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Role of Environment, Nutrition, Microbiota, Mammalian Target of Rapamycin and Dietary Supplements in Autism

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Abstract

Autism Spectrum Disorder (ASD) is a developmental disorder with the age of onset under 3 years old. It is characterized by definite impairments in social interactions, speech abnormalities, and stereotyped patterns of behaviors. Although the exact pathology and etiology of ASD are not fully elucidated, exposure to environmental toxins, micronutrients deficiency, dysbiosis and mutation in genes of mammalian target of rapamycin (mTOR) signaling pathway are emerging as risk factors for ASD. Maternal exposure to heavy metals, air pollutants, and pesticides markedly increases the risk of ASD. Many clinical and experimental trials documented that gastrointestinal symptoms and disturbances of the gut microbiota usually accompanied cerebral disorders in autistic patients. Furthermore, studies showed that gene mutations causing hyperactivation of mTOR significantly lead to autistic symptoms. Pharmacological and nutritional interventions revealed a significant improvement in autistic individuals. The use of dietary supplements and the elimination diets exhibit minor or no adverse effects as compared to conventional drugs. In this review article, we tried to summarize some of the etiological factors that predispose to autism. We discussed the possible mechanisms that potentiate autistic symptoms by such factors. Also, we focused on the role of interventions either by various dietary supplements or by elimination diets in the management of autism.

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with age of onset under 3 years old. It is estimated that 1 in 160 children worldwide suffers from ASD. This estimate represents an average figure, and reported prevalence varies substantially across studies. The prevalence of ASD in many low- and middle-income countries is so far unknown (**WHO, 2018**). Based on epidemiological studies conducted over the past 50 years, the prevalence of ASD appears to be increasing globally. The disease affects males more than females (4:1), however, in girls the symptoms may be more intensified (**Rapin 1999; Solomon *et al.*, 2012**). ASD is characterized by a noticeable impairment of social interaction, delayed usage of language and behavioral disturbances such as self-injurious behavior (**Minshawi *et al.*, 2014**). Besides, the behavioral impairment, autistic patients has marked prevalence of gastrointestinal (GI) disease and dysbiosis (**White, 2003; Hsiao, 2014; Li *et al.*, 2017**), autoimmune disease (**Keil *et al.* 2010**) and mental retardation (**Noterdaeme and Wriedt, 2010**).

The etiology of autism is not clear, although various genetic, environmental, metabolic, neurologic and immunologic factors are probably involved. Environmental factors such as exposure to some toxic chemicals (heavy metals, pesticides, persistent and nonpersistent organic pollutants) can lead to neurological disorders (**Saghazadeh and Rezaei, 2017; Voorhees *et al.*, 2017; Guo *et al.*, 2018 b; Jeddi *et al.*, 2016**) respectively.

Early diagnosis and treatment for autistic patients exactly appear to improve their outcomes like a decreased need for special education and an increase in their independence (**Elder *et al.*, 2017**). Pharmacological, behavioral and nutritional interventions have been identified to minimize symptoms in autistic children (**Fung *et al.*, 2016; Cekici and Sanlier, 2019**). Meanwhile, the GI disturbances, intestinal mucosal abnormalities and altered intestinal microbiome (**Fulceri *et al.*, 2016; Li *et al.*, 2017**) extremely potentiate dietary intervention together with minimal or no side effects.

Adequate nutrition ensures normal central nervous system development and a diet rich in certain nutrients like omega 3 fatty acids keep mental function (**Lyall *et al.*, 2014**). Besides, adequate nutrients and nutrition have been documented to be essential in regulating molecular mechanisms that maintain synaptic function and plasticity **(Maynard and Mantini, 2017**). Moreover, cell proliferation, hormones and neurotransmitters' metabolism, and deoxyribonucleic acid (DNA) synthesis in the brain noticeably depend on sufficient nutrients (**Onaolapo and Onaolapo, 2018**). Previous studies have indicated that protein malnutrition and deficiencies of iron and iodine in early life predispose children to compromised growth and cognition (**Dosman *et al.*, 2007**). In this context, **Fujiwara *et al.* (2016)** found a strong correlation between malnutrition and autism.

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Ornoy *et al.* (2015) agreed with Fujiwara, they reported that obese mothers are liable to have autistic offsprings, On the other hand, specific maternal nutritional deficiencies are associated with increased ASD in their offsprings (**Surén *et al.*, 2013**). Additionally, experimental studies have demonstrated that propionic acid, a dietary short-chain fatty acid, induced a neuroinflammatory response and behavioral changes similar to those of ASD (**El-Ansary *et al.*, 2012**). Collectively, autistic children have higher food selectivity with a concomitant imbalance of nutrients levels in their blood (**Esteban-Figuerola *et al.*, 2019**) and the importance of dietary intervention is a must. So, our aim is to discuss nutritional and environmental factors involved in the occurrence of autism focusing on dietary intervention either by various dietary supplements or by elimination diets that may be effective for children with ASD.

2. Materials and Methods

PubMed database was used for the collection of data for the study in 2019. No limitation for the publication period was considered. Keywords were selected from the Medical Subject Headings and combined as "Autism Spectrum Disorder", "environmental factors", "nutritional factors", microbiota","mTOR", "dietary supplements" and "elimination diets".

Etiology of ASD

Environmental Factors

Recently, environmental factors have been involved in the etiology of ASD. There is an increasing growth in studies associating with environmental factors with ASD. Maternal environmental and or occupational exposures or both are non–genetic factors that may act during the prenatal period and cause neurodevelopmental deficits among which ASD has been introduced. Prenatal exposure to ambient air pollutants has been associated with the risk of ASD (**Gong *et al.*, 2017**). Residence in the proximity of areas with high levels of air pollutants emitted from industrial processes, biogenic sources, vehicular exhaust, and combustion products increases maternal risk of giving birth to an autistic child (**Weisskopf *et al.*, 2015**). It is obvious that exposure to air pollution and its components, both in the prenatal period and in early postnatal life, has been linked to poor developmental outcomes. **Kalkbrenner *et al.* (2014) and Karimi *et al.* (2017)** stated that some environmental exposures are linked with autism, especially traffic-related air pollutants, some metals, and several pesticides, with suggestive trends for some volatile organic compounds (e.g., methylene chloride, trichloroethylene, and styrene) and phthalates. They also concluded that other chemicals could not be ruled out. **Jeddi *et al.* (2016**) found a significant association between phthalate exposure and risk of ASD, whereas **Brown *et al*. (2018)** found no association between polychlorinated biphenyls (PCB) and risk of ASD.

Heavy metals are recognized as neurodevelopmental toxins which can lead to neurological defects, developmental delays, learning disabilities and behavioral abnormalities **(Gorini *et al.*, 2014). Mortazavi *et al.* (2016)** introduced the hypothesis that maternal exposure to electromagnetic fields increases the release of mercury from dental amalgam fillings. They suggested that such a rise in the level of mercury may be a possible mechanism for high rates of autism in offsprings. Blood levels of mercury, arsenic, cadmium, and lead has been investigated in one hundred eighty unrelated children with ASD and 184 healthy controls (**Li *et al.*, 2018**). Data showed that the children with ASD had significantly ($p < 0.001$) higher levels of mercury and arsenic and a lower level of cadmium. The levels of lead did not differ significantly between the groups. The results of this study are consistent with several previous studies, supporting an important role for heavy metal exposure, particularly mercury, in the etiology of ASD (**Talbott *et al.*, 2015; McCaulley, 2019**). This could be explained by the possibility that women with chronic metal exposure accumulate high tissue levels of mercury and other metals. Consequently, they may pass potentially toxic metals to their fetuses or intoxicate infants during nursing. Molecular mechanisms by which metals trigger neurobehavioral disorders and, specifically, ASDs are still not completely clear. In general, some environmental factors or perinatal complications might cause a pro-inflammatory state and oxidative damage in the brain and subsequently lead to alterations in neural growth and development (**Goines and Ashwood, 2013**). High body burden of toxic metals in autistic patients is associated with oxidative stress and increased levels of the ratio of glutathione disulfide (GSSG) to glutathione (GSH) (**Adams *et al.*, 2011**). Glutathione-S-transferase (GST) genes and enzymes play a major role in detoxification of several heavy metals. A recent systematic review suggested gender-related differences in the susceptibility to metals, with boys generally more susceptible than girls (**Llop *et al.*, 2013**). Metals such as lead, mercury, and cadmium have the ability to interfere with physiological thyroid hormone levels (**Chen *et al.*, 2013**). The interaction of metals with hormones and neurotransmitters may represent one of the neurotoxicity mechanisms involved in ASDs (**Hall and Kelley, 2014**). **Kinney *et al.* (2010)** reported that some environmental factors such as certain toxins and vitamin D deficiency increase the risk of a gene mutation that in turn can lead to an increased risk of ASD. Moreover, other environmental factors interact directly with neurotransmitter pathways. For example, lead disrupts the activity of N-methyl-D-aspartate receptors (**Neal *et al.*, 2010**).

More work is mandatory to estimate the effect of various chemicals and xenobiotics on the occurrence of ASD. Exposure to harmful environmental factors can alter the expression of developmental key genes in critical periods of embryo formation and raises the potentiality of genomic imprinting diseases such as autism (**Karimi *et al.*, 2017**). None of the environmental factors alone are sufficient to induce autism, but rather a collection of them can be involved in the incidence of autism (**Kim and Leventhal, 2015**).

Nutritional Factors

The role of nutrition in ASD etiology begins as early as the prenatal period. Maternal nutrients deficiency has been strongly associated with increased risk of schizophrenia, neural tube defects and many neurodevelopment disorders (**Furuse *et al.*, 2017**). Nutritional deficiencies are particularly increased during gestation due to elevated metabolic needs imposed by the fetus, placenta, and maternal tissues and have proven to influence fetal brain development in terms of function and structure. Therefore, a pregnant woman's diet could affect brain development which may increase autism risk. However, relatively few studies have been dedicated to understanding how maternal dietary factors could influence offspring brain development. **Schmidt *et al.* (2011)** suggested strong protection of prenatal high levels of folic acid from autism. Moreover, the risk of autistic disorder was 40% lower among those whose mothers administered folic acid supplements, 6 weeks before and after conception (**Surén *et al.*, 2013**). Also, it was proven that pregnant women who are obese or suffering from diabetes may give birth to an autistic child (**Connolly *et al.*, 2016**).

Maternal fish intake may also be important for ASD, as a source of essential fatty acids and vitamin D. Children of mothers with increased intake of omega 3 fatty acids before and during pregnancy had reduced risk of ASD relative to children of

mothers of low intakes of omega 3 fatty acids (**Lyall *et al.*, 2014**). In contrast, **Lyall *et al.* (2013)** found no association between prenatal fish oil and ASD.

Low maternal and fetal vitamin D levels have been proven as a risk for ASD (**Ali *et al.*, 2019**). Vitamin D insufficiency in mothers has been linked to impaired language skills in their children at ages 5-10 years (**Whitehouse *et al.*, 2013**). Vitamin D influences neuronal differentiation, metabolism of neurotrophic factors and neurotoxins, protection from brain inflammation, endocrine function and fetal brain growth. Also, vitamin D can decrease the risk of viral infection for pregnant woman. Infectious disease during pregnancy adversely influences brain development that may lead to autism. Vitamin D level is important, especially in the third trimester of pregnancy when the fetal brain develops (**Larqué *et al.*, 2018**). Thus, vitamin D supplementation during pregnancy can confer protection against autism either directly or indirectly by aiding proper brain development and enhancing the immune system, respectively. Moreover, during the perinatal period, maternal diet plays a crucial role in the maturation of the vital organs and neuronal connections. If nutrition is deficient of specific micro or macronutrients or overloaded with excess calories, many developmental disorders can be devastating and long-acting because the brain is especially sensitive to prenatal nutrition. Autism is hypothesized to be attributed in part to such factors that may date back to very early life (**Moody *et al.*, 2017**).

The gut microbiota

The human gut contains up to 100 trillion micro-organisms including different species of bacteria. Bacteroidetes and Firmicutes phyla are the most predominant bacterial species in the human microbiota (**Eckburg *et al.*, 2005**). Although the exact etiology and pathology of autism still not obvious, gut-brain interactions have received certain attention. There is a complex bidirectional axis between the gut microbiota and the brain (**Liu *et al.*, 2019**). Communication along this axis principally shows how signals from gut microbiota affect brain function, and on the other hand, brain messages influence microbiota activity and other GI functions. This bidirectional communication is mainly achieved through neuroendocrine and neuroimmune mechanisms (**Mayer, 2011**). In autistic patients characteristic neurological deficits are generally associated with various GI symptoms (**Adams *et al.*, 2011**). It was documented that in ASD, the intestinal inflammation is often accompanied by elevated neuroinflammatory markers and reduced serotonin levels in the brain (**de Theije *et al.*, 2014**). Such deficits and symptoms may be initiated from dysbiosis or microbial imbalance which could disturb the coordination of the gut microbiota-bran axis (**Pulikkan *et al.*, 2019**). Metagenomic analysis proved that autistics have a decrease in Bacteroidetes, an elevation in the ratio of Firmicutes to Bacteroidetes, and an increase in Betaproteobacteria (**Parracho *et al.*, 2005**). Moreover, Clostridia, Bacteroidetes, and Desulfovibrio are common bacteria that may promote GI symptoms and neurological autistic behaviors (**MacFabe, 2012**). They not only modulate the intestinal immune system but also produce certain metabolites that contribute directly to autism pathology. For instance, Colistridium produces a potent neurotoxin that manifests a wide variety of behavioral deficits seen in autism (**Bolte, 1998**). Besides, it's another metabolite,3-hydroxyphenyl-3-hydroxy propionic acid (HPHPA) depletes catecholamines in the brain, consequently, genus Clostridium is strongly correlated with the etiology of autism (**Kesli *et al.*, 2014**).

Generally, the colonization of gut microbiota commences at the time of birth on exposure to vaginal microbiota. The host genomic significantly influences microbiota activity and diversity. Also, environmental factors that include, infections, diet, stress, diseases and antibiotics may alter microbiota natural composition (**Nicholson *et al.* 2012**). Additionally, adult maternal immune activation results in fetuses that have ASD features, dysbiosis in gut microbiota and an altered blood metabolic profile (**Hsiao *et al.*, 2013**). Besides, recent studies have documented that probiotics and prebiotics administration may be an effective therapy for autistics via modulation gut microbiota (**Parracho *et al.*, 2010**; **Liu *et al.*, 2016**) respectively.

The mammalian (mechanistic) target of rapamycin (mTOR)

ASD exhibited aberrant expression of various genes, but those involved in the mTOR signaling pathway like Neurofibromatosis type 1 (NF1), Tuberous sclerosis proteins 1 and 2 (TSC1, TSC2), Phosphatase and Tensin homologue (PTEN), and Fragile X mental retardation protein (FMRP) are the most associated with autism. Single gene mutations result in enhanced mTOR activity in the brain of the ASD model (**Ehninger and Silva, 2009**). Consequently, an increase in the phosphorylation of proteins is observed with concomitant elevation of neuroligins that participate in the formation and maintenance of synapses between neurons. This caused an increased synaptic excitation/inhibition ratio which may be a risk for ASD (**Wang and Doering, 2013**). Additionally, the mTOR-signaling pathway has a potential role in directing the immune responses. **Kim *et al.* (2008)** proved that mTOR complex1 (mTORC1) when activated in mast cells results in survival, differentiation and cytokine production. Moreover, mTOR activation is shown to markedly attenuate autophagy (**Yu *et al.*, 2010**) in the intestine that is essential to limit intestinal inflammation. Therefore, a loss of both immune regulation and intestinal barrier integrity resulted from mTOR hyperactivity, thus, in ASD distribution of the mTOR signaling pathway could disrupt immune response, gastrointestinal tract, and brain. By this way, mTOR signaling in ASD may be considered as an important factor in the gut-immune-brain axis (**Van Sadelhoff *et al.*, 2019**). Therapeutic strategies for autism could manage the signaling pathway. **Kotajima-Murakami *et al.* (2019)** documented that pharmacological treatment by rapamycin successfully recovered mutations in the expression of some mTOR genes with consequence improvement in social communication in the ASD model. However, intervention with some nutrients like amino acid may manipulate the mTOR signaling pathway with minor or no adverse effects. They are capable to inhibit mTOR, inflammation, improve gut barrier function and normalize microbiota composition and immunity in the ASD patients (**Van Sadelhoff *et al.*, 2019**). Interestingly, cellular levels of different amino acids are maintained through the mTOR signaling pathway and the disturbance of this pathway significantly dysregulates amino acids that is strongly associated with autism (**Maynard and Manzini, 2017; Smith *et al.*, 2019**)

Nutritional assessment of autistic patients

Several studies have high lightened that individuals with autism are nutritionally vulnerable because they have a picky eating pattern and sensory sensitivity; both predispose them to restricted dietary intakes (**Emond et al., 2010**). Nutritional assessment includes dietary, anthropometric and biochemical evaluations for autistic patients. In addition, clinical examination that assesses the patients for signs which are consistent with nutrient deficiencies. Finally, the environmental factors assessments that affect the nutritional status, such as socioeconomic status and lifestyle (**Ranjan and Nasser, 2015**).

Dietary measurements

They include both qualitative and quantitative assessments of dietary intake which could determine adequacies and inadequacies inpatient nutrient intake. Consequently, nutritional status can be evaluated. The majority of autistic children exhibited nutritional challenges that include difficulty accepting new foods, late acceptance of solid food, restricted intake of food based on its color, texture, appearance etc, meal time presentations difficulties like position of food on a plate or even the type of plate, increased sensory sensitivity, disruptive mealtime behaviors such as not eating with family, eating the same foods and refusing the change and finally, the pica behavior. Autistic children are called picky eaters; however, this habit does not correlate with a lack of appetite (**Williams *et al.*, 2000**). They more frequently accept food of low texture and highly energetic and particularly refuse vegetables. Although they eat less variety of foods, their total calories, carbohydrates or fat intakes are not statistically different as compared with normally developing children (**Emond *et al.*, 2010**) indicating that their satiety mechanisms are not impaired. Their protein intake is approximately similar to normal children, but the nondairy protein intake was increased in ASDs children (**Zimmer *et al.* 2012**). Therefore, it is obviously clear that macronutrient deficiencies are often not present in children with autism (**Herndon *et al.,* 2009**). Besides, a substantial number of autistic children had inadequate intakes of calcium, zinc, iron, and vitamins; D, C, E, riboflavin, B12, and folic acid and choline, which may be attributed to less consumption of vegetables, fruits and salads with concomitant reduction of these essential micronutrients (**Bandini *et al.,* 2010**). In the contrary vitamin B6 intake was found to be significantly higher in autistic children than typically developing children. However, deficiencies of micronutrients in autistic children could be overcome by giving those fortified foods rather than additional vitamins or food supplements.

Anthropometric measurements

Anthropometric assessments include height, body mass index (BMI) and head circumference (HC). Early recognition of such measurements will serve as a noninvasive, inexpensive, and objective method of nutritional status evaluation. Such measurements have been carried out with autistic children and compared them with typically developing controls. An early warning signal of vulnerability to autism may be an abnormally accelerated rate of growth (**Courchesne *et al.,* 2001**). This growth abnormality may be attributed to nonspecific expression of biological abnormalities that are present in these disorders. There are inconsistent results in the prevalence of obesity in autistic children. Some studies reported an overweight or obesity prevalence in ASD patients, which was similar to nonautistic children (**Curtin *et al.,* 2010**). Other studies found a higher prevalence of obesity in autistic children than controls (**Egan *et al.,* 2013**) which may be explained by their unusual dietary patterns that are accompanied by decreased access to appropriate physical activity. Concerning HC, studies showed that autistic children have an atypical head growth pattern that is at birth, they have a normal HC, followed by an increase in the rate of growth of HC until one year of age. Then, there is a rapid decrease in HC between 12 and 24 months, which is normal as compared with controls (**Redcay and Courchesne, 2006**). Many authors have postulated that this abnormally accelerated growth in HC is attributed to dysregulation of growth in general. The relation between HC and height is inconsistent. Some studies stated that HC is relatively increased in comparison with height (**Grandgeorge *et al.,* 2013**), others reported that HC is normal (**Mraz *et al.* 2007**) or smaller (**Schrieken *et al.* 2013**) relative to height.

Biochemical measurements

Estimations of nutrients and nutrient-related substances in biological specimens are important in the diagnosis of autism before the clinical signs or symptoms are apparent. Besides, the obtained knowledge of this analysis helps to determine the treatment plan and monitor its effectiveness. Autistic children have decreased concentrations nearly below the reference range of folate and vitamin B_{12} (**Ali *et al.,* 2011**), pantothenic acid, biotin and vitamin E (**Adams *et al.,* 2011**) and vitamin D (**Meguid *et al.* 2010**). Meanwhile, vitamin B6 has an elevated and broad distribution in autistic children that is very fascinating. The highest concentration of vitamin B6 may be explained by a low activity of pyridoxal kinase that converts pyridoxal and pyridoxine into the active form pyridoxal 5-phosphate (PSP). Compared with controls, autistic children have lower concentrations of lithium, calcium, magnesium (**Adams *et al.*, 2011**), iodine and chromium (**Adams *et al.,* 2006**) and selenium (**Lakshmi Priya and Geetha, 2011**). The significant correlation between vitamin D (25-hydroxyvitamin D) and calcium supports the idea that autism is a vitamin D deficiency disease (**Meguid *et al.*, 2010**). By contrast, copper, phosphorus, and boron were elevated in autistic children (**Adams *et al.*, 2011; Ranjan and Nasser, 2015**) and also mercury and lead. On the other hand, the prevalence of iron deficiency and anemia in ASD patients is reported (**Bilgiç *et al.*, 2010**). However, iron deficiency results in impaired cognition and developmental defects which could further compromise their behavior and communication. Concerning amino and fatty acids, autistic children have lower levels of plasma tyrosine and tryptophan (**Adams *et al.*, 2011**) which may impair serotonin synthesis that has an important role in the neurogenesis and also in neurotransmission. The contributory factors to such decreased amino acid levels may be decreased protein intake or digestion by autistic children. Autistic patients have elevated glutamate levels in plasma (**Aldred *et al.*, 2003**) that may be closely related to the behavioral changes in autism. Moreover, omega 3 polyunsaturated fatty acid concentration is significantly lower in children with autism (**El-Ansary *et al.*, 2011**).

Dietary interventions

Dietary intervention mostly includes either: Dietary supplements or Elimination diets or both together (**Adams *et al.*, 2018; Fraguas *et al.*,2019**). Successful dietary interventions could quickly relieve the autistic symptoms and are usually used as complementary with conventional pharmacological drugs.

Dietary supplements

Folic acid and vitaminB_{12} supplements

Both folic acid and B_{12} participate in the methionine cycle that involves the regeneration of methionine through the transfer of the methyl group from 5-methyltetrahydrofolate.
Methionine forms S- adenosylmethionine (SAM) which is the primary methyl donor for DNA, Ribonucleic acid (RNA), phospholipids, proteins, and neurotransmitters. Another important role for the methionine cycle is the production of glutathione, a crucial antioxidant compound. Vitamin B_{12} and folic acid deficiencies were seen in many autistic children (**Ali *et al.* 2011**). Moreover, patients with autism exhibited cerebrospinal fluid (CSF) deficiency of folic acid that may be attributed to the action of serum antibodies against foliate receptors. These antibodies bind folic acid receptors with

concomitant inhibition of folic acid synthesis and reduction level in CSF (**Ramaekers *et al.,* 2005)**. In this view, **Mckee *et al.* (2017)** pointed out that dietary methyl donor supplementation in early life can change cognitive performance and motivation. Meanwhile, vitamin B_{12} deficiency may result from a digestive cause, especially rare consumption of animal sources. **Pineles *et al.*** (2010) demonstrated reversible optic nerve neuropathy in autistic patients via vitamin B_{12} replenishment. Hence early detection of folic acid and vitamin B_{12} deficiencies may be an essential contributory factor in preventing ASD or in determining the therapeutic interventions for autistic ones. Also, a diet rich in these nutrients or supplements may be supportive of pharmacotherapy.

Vitamin C supplement

Vitamin C is essential for the synthesis of neurotransmitters and via its antioxidant power, it protects the brain and nervous tissue against free radicals. **Planerova *et al***. (2017) demonstrated that ASD patients may have scurvy, which results from vitamin C deficiency which was a consequence of typical food consumption by those patients. **Malhi *et al.*** (2017) agreed with the previous authors in that ASD children failed to achieve vitamin C requirements. Moreover, moderate doses of vitamin C with other vitamins may affect sleep disorders and gastrointestinal troubles in ASD patients (**Adams and Holloway, 2004**). Besides, vitamin C supplementation in an autistic patient with normal or low serum vitamin C level, may exhibit a positive impact concerning the pathological behavior through prevention of dysregulation of glutamatergic signaling of the brain, consequently, reducing brain inflammation (**Blaylock and Strunecka, 2009**). Vitamin C could be complementary to conventional therapy taking into consideration its tolerance in autistic patients, therefore, continuous monitoring is essential.

Vitamin B_6 supplement

Dietary vitamin B_6 supplements were proven to improve behavior in autistic children (**Martineau *et al.*** 1985). Meanwhile, **Wong and Smith (2006)** demonstrated that no marked benefits of vitamin B_6 administration in ASD children. However, vitamin B_6 has an essential role in CNS as it participates in neurotransmitter synthesis like serotonin, dopamine, and epinephrine which may be altered in autistic children. Therefore, vitamin B_6 supplementation could be beneficial for autistic patients, taking into consideration to adjust its doses because high blood levels are accompanied by low activities of both kinase and oxidase enzymes that transform vitamin B_6 to the active form PSP.

Vitamin A and D supplements

Many autistic patients have vision problems besides other behavioral and clinical symptoms. Hence, the administration of vitamin A may be helpful (**Uyanik, 2006**). In agreement with the previous author, **Megson (2000)** demonstrated that vitamin A supplement is effective in reducing autistic symptoms since there was an absence of specific genes in ASD patients encoding an essential protein for vitamin A synthesis. Moreover, **Guo *et al.* (2018 a**) proposed that vitamin A supplementation may improve symptoms and reduce 5-hydroxytryptamine(5-HT) levels in autistic children. Hence, vitamin A supplementation is a rational therapy for children with autism. Concerning vitamin D, it was proven that adequate intake of this vitamin from a diet or as a supplement may reduce the risk of autism by providing the proper development of the brain and immune system. It also has a neuroprotective effect and influence many neurotransmitter interactions (**Meguid *et al.*, 2010**). **Jia *et al.* (2015**) concluded that vitamin D3 may play a significant role in the etiology of ASD. In this context, **Feng *et al.* (2017)** reported that autistic children exhibit clinical improvement after vitamin D_3 supplementation. While **Kerley *et al.* (2017)** reported that vitamin D supplementation did not affect the primary outcome with limited and incompatible effects in children with ASD. This may be attributed to vitamin D activity and dose.

Probiotics supplement

Autistic patients frequently suffered from several gastrointestinal disturbances like diarrhea, constipation, and inflammation. Therefore, the use of probiotics may be beneficial as they help to restore the normal intestinal microflora and normal intestinal epithelial cells, consequently reducing gastrointestinal disturbances. Moreover, probiotics could increase the utilization of food ingredients and vitamin synthesis by the body that may be helpful for autistic patients who have multiple nutrient deficiencies. Besides, probiotics enhance immunity and inhibit many pathogens developments (**Galdeano *et al.*, 2019**). Probiotics are also supposed to improve intestinal permeability, enhance the attainment of a balanced intestinal microflora, and alter the mucosal immune response (**Critchfield *et al.*, 2011**).

Mineral supplement

Disturbed cognitive function and concentration, mood changes, and slow growth may be associated with iron deficiency in autistic children. In addition, decreased serum iron levels can cause sleep and nervous system disorders which were significantly improved with oral iron supplements (**Dosman *et al.*, 2007**). Also, autistic patients appear to be at risk for zinc (Zn) deficiency (**Sweetman *et al.*, 2019**), Copper (Cu) toxicity and have low Zn/Cu ratio that predisposes the body to oxidative stress. Hence, zinc supplementation is required treatment for autism (**Isaacson *et al.*** 1996). Taking into consideration that it is important to estimate and follow the levels for both Cu and Zn together during Zn therapy because these two trace elements are antagonistic in function, and essential for living cells (**Bjørklund, 2013**). Moreover, selenium deficiency was documented in autistic children (**El- Ansary *et al.*, 2017**) therefore, it should be supplied.

Amino acid supplement

Amino acids play a crucial role in the brain as they are precursors to neurotransmitters or they behave as neurotransmitters themselves. Serotonin plays essential role in both brain and intestinal development. Since the brain-gut axis disturbances are a major complication in autistic children; serotonin may modulate these changes (**Margolis, 2017**). Tryptophan supplementation potentiates the production of serotonin. Taurine has antioxidant properties and is associated with improved visual symptoms. Also, L-carnosine has been shown to improve vocabulary, total score and behavior in autistic children (**Chez *et al.*, 2002**). It is obvious that the neurotransmitter imbalance in the central nervous system (CNS)could participate in autism pathophysiology. There was an increase in glutamate levels in autistic children as a result of the upregulation of glutaminergic gene expression. In this concern, the oral administration of N-acetylcysteine exerts an anti glutaminergic effect besides its antioxidant power through glutathione production (**Dean *et al.*, 2011**). Besides, the aforementioned potential role on mTOR that exerted by many amino acids.

Polyunsaturated fatty acids (PUFA) supplement

Phospholipids in the brain and retina are rich in PUFA, especially n-3 PUFA such as docosahexaenoic (DHA). DHA converted to oxylipins by 15-lipoxygenase. Oxylipins could regulate cell redox homeostasis and neurotransmitters signaling pathways. There is evidence that inadequate consumption of maternal DHA may be a risk of impaired CNS function. Also. DHA intake above the nutritional requirement may modify the risk of many CNS diseases (**Sun *et al*., 2017**). It was found that ASD populations have decreased DHA levels and, hence, n-3PUFA supplementation can noticeably improve ASD symptoms (**Mazahery *et al*., 2017**). In contrast, **Politi *et al.* (2008)** observed no significant improvement of disease severity and frequency, after omega-3supplementation in adult patients with ASD. On the contrary, **Cheng *et al.* (2017)** suggest that supplementation of omega 3 fatty acids may improve hyperactivity, lethargy, and stereotypy in ASD patients. Also, a comprehensive nutritional and dietary intervention with DHA and eicosapentaenoic acid (EPA), vitamin A, B complex, folic acid and coenzyme Q10 for autistic children, showed a significant improvement in autism symptoms and developmental age **(Adams *et al.*, 2018)**. Further trials are required to explore the potential advantages of omega 3 fatty acid supplementation in ASD patients.

Elimination diets

These diets were designed to reduce or even completely remove foods or food additives in ASD patients, they include:

Gluten-free diet and/or casein-free diet

Patients with ASD have gastrointestinal tract troubles that might be due to increased intestinal permeability. Digestion of casein and gluten generates peptides that can reach the bloodstream through the leaky gut and bind to the opioid receptors, causing deleterious CNS effects (**Reichelt *et al*., 1990). Hyman *et al*. (2016)** do not provide evidence to document the general use of the gluten-free /casein-free diet**.**

Ketogenic diet

It contains high fat, low carbohydrate, and low protein concentrations consequently provide about 90% of energy from fat. The ketogenic diet could reduce the symptoms in the autistic patients with a significant improvement in communication ability (**Evangeliou *et al*. 2003**). The ketogenic diets may improve social effect in children with ASD (**Lee *et al*., 2018**).

Specific carbohydrate diet

This diet mainly contains monosaccharides from fruits, honey or vegetables, while complex carbohydrates are limited. It is used to alleviate the malabsorption and growth of pathogenic intestinal microorganisms (**Gottschall, 2004). Barnhill *et al.* (2020)** declared at the 16-week intervention with the specific carbohydrate diet protocol was well tolerated in a 4-year-old child diagnosed with ASD and Fragile X syndrome, improving growth status, gastrointestinal symptoms, and behaviors.

Low oxalate diet

In autistic children, gastrointestinal dysfunction permits some substances like oxalate to cause abnormalities in their CNS. Autistic patients have high blood levels of oxalate about 3 fold the reference value with concomitant increased risk for ASD (**Konstantynowicz *et al.*, 2012**). Therefore, the autistic patients should restrict oxalate containing food like spinach, figs and green apples.

3. Conclusion

ASD accounts for substantial social and financial burden across the lifespan. The plausibility of the role mTOR signaling pathway of nutrition and environment as risk factors for ASD is growing. It is obvious that both factors appear to be causal and not co-founded. There is a link between autism and gastrointestinal disorders. Therefore, parents and their assistants should take into consideration the benefits of nutritional intervention for their autistic patients. Dietary supplements of the nutritional deficiencies of autistic patients are essential. Supplements of omega-3 fatty acid, amino acids, probiotics, minerals, and vitamins may be required in combination with medical and psychological treatments. Besides, a suitable elimination diet that were tailored to each patient according to one's symptoms may relief both autism symptoms and gastrointestinal disorders. It is desirable to continue future research into the relationship between ASD and maternal environmental pollutants' exposure and nutritional status. The reduction of environmental chemical exposures and considering nutrition as an important determinant in autism opens new avenues for lowering the risk of ASD. Large-scale epidemiological studies are needed to confirm the existing findings.

Declaration of interest

The authors have no conflicts of interest.

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