Section A -Research paper

Serum Magnesium, Zinc and Copper levels in ASD Children: Review Article

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Abstract:

Neural and cognitive processes require zinc and copper homeostasis and a normal zinc/copper ratio. Ceruloplasmin, an intrinsic antioxidant protein, maintains copper homeostasis, which might also influence autism spectrum disorder (ASD). ASD children are frequently reported with altered levels of these elements with wide geographical variations. This review will evaluate any alteration in plasma magnesium, zinc and copper levels in ASD children.

Keywords: Magnesium, Zinc, Copper, ASD.

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Introduction:

Autism is a behaviorally defined condition with a limited range of interests and frequently stereotyped repetitive behaviors and mannerisms. It is marked by qualitative impairments in social communication, social interaction, and social imagination. Since autism is a complex, heterogeneous disorder with a range of severity, the etiology has remained unknown despite decades of study and inquiry. Increasing conceptual, genetic, cognitive neuroscience, and clinical integration is necessary to improve our understanding of the origins, nature, and treatment of autism **(1).**

The etiology of autism has been linked to both genetic predisposition and environmental toxins and toxicants; the effects of these environmental triggers are linked to increases in oxidative stress, and are further exacerbated when coupled with hereditary susceptibility. At the moment, issues like prenatal stress, melatonin deficiency, immune system changes, maternal diabetes, and prenatal infections are addressed. It has not yet been possible to pinpoint the underlying process or a particular metabolic target that is pertinent to ASD. It is more probable that all of these variables have an impact on how synapses work and change, which may be the common denominator **(2).**

The role of metals in ASD:

Bioelements play important roles in the central nervous system. The lack or excess

of essential minerals and trace elements are known to cause a variety of health problems, and could contribute to the etiology of ASDs. Children are, due to their behavior, more exposed to environmental toxins than adults, but often also have higher intestinal absorption rates and lower detoxification ability Many studies show that autistic children have a higher body burden of toxic metals compared to neurotypical controls. These studies indicate that children with autism have a decreased ability to excrete toxic metals, leading to a higher body burden **(3).**

Toxic metal exposure before or during pregnancy has been linked to typical ASD traits like cerebral disability and language difficulties. According to an analysis of almost 100 studies, 74% of them point to physiological mercury levels as a risk factor for ASD, most likely as a result of oxidative stress, autoimmune activation, and subsequent neuroinflammation **(4).** Both the white and gray matter of ASD patients contain high amounts of aluminum, which is thought to be able to pass through blood-brain barriers and be absorbed by microglial cells **(5).** Additionally, research on animals looking at how metals affect neurodevelopment discovered alterations in brain architecture linked to ASD **(6).**

Aside from heavy and toxic metals, which actively disrupt neurodevelopmental processes, dyshomeostasis of metal micronutrients may also be involved in ASD etiology. Zinc, copper, selenium, iron, and magnesium levels have been associated with ASD incidence **(7).**

Magnesium:

Since magnesium (Mg^{2+}) is necessary for optimal nerve transmission and participates in the creation of membrane phospholipids, it is one of the micronutrients that has a significant impact on brain activity and mood. Because of this, it is essential to the proper operation of the central nerve system. Magnesium has also been investigated as an adjunct treatment for mental disorders, particularly in anxiety and mood disorders, both in the form of enriched diet and supplementation at high doses. It is possible that methodological heterogeneity, which includes, among other things, measuring techniques (extracellular vs. ionized magnesium) and supplementation modalities, is the reason why findings from reports on both magnesium levels and supplementation in psychiatric illnesses are frequently contradictory (dose, posology, magnesium form used) **(8).**

- **Biological Plausibility of Magnesium for Brain and Psychiatric Disorders:**

Magnesium is the fourth most common mineral ion, and leafy green vegetables, whole grains, nuts, and fish are the primary sources of intake. The kidney system and the digestive tract both aid in the absorption of magnesium. Although the parathyroid hormone controls both of these ions and this element aids calcium (Ca^{2+}) uptake, free ion concentration is not always correlated with total concentration. Magnesium is mainly found in cells; extracellular magnesium makes up only 1% of the overall magnesium in the body. Additionally, there

are three forms of magnesium in serum, with ionized magnesium having the greatest biological activity **(9).**

Magnesium is essential to ensure the correct functioning of all human cells, neurons included; it is involved, among others processes, in hundreds of enzymatic reactions, intracellular transmission, myelination process, synapses formation and maintenance as well as in the regulation of serotoninergic, dopaminergic and cholinergic transmission. Because it has been demonstrated to reduce apoptosis in an animal model of induced hypoxia-ischemia and to prevent synaptic loss in a mouse model of Alzheimer disease, magnesium is therefore an element required to keep neurons healthy and viable **(8).**

There is also some proof that magnesium plays a role in neurogenetic processes and the maturation of newly formed neural cells; in fact, magnesium has been shown to effectively promote neural stem cell proliferation and neurite outgrowth. Magnesium has also been shown to improve learning skills, working memory, short- and long-term memory in rats by inducing synaptic plasticity and potentiating synaptic transmission **(10).**

Magnesium's antidepressant effect is probably transmitted by a number of mechanisms. The glutamatergic N-methyl-D-aspartate receptor (NMDAR) blockade appears to be the most significant one; curiously, this is also the target of the NMDAR antagonist and fast-acting antidepressant ketamine. However, magnesium also appears to influence other aspects of glutamatergic signaling, including the -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid AMPA receptor**(11).**

The discovery that magnesium deficiency is associated with dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis, which is well known to be involved in the pathogenesis of anxiety disorders and depression **(12).** Is another pertinent finding that supports the idea that magnesium has antidepressant action. Conversely, increased levels of brain magnesium have been shown to enhance (a) the retention of the extinction of fear memory, through increased NMDA signaling, (b) the brain-derived neurotrophic factor (BDNF) expression and (c) synaptic plasticity in the prefrontal cortex (PFC); notably, these effects were absent in another region closely linked to depression pathogenesis **(13).**

The antidepressant action of magnesium appears to be, at least partially, mediated by a modulation of the serotoninergic system; in fact, it seems that magnesium has a synergistic effect when administered with molecules of the selective serotonin reuptake inhibitor (SSRI) class and that the antidepressant action of magnesium is impaired when animals are pre-treated with a compound that inhibits the serotonin synthesis **(14).**

Additionally, magnesium appears to play a role in the avoidance and treatment of movement disorders brought on by prolonged use of conventional antipsychotics. Additionally, magnesium was discovered to lessen the severity of movement disorders in an animal model by preventing the creation of reactive oxygen

species in cortical regions. the substantia nigra and striatum **(15).**

Magnesium deficiency has been observed in children with ASD. **(1).** Magnesium has been proposed as a possible nutritional intervention for ASD when combined with vitamin B6. But from the late 1990s to the early 2000s, a number of systematic reviews showed little support for the use of magnesium and vitamin B6 as ASD treatments **(16).**

Few studies have looked at magnesium's involvement in ASD since that time. However, a more recent review discovered a substantial magnesium deficiency in ASD patients, and it advises patients with ASD to have their magnesium levels monitored **(17).**

Zinc:

The second most common metal in the human body, zinc, is essential for numerous cellular processes. In vivo zinc binding is possible by about 2,800 proteins, or 10% of the human proteome. During embryonic and childhood development, zinc plays a special role in glutamatergic transmission (also known as the GABA route). Through the action of SHANK proteins, a family of postsynaptic scaffolding proteins, zinc deficiency in mouse models causes altered neural tube closure and ASD-related behavior such as decreased vocalization and social behavior **(18).**

In SHANK3-mutant rodents, non-ASD behavior is recovered when dietary zinc is added. **(19).** According to **(20),** brain zinc levels are 10 times higher than serum zinc levels, suggesting a bigger role for zinc in neurodevelopment. Zinc is more prevalent in neuronal-rich regions and is essential for learning, memory, synaptic plasticity, and neuronal modulation.. It should be noted that current research links zinc to ASD in the setting of the central nervous system, so there is still much to learn about the peripheral nervous system. The importance of zinc homeostasis in the brain is demonstrated by the fact that new pharmaceutical treatments for brain injury aim to restore homeostasis by lowering amounts of free zinc in the brain **(21).**

Zinc &Gut-Brain Interaction in ASD:

The immune system's nearly all of its components are negatively impacted by zinc insufficiency. Even a slight zinc shortage causes a severe immune system impairment. According to research done on humans and animal models, infection susceptibility rises as zinc status declines. An impaired T and B lymphocyte growth and differentiation as well as their decreased activity are linked to infection susceptibility. Reduced frequency of acute lower respiratory infections and shorter length of acute and persistent diarrhea are two positive effects of zinc supplementation in diseases **(22).**

There has long been speculation that ASD may be caused by immune system dysfunction. Similar to some animal models, postmortem brains of people with ASD showed activation of astroglia and microglia, suggesting some degree of neuroinflammation **(23)**. But GI system anomalies can also act as mediators of inflammatory events. Natural killer cells, polymorphonuclear leukocytes, and the

complement system are typically what start an organism's defense against viruses. Zinc deficiency suppresses all of these defense systems, which leads to a protracted inflammatory response. Both diarrhea and inflammatory bowel illness, both effects of zinc deficiency, are linked to the disruption of these processes **(24).**

Additionally, there is a connection between physiological and psychic stress and gut inflammation and zinc deficiency. Psychological stress decreased serum zinc levels, according to animal research **(25).** The production of glucocorticoids is influenced by both decreased zinc levels and psychological stress. Reduced B lymphocyte counts and thymic atrophy have been linked to increased amounts of glucocorticoids.

Furthermore, long-term elevated glucocorticoid concentrations may cause glucocorticoid receptors to become resistant, which would prevent the immune system from being downregulated. To prevent long-lasting inflammation processes, this downregulation is required. When considered as a whole, immune system issues, stress, and zinc insufficiency are closely related processes (Figure 1). The end effect might be altered signaling to and within the developing brain, which might help explain how ASD develops.

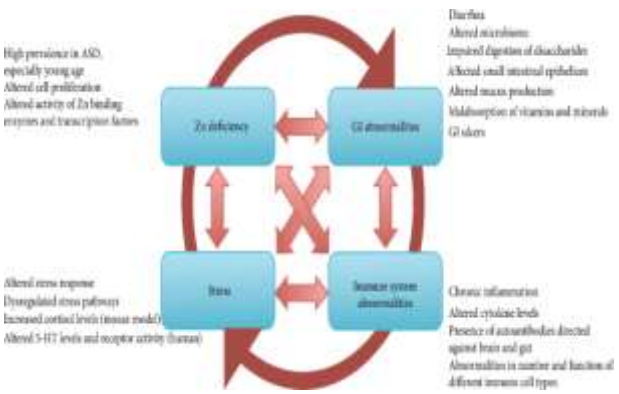

Figure (1): Zinc in Gut-Brain Interaction in Autism**(22).**

There is considerable evidence for an association between zinc deficiency and ASD. Zinc-binding genes associated with ASD are up-regulated in all neurodevelopmental stages. In a study examining 1,967 children with ASD, almost 30% had low zinc concentration in hair samples . Another small study found lower zinc levels in saliva of autistic children when compared to healthy controls. Zinc levels may also be correlated to severity of ASD presentation. It is important to note, however, that significant variance is observed when comparing zinc from hair and nails suggesting that serum may be a better source for zinc measurement. When serum was evaluated in 78 children with autism, 71.8% of children had zinc levels either in the lowest 10% or below the reference range **(7).**

Copper:

Copper also has important roles in the human body, and is involved in cell growth, among many others Copper is involved in reactions connected to neurological diseases, and dyshomeostasis of copper has been seen in disorders such as Parkinson’s,

Alzheimer's, and Huntington's Diseases **(26).** Further, copper is integral in several autism-related biological processes, such as immunity and placental development. Copper levels are typically higher than average in ASD patients **(27).**

Common symptoms of Cu deficiency include hypocupremia, impaired iron mobilization, anemia, leukopenia, neutropenia, decreased superoxide dismutase, ceruloplasmin as well as cytochrome c oxidase, but also increased plasma cholesterol and LDL/HDL cholesterol ratio and abnormal cardiac function **(28).** Low copper levels can result from Menke's kinky hair condition, malabsorption, and undernutrition. An excessive diet, infections, inflammation, Wilson's disease, injuries, systemic lupus erythematosus, and autism are all linked to elevated Cu levels **(29).** Neurological disorders such as amyotrophic lateral sclerosis, Alzheimer's disease, and Creutzfeldt-Jacob disease are associated with copper-cofactor-containing proteins**(26).**

Interestingly, copper and zinc play competing roles physiologically, such that an increase in copper leads to zinc deficiency. The zinc/copper ratio has therefore been examined in the ASD setting. Patients and children with ASD tend to have lower zinc/copper ratios than controls even if differences are not seen in copper levels alone. Such differences are not known to be sex-dependent, though copper levels alone may differ by sex due to oral contraceptive use. One study found that the zinc/copper ratio could be used as a diagnostic biomarker. Zinc/copper cycles may play a role in ASD occurrence, and the rhythmicity of these cycles can be used as a diagnostic tool to classify ASD **(7).**

❖ **Zinc to copper ratio:**

In the blood, where changes in these two trace elements typically have an inverse relationship, zinc and copper keep a balance. This can be partially explained by the fact that cytokines control how the two elements are metabolized, with the same cytokines causing increased Zn uptake by cells and increased ceruloplasmin synthesis in the liver. A high serum Cu content is almost always correlated with a low plasma Zn concentration. Published research show that children and adults typically have a Zn to Cu ratio close to 1:1. The plasma Zn/serum Cu ratio has been suggested as a potential quick technique for assessing the functional state of the metallothionein system **(30).**

In 2009, Faber and colleagues conducted a retrospective analysis of plasma Zn, serum Cu, and Zn/Cu on data from 230 kids [179 boys, 51 girls, mean age 6.3, SD 3.67] with Asperger syndrome, pervasive developmental delay, and autism disorder. The mean Zn level for the total cohort was 77.2 g dl1, the mean Cu level was 131.5 g dl1, and the mean Zn/Cu ratio was 0.608, which was under the 0.7 cut-off for the lowest 2.5% of healthy children **(26).**

Lower Zn/Cu ratios may indicate a Zn deficiency throughout the body or a buildup of toxic metals that are antagonistic to Zn. According to some theories, Hg toxicity may be a significant factor in individuals

with ASD having MT dysfunction, which may be reflected in the Zn/Cu ratio. It is not impossible that the toxic metals Hg and Cd, similar to that proposed above for oxidative stress due to genetic disturbances, might have opposite net effects on Zn and Cu metabolism because enhanced MT induction in the liver might affect Cu excretion via the bile more than it affects the mobilization of this element from the liver to the blood, while for Zn it is the rate of mobilization to the blood which is more strongly affected**(31).**

Association Between Metal Micronutrients and Biological Processes:

Iron, zinc, and copper have been considered as essential metal nutrients for neurodevelopment processes.. The enzyme ribonucleotide reductase, which controls the central nerve system, needs iron to function. In the creation of myelin, iron also functions. Due to its function in the synthesis of DNA, zinc is essential for cell growth. Additionally, zinc is needed to modify postsynaptic learning, activate GABA receptors, and NMDA receptors for glutamate **(32).**

For instance, studies using mice show that variations in zinc levels affect both synaptic learning and neurogenesis. Rodent pups show impaired DNA synthesis and improper thymine incorporation in brain DNA when mothers are deficient in zinc early in pregnancy or after delivery. Early-stage zinc deficiency also causes morphologic brain abnormalities, including in the hippocampus, a region of the brain that is particularly important for working memory **(7).**

Along with folic acid and vitamin A, copper is needed for the formation of the neural plate and neural tube very early in the development. Copper is also a cofactor of dopamine-β-hydroxylase, peptidyl-a-monooxygenase and many other enzymes which are involved in vital central nervous system processes **(33).**

Copper is particularly involved in neurotransmitter synthesis and neuromodulation. Subsequently, several neurodegenerative diseases associate with copper dyshomeostasis. Other essential trace metals, such as Mn, Mo, and trace elements such as Se ions also play a critical role in neurodevelopment. Metal ion contents also vary with age**.** Deficiency or dyshomeostasis of any of these metal ions will affect the neurodevelopmental process and may not be corrected even after the repletion of these metal ions **(34).**

Considering the importance of metal ions as integral part of various metalloproteins and enzymes, perturbation of metal ions homeostasis either through dietary deficiencies or via genetic alterations in metalloproteins and enzymes can have detrimental effect on neurodevelopment. There are many zinc-binding genes within each neurodevelopmental pathway**(7).**

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