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Review Article

The Effects of Dietary Approaches and Nutritional Supplements in Autism

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Abstract

ASDs (Autism Spectrum Disorders); is a neurodevelopmental disorder in which the symptoms and effects are different in each individual and there is an increase in prevalence worldwide. The increase in ASDs prevalence may be due to the increase in diagnostic criteria. The causes of ASDs are thought to be genetic, environmental and neurobiological factors. In recent years, studies have been conducted on the effect of dietary approaches on improving ASDs symptoms. Researchers are studying on dietary approaches based on the interaction between the brain and the gut. This review includes studies on the most popular dietary approach, the gluten-free and casein-free diet, and the effects of Omega 3, vitamin D, vitamin B12, folic acid and probiotic supplements on ASDs core symptoms. The results of the studies are evaluated according to special ASDs symptom criteria scores. The limited number of studies, the low number of participants in some studies and the lack of significant improvements are seen as limiting aspects of the studies.

Keywords: Autism, ASDs, Diet, Supplementation.

Beslenme Yaklaşımlarının ve Besin Takviyelerinin Otizm Üzerindeki Etkileri

Öz

ASDs (Autism Spectrum Disorders); semptomların ve etkilerinin her bireyde farklı olduğu nöro-gelişimsel bir bozukluktur ve prevelansında dünya genelinde artış söz konusudur. ASDs prevelansındaki artışın sebebi tanı kriterlerinin artışı ile olmuş olabilir. ASDs'nin sebeplerinin genetik, çevresel ve nörobiyolojik faktörler olduğu düşünülmektedir. Son yıllarda, beslenme yaklaşımlarının ASDs semptomlarını iyileştirmeye etkisi üzerine çalışmalar yapılıyor. Araştırmacılar beyin ve bağırsak arasındaki etkileşimi baz alarak beslenme yaklaşımları üzerine çalışıyorlar. Bu inceleme en popüler diyet yaklaşımı olan glutensiz ve kazeinsiz diyet ve omega 3, D vitamini, B12 vitamini, folic acid ve probiyotik takviyesinin ASDs çekirdek semptomları üzerine etkisinin incelendiği çalışmalar içeriyor. Çalışmaların sonuçları özel ASDs semptom kriter skorlarına göre değerlendiriliyor. Yapılan çalışmaların azlığı, bazı çalışmaların katılımcı sayısının azlığı ve önemli gelişmeler görülememesi ise çalışmaların sınırlandırıcı yönleri olarak görülüyor.

Anahtar Kelimeler: Otizm, ASDs, Diyet, Takviye.

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1. Introduction

Autism spectrum disorders (ASDs) are a complicated developmental disorder that include permanent challenges in speaking, nonverbal communication, social interaction and repetitive/restricted behaviors. Symptoms' severity and the effects of ASDs are diverse in each individual (American psychiatric association, 2022). Diagnosing autism spectrum disorder (ASD) presents challenges. There is no blood test or medical test available for the purpose of diagnosing this condition. (Centers for Disease Control and Prevention, 2022). Savant skills ,which is special skills, like high memory power, extraordinary artistic and musical abilities and mathematical calculations are seen around 10% of the autistic people (Bhat, et al., 2014).

1.1.Prevalance

One in 100 children has ASDs worldwide according to data. (World Health Organization, 2023). Number of cases in low-income countries are undetermined due to a lack of diagnostic tools and assessment (Sauer, et al., 2021).

The prevalence of autism spectrum disorders (ASDs) is approximately four times higher in boys compared to girls and it occurs in all socioeconomic, racial and ethnic groups (Baio and friends, 2018). Owing to the lack of accurate statistics in Turkey, it is though that there are an estimated 550,000 people with autism and roughly 150,000 children with autism in the group of 0-14 age according to the data predicted by the Autism Platform in previous years (Otizim Dernekleri Federasyonu, 2013).

1.2.Etiology

It is believed that autism is a neurobehavioral disorder and many factors contribute to development of ASDs. (Inglese MD, Elder JH,2009). The available data indicate that autism is believed to result from a combination of various causal factors, including genetics, neurobiology, and environmental influences, which manifest in distinct behavioral symptoms (American Speech-Language-Hearing Association, 2022).

1.2.1. Genetic Factors

In family studies, it has been reported that there is a 60% concordance rate for classic autism among monozygotic twins, whereas there is no concordance (0%) among dizygotic twins. However, when a broader phenotype for autism is considered, the concordance rate among monozygotic twins increases to 92% (Inglese, et al., 2009). Furthermore, if a family already has a child with autism, the likelihood of having another child with autism increases by 25 times compared to the general population (Almandil, et al., 2019). These results indicate that genetics plays a significant role in the development of autism. Moreover, the findings emphasize that there are also likely to be environmental or non-genetic factors that influence the expression and severity of autism traits (Inglese, et al., 2009).

1.2.2. Neurobiological Factors

Children with ASD have abnormal brain connectivity which is shown with neuroimaging techniques. The brain's intrinsic connectivity is different in ASD subjects and normal subjects. The brain's intrinsic wiring potential is lower and this causes shorter geodesic distances (Bhat, et al., 2014).

Between the ages of 2 and 4, an atypical pattern of brain overgrowth occurs in specific regions such as the frontal lobe, cerebellum, and limbic structures. This abnormal growth is followed by a deceleration in brain growth. These regions of the brain are closely involved in the development of social, communication, and motor skills, which are impaired in individuals with autism spectrum disorders (ASDs). Abnormalities in the frontal brain mechanisms responsible for associating rewards with goal-directed activity have been found to be linked to social orienting deficits in individuals with autism spectrum disorders. In a clinical study, fronto-occipital head circumference was measured in 241 nonsyndromic autistic patients (Sacco R, Militerni R, 2007) and having a head circumference greater than the 75th percentile is associated with more pronounced impairments in adaptive behaviors. However, it is also associated with less impairment in measures of IQ, motor skills, and verbal language development. (Pardo and Eberhart, 2007).

1.2.3. Environmental Factors

Environmental factors may contribute to ASDs. A specific environmental factor hasn't been scientifically demonstrated that cause to ASDs until now. On the other hand, a few research projects have investigated the possible gene and environment interaction of ASDs. The most frequently mentioned factors are medications from environment, exposure to toxins and heavy metals (mercury, cadmium, or lead), intolerance to food (primarily those containing casein and gluten), immunization of child and mother during pregnancy, and perinatal incidence such as low birth weight, prematurity or anoxia. Additionally, other prenatal, perinatal and postnatal factors are investigated. (Inglese, et al., 2009).

The occurrence of stress during labor is connected to an elevated risk of hypoxia and cerebral hemorrhage in newborns. A study has demonstrated that individuals who survive cerebellar hemorrhagic injury are notably more prone to developing autism (Grabrucker, 2013). One of the critical factor for normal brain development is maternal nutritional status during pregnancy. A deficiency or a an excess of nutrients like omega-3, folic acid, vitamin D, zinc or iron can cause impaired neurodevelopment (Sauer, et al., 2021).

1.2.4. Vaccines

Vaccination is a hot topic discussed in many different situations around the world. Relationship between vaccination and autism is investigated in many research and various studies (Almandil, et al., 2019). There is not an evidence of a causal association between ASDs and rubella, mumps and measles vaccine according to the available epidemiological data's conclusion. Lots of methodological flaws found in previous studies that suggest a causal link between ASDs and vaccine. Also, there is not an evidence shows that another childhood vaccine may increase the risk of ASDs. Evidence reviews of the potential relationship between the risk of ASDs and inactivated vaccines which contain preservative aluminum and thiomersal adjuvants strongly concluded that vaccines do not increase the risk of ASDs (World Health Organization, 2023).

Meta-analysis by Taylor et al. included five cohort studies involving 1,256,407 children, and five case-control studies involving 9,920 children. The results of this meta-analysis indicate that there is no association between vaccinations and the development of autism or autism spectrum disorder. Additionally, the components of vaccines such as thimerosal or mercury, or multiple vaccines like MMR (measles, mumps, and rubella), are not linked to the development of autism or autism spectrum disorder. Taylor, et al., 2014).

2. Dietary Approaches and Nutritional Supplementation

Alternative medicine and complementary treatment strategies are searched by many patients with ASDs and their relatives. For example, dietary interventions used by 25% of people with ASDs such as elimination diets (gluten- and casein free diet is the most common one) and supplementation of omega-3, amino acids, vitamins, minerals, and herbal compounds. However, dietary interventions' results on the efficacy of in ASDs are still moot (Fraguas, et al., 2019).

Children with ASD generally do not consume enough of vitamins A, D and E, calcium, and folic acid. These children consume too many calories from either carbohydrates, protein, or fat (or the combination of these three nutrients) (Ismail, et al., 2020).

2.1. Gluten and Casein Free Diet

The hypothesis proposing that abnormal metabolism of gluten and casein may result in an excessive opioid activity in the central nervous system and subsequent alterations in its function has sparked interest in exploring the role of these two proteins. An increased intestinal permeability or ‘leaky gut’ is another potential mechanism. Leaky gut is a digestive condition characterized by the permeability of the intestinal wall, allowing bacteria and toxins to leak into the bloodstream. As a result of the abnormal function of the gut barrier, and potentially the blood-brain barrier, there is an enhanced permeability that allows the passage of gluten, casein, and their metabolites into the bloodstream and subsequently into the central nervous system. This proposed mechanism suggests a potential link between the gut and the central nervous system in the context of gluten and casein metabolism. Autistic symptoms development may be caused by that combined metabolic defect. In conclusion, GFCF (a gluten- free and casein-free) diet has been suggested as being potentially helpful for patients with ASDs (Piwowarczyk, et al., 2017).

Eliminating gluten from the diet involves avoiding all food items that contain wheat, oats, barley, or rye. This includes products such as bread, flours rusks, pasta, pastries, and other baked goods made with these cereals. Similarly, eliminating casein requires avoiding the intake of dairy products, including milk (including breast milk), cheese, yogurt, cream, butter and ice cream, among others (Marí-Bauset, et al., 2014).

In current systematic review by Piwowarczyk, et al., main purpose was resolving dubiousness about the role of GFCF diet in children with autism. Although new data may have been obtained, the previous overall conclusions have remained unchanged. There isn't consistent evidence that supporting the use of a GFCF diet in children with autism according to the limited available evidence. When existing evidence is interpreted, it should have been careful since the evidence is finite. High quality randomized control trials which involve multidisciplinary teams are needed for the clarified effects of GFCF diet on functional results and performance in children with autism (Piwowarczyk, et al., 2017).

In randomized control trial by Elder, et al., 15 autistic children which chronological age ranges between 2 and 16 selected as participants and the study continued for 12 weeks. No significant differences in CARS (Childhood Autism Rating Scale), urinary peptide levels of gluten and casein, or behavioral frequencies were observed according to the group analysis results. Besides that, there weren't significant differences in parent behaviors that observed statistically. Interestingly, there were a few anecdotal reports which were different from non-significant findings. For instance, decreased hyperactivity, improvements in child language and decreased tantrums were reported by seven children' parent. Also, it was decided by the parent of nine children to continue the child's GFCF diet although there wasn't a support. According to the unasked reports of respite worker and a teacher, behavioral and language improvements of two of the children were observed. Before unblinding, parents were asked whether their child was on the gluten-free casein-free (GFCF) diet during the first six weeks or the second six weeks. Five answers were right, two were "no idea" and six were wrong (Elder, et al., 2006).

Systematic review by Marí-Bauset, et al. included 32 studies involving 1346 individuals. It is concluded that supporting evidence of GFCF diets is weak and limited. Also, there are biomedical adverse effects of dietary restrictions such as stigmatization, social rejection, integration and socialization deficiency, and malfeasance of resources. Therefore, it is not recommended that using elimination diets to treat ASDs symptoms by this review's authors. It is generally not recommended to implement gluten and casein-free diets for individuals with autism spectrum disorder (ASD) unless there is clear evidence of the benefits, or if there is a diagnosed allergy or intolerance to these food components. As it turns out, the obtained results don't support the opioid theory. (Marí-Bauset, et al., 2014).

In study by Winburn, et al., it is explained that gluten and casein free diet prevalence in families of autistic children is investigated in several survey studies. These studies evaluate the parental perceptions regarding the impact of dietary interventions on the symptoms of ASDs. It is found that 41–69% of parents report positive effects when categorical questions were asked. In this survey, the aim was to survey 246 UK child health professionals working with autistic children and 246 UK parents of autistic children. The duration of the study was more than 10 months. For 76 children who have already started a GFCF diet and are still on a diet, it is reported that 'significant improvements' in the autistic behaviors according to the 5-point scale (5-point scale: significant improvement, improvement, no change, decline, significant decline). The most reported was gastrointestinal symptoms and it was 54%. Other symptoms that have been reported to have 'significantly improved' includes 42% attention and concentration, 29% communication, 25% social interaction and 20% repetitive behaviors and interests. Some improvement in gastrointestinal symptoms was reported by 22 parents of 29% of children on the GFCFD. Therefore, at least some improvement of gastrointestinal symptoms was experienced by the 83% of children (n = 63) on the gluten and casein free diet. No chance in social interaction was reported in 8 children and no change in communication was reported in 13 children from all 63 children. No change in repetitive behaviors and interests was reported in 20 children as a higher figure. Worsening aggression and anxiety were observed in 10 children. According to the statement, 79% of professionals express the opinion that there is insufficient evidence to support the benefits of a gluten-free and casein-free diet. Almost two thirds of professionals thought that they had enough information about gluten and casein free diet to discuss with the children' families. They reported that they would support families who wish to continue gluten and casein free diet, but they wouldn't recommend it to families specifically. Only 19% reported that they would recommend parents against the implementation of a diet that excludes gluten and casein. (Winburn, et al., 2013).

In controlled clinical trial by Hyman, et al., 14 autistic children were included, and their ages were between 3-5. Participants followed the diet for 4-6 weeks. Then, while they were continuing the diet, they were conducted a double-blind, placebo-controlled study lasting for a duration of 12 weeks. After that, they were followed for 12 weeks. This study findings must be evaluated with caution because sample size of study was small and there were limitations. As a result, this study doesn't provide evidence to support GFCF for the management of ASDs. In addition, findings of study showed that GFCF can be safe for autistic children if appropriately applied and followed for adequate nutrition although potential use of the diet for autistic children who have gastrointestinal symptoms is a question mark. The design of this study has been checked for the simultaneous use of therapy, which is known to be effective so that the effect of the diet is not associated with other interventions a child can receive. (Hyman, et al., 2015).

In randomized controlled trial by Knivsberg, et al., 20 participants were included. 10 children were in the diet group and other 10 children were in the control group. Diet group's mean age was 91 months and control group's mean age were 86 months. All participants were autistic children and their urinary peptide patterns were abnormal. Positive development was observed in all children on diet. Some of them were more than others. After one year, they had fewer autistic features which we saw as a direct result of dietary intervention. As expected in the control group, it is reported that autistic behavior fluctuated normally, good periods followed periods with more abnormal behavior, and opposite way. An exception was observed which is positive in one child in control group. The autistic features of the child disappeared in the experiment period according to his parents. Full recovery is uncommon for autism. It is assumed that, since the diet group's mean age is high, children's attention has increased, and the results of the nonverbal cognitive assessment have affected. It is likely that they were in a better position since they could utilize their skills due to the decrease in opioid activity and its associated impact. It was expected to have nearly the same result before and after the experiment duration in the control group and there wasn't an explanation for the reduce recorded. As far as is known, this hasn't been reported for autistic children previously. Communication and language education have been a positive development to be expected, as it is an important part of remedial programs for autistic children, for both groups of children. The improvement observed in the diet group was considerably more significant compared to the improvement seen in the control group. However, it can be speculated if the group's significant difference was seen in followed years. Motor impairments and strange motor behaviors are observed in autistic children. Many participants have severe motor impairments according to the results of tests, but this may also show that imitating motor behaviors is difficult for children. Combination of improved ability to imitate, to learn and improved skills may be a reflection of the diet group's improvement. Although the slight reduce in the control group cannot logically be clarified, two groups' development was importantly different. It is found promising that the improvement in diet group when the results were evaluated. However, further replications are necessary, and hopefully future investigations may bring more detailed and refined answers to how much it can be gained through diet intervention, that a subset of children with autistic syndrome can respond better than another (Knivsberg, et al., 2002).

2.2. Omega 3 Fatty Acid Supplementation

HUFA (highly unsaturated fatty acids), basically DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), must be supplied by nutrition because they can't be produced by the human body although the central nervous system is rich in HUFA. For brain's normal development and function, HUFA are essential. Certain adult neuropsychiatric disorders risk may be modified by dietary consumption of the long-chain omega-3 fatty acids, DHA and EPA (eicosapentaenoic acid), found in fish and fish oil (Cold-water fatty fish, such as mackerel, salmon, tuna, sardines, and herring contain high amounts of omega 3) in general. Evidence is increasing that fatty acid imbalances and insufficiencies can conduce to childhood neurodevelopmental disorders, including autistic spectrum disorders (Amminger, et al., 2007).

According to the review conducted by James S, et al., it is concluded that two trials with a total of 37 autistic children who were randomized into groups. One group received omega-3 fatty acid supplements, while the other group was given a placebo.

Outcome of social interaction: The results of both trials for social lethargy/withdrawal were evaluated according to ABC (Aberrant Behavior Checklist) subscale score (Bent 2011, Amminger 2007). There wasn't significant difference in the social

lethargy/withdrawal scores of ABC between the control group and the experimental group after combining results from both trials. One trial (Bent 2011) results showed no significant difference between the experimental group and control group, according to SRS (Social Responsiveness Scale) score.

Outcome of communication: the result of both trials for inappropriate speech score were evaluated according to ABC (Bent 2011, Amminger 2007). There wasn't significant difference in inappropriate speech scores of ABC between the experimental group and control group after combining results from both trials. One trial (Bent 2011) results showed no significant difference between the experimental group and control group, according to EVT (Expressive Vocabulary Test) scores and PPVT (Peabody Picture Vocabulary Test).

Outcome of stereotypy: The results of both trials for stereotypy were evaluated according to ABC subscale score (Bent 2011, Amminger 2007). There wasn't significant difference in stereotypy scores of ABC between the experimental group and control group after combining results from both trials.

Outcome of hyperactivity: The results of both trials for hyperactivity were evaluated according to ABC subscale score (Bent 2011, Amminger 2007). There wasn't significant difference in hyperactivity scores of ABC between the experimental group and control group after combining results from both trials.

Outcome of irritability: The results of both trials for irritability were evaluated according to ABC subscale score (Bent 2011, Amminger 2007). There wasn't significant difference in irritability scores of ABC between the experimental group and control group after combining results from both trials.

For these reasons, several rigorous studies on omega-3 fatty acids for ASDs have been completed and reported. This review included only two trials with a total of 37 participants. Data of the primary outcomes of social interaction, communication, and stereotypy, and the secondary outcome of hyperactivity were included and reported in this review. There wasn't a significant improvement statistically, but the largest positive effect was reported for the treatment of hyperactivity (James, et al., 2011).

In study by Meiri G, et al., 10 autistic children were included, and their ages were between 4 and 7. One gram of omega-3 fatty acids were given daily for the duration of 12 weeks. ATEC (Autism Treatment Evaluation Checklist) were used to measure the main outcome. According to ATEC, the improvement was averaged 33% from beginning to 12 weeks in 8 participants. In the first 6 weeks most of the improvement was observed. The same trend was observed in the CGI (Clinical Global Impression), CARS (Childhood Autism Rating Scale), and CPRS (Computerized Patient Record System). Only one child didn't respond and none of them worsened. In the autistic core symptoms, such as speech and sociability, some improvements were noted. There wasn't a specific parameter which is the improvement of children. However, a general improvement was observed in all of the symptoms of autism (Meiri, et al., 2009).

In a systematic review by Bent, et al., 143 potentially relevant articles were yielded by experts from bibliography reviews, database searches, and contact with experts. Six articles met all of the inclusion criteria and none of the exclusion criteria, thus all six were included in this systematic review. Out of all the studies, only one was a randomized controlled trial. (Amminger, 2007). 13 autistic children were got involved in this study. For a period of 6 weeks, they were assigned at random to consume either 1.5 g of omega-3 fatty acids daily or an identical placebo. The result was evaluated according to ABC. A greater progress was seen in the omega-3 group compared to the placebo group according to every subscale but, these changes didn't reach statistical significance. The stereotypy and hyperactivity showed the largest changes. The study was sound methodologically. On the Jadad scale, it received a rating of four out of five points. The randomization method wasn't described so the score was decreased by one point. Four open-label studies that included young adults or children with Asperger's or autism and, they were uncontrolled. In an open-label study (Politi et al. 2008), participants were 19 young adults with an average age of 29 and they were with moderate to profound intellectual disability, severe maladaptive behaviors and severe autism. All subjects received a daily dose of 0.93 g of omega-3 fatty acids (DHA, EPA) and a vitamin supplement containing 5 mg of vitamin E for a period of 6 weeks. Problematic behaviors' severity and frequency was assessed according to Rossago Behavioral Checklist for 18 weeks total. (6 weeks before, during, and after treatment). The authors did not find any improvement in the score measuring problematic behavior between the pre-treatment and treatment periods. Curiously, an improvement in both symptoms' severity and frequency were appeared in the period after treatment. Since there was no control group, it is unclear whether the lack of improvement can be attributed to the beneficial effects of omega-3 fatty acids or other factors. In another study (Meguid, 2008), Participants were 30 autistic children in National Research Center in Egypt. A combination of omega-3 (240 mg DHA, 52 mg EPA daily), omega-6 fatty acids (68 mg), and Vitamin E was given to them for a duration of 3 months. According to the reports, 20 out of 30 children showed improvement based on the CARS assessment. However, the average change in the overall group of 30 children was not reported. In another study (Patrick and Salik, 2005), participants were 22 children. They were given 247 mg of omega 3 fatty acids on a daily basis for a period of 90 days. Although a statistically significant increase was observed in subscales of learnin skills and basic language from day 0 to 90 by authors, raw data weren't presented. In another open-label study (Bell, 2004), Nine children with Asperger's or autism were given one of two different omega-3 supplements with varying doses for a minimum of 6 months. Any structured outcomes weren't assessed. On the other hand, progress in general health and various outcome measures were reported by parents. In a case report (Johnson and Hollander, 2003), participants were 11 autistic children. They had high levels of agitation and anxiety associated with compulsive rituals. Fish oils were given first and then increased up to 3 g/day (540 mg EPA). Clinicians and parents reported complete elimination of agitation and anxiety after only one week. This improvement remained stable over the 8-month follow-up period (Bent S, et al., 2009).

In a study by Ooi, et al., autistic 41 children and adolescents aged 7–18 years were included. At the post-treatment stage, significant improvements were observed in the social and attention problem syndrome scales of the CBC (Child Behavior Checklist)

and all subscales of the SRS (Social Responsiveness Scale). Changes in the autism core symptoms were correlated with fatty acid levels in blood significantly. In addition, the response to the omega-3 treatment was predicted by the baseline levels of fatty acid levels in blood. Participants tolerated supplementation of omega 3 fatty acids well and no serious side effects were observed. Findings of this study shows effectiveness of omega 3 fatty acids on ASDs (Ooi, et al., 2015).

In randomized controlled double-blind trial by Voigt, et al., 48 children between the ages of 3 and 7 were included. For half a year, they were given with either a placebo or a supplement that contained 200 mg of DHA. After 3 and 6 months, the investigator and the parents completed the CGI-I (Clinical Global Impressions-Improvement) scale to rate autism core symptoms' changes. Child Development Inventory and the Aberrant Behavior Checklist were completed by their parents, and BASC (Behavior Assessment Scale for Children) were completed both teachers and parents at the end of 6 months. There were 48 children and 24 placebos and 24 received DHA. Although there was an average rise of 431% in total plasma DHA levels over a span of 6 months, the DHA group did not exhibit any notable enhancement in the core symptoms of autism in contrast to the placebo group as evaluated by CGI-IIn accordance with the analysis of models adjusted for baseline rating scores, parents observed that children in the placebo group had a greater mean rating of social skills on the BASC than those in the DHA group, although this was not the case according to the teachers. Conversely, teachers reported that children in the DHA group exhibited a higher mean rating of functional communication on the BASC compared to those in the placebo group, as perceived by parents. In conclusion, autism core symptoms don't improve with daily 200 mg dietary DHA supplementation for 6 months (Voigt, et al., 2014).

2.3. Vitamin D Supplementation

Only a limited number of foods contain vitamin D, which is a fat-soluble vitamin. To increase the intake of this vitamin, it is often added to other foods or taken as a dietary supplement. Vitamin D can also be synthesized within the body when the skin is exposed to ultraviolet rays from the sun, which triggers vitamin D synthesis. However, vitamin D obtained from food, supplements, or sun exposure is biologically inert and requires two hydroxylations in the body for activation. The first hydroxylation takes place in the liver and converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also called calcidiol. The second hydroxylation occurs mainly in the kidney and produces the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as calcitriol. Vitamin D plays various roles in the body, such as regulating cell growth, immune function, and neuromuscular activity. It also has anti-inflammatory effects. Additionally, many genes that are involved in cell proliferation, differentiation, and apoptosis are regulated, at least in part, by vitamin D. Many cells have vitamin D receptors, and some convert 25(OH)D to 1,25(OH)2D. Vitamin D is naturally present in very few foods. Some of the best sources of this vitamin include the flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils. Beef liver, cheese, and egg yolks also contain small amounts of vitamin D. The vitamin D, which is in these foods, is mainly in the form of vitamin D3 and its metabolite 25(OH)D3. For most people, exposure to sunlight is the primary source of vitamin D. When the skin is exposed to ultraviolet (UV) B radiation, it converts a precursor molecule called 7-dehydrocholesterol into previtamin D3, which then gets converted into vitamin D3 (National Institutes of Health, 2020).

Vitamin D can have a major role in the etiology of ASD. It is regarded that brain function and development is affected by vitamin D which is a neuroactive steroid. Vitamin D's role is essential for connectivity in brain which is called myelination. Risk for ASDs might increase when the vitamin D levels decrease in patients, maternal levels of vitamin D decrease during pregnancy, and solar UVB exposure decrease according to the studies (Wang, et al., 2016).

In study by Feng, et al., 215 autistic children and 285 healthy children were included in total. Vitamin D treatment were given to 37 of 215 autistic children. To evaluate the autistic symptoms, CARS (Childhood Autism Rating Scale) and ABC (Autism Behaviour Checklist) were used. The serum 25(OH) D [25-hydroxyvitamin D] was assessed with high-performance liquid chromatography. Before and after 3 months of treatment; CARS, ABC, and serum 25(OH) D levels were evaluated. Autistic children' serum levels 25 (OH) D serum levels were lower than typically developing children significantly. Scores of language subscale and ABC subscale were negatively correlated with 25(OH) D serum levels. CARS and ABC symptom scores were reduced significantly after supplementation of vitamin D. Besides that, the data showed that effects of treatment were more evident in younger autistic children. In summary, autism etiology might be based on deficiency of vitamin D. Also, vitamin D supplementation is a safe and cost-effective treatment, and it improve the outcomes of autistic children (especially younger ones) significantly (Feng, et al., 2017).

In meta-analysis by Wang, et al., 11 studies were included. There were 1652 participants (782 healthy controls and 870 autistic patients) in total. In these studies, serum 25(OH) D levels of in both healthy controls and autistic patients were evaluated and included. Lower serum levels of 25(OH) D were reported in autistic patients compared to healthy controls in all of the studies. The findings of this meta-analysis seem quite robust despite limitations. There are clinical consequences of vitamin D reduction observed in autistic children probably. Firstly, it is believed that to assess vitamin D status routinely and a clear vision on supplementation of vitamin D is necessary. The brain damage due to vitamin D deficiency may be malleable according to the animal evidence. Therefore, the brain damage can be partially reversed by vitamin D given in adequate doses. Besides that, there are indications that vitamin d supplementation may improve symptoms of autism directly. There is a debate on whether children with autism should receive higher doses of vitamin D supplements to maintain optimal 25(OH)D levels. While the 25(OH)D levels of people who spend significant time in the sun or work outdoors can reach around 50 ng/mL, it is unclear what the natural 25(OH)D levels are for the general population. Large-scale, controlled clinical trials that span over an extended period of time will be necessary to validate the potential benefits of vitamin D supplementation for children with autism (Wang, et al., 2016).

In study by Mostafa and Al-Ayadhi, the relationship between anti-MAG (anti-myelin-associated glycoprotein) autoantibodies and serum levels of 25(OH)D in children with autism was investigated for the first time. Anti-MAG autoantibodies and 25(OH)D serum levels were measured in 50 children with autism between the age of 5-12 and 30 healthy children. Serum 25(OH)D serum levels < 10

ng/mL and 10-30 ng/mL were defined as deficiency and insufficiency, respectively. Significantly lower 25(OH)D serum levels were found in children with autism than healthy children. 48% vitamin D insufficiency and 40% vitamin D deficiency were observed in children with autism. CARS had negative correlations with 25(OH)D serum levels significantly. Additionally, 70% of the children with autism had increased levels of serum anti-MAG autoantibodies, which also had a significant negative correlation with 25(OH)D serum levels. The study suggests that vitamin D deficiency may contribute to the production of serum anti-MAG autoantibodies in children with autism (Mostafa and Al-Ayadhi, 2012).

In randomized controlled trial by Dong, et al., there were 87 subjects in the autism group and 75 subjects were male and 12 subjects were female. There were 301 subjects in the control group and 249 subjects were male and 52 subjects were female. Autistic children had lower serum vitamin D level than the control group. It was statistically significant that the between-group percentage difference of deficient, insufficient and normal levels of vitamin D. There were negative correlations between ABC subscale scores (self-care, social interaction, body behavior and language) or total ABC score and serum vitamin D level. There were negative correlations between CARS subscale scores (nonverbal communication, imitation and general impression) or total CARS score and serum vitamin D level in autistic children. There were negative correlations between ATEC social interaction subscale or SRS behavior subscale and serum vitamin D level in autistic children. In conclusion, children with autism have lower 25(OH)D level than the healthy group. In addition, there are negative correlations between ASDs core symptoms and vitamin D levels (Dong, et al., 2017).

In randomized controlled trial by Kerley, et al., 42 autistic children were included. 2000 IU vitamin D3 were supplemented daily or placebo for a period of 20 weeks. Evaluations were done at the beginning and after supplementation for 20 weeks. ABC's stereotypic behaviour subscale was the primary outcome. Secondary exploratory outcomes included additional subscales from the ABC, SRS and DD-CGAS (Developmental Disabilities-Children's Global Assessment Scale). In addition, there were biochemical parameters of total vitamin D status, systematic inflammation and immunity. As a result, 38 children complemented the trial. At the beginning, 25(OH)D was 54.2±19.7 nmol/L. Following vitamin D supplementation, a significant increase in 25(OH)D levels to 83.8 nmol/L was observed, although no effect on the primary endpoint was noted. However, there was an improvement in DD-CGAS self-care scores. Also, placebo group showed a trend towards decreased of inappropriate speech. In conclusion, there wasn't effect of vitamin D supplementation on the primary outcome with inconsistent and limited effects in autistic children (Kerley, et al., 2017).

2.4. Omega3 and Vitamin D Supplementation

In double-blind, randomized controlled trial by Mazahery, et al., 111 autistic children between the ages of 2.5 and 8 were included in the study for 12 months. Omega-3 LCPUFA (722 mg/day DHA, OM), vitamin D (2000 IU/day, VID) or both (2000 IU/day vitamin D+722 mg/day DHA) were given to the children. ABC's hyperactivity and irritability scores were the primary outcomes. Primary outcomes and biomarkers (omega-3 and serum 25(OH)D) were measured before and after 12 months. 66% of 111 children who completed basic data collection completed the study (Omega 3=23, vitamin D=19, both=15, placebo=16). Children receiving omega 3 and vitamin D had higher reduction in irritability than placebo after 12 months. Also, children receiving vitamin D had higher reduction in hyperactivity compared to placebo. There was an increase of serum 25(OH)D concentration in vitamin D group and group taking both vitamin D and omega 3 which indicated a good compliance rate. According to the results, omega3 LCPUFA and vitamin D decreased irritability symptoms in autistic children. Also, these children' hyperactivity symptoms were reduced by vitamin D (Mazahery, et al., 2019).

2.5. Vitamin B12 Supplementation

Vitamin B12 is a water-soluble nutrient that found naturally in some foods, is added to others, and can be taken in supplement or prescription form. It plays a critical role in proper formation of red blood cells, neurological function, and DNA synthesis. Vitamin B12 acts as a cofactor for both methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase facilitates the conversion of homocysteine to methionine, a crucial component in the creation of S-adenosylmethionine, which acts as a universal methyl donor for nearly 100 different substances, such as DNA, RNA, hormones, proteins, and lipids. While animal products, like fish, meat, poultry, eggs, milk, and milk products, are naturally rich in vitamin B12, plant-based foods do not typically contain significant amounts of the nutrient. However, vitamin B12 can be found in fortified breakfast cereals, which offer a convenient and highly bioavailable source of the nutrient (National Institutes of Health, 2020).

In randomized controlled trial by Hendren, et al., 57 autistic children were included. Methyl B12 (75 lg/kg) were given to children or saline placebo for 8 weeks. Subcutaneous injection was done every 3 days. When the primary outcomes were measured according to CGI-I (Clinical Global Impressions-Improvement) score, overall improvement in autism symptoms was observed. Secondary outcomes were measured according to ABC and SRS. Laboratory measures of antioxidant glutathione metabolism and methionine methylation were evaluated at the beginning and end of 8 weeks. The study was completed by 50 children totally. The primary outcome measure: statistically. The group receiving methyl B12 demonstrated a significantly lower CGI-I score (2.4) compared to the placebo group (3.1), indicating a better outcome. The improvement observed in the methyl B12 group was 0.7 greater than that seen in the placebo group. There was a positive correlation between clinical improvement and plasma methionine increase, SAH (S-adenosyl-lhomocysteine) decrease, and improvement in the ratio of SAM (S-adenosylmethionine) to SAH observed in children treated with methyl B12. That indicates an improvement in cellular methylation capacity. There were no improvements in parent-rated SRS or ABC. Clinician-rated autism symptom measures and cellular methylation capacity were improved with methyl B12 treatment (Hendren, et al., 2016).

In randomized controlled trial by Bertoglio, et al., 30 autistic children between the ages of 3 and 8were included. The study took 12 weeks. 6 weeks of methyl B12 at a dose of 64.5 mcg=kg every three days and 6 weeks of placebo were received by all subjects. Methyl B12 were administered subcutaneously into the buttocks. All subjects completed the 12-week, double-blind study. In addition,

6-month extension study was completed by 22 subjects. Significant mean differences between placebo and active groups were not identified statistically in glutathione status or in behavior tests. Clinically significant improvement was demonstrated in 9 children (30%) on the CGI-S and at least two additional behavioral measures. In conclusions, methyl B12 is ineffective in treating autism behavioral symptoms as it turns out comparison of the overall means between groups (Bertoglio, et al., 2010).

2.6. Vitamin B12 Supplementation

Folic acid is not able to be synthesized by human body and thus people depend on dietary sources to meet their need. Folate can be found naturally in a diverse range of foods, however, the synthetic form of the vitamin that is added to foods and supplements, folic acid, is more readily absorbed by the body. Good sources of folate include dark green leafy vegetables, beans, peanuts, fresh fruits, sunflower seeds, fruit juices, whole grains, seafood, liver, eggs and fortified foods and supplements (Harvard T.H. Chan, 2020). According to the evidence from studies, insufficient dietary intake and micronutrient deficiencies are observed in autistic children because they have gastrointestinal problems and/or they are picky eaters. Cellular methylation is affected by folic acid deficiency and cellular redox homeostasis is unbalanced indirectly. Besides that, oxidative stress and methylation impairment may be contributing factors to autism pathology according to the experimental studies (Sun, et al., 2016).

In open-label trial by Sun, et al., 66 children with autism were included for 3 months of structured teaching. 400 µg folic acid were given to 44 children (intervention group) for a period of three months during their structured teaching. The remaining 22 children (control group) were not given any supplement for the duration of the study. PEP-3 (Psychoeducational Profile-third edition) and ATEC were evaluated at the beginning and at the end of 3 months. In the study conducted on 29 autistic children selected at random from the intervention group, folic acid, glutathione metabolism, and homocysteine in plasma were measured at the beginning and end of treatment. Results were compared with those of 29 age-matched children with typical development. The findings suggest that folic acid intervention helped improve autism symptoms related to cognitive verbal/preverbal skills, sociability, receptive language, and affective expression and communication. Additionally, the treatment led to improved levels of homocysteine, folic acid, and glutathione redox metabolism. In conclusion, folic acid supplementation may play a certain role in the treatment of children with autism (Sun, et al., 2016).

2.7. Probiotics Supplementation

Probiotics refer to live microorganisms that, when consumed or applied to the body, are believed to offer health benefits. They can be found in fermented foods like yogurt, as well as in dietary supplements. Probiotics may consist of a variety of microorganisms, although the most commonly used types belong to the bacterial groups Lactobacillus and Bifidobacterium. Yeasts like Saccharomyces boulardii may also be used as probiotics. It's important to note that different types of probiotics may have varying effects on the body (National Center for Complementary and Integrative Health, 2019). Probiotics have the potential to stabilize the mucosal barrier by increasing mucin expression, reduce the overgrowth of harmful bacteria, stimulate mucosal immunity through secretory IgA production, and generate antioxidant substances. This makes the use of probiotic bacteria an effective therapeutic tool for altering the microbiota. By restoring the normal gut microbiota, improving epithelial barrier function, and reducing inflammation, probiotics may also improve some behavioral symptoms associated with autism (Shaaban, et al., 2018)

In study by Shaaban, et al., 30 children with autism between the ages of 5 to 9 were included. Probiotics nutritional supplement formula (each gram contains 100 × 106 colony forming units of three probiotic strains; Lactobacillus rhamnosus, Bifidobacteria longum and Lactobacillus acidophilus) were given to them. GI (Gastrointestinal) flora were evaluated by real-time PCR of stool samples of participants. Autistic children' GI symptoms were evaluated with a modified 6-GSI (six-item Gastrointestinal Severity Index) questionnaire, and autistic symptoms were evaluated with ATEC at the beginning and end of 3 months of supplementation. Significant decrease was observed in the total ATEC scores with supplementation of probiotics among autistic children. This indicated a decrease in severity of autism symptoms. Also, significant improvements were observed in all four categories of the ATEC. Sensory/cognitive awareness, sociability, and health/physical/behavior domains of ATEC scores also saw significant improvements. Moreover, supplementation with probiotics led to a significant reduction in stool consistency, constipation, abdominal pain, and flatulence. It is worth noting that no serious adverse effects were reported by families who used probiotics in this study (Shaaban, et al., 2018).

In randomized controlled trial by Liu, et al., 80 males with autism were included. The effects of Lactobacillus plantarum PS128 (PS128) were investigated for four weeks. The ABC-T (Autism Behavior Checklist-Taiwan version), the CBCL (Child Behavior Checklist), the SNAP-IV (The Swanson, Nolan, and Pelham-IV-Taiwan version) and SRS were used to evaluate the primary outcomes. The CGI-I) were used to evaluate secondary outcomes. The improvement in CGI-I scores was minimal for both groups. Any difference between the PS128 group and placebo group were not observed at the beginning and at the end of four weeks according to the total ABC-T-score and subscale scores. Further exploratory analysis demonstrated that the consumption of PS128 for a period of four weeks was associated with a trend towards lower scores for object and body use. According to CGI-I and CGI-S scores, similar severity at baseline and improvements at week 4 were observed in subjects from both the placebo and PS128 groups. This suggested that autism traits didn't change between the two visits. Except that, nominal improvement in several elements were observed in the PS128 group subjects, including SRS-total score, ABC-T-body and object use, CBCL-rule breaking behavior, CBCL-anxiety, SNAP-IV-total score, SNAP-IV-opposition/defiance, and SNAP-IV-hyperactivity/impulsivity. Also, there were improvements in SNAP-IV-total score and SNAP-IV-opposition/defiance in younger subjects between the age of 7 and 12, while there were not in the counterpart placebo group. In conclusions, this study's data indicates that the psychobiotic Lactobacillus plantarum PS128 can alleviate certain symptoms of autism, particularly those related to rule-breaking behavior, disruptive behavior, and hyperactivity/impulsivity. In addition, the effectiveness of PS128 supplementation appeared to be contingent on age, with more

significant effects observed in younger children than in older children. This emphasizes the significance of intervening early. In summary, it appears that the psychobiotic PS128 may be advantageous for children with autism, but additional research is needed (Liu, et al., 2019).

3. Conclusion

As we know there is no current cure for ASDs which is neurological disorder. Dietary interventions aim to improve in behavior, communication skills and speech. At the same time, individuals with ASDs have GI symptoms and disturbances and interventions focuses on these ailments. Also, researchers think that there is a link between gut and brain due to the substances leaking from the intestine and these substances worsen the core symptoms of ASDs. Thats's why researchers focus on elimination diets (especially gluten and casein free diet) and supplementations which are omega 3, vitamin D, vitamin B12, folic acid and probiotics in their studies.

In conclusion, gluten and casein free diet, supplementation of omega 3, vitamin D, vitamin B12, folic acid and probiotics can't be recommended for improvement of ASDs behavioral symptoms due to the lack of evidence. Gluten-free and casein-free diet can be effective in individuals with allergies, and the probiotic supplement can be effective in individuals with GI symptoms. The effect of any supplement or elimination diet in improving behavioral symptoms is low. We need more randomized controls trials to determine the effectiveness of new approaches.

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