerous enzymes, impair cell signaling processes, and generate oxidative stress leading to apoptosis, they can significantly contribute to autism etiology (Modabbernia et al., 2017; Fujiwara et al., 2016).

Metallomic studies conducted on the hair of patients with ASD reveal high toxic loading with metals such as aluminum in 17.2 %, cadmium in 8.5 % and lead in 4.8 % of patients, and slightly less contamination related to mercury and arsenic, which was detected in 2.8 % and 2.6 % of patients with ASD, respectively (Yasuda et al., 2013). Most studies confirm that children with autism present elevated average levels of toxic metals that are strongly correlated with the degree of severity of autism symptoms (Adams et al., 2013a).

De Palma et al. (2012) conducted a case-control study in order to assess the concentration of metallic elements in the hair of children diagnosed with autism. The results revealed higher molybdenum, lithium, and selenium concentrations in autistic children. Logistic regression analysis confirmed the role of the risk factor for male sex and proved a negligible association with molybdenum concentrations. Meta-analysis ruled out any association of autism with the concentration of mercury, cadmium, selenium, lithium, and copper in the hair, which was only found for lead at a negligible statistical significance (De Palma et al., 2012).

2.1. Mercury (Hg) levels in children with ASD

The most common sources of mercury are inorganic mercury compounds (e.g. mercuric chloride), organic mercury compounds such as: methylmercury, which occurs in sea fish, or ethylmercury found, e.g., in vaccines containing Thimerosal (ethyl mercury thiosalicylate containing 49.55 % mercury by weight) and elemental mercury in the form of vapors released from dental amalgams. All forms of mercury are toxic, but organic forms exert stronger cytotoxic and neurotoxic effects (Environmental Protection Agency, 2016; Lohren et al., 2015). In the past, organic mercury poisoning was quite frequent, as for example in the tragedy in Minamata, or Iraq in 1960 (Harada et al., 1999; Bal-Price et al., 2010).

The source of exposure to mercury poisoning is Thimerosal, used in many vaccines, e.g. influenza, tetanus, meningococcal vaccine, as an adjuvant increasing the body’s immune response to the introduced antigen (Rosenblatt and Stein, 2015; Geier et al., 2015; Harry et al., 2004). Research conducted by Rodrigues et al. (2010) proved that mercury from Thimerosal is accumulated in the brain, kidneys, and liver, with much higher levels compared to the ones in the blood. After exposure to Thimerosal, mercury is accumulated in the brain mainly in an inorganic form.
form (63 %), whilst, the rest remains in highly toxic forms of organic mercury: ethylmercury (13.5 %) and methyl mercury (23.7 %).

Numerous studies confirm the relationship between mercury exposure and ASD. Research conducted by Vojdani et al. (2003) revealed that xenobiotics such as Thimerosal are capable of binding to lymphocyte receptors and tissue enzymes, causing autoimmune reaction. This hypothesis was also confirmed by the research of Mostafa et al. (Mostafa and Al-Ayadhi, 2015; Mostafa and Refai, 2007) who proved that the level of mercury in children with ASD correlates with the level of serum anti-myelin basic protein auto-antibodies that are capable of altering brain functioning (Elamin and Al-Ayadhi, 2014), which in turn is associated with disturbed cognitive and behavioral profiles (Piras et al., 2014).

Havarrinassab et al. (2004) confirmed, in mice studies, that Thimerosal is capable of triggering a systemic autoimmune syndrome. Increased serum mercury levels were confirmed in 78.3 % of children with ASD, who also had an elevated serum level of pro-inflammatory neuropeptide-neurokinin A. Moreover, a positive correlation was found in the study between the serum neurokinin A level in children with ASD and the levels of mercury in the blood (Mostafa et al., 2016a). There was also a correlation between mercury levels and the neurotrophin-3 (NT-3) oxidative marker in the cerebellar areas of individuals with ASD (Sajdel-Sulkowska et al., 2008).

There also exist several studies suggesting that mercury does not constitute a risk factor for ASD. Khan et al. (2014) compared mercury levels in the brainstem and cerebellum areas and levels of 3-nitrotrosine (3-NT) an oxidative stress marker, in the control group, and an ASD group. The results of the study prove that in individuals with ASD, 3-NT levels increased, while mercury levels did not differ between the cases and the controls. In the study of Pampphlet et al. (Pampphlet and Kum Jew, 2016), control samples from healthy individuals from the cerebus locus area presented even more elevated mercury levels than in those individuals with ASD.

The impact of pregnant women’s exposure to mercury on the risk of ASD in children was also investigated. However the results also seem to be contradictory. Geier et al. (2009a) reported that dental fillings with amalgam in pregnant mothers are a risk factor for ASD, and Austin and Shandley (Shandley and Austin, 2011) observed a higher ASD rate in the descendants of the mercury-poisoned population. In turn, Van Wijngaarden et al. (van Wijngaarden et al., 2013) postulate that there exists no relationship between prenatal mercury exposure and ASD phenotypic behavior. Thimerosal, used as a preservative in Rho (D) immune globulin (RhoGAM), administered to pregnant mothers in the event of a serological conflict, raises similar controversies. Some authors confirmed that Thimerosal in RhoGAM is a risk factor for ASD (Bauman et al., 1997; Courchesne et al., 2007), while in another study (Eissa et al., 2006) no such association was observed. Due to controversies and a lot of intense polemics in 2001, Thimerosal was eventually removed from Rh immunoglobulin (Institute of Medicine (US) Immunization Safety Review Committee, 2001).

There is no doubt that the majority of studies (74 %) carried out in recent decades substantiate the existence of a link between elevated mercury levels and ASD risk factors. Mercury exposure has been demonstrated to exert both direct and indirect effects that include autoimmunity, oxidative stress, neuritis, neuronal damage, and loss of neuronal connectivity (Kern et al., 2016a). Studies have also proved that children with ASD exhibit high levels of mercury in various body tissue like skin, blood, urine, teeth, hair, and nails which correlates with the severity of autism symptoms (Kern et al., 2016b). The particular susceptibility of individuals with ASD to toxic substances, including mercury, is derived from the fact that their detoxification ability is impaired. In the population of individuals with ASD, limited availability of cell thiol, necessary for mercury detoxification, and reduced levels of glutathione in the cerebellar and temporal cortex (GSH) samples were detected (James et al., 2009a; Chauhan et al., 2012; James et al., 2009b, 2006). It was confirmed that the risk of ASD is greater in those geographical areas where higher levels of mercury in the environment are present, for instance, in China (Zhang and Wong, 2007; Palmer et al., 2006, 2009; Blanchard et al., 2011; Dickerson et al., 2015; Windham et al., 2006; Roberts et al., 2013).

Table 1 presents the information on published papers, concerned with the studies of various tissues, that track the relationship between mercury levels in ASD patients against control group or the groups with other neurological, neuropsychiatric disorders or learning deficiency cases, and the relationship between mercury content and severity of symptoms in ASD.

In addition, it has been demonstrated that the levels of some biomarkers of toxic tissue load allow it for mercury exposure to be assessed. Urinary levels of porphyrians in urine, urinary coproporphorhine (eP), pentacoproporphorin (5oxP), and especially preproporphorhine (preP), which is a specific marker for assessing mercury exposure, are considered to be such markers (Heyer et al., 2006; Woods, 1996; Woods et al., 2005; Nataf et al., 2006; Geier and Geier, 2006a). Most studies have agreed on a relationship between porphyrian biomarkers, mercury content, and ASD staging (Jafari et al., 2017; Geier et al., 2009b, c; Kern et al., 2010; Heyer et al., 2012; Ip et al., 2004; Erratum, 2007).

2.2. Arsenic (As), cadmium (Cd) levels in children with ASD

Toxic metals can play an epigenetically pathogenic role in the etiology and the development of autism. The presence of toxic metals, e.g. arsenic, cadmium, and others in various biological samples from children with ASD depends on the context of origin and the period of the test done. This hypothesis is affirmed by the fact that higher concentrations of some metals, e.g. cadmium, but also mercury and lead in the blood plasma of Shenzhen children, may be associated with their diet, rich in seafood (Qin et al., 2018). In blood sample tests performed in Romania by Hessabi et al. (2019) on a group of 60 children with ASD in 2015–2017, more than 90 % of the samples demonstrated As and Cd levels to be below detection limits. None of the children had elevated arsenic levels, and only 1.7 % of children with ASD had elevated cadmium levels. The geometric mean blood arsenic concentration for Jamaican children with ASD was 2.48 μg/l, much more than for Romanian children with ASD.

In the study of metal content in hair, performed in Japan by Yasuda et al. in 2013, on a group of 1967 children with ASD, it was found that in the case of arsenic, the maximum load levels in children with ASD were 33.5 times higher as compared to the reference level (Yasuda et al., 2013).

An interesting observation was made by Blaurock-Busch et al. (2011) whose study pointed to higher cadmium levels in the hair of children with ASD as compared to controls. At the same time, urinary excretion levels were higher in healthy children, which may indicate that the accumulation of this element in tissues affects autistic children to a larger extent.

2.3. Aluminium

The neurotoxicity of aluminum has been acknowledged for decades. The source of exposure to aluminum comes from the natural environment, in which aluminium occurs abundantly in waters and the earth’s crust (EFSA (European Food Safety Authority), 2008), but perhaps predominantly from food packaging that contain Al.

Under physiological conditions, intestinal absorption of aluminum is impossible, since bioactive metals are absorbed only in the 2+ state, while the existence of Al3+ was found naturally only in the gas phase after the explosion of aluminum grenades and in the interstellar space. In the acidic stomach environment, aluminum exists as the hydrated Al(OH)3 ion [Al(H2O)3]3+. Insoluble aluminum hydroxide is formed in the intestines due to an increase in pH and then excreted in the faeces (Ra et al., 2008). Trivalent Al can be absorbed by simple diffusion only through the damaged intestinal mucosa (infection, inflammation,
The results of human tissue mercury levels and ASD symptoms.

| Human tissue | Measured biomarkers | Obtained results | Sample size | Ref. |
|--------------|---------------------|------------------|-------------|------|
| ASD versus HC |                      |                  |             |      |
| Hair Hg      | No association between ASD and hair Hg. |             | ASD 44, HC 61 | (Hertzi-Picciotto et al., 2010) |
| Hair, blood Hg | No association between ASD and Hg. |             | ASD 82, HC 55 | (Yato et al., 2014) |
| Blood Hg     | No association between ASD and Hg. |             | ASD 452 | (Rahbar et al., 2021) |
| Blood Hg     | No association between ASD and Hg. |             | ASD 109, HC 109 | (McKean et al., 2015) |
| Blood Hg     | No association between ASD and Hg. |             | ASD 164 DD 35, HC 58 | (Soden et al., 2007) |
| Urine Hg     | 24-h provoked urine excretion test for heavy metals in children with ASD. Excess chelatable body burden of As, Cd, Pb, or Hg is zero. |             | ASD 15, HC 4 | (Wood et al., 2021) |
| Urine Hg     | No differences were found. |             | ASD 64, PDD 19, HC 114 | (Holmes et al., 2003) |
| Serum Hg, antineuronal antibodies | ASD cases: higher level of Hg, higher seropositivity for antineuronal antibodies, behavioral abnormalities, EEG abnormalities. |             | ASD 40, HC 40 | (Montali and Al-Ayadhi, 2015) |
| Blood Hg, seropositivity of anti-MBP autoantibodies | Increased levels of Hg in 48 % ASD, anti-MBP auto-antibodies in 72 % ASD. |             | ASD 50, HC 30 | (Montali and Refai, 2007) |
| Brain 3-NT, Se, Hg | Increase in the mean cerebellar levels of 3-NT and in the ratio of Hg/Se. |             | ASD 9, HC 10 | (Sajdel-Sulkowska et al., 2008) |
| Brain Hg     | No differences between Hg levels in extra-cortical regions of brain in ASD children vs. HC. |             | ASD 10, HC 11 | (Khan et al., 2014) |
| Brain Hg     | Lower levels of Hg in locus ceruleus of ASD vs. HC. |             | ASD 6, HC 11 | (Pamphlett and Kum Jew, 2016) |
| First baby hair Hg | The level of Hg was significantly lower in ASD (0.47 ppm) vs. HC (3.63 ppm). |             | ASD 94, HC 45 | (Lakshmi Priya and Geetha, 2011) |
| Hair, nails Hg, Pb | Significant elevation in the levels of Hg in both hair and nail samples in autism vs. HC. |             | ASD 45, HC 50 | (Majewska et al., 2010) |
| Hair Hg      | Autistic children significantly differed from healthy peers as for the concentrations of Hg in hair. |             | ASD 91, HC 75 | (Elisheshetawy et al., 2011) |
| Hair Hg, Pb, Cu, Zn | The level of Hg was significantly lower in cases (0.55 ± 0.06 μg/mg) than in HC (3.2 ± 0.2 μg/mg). |             | ASD 32, HC 32 | (Geier et al., 2012) |
| Hair Hg      | Hair Hg levels were higher in children with ASD compared to HC. |             | ASD 100, HC 100 | (Adams et al., 2013a) |
| Hair Trace elements and heavy metals | High contamination to heavy metals in ASD compared to HC detected for Pb (78 % and 16), Hg (43 % and 10 %) and Cd (38 % and 8 %). |             | ASD 30, HC 30 | (Alabali et al., 2014) |
| First baby hair Hg | Children with lower levels of Hg in hair were 2.5 times more likely to suffer from ASD. |             | ASD 78, HC 31 | (Mohamed Fel et al., 2015) |
| Hair, blood Hg | Significant relationship exists between the blood levels of Hg and ASD with ASD exhibiting higher blood Hg levels. |             | ASD 82, HC 55 | (Adams et al., 2008) |
| Hair Hg      | Elevated hair concentrations were noted for Hg in autism vs. HC. |             | ASD 30, HC 30 | (Tabatabaz et al., 2015) |
| Hair Hg      | Hg levels were markedly elevated in the hair of autistic subjects vs. control subjects. |             | ASD 27, HC 27 | (Desoto and Hitman, 2007) |
| Hair Toxic metals | Significant alteration in deposition of several heavy metal species, including Hg in hair samples between the groups. |             | ASD 22 HC 39 | (Blaurock-Basch et al., 2012) |
| Hair metals | Higher levels of Hg in ASD vs. HC. |             | ASD 77, HC 77 | (Hodgson et al., 2014) |
| Blood Hg, hair Hg | Children with autism had significantly (p < 0.001) higher in-hair concentration levels of Pb, Hg and U. |             | ASD 40, HC 40 | (Obernovich et al., 2011) |
| Blood, hair Hg | High level of Hg and lead among kids with autism, with significant decline in the blood level of Pb and Hg with the use of DMSA as a chelating agent. |             | ASD 45, HC 45 | (Al-Ayadhi, 2005) |
| RBC Hg, Pb, Vit.E, GSP | ASD had significantly higher lead and Hg levels and lower GST activity and vitamin E concentrations as compared to HC. |             | ASD 20, HC 20 | (Fido and Al-Saad, 2005) |
| RBC Hg      | Mean Hg levels were 1.9-fold significantly increased in ASD (21.4 microg/L) vs. HC (11.4 microg/L). |             | ASD 83 HC 89 | (Yassa, 2014) |
| Blood Hg    | Blood Hg levels were higher in ASD |             | ASD 20, HC 20 | (Geier et al., 2010) |
| Urine Hg    | Children with ASD excreted six-fold greater Hg content after treatment of DMSA compared to HC in their urine. |             | ASD 221, HC 18 | (El-Enany, 2016) |
| Urine GSH, GDH, Hg GSH/GSSG, GR, GST, Trx, TrxR, Prx I, III, glutamate, glutamine, | Statistically significant differences in the mean urine levels of Hg. |             | ASD 44, HC 146 | (Bradstreet et al., 2003) |
| Baby teeth Hg, Pb, Zn | ASD had 2.1-fold higher levels of Hg. |             | ASD 15, HC 11 | (Macedoni-Lukic et al., 2015) |

ASD versus other neurodevelopmental disorders

Blood, urine, hair Hg | No significant differences between the groups, ASD group had significantly elevated blood Cu/Zn ratio, the levels of coproporphyrin I and coproporphyrin III were lower in the ASD group. |             | ASD 52, others 22 | (Albizzati et al., 2012) |
| Blood, urine, hair Hg, Pb, Cd, Al | No significant difference between the groups was found. |             | ASD 17, others 20 | (Wright et al., 2012) |
| Urine Hg, creatinine | No significant differences in creatinine levels, in uncorrected urinary Hg levels or in levels of Hg corrected for creatinine. |             | ASD 56, siblings 42, HC 121, delayed 34 | (De Palma et al., 2012) |

**Hg level versus symptom severity**

Correlation of proinflammatory Neurokinin A and Hg in moderate and severe ASD, but not HC. |             | ASD 84 HC 84 | (Montafa et al., 2016) | (continued on next page)
Table 1 (continued)

| Human tissue | Measured biomarkers | Obtained results | Sample size | Ref. |
|--------------|---------------------|------------------|-------------|------|
| Hair         | Hg                  | Lower Hg levels in ASD than HC, inverse correlation of Hg levels with symptom severity. | ASD 94, HC 45 | (Holmes et al., 2003) |
| Hair, nails  | Pb, Hg              | The higher the Hg concentrations, the worse the autism symptoms. | ASD 45, HC 50 | (Gaskhni Priya and Geetha, 2011) |
| Hair         | Pb, Cu, Zn          | Positive correlation of CARS score with Hg and Cu. No correlation of zinc with CARS score, intelligence quotient. | ASD 32, HC 32 | (Elheshstawy et al., 2011) |
| Hair         | Hg                  | Positive correlation between Hg concentrations and increased ASD severity. | ASD 18 | (Geier et al., 2009a) |
| Blood, urine, RBC | Hg              | A strong association in the degree of ASD with Hg. | ASD 51, HC 40 | (Adams et al., 2013b) |
| RBC          | Hg, Pb, GST, vitamin E | Levels of mHg GST, and vitamin E were correlated with ASD symptoms. Decrease in REEs (La, Ce, Gd, Eu) seemed to be correlated with the severity of autistic syndrome. | ASD 30, HC 30 | (Alabdali et al., 2014) |
| RBC          | 34 detected elements | | ASD 50, HC 50 | (Wu et al., 2018) |

Abbreviations: Childhood Autism Rating Scale (CARS), glutathione-s-transferase (GST), Red Blood Cells (RBC), glutathione status (GSH/GSSG), glutathione reductase (GR), glutathione-s-transferase (GST), thioredoxin (Trx), thioredoxin reductase (TrxR), peroxidoxins (Prxs I and III), glutamate dehydrogenase (GDH), developmental delay (DD), pervasive developmental disorder (PDD), Antibody anti-maltose-binding protein (Anti-MBP), 3-nitrotyrosine (3-NT), healthy controls (HC).
adverse effects of the exposure (Arora et al., 2017).

3. Essential elements imbalances in autism spectrum disorders

ASD is considered a multifactorial disorder in which trace elements, primarily toxic ones, constitute an important environmental factor. Essential elements, especially macroelements, are still a margin of research devoted to the problem of the pathophysiology of autism. Table 2 summarizes the results of the studies on the determination of essential metals in various tissues of patients with ASD.

3.1. The role of zinc (Zn) and copper (Cu)

Crucial to the development of the central nervous system (CNS) are Cu and Zn, which are involved in a complex regulatory network that maintains CNS homeostasis from the very early stages of life. Copper (Cu) is an essential element in mammalian nutrition. Health problems can be caused by both a deficiency and the excess of copper intake. The most important proteins that control copper balance include Cu-transporting P-type ATPases (copper-ATPases), encoded by ATP7A, and the ATP7B gene. ATPase 7A and ATPase 7B control Cu transport across the blood-brain barrier and the cerebrospinal fluid barrier (Telianidis et al., 2013). The first one participates mainly in absorption and the second one in the elimination of copper. ATPase 7A and ATPase 7B are very important in the development of the CNS since ATPase 7A is involved in synaptogenesis and axonal growth and mediates Cu transport in glutaminergic neurons. Cu release is stimulated by the N-methyl-D-aspartate receptor (NMDAR), thus protecting neurons from excitotoxicity. In turn, ATPase 7B is a mediator of the synthesis of Cu-dependent enzymes indispensable for CNS development (Barnes et al., 2005). Inactivation of ATPase 7A in Menkes disease causes a sharp decrease in Cu transport in brain barrier cells, resulting in severe neurological symptoms including neurodegeneration. Defects in the ATP 7B gene cause, among others, Wilson’s disease, which, like amyotrophic lateral sclerosis, Alzheimer’s disease, and Creutzfeldt-Jakob disease, all leading to a toxic accumulation of metal in tissues, causing the damage of numerous organs, mental illness, and a variety of neurological symptoms (Hooenraad and Kinnier Wilson, 2001).

80–95 % of plasma copper binds to ceruloplasmin. Other copper-containing enzymes are tyrosine hydroxylase and dopamine hydroxylase which produce dopamine and noradrenaline, and cysteoxime oxidase, critical for mitochondria, where electron transport and energy production take place. In addition, Cu is included in superoxide dismutase, which is associated with free radical neutralisation. The main copper storage protein is metallothionein. Copper is involved in maintaining hemato poetic function by participating in the formation of hemoglobin and cross-linking of collagen and elastin (lysyl oxidase); keratin cross-linking (sulfhydryl oxidase); skeletal demineralization (ascorbate oxidase), controlling iron flow to various tissues and developing myelin. Premature infants and low birth weight infants are the populations vulnerable to Cu deficiency (Sugahazadeh et al., 2017).

However, high levels of copper are equally dangerous. Cu excess overload is the causative agent of synaptic pathology discovered in ASD. Copper can effectively block dopamine beta-hydroxylase function at the coeruleus locus, which, in turn, leads to alteration of noradrenaline synthesis mechanisms (Li et al., 2014a).

The potential neurotoxic effect of copper (Madsen and Gitlin, 2007) is associated with the fact that copper is a cofactor for dopamine β-hydroxylase (DBH) (Deinum et al., 2004; Rahman et al., 2009). An increase in norepinephrine levels in autistic individuals (Lake et al., 1977) can be partly explained by analyzing the state of hypercupremia. Increased levels of Cu and ceruloplasmin are associated with inhibition of decarboxylase hydroxytryptophan enzyme, which reduces the production of serotonin (Lakshmi Priya and Geetha, 2011). Redox imbalance is attributed to overexpression of Cu/Zn superoxide dismutase (SOD-1), encoded in trisomal chromosome 21. Moreover, copper is involved in the regulation of MT protein synthesis. Thus, in case of high Cu loads, MT levels also increase (Faber et al., 2009a). MTs capture Cu in intestinal cells and prevent their absorption (Russo and DeVito, 2011). The same is characteristic of Zn, therefore, excess copper hinders intestinal absorption of Zn and vice versa.

Zinc is one of the essential trace elements. It is crucial in the functioning of the immune system, protein and DNA synthesis, and cell division (Prasad, 1995; Heyneman, 1996). Zinc is a component of many transcription factors and enzymes, where it both plays the role of a stabilizer of spatial structure and a cofactor, participating in the catalysis of substrates (Oteiza and Mackenzie, 2005; Prasad, 2012). Zinc enzymes include, for example, carboxypeptidase A, superoxide dismutase, carbonic anhydrase, or alcohol dehydrogenase. Zinc activated enzymes are enolase and dehydropeptidase. Absorption of zinc occurs in the small intestine and duodenum. However, the bioavailability of zinc is low and it is estimated to be only 20–40 %. The divalent metal transporter 1 (DCT1) located on the brush border membrane is responsible for the transport of zinc (Gapps et al., 2014). After entering the cell, zinc ions are stored in secretory vesicles or bind to metallothioneins (MT) through nitrogen atoms, SH groups, carboxyl groups of amino acids, mainly cysteine and histidine. In plasma, zinc ions are bound to albumin and α2-macroglobulin (Reyes, 1996). Metallothioneins, thanks to the possibility of binding excess zinc ions in the cell space, protect cells against their toxicity and other dangerous consequences in the nervous system. Zinc is a structural component of zinc finger proteins and also it affects some genes recognized to be associated with the development of autism (Krishna et al., 2003).

Zinc through the stabilization of zinc finger structures in the cell participates in the regulation of DNA replication and repair, transcription and translation, cell proliferation and maturation, apoptosis (transcription initiation factor (TFIIIB), transcriptional SI elongation factor (TIIS), RNA polymerase, topoisomerase, ribosomal proteins) as well as responses to metals (factor 1 (MTF-1) (Wang et al., 2004; Wimmer et al., 2005).

Congenital metabolic abnormalities or the accumulation of mitochondrial DNA mutations lead to elevated ROS production and increased expression of metallothionein, which by binding to Zn causes a decrease in its plasma concentration. In turn, increased expression of metallothionein in enterocytes most probably impacts impaired absorption of Zn in the intestines. Some researchers suggest it to be the mechanism responsible for Zn deficiencies in ASD (Bjorklund, 2013).

The deficiency of this metal also significantly affects the functioning of the immune system which consequently might result in immunodeficiency. However, literature data investigating the effect of zinc on cytokine levels are not consistent. The opposite effect of zinc supplementation on the level of proinflammatory cytokines produced by peripheral mononuclear cells such as IL-1β, IL-6, and TNFα has been described (Chang et al., 2006). In addition, the concentration-dependent effects of zinc on immune function have been demonstrated. Zinc concentration from 1 to 100 μM increased the synthesis of pro-inflammatory cytokines, as well as the induction of proapoptotic proteins (caspase 3, Fas, and FasL), while concentrations above 100 μM reduced the synthesis of cytokines and anti-apoptotic factors NFκB, Bcl2, Bcl-XL (Chang et al., 2006).

It has been confirmed that people suffering from severe Zn deficiency could present neuropsychological disorders (Russo and DeVito, 2011; Lakshmi Priya and Geetha, 2011) and cognitive dysfunctions, as well as mental retardation (Prasad, 2012). This is due to the fact that Zn is a very important metal indispensable for the proper functioning and development of the central nervous system (CNS). The largest amounts of zinc are located in synaptic vesicles. Glutamatergic NMDAR terminals, in which it inhibits postsynaptic GluN2A (GRIN2A subunit) -NMDAR, are responsible for synaptic integration and plasticity of neurons.

Numerous studies prove that zinc deficiency during pregnancy, fetal development, and childhood, may generate anomalies in the process of shaping cognitive functions and may induce the development of autism.
Table 2
Status of toxic and essential elements in autism spectrum disorder.

| The type of sample | Biomarkers investigated | Results                                                                 | Number of patients | Ref.                  |
|--------------------|-------------------------|-------------------------------------------------------------------------|---------------------|-----------------------|
| Hair               | trace elements          | Lower level of Zn (29.7 %), Mg (17.6 %), Ca (5.8 %), other essential metals (2.0 %), High level of Al (17.2 %), Cd (8.5 %), Pb (4.8 %), Hg, As (2.8 %) | 1 967 ASD           | (Yasuda et al., 2013) |
| Hair               | Hg, Pb, Al              | Levels of mercury, lead, and aluminum in the hair of autistic children are higher than in controls. | ASD 100, HC 100     | (Mohamed Fel et al., 2015) |
| Blood              | GST genes, Al           | A marginally significant interaction between GSTP1 Ile105Val (rs1695) and ASD status (p = 0.07); none of other effects were statistically significant | ASD 116, HC 116     | (Babbar et al., 2016) |
| Brain              | Al                      | The aluminium content was consistently high. The mean (standard deviation) aluminium content were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes, respectively. Higher levels of Pb in RBC (+41 %) and higher urinary levels of Pb (+74 %), Ti (+77 %), Sn (+115 %), and W (+44 %). Lower levels of Cd in whole blood (–19 %). | ASD 5                | (Skalny et al., 2017a) |
| Whole blood, RBC, urine. | metal status | A strong association of levels of toxic metals with variation in the degree of severity of autism, Cd (whole blood), and Hg (whole blood and RBC) were the most consistently significant variables. Children with autism had significantly (p < 0.001) higher levels of lead, mercury and uranium. | ASD 55, HC 44        | (Adams et al., 2013a) |
| Hair               | Pb, Hg, U, Sh, As, Be, Cd, Al | There was no significant difference between the two groups as for Sh, As, Be, Cd, Al Urinary Hg (p b 0.05, AOR – 2.90; 95 % CI: 1.39, 6.07) and Pb (p b 0.05, AOR – 1.95; 95 % CI: 1.01, 3.77) positively associated with ASD, Cu associated with GSTM1 positive genotype (p b 0.05, AOR – 1.05; 95 % CI: 1.00, 1.10). C, As, Pb and Hg associated with ASD prevalence. Hair (Ca) and (Se) levels lower in ASD patients. (Hg) 3-fold and 2-fold higher as compared to the controls and children with catatonia in ASD, (I) and (Mn) the lowest and the highest in ASD > Catatonia respectively, Hg and serum Al and Cd negatively associated with catatonia in ASD Serum (Al) and (Cd) levels in healthy controls significantly higher in comparison to patients of both groups, (Cr), (Ca) levels higher in patients with ASD and catatonia, (V) levels higher in patients both with and without catatonia. | ASD 40, HC 40        | (Fido and Al-Saad, 2005) |
| Hair, urine, blood, | As, Zn, Ni, Pb, Hg, Ca, Cd, and Co | | ASD 90, HC 76 | (Amen et al., 2020) |
| Hair and serum     | Ca, Hg, Se, I, Mn, Al, Cd, Cr, Ca, V | Serum (Al) and (Cd) levels in healthy controls significantly higher in comparison to patients of both groups, (Cr), (Ca) levels higher in patients with ASD and catatonia, (V) levels higher in patients both with and without catatonia. | ASD 30, HC 30        | (Tinkov et al., 2019) |
| Erythrocytes       | 34 metals: toxic, essential, REE | Five elements including Pb, Na, Ca, Sh, and La are associated with the CARS total score. Iodine levels were 45 % lower in the children with autism (p = 0.005). Autistic children with pica had a 38 % lower level of chromium (p = 0.002). Autistic children with low muscle tone had very low levels of potassium (-66 %, p = 0.01) and high zinc (31 %, p = 0.01). The mothers of young children with autism had especially low levels of lithium (56 % lower, p = 0.005), and the young children (ages 3–6 yr) with autism also had low lithium (-30 %, p = 0.04). | ASD 50, HC 50        | (Wu et al., 2018) |
| Hair               | 39 toxic metals and essential minerals | | | (Adams et al., 2006) |
| Hair               | 20 metals               | Lower levels of Cr, I, V, Be, Sn, As, B, no significant difference in Hg, Zn, and Ca; Se was higher. | ASD 74, HC 74        | (Skalny et al., 2017b) |
| Hair               | Pb, Cd, As, Cu, Zn, Fe, Hg, Ca, Mg | Concentrations of lead, arsenic, copper, zinc, mercury, calcium and magnesium were significantly higher in the ASD group than in control group. Significantly lower Ni, Cr, and Se levels, significantly decreased serum Ni and Se in patients with childhood autism, atypical autism is associated with lower serum Al, As, Ni, Cr, Mn, and Se levels in comparison, Al and Mn concentration in this group was also lower than that in childhood autism patients. Higher Pb (ASD 31.9 μg/L, unaffected children 18.6 μg/L), Hg (3.83, and 1.09 μg/L), Cd (0.70 and 0.26 μg/L); lower Zn (ASD 4552.0 μg/L, and 5118.6 μg/L), Se (61.7 and 90.6 μg/L), Mn (13.5 and 21.4 μg/L); the children exposed to passive smoking had higher Cd (passive smoking 1.08 μg/L, non-passive smoking 0.22 μg/L); positive associations were found between levels of Hg or Pb and seafood consumption as well as body mass index (BMI). | ASD 78, HC 58        | (Zhai et al., 2019) |
| Serum              | Al, As, Ni, Cr, Cu, Ca, Fe, I, Mn, Se, V, Zn, Ca, K, Mg, Na | | ASD 48, HC 48 | (Skalny et al., 2016) |
| Plasma             | Pb, Hg, Cd, Zn, Se, Mn | | ASD 34, HC 38 | (Qin et al., 2018) |
| Blood              | Zn, Cu, Al, Pb, Hg | No significant difference between the groups was found, ASD group had significantly elevated blood Cu/Zn ratio; no significant difference between the groups was found as for uroporphyrins in the urine. Positive correlation among metals: Pb, Al, As, Cd and severity of ASD | ASD 52, HC 22        | (Macedoni-Lukstic et al., 2015) |
| Hair               | Li, Be, Al, Ni, As, Mo, Cd, Hg, U, Pb Cr, Co, Mn, Zn, Cu, Se | Symptoms, Zn level inversely related with age while there was a negative, association between Zn level and severity of autistic symptoms. Pb, Mo, Mn inversely correlated with cognitive level | ASD 48                | (Fiore et al., 2020) |
| Hair               | Pb, Al, Si, Mo, V, Cr, Cd, Co, Ni, B, Ba and 10 minerals | Higher levels of all, 11 analyzed heavy metals (p < 0.05), ranging from 150 to 365 % of control levels, higher levels of S, Na, Mg, K, Zn, Fe; lower levels of Ca, Cu. | ASD 27, HC 27        | (Al-Farsi et al., 2013) |
| Teeth              | Pb, Hg, Mn | No significant differences, marginally lower manganese Hg (p < 0.001) was significantly lower, As (p < 0.0001) was significantly elevated in ASD, no statistically significant differences in most of the other elements, Hg(p < 0.001), Cu(p < 0.05), Fe (p < 0.07) were lower in the ASD group, Na/k, Mg/Ca ratios were not significantly different, but were | ASD 22, HC 22        | (Abullah et al., 2012) |
| Hairs, 22 heavy metals and minerals | | | ASD 26, HC 39 | (Obrenovich et al., 2011) |

(continued on next page)
Table 2 (continued)

| The type of sample | Biomarkers investigated | Results | Number of patients | Ref. |
|-------------------|-------------------------|---------|--------------------|------|
| Hair, urine       | 23 metals               |         | ASD 25, HC 25      | (Blaurock-Busch et al., 2011) |
| Hair              | 17 metals               |         | ASD 44, HC 61      | (De Palma et al., 2012) |
| Urine             | Cr, Cd, Pb              |         | ASD 30, HC 20      | (Yorbik et al., 2010) |
| Hair, nails       | Cu, Zn, Mn, Se, Pb, Hg  |         | ASD 40, HC 40      | (Fido and Al-Saad, 2005) |
| Blood             | Hg, As, Cd, Pb          |         | ASD 45, HC 45      | (Kern et al., 2007) |
| Baby teeth        | Hg, Pb, Zn              |         | ASD 15, HC 11      | (Adams et al., 2007) |
| Hair, nails       | Cu, Zn, Mg, Se, Pb, Hg  |         | ASD 45, HC 50      | (Lakshmi Priya and Geetha, 2011) |
| Blood             | Pb, Hg, As, Cd, Mn, Al  |         | ASD 180, HC 184    | (Li et al., 2018) |
| Tooth enamel      | Mn                      |         | ASD 4, Other disorders 56 | (Jensabi et al., 2019) |
| Blood, BMC        | Mn                      |         | ASD 109, HC 109    | (Rahbar et al., 2014) |
| RBC               | Se, Pb, Hg              |         | ASD 35, HC 30      | (El-Ansary et al., 2017) |
| Hair, nail        | Cu, Zn, Mg, Se, Pb, Hg  |         | ASD 45, HC 50      | (Lakshmi Priya and Geetha, 2011) |
| Urine             | Cr, Cd, Pb              |         | ASD 30, HC 20      | (Yorbik et al., 2010) |

Abbreviations: Autism Spectrum Disorder (ASD), Healthy Controls (HC), Glutathione-S-transferase (GST), Childhood Autism Rating Scale (CARS), Red blood cells (RBC), Rare earth elements (REE), Blood manganese concentrations (BMC), typically developing children (TD).

(Copper and Zn are metabolic antagonists (Underwood, 1977; Baecker et al., 2014) and the proper functioning of the body depends on their mutual balance in the extracellular space (Björklund, 2013). Copper absorption is reduced when Zn is administered in excess, leading to impaired iron utilization and heme synthesis, and eventually anemia. Low plasma Zn levels are almost always associated with high serum Cu levels and increased copper toxicity (Plum et al., 2010b). Excessive plasma Cu levels can disturb Zn homeostasis, especially if it is associated with genetic deletion or knockdown of the Cu-transported complex COMMD1. Some argue that, in fact, the toxic effect of Zn is directly due to Cu deficiency (Baecker et al., 2014; Plum et al., 2010b). On the other hand, low Zn levels exacerbate Cu toxicity (Blaurock-Busch et al., 2012). The ratio of Zn to Cu in children and adults is close to 1:1 (Faber et al., 2009b; Van Weyenberg et al., 2004). (Faber et al., 2009b) proposed that the serum Zn/Cu ratio should be used as a rapid ASD diagnostic method reflecting the condition of the metallothionein system. The reduced Zn/Cu ratios observed in ASD may be due to a deficiency of Zn in the body or the accumulation of toxic antagonist metals. Some authors argue that a low Zn/Cu ratio may be the result of the toxicity of such metals as Hg, Cd (Faber et al., 2009b; Aschner et al., 2006). Therefore, the Zn/Cu ratio was considered an important biomarker of ASD (Midvtedt, 2012). The cut-off value for the Zn/Cu ratio in serum was determined as an indicator of the auxiliary diagnosis of autism at the level of 0.665 (Takeda et al., 2001). Table 3 collected published papers on the study of Cu and Zn levels in different tissues.)
and electron transport, and its levels definitively affect brain functioning. Iron is necessary for early neurodevelopmental processes that seem to be deregulated in an ASD. There is significant evidence of the important role of iron for cognitive, behavioral, and children physical activity development. One has to remember that iron is a component of a multitude of enzymes involved in the synthesis of neurotransmitters. The decrease in iron concentration in the brain is accompanied by alterations in serotonergic and dopaminergic systems in cortical fiber conductivity and myelogenesis. Therefore, iron deficiencies might contribute to negative effects on cognitive development and functionality in children with ASD.

The reason behind iron deficiency in the body is an inadequate diet, and absorption disorders resulting, among others, from poor bioavailability of the chemical form of this element. Therefore, iron consumption is not always related to its status in the body. For example, the bioavailability of iron from non-heme sources is poor, the presence of vitamin C improves absorption; while, the state of chronic inflammation, the presence of calcium or other divalent cations may inhibit it (Geisler and Singh, 2011). The status of iron in children with ASD has been taken into account in numerous studies (Table 3). Anemia was found in 1%–15% of children with ASD, but the studies presented did not always provide sufficient markers for the diagnosis of iron deficiency (ID).

Hemoglobin (Hgb) levels are a common anemia marker, however, iron deficiency took place before reducing Hgb. Other markers commonly used for ID assessment are transferrin (TS) and ferritin (SF) saturation, as it is an iron storage protein and the first value to decrease in case of ongoing deficiency. However, it should be remembered that serum ferritin (SF) levels may return to normal levels after iron supplementation. Anemia was found in 1%–15% of children with ASD, but the studies presented did not always provide sufficient markers for the diagnosis of iron deficiency (ID).

Table 3

| The type of sample | Description | Number of patients | Ref. |
|--------------------|-------------|--------------------|------|
| Serum, plasma, erythrocytes | The difference between ASD patients and controls did not reach the level of significance. | ASD 13, HC 13 | (Tórsdóttir et al., 2006) |
| Hair, nails | Autistic individuals had elevation level of Cu, Pb, Hg; lower level of Mg and Se | ADS 45, HC 50 | (Blaurock-Buch et al., 2012) |
| Serum | Autistic individuals had lower Zn and Zn/Cu ratio, higher Cu, negative correlation between Zn/Cu and CARS scores (r = −0.345, p = 0.007). | ASD 60, HC 60 | (Li et al., 2014b) |
| Plasma | Autistic individuals had elevated plasma levels of copper and Cu/Zn and lower, but not significantly lower, plasma Zn compared to neurotypical controls | ASD 102, HC 18 | (Russo et al., 2012) |
| Serum | The entire cohort’s mean zinc level was 77.2 μg/dl, mean copper level was 131.5 μg/dl, and mean Zn/Cu was 0.608, which was below the 0.7 cut-off of the lowest 2.5% of healthy children. | ASD 230, NOS and Asperger’s syndrome | (Faber et al., 2009b) |
| Whole blood | ASD had 10% (p = 0.005) and 12% (p = 0.015) lower levels of Zn and Zn/Cu ratio, respectively, no significant difference in Cu was observed. Cu/Zn ratio was 15% (p = 0.008) higher in ASD. | ASD 26, HC 28 | (Cracium et al., 2016) |

Abbreviations: Autism Spectrum Disorder (ASD), Healthy Controls (HC), Pervasive Developmental Disorder (NOS), Childhood Autism Rating Scale (CARS).

3.3. Magnesium and calcium deficiency

Magnesium (Mg) is the fourth most common cation in the body which performs many different functions, partly overlapping with the role of calcium. Magnesium is mainly located in the intracellular space (Altura, 1991). It plays an important role in brain development and its functioning by activating copper and zinc superoxide dismutase (CuZn-SOD) and releasing nitric oxide from body cells (Johnson, 2001). Magnesium is indispensable in diverse enzymatic reactions involving ATP, among others, as a co-factor for thiamine pyrophosphate and in many metabolic pathways, e.g. of neurotransmitters such as serotonin, γ-aminobutyric acid (GABA), dopamine, adrenaline, and noradrenaline (Kidd, 2002a, b).

According to Johnson (2001), Mg plays a fundamental role in the process of converting thiamine into thiamine pyrophosphate, whose lack of secretin content in gastric juice leads to autism. This fact seems to be confirmed by the study of Rimland (2002), who observed an improvement in the clinical profile of patients with autism following vitamin B6 and Mg supplementation.

Mg deficiency has been associated with personality changes, apathy, depression, and anxiety. Studies on magnesium levels in children with ASD are, however, inconclusive (Bener et al., 2017; Mousain-Bosc et al., 2006; Skalny et al., 2018). Magnesium profile in plasma and erythrocytes is discussed in the study of Strambi et al. (2006).

In a group of 12 children with ASD and 17 children with a different spectrum of disorders, there was no significant difference in intracellular magnesium levels, however plasma levels were statistically lower in children with ASD, as compared to controls.

Yasuda et al. in 2013, described variations in magnesium and calcium levels in the hair of almost 3,000 children with autism. Based on measurements of 26 elements in the hair, metallokins profiles were created (Yasuda et al., 2013). The aim of the study was to find a

biochemical results (Centers for Disease Control and Prevention (CDC), 2002). The suggestions of Luck et al. seem interesting (Luck et al., 2013), namely: that large amounts of oxalate in the plasma may play a role in autism by binding to the bilobal transferrin iron transport protein (HTP), thereby interfering with iron metabolism by inhibiting iron supply to cells.

However, there exist studies that, using various statistical tools, provide results suggesting the absence of relationship between ASD and iron deficiency (ID) (Tseng et al., 2018). The result of the meta-analysis performed on data until 2017 by Pao-Yen Lin et al. states that peripheral iron levels, serum ferritin, Fe in hair, and iron uptake with food do not differ significantly between ASD and non-ASD groups. However, the coexistence of ASD and ID was significantly higher in children with ASD than in children without these disorders. The results of other investigations on iron status in ASD are collected in Table 4.
relationship between mineral imbalance and the pathogenesis of autism. 17.6 % of children with ASD were found to reveal manganese deficiency. Calcium deficiency was observed only in 5.8 % of cases. Deficits of other essential metals were smaller or equal to 2%. An interesting observation by the authors of this study was the decrease in magnesium deficit with age (r = 0.362, p < 0.0001). The largest deficit was observed in children aged 0-3 years (27 %), while, calcium deficiency only occurred in children under 10 years of age (Yasuda and Totsui, 2013).

Determining the level of magnesium in the whole blood provided slightly different results. Wu et al. in their study of 113 blood samples of children with ASD, demonstrated no differences in magnesium levels when compared to controls. There was also no relationship between age and blood magnesium levels in children with ASD (Wu et al., 2019).

Several studies have also shown abnormal levels of ions such as Na, Ca, and K in the blood, which may play a role in oxidative stress and energy metabolism, and may also indirectly contribute to the development of autism (Krey and Dolsmetsch, 2007; El-Ansary et al., 2010).

### 3.4. Manganese, selenium, chromium, cobalt, disturbances

Manganese (Mn) is a naturally occurring trace element. Adequate amounts of manganese are usually maintained thanks to a properly balanced diet. Excessive exposure to manganese, on the other hand, may result in neurotoxicity, affecting cognition and creating behavioral problems (Zoni et al., 2007). Thus, both low and high levels of manganese can have a negative impact on neurological development in early human life (Bhang et al., 2013; Claus Henk et al., 2010). In individuals exposed to higher levels of manganese, increased behavioral problems, i.e. mood changes and compulsive behavior (Bouchard et al., 2007; Ericson et al., 2007; Khan et al., 2011; Takser et al., 2003), reduced verbal abilities, intellectual deficits, and finally irreversible extrapyramidal disorders (Wright et al., 2006; Wasserman et al., 2011; Kim et al., 2009; Menezés-Filho et al., 2011; Bouchard et al., 2011; Khan et al., 2012) are noted. The cause of manganese can derive not only from the body. There is no doubt, however, that exposure to manganese compounds, especially in the air, is worrying because the neurotoxicity of inhaled manganese is greater than that of the one taken orally (Bounds, 2021; Boyes, 2010; Environmental Protection Agency (EPA), 2007). Considering the fact that the occurrence of inorganic manganese compounds is common, during carbon combustion processes, generated by industrial facilities and car engines, there exists a dire need to identify sensitive manganese status biomarkers (Environmental Protection Agency (EPA), 2007).

The body is protected against manganese toxicity primarily by low absorption and/or elimination of manganese by the liver, the processes which are disrupted in ASD. Serum manganese concentrations combined with the activity of specific manganese dependent enzymes such as superoxide dismutase (MnSOD) and blood arginase may be useful for assessing manganese status in the body. However, only brain MRI scans in combination with serum manganese levels offer the most reliable manner of monitoring of exposure to manganese (Greger, 1999).

The last few years have shown that selenium also has been an effective anti-oxidant, thereby playing a significant role in neuroprotection. Antioxidant and anti-inflammatory properties of selenium are provided by the catalytic function of selenoproteins (Schweitzer et al., 2004) and selenium-dependent enzymes, particularly glutathione peroxidases (GPX) and thioredoxin reductases. Taking into account the major importance that oxidative stress plays in ASD pathogenesis (Chauhan and Chauhan, 2006), selenium is considered a protective agent in ASD. A significant association has been demonstrated between genetic variants of glutathione peroxidase 1, antioxidant selenoprotein, and susceptibility to ASD (Ming et al., 2010).

As in the case of other elements, the metallic data regarding selenium status in ASD are not consistent, showing simultaneously a decrease (Lakshmi Priya and Geetha, 2011; Blaurock-Busch et al., 2012; Skalny et al., 2016; Jory and McGinnis, 2008), an increase (Yasuda et al., 2013; Skalny et al., 2017b; Lukowska and Sobieraj, 2009) or no changes in Se level in various tissues. An interesting aspect appears to be the ratio of selenium to the level of mercury or lead exhibiting antagonistic action against selenium. In patients with ASD, a significantly lower Se/Hg (Sajdel-Sulkowska et al., 2008) and Se/Pb (El-Ansary et al., 2017) ratios were observed as compared to controls. This confirms the hypothesis that selenium deficiency in ASD increases the susceptibility to Hg and Pb neurotoxicity. This is of particular relevance in case of accumulation of many toxic elements in the body tissue, due to possible inadequacy of a synergistic action. Selenium protective mechanism against oxidative stress caused by mercury and lead toxicity was described in 1998 by Othman and El Missira (Othman and El Missira, 1998) and includes the formation of an inactive Se-Pb complex; activation of superoxide dismutase (SOD), and increased glutathione reductase activity. A reduced Se/Pb ratio is associated with cell membranes susceptibility.
to oxidative stress caused by the destruction of their integrity due to the damage of fatty acids (Yiin and Lin, 1995; El-Ansary and Al-Ayadh, 2014) and increasing the level of other markers of oxidative stress in patients with ASD (Al-Yafee et al., 2011).

Chromium (Cr) occurs in two oxidation states: +3 and +6. Only Cr (+3) is an essential trace element that plays an important role in glucose and cholesterol metabolism (Broadhurst and Domenico, 2006). Research on mouse embryos has shown that an increase in the level of this element affects neural tube defects (Ijima et al., 1983). Metallomic studies in children with autism vary depending on the kind of tissue that was examined. Most studies on the level of chromium in hair find lower levels of this element in children with ASD compared to controls. On the contrary, urine Cr levels generally show higher levels in autistic children (Yorbik et al., 2010).

Cytotoxic Cr (+6) through oxidative stress leads to lipid peroxidation, oxidative degradation of proteins, damage to genomic DNA, loss of membrane integrity, cellular dysfunction, and ultimately cell death (Valko et al., 2006; Bertin and Averbeck, 2006; Rana, 2008). Various studies have shown that Cr (+6) lowers GSH and cysteine glutathione levels (Valko et al., 2006), which is being frequently noted in autism (James et al., 2009b; Geier and Geier, 2006b; Geier et al., 2009d; Vojdani et al., 2008). Yorbik et al. observed that the activity of antioxidant enzymes is much lower in children with autism, hence the hypothesis that oxidative stress may play a major role in the pathogenesis and development of autism (Yorbik et al., 2002). Many independent studies have confirmed this hypothesis (Chauhan et al., 2004; James et al., 2004; McGinnis, 2004; Ming et al., 2005; Yao et al., 2006).

Cobalt is a key ingredient in vitamin B12, which is also important for the proper functioning of the nervous system. The activity of some enzymes depends on cobalt, such as isomserases, methyltransferases, and reductive dehalogenases. These enzymes are involved in the synthesis of DNA, fatty acids, energy production, and the elimination of oxidative stress. Only few research groups working on the etiology of autism, elaborate on this element in individual manner. One example is the study of Geier et al. (Geier and Geier, 2010) which assessed the effect of methylcobalamin (vitamin B12) injection (75 μg/kg) on cobalt levels in autism spectrum disorders (ASD). Researchers observed that the mean of plasma and urinary cobalt levels have significantly increased (6.83-fold and 51-fold respectively) after methylcobalamin injections, as compared to controls.

4. Conclusions

The levels of elements, in the blood or blood fractions, are widely recognized as the basic bioindicator, representing their current status in the body. However, the content of toxic metals and essential minerals can also be tested in hair, nails, teeth, or urine.

Regarding essential minerals in children diagnosed with autism, numerous deficiencies of zinc, magnesium, and calcium, etc. have been documented. Among toxic metals, elevated levels of metals such as mercury, lead, and cadmium are most commonly observed in ASD. Thimerosal, containing mercury as a preservative, was regarded as the main source of mercury exposure. However, recent studies have arrived at conflicting results as to the effect of this element on the increased risk of autism. Also lead and cadmium are confirmed to have affected cognitive and behavioral development. The area that is still little explored is rare earth elements (REE). Only one publication concerns REE determination in erythrocytes (Wu et al., 2018). In this paper written by Wu et al., the lanthanum (La) level was associated with the Childhood Autism Rating Scale (CARS) total score. The concentrations of the other three REEs (Ce, Gd, and Eu) presented the same range as in control group. It should be emphasized, however, that the proper determination of these elements at such a low pg/mL level required advanced analytical techniques such as ICP-OES, ICP-MS, or LA ICP-MS, thanks to which it is possible to perform micro-sampling, distribution, and mapping of elements in tissues. Moreover, the lower the concentration of the element in the sample, the greater the contribution of the measurement error, which raises various doubts when interpreting the results.

Based on the collected literature, it can be inferred that autistic children suffer from an imbalance of elements, however, their metallomic profiles could be very diverse. The reasons for this state of affairs can be seen, among others in the methods used to recruit the controls/study groups, the size of the cohort, methods for determining elements, types of tissue used for research, and perhaps also in various causes behind the general notion of autism. The country of origin is also significant, due to the incomparable environmental contamination that determines the toxic metal burden in the body. This is especially true in Asian countries, where there is a higher consumption of seafood contaminated with mercury, hence the significantly increased concentration of mercury in Asian children’s blood plasma. Meta-analyses also reveal that patients with ASD in non-Asian countries have, for instance, lower levels of chromium, and higher levels of zinc in their hair compared to patients with ASD in Asian countries. Thus, given the fact that ASD is a multifactorial disorder associated with heavy metal pollution, instead of absolute levels of individual elements, many authors considered an application of "relative concentrations" e.g. Se/Hg, Cu/Fe, Ca/Mg for an early stage of diagnosis.

There is no doubt that deficiencies of essential elements or toxic metal burden may induce epigenetic changes that disrupt the maturation of neurons causing neurodevelopmental disorders during early development. The pathogenesis of autism has not been fully elucidated, but early intervention is considered absolutely vital. Most authors emphasized the importance of the "infantile window" as a critical factor that plays a significant role in neurological development.

Therefore, in the treatment/prevention of ASD, early screening is necessary to detect any metabolic and mineral imbalances. The conclusions from those studies that take into account the limited age of children can constitute a reliable diagnostic factor scheme. A reliable type of metallomic profile for a specific “time window” and type of sample that would correspond to behavioral/neurological deficits in autism spectrum disorders still remains to be determined. This should, undoubtedly, enable a better future diagnosis, effective prevention, and treatment of autism spectrum disorders.

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