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Autism and Vitamin D

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Abstract

Autism spectrum disorder (ASD); It is an extremely heterogeneous neurodevelopmental disorder that occurs in early childhood. Although the number of cases of Autism diagnosed during the last four decades has increased significantly, there is still considerable debate about the underlying pathophysiology of ASD. The aim of this retrospective study was to evaluate the biochemical blood parameters of children with autism.The study included 30 children with autism aged between 10 and 18 years and 30 healthy children as a control group. The autism group was selected from the children with autism diagnosis in the Department of Pediatric Neurology at Gazi University Faculty of Medicine between 2018-2019 and the control group was selected from the Children's Health Unit. Control group; was selected from patients who were in the same age range as the autism group and who did not have any neurological disease. For the study, the ethics committee permission was obtained from the meeting on 28.05.2018 (decision number: 430) from Gazi University Clinical Research Ethics Committee. In two groups, routine biochemical parameters were evaluated retrospectively. Autism is now seen that it affects 1% of the world's population and disproportionately affects men According to the our study, the ratio of boys to girls was 1,5 in children with autism and 0,87 in the control group. The difference between vitamin 25-OHD (µg/L) levels of autism and control group was statistically significant p = 0,025 (p<0,05). We believe that randomized controlled trials using adequate doses of vitamin D3 are necessary in children with autism, and that further studies on nutritional disorders and behavioral problems, metabolic differences, and biomarkers are necessary.

1. Introduction

Appetite Autism is a developmental disorder that usually occurs in the first two years of life and negatively affects social and communication skills (Nogay, & Nelms, 2019). Autism is more common in boys than in girls (Ding, Taur, & Walkup, 2017). Abnormal functioning, depending on the type of psychopathology, manifests itself in all three areas: interactions, communication, and stereo repetitive behavior (Gevi, Zolla, Gabriele, & Persico, 2016). In addition to these specific diagnostic features, there are a number of other non-specific problems; phobias, sleep and eating disorders, anger attacks and aggression are common (American Psychiatric Association, 2013). The estimated global prevalence of autism spectrum disorder (ASD) is approximately 1.0% (Tseng et al., 2018). Although the pathogenic mechanism of autism has not yet been clearly elucidated, changes in cellular methylation capacity and antioxidant capacity indicate metabolic disorders in autism (Sevim, &Ayaz, 2017).

In the emergence of the disease;

• Neurological factors (Gvozdjakova et al., 2014),

• Metabolic factors (Zhang, Zhang, & Zhang, 2016),

• Gastrointestinal factors (Newell et al., 2016),

• Prenatal, postnatal factors (Volkmar, & Pauls, 2003),

• Psychosocial factors (Ford, Nibbs, & Crewther, 2017),

•Genetic predisposition (Lintas, & Persico, 2009),

• Environmental and immunological factors (Sathe, Andrews, McPheeters, & Warren, 2017),

Interactions between these factors are shown (Howsmon, Kruger, Melnyk, James, & Hahn, 2017).

Many studies in recent years show that nutritional problems are quite common in children with autism. It is thought that limited interest and repetitive behaviors, which are the main diagnostic component of Autism Spectrum Disorder (ASD), are the sources of 'food selectivity' behavior in nutrition problems (Sathe et al., 2017).There are case reports on the clinical consequences of vitamin deficiencies in patients with autism (Nogay, & Nelms, 2019).

It is emphasized that gene-gene and gene-environment interactions are effective in the emergence of the disease. Genetics plays an important role in the etiology of autistic disorders. Increased risk was also identified among monozygotic twins and siblings of children with autism. In addition, autistic symptoms; Regression syndrome has been associated with many genetic diseases such as fragile X, phenylketonuria, S nucleaidosis hyperactivity. However, these genetic diseases were responsible for 10% of autism, many recent studies have identified only rare de novo mutations (newly formed, non-hereditary)in autism, thus pointing to secondary mutations rather than hereditary genetic syndromes (Sevim, &Ayaz, 2017).

Due to environmental rooted FOCM / TS (Folate-dependent single carbon metabolism / transsulfuration) dysfunction, mother / father age, the lack of folate supplementation as well as exposure to toxic chemicals such as prenatal heavy metals, ethyl alcohol, pesticides, phthalates, polychlorinated biphenyls and traffic-related air pollution relieved basic neurodevelopmental processes, maternal rubella infection, valproate use in pregnancy is associated with ASD rates. These toxins create

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oxidative stress and affect heavy metal transsulfiration, glutathione, and intracellular processing of methylcobalamin (Howsmon et al., 2017).

Neurochemical studies have reported differences in glutamate, seratonin, dopamine, opioid, and gamma amino butyric acid levels in children with autism. Diabetes mellitus has been associated with an increased rate of ASD during pregnancy. The adverse effects of maternal diabetes in the brain have been associated with increased fetal oxidative stress, epigenetic changes in the expression of several genes (Stubbs, & Cheng, 2005).

In order to maximize brain function in autistic children, it is important that nutritional cofactors required for neurotransmitter synthesis and enzyme activation are taken regularly and adequately (Rossignol, & Frye, 2012). In particular, cofactors that are necessary for the regulation of genes encoding polymorphism-causing enzymes are even more important at this stage. Children with autism have many dietary deficiencies such as calcium, iron, vitamin D and vitamin E in their daily diet (Sathe et al., 2017)

Vitamin D is metabolized into a seco-steroid hormone that regulates about 3% of the 26,000 genes in the coding human genome.It is also a neurosteroid that is active in brain development, having effects on cellular proliferation, differentiation, calcium signaling, neurotrophic and neuroprotective actions; it also appears to have an effect on neurotransmission and synaptic plasticity (Cannel, 2017).

Vitamin D deficiency is seen as a risk factor for autism, but the biological mechanism has not yet emerged clearly. In addition to maintaining calcium/phosphate homeostasis, vitamin D assist several health benefits including neuroprotective effects, especially by antioxidant activity, neuronal calcium regulation, neurotransmitter regulation, influence on several neurotrophic factors and immunomodulation. Vitamin D deficiency leads to disturbance of these processes and may be involved in the development of autism. It seems likely that vitamin D exerts the strongest effects on nervous system during the prenatal development and early infancy, but also relate to the adult subject´s mental state (Máčová, Bıčíková, Ostatníková, Hıll, Stárka, 2017).Vitamin D is a neuroactive steroid affecting brain development and function. It plays an important role in myelination, which is important for connectivity in the brain. Studies have shown that decreased vitamin D levels, decreased maternal vitamin D levels during pregnancy, and decreased exposure to solar UVB might increase the risk of ASD (Fernell et al., 2015).

2. Materials and Methods

The study included 30 children with autism aged between 10 and 18 years and 30 healthy children as a control group. 18 of the children with autism were boys and 12 were girls. The control group children were 14 boys and 16 girls. The autism group was selected from the children with autism diagnosis in the Department of Pediatric Neurology at Gazi University Faculty of Medicine between 2018-2019 and the control group was selected from the Children's Health Unit at Gazi University Faculty of Medicine between 2018-2019. Control group; was selected from patients who were in the same age range as the autism group and who did not have any

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neurological disease. For the study, the ethics committee permission was obtained from the meeting on 28.05.2018 (decision number: 430) from Gazi University Clinical Research Ethics Committee. In two groups, routine biochemical parameters were evaluated retrospectively. Since our study was retrospective, no financial support was required.

Children with autism and control groups participated in the study; Ferritin, TSH, T4, D, B12, Glucose, BUN, Creatinine, Calcium, AST, ALT, GGT, Sodium, Potassium, Chlorine, Total cholesterol, Triglyceride values were analyzed retrospectively.

Ferritin, TSH, T4 Vitamin D, B12 tests were run on Beckman Coulter DXI 800 autoanalyser. All of these tests use the paramagnetic particle chemiluminescence immunoassay method for the quantitative determination of these tests in human serum.

Glucose Hexokinase, Urea: Urease; Creatinine: Jaffe,; Calcium: Arsenazo III; AST, ALT, GGT: Enzymatic; Sodium, Potassium, Chlorine: Indirect ISE (Ion Selective Electrote), Total Cholesterol: The spectrophotometric measurement of the absorbance of the chromophore formed after enzymatic destruction of cholesterol esters with Cholesterol Esterase; Trigiliserid: It is based on the spectrophotometric measurement of the absorbance of the chromophore formed by a series of enzymatic reactions with the enzymatic destruction of triglyceride with Lipase. All these above measurements were carried out on Beckmac Coulter AU 5800 autoanalysers.

3. Results

Thirty (30) children with autism and 30 healthy controls aged between 10 and 18 years were included in the study. 18 of the children with autism were boys and 12 were girls. The control group children were 14 boys and 16 girls.

Children with autism and control groups participated in the study; Ferritin, TSH, T4, D, B12, Glucose, BUN, Creatinine, Calcium, AST, ALT, GGT, Sodium, Potassium, Chlorine, Total cholesterol, Triglyceride values were analyzed retrospectively.

In our study the difference between vitamin 25-OHD levels of autism and control group was statistically significant p = 0,025 and vitamin 25-OHD levels in the autism group were lower than in the control group ($p<0,05$) . The average vitamin D levels of children diagnosed with autism was Mean=18,3028 (µg/L), while the average vitamin D levels of the control group were Mean=25,1579 (µg/L). When evaluating Vitamin D levels, the reference interval in our hospital was taken into consideration. Reference ranges used in Gazi University Hospital; Vitamin D deficiency if 25 (OH) D level is less than 20 µg / L, vitamin D insufficiency if between 20 and 30 µg / L, adequate level between 30 and 100 µg / L, vitamin D higher if higher than 100 µg / L is considered as the level.

The difference between ALT levels of autism and control group was also statistically significant, p = 0,049 ($p <0,05$).

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Table 1: Comparison of study and control groups in terms of biochemistry laboratory examination results; non-parametric data

	Autism Group N=30 Mean Rank	Control Group N=30 Mean Rank	p-value
ALT (U/L)	27.23	19.43	0.049*
TSH (µIU/mL)	18.64	18.36	0.938
Ferritin (ng/ml)	18.68	17.55	0.749

Notes: Student t-test was used for parametric data and Mann–Whitney U test was used to compare the non-parametric data. N (Number of people)=30 autism group, N (Number of people)=30 control group. ALT= Alanine Aminotransferase , TSH=Thyroid Stimulating Hormone.

Table 2: Comparison of study and control groups in terms of biochemistry laboratory examination results; parametric data

	Autism Group N=30 Mean Rank	Control Group N=30 Mean Rank	p-value
Glucose (mg/dl)	86.0857±10.37662	87.1000±10.81373	0.761
Creatinine (mg/dl)	0.5292±0.13558	0.4873±0.19560	0.398
D (µg/L)	18.3028±9.53339	25.1579±8.32175	0.025*
T4 (ng/dl)	0.8105±0.15241	0.9039±0.14725	0.067
Calcium (mg/dl)	9.8848±0.35437	10.0894±0.51769	0.141
AST (U/L)	27.2542±10.47660	31.4545±15.26817	0.297
Sodium (mmol/L)	139.8750±2.50760	138.6842±1.73374	0.086
Potassium (mmol/L)	4.2638±0.29609	4.3228±0.43516	0.604

Notes: Student t-test was used for parametric data and Mann–Whitney U test was used to compare the non-parametric data. N (Number of people)=30 autism group, N (Number of people)=30 control group. D=Vitamin D, T4=Thyroxine, AST=Aspartate Aminotransferase.

4. Discussion

In the brain, vitamin D has important roles in proliferation and differentiation, calcium signaling and neurotrophic and neuroprotective actions; it may also alter neurotransmission and synaptic plasticity. Vitamin D has a major role in inducing T regulatory cells which have an effect on controlling antibodies contributing to autoimmune conditions . Thus, the vitamin D induced T regulatory cells may have a role in reducing autoimmune conditions and protecting the fetus (Macova, Bıcíkova, Ostatníkova, Hıll, & Starka, 2017).

In a clinical trial study by Feng and colleagues on 37 children with autism, for three months, these children received 150,000 IU as intramuscular

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injection(monthly) and 400 IU orally (daily). These researchers reported that disease symptoms and behavioral checklist in children (3 years old and older) with autism improved (Feng et al.,2017). Overall, studies of nutritional supplements or specialized diets were typically small and short-term (<6 months) and provided little evidence regarding the potential effects of these approaches (Sathe et al., 2017).

In the study conducted by Biçer & Alsaffar, 80 children with autism aged between 12 and 18 years were studied on the nutritional status of overweight or obese children with autism. Of the 80 participants, 25 were defined as overweight and 55 as obese. In both groups, carbohydrate and sodium uptake was high and vitamin D and dietary fiber uptake were low (Biçer, & Alsaffar, 2016).

According to the results of the study conducted by Mazaheri and colleagues with children diagnosed with autism; Vitamin D reduced symptoms of hyperactivity. The results indicate that vitamin D and omega-3 LCPUFA reduced irritability symptoms in children with ASD (Mazahery et al., 2019).

Vitamin D deficiency has recently been considered a potential environmental risk factor for ASD. Vitamin D is potent neurosteroid, which mediates numerous actions in several body tissues including brain.

In our study the difference between vitamin 25-OHD (µg/L) levels of autism and control group was statistically significant p = 0,025 and vitamin 25-OHD(µg/L) mean levels in the autism group were lower than in the control group (p<0,05) . The average vitamin D levels of children diagnosed with autism was Mean=18,3028 (µg/L), while the average vitamin D levels of the control group were Mean=25,1579 (µg/L). When evaluating Vitamin D levels, the reference interval in our hospital was taken into consideration. Reference ranges used in Gazi University Hospital; Vitamin D deficiency if 25 (OH) D level is less than 20 µg / L, vitamin D insufficiency if between 20 and 30 µg / L, adequate level between 30 and 100 µg / L, vitamin D higher if higher than 100 µg / L is considered as the level.

The difference between ALT (U/L) levels of autism and control group was also statistically significant, p = 0,049 (p <0,05).Alanine aminotransferase is an enzyme produced in the liver. When the serum level is measured, it provides a marker of hepatic disease (Sherman, 1991). Measurement of serum alanine aminotransferase (ALT) is a common, readily available, and inexpensive laboratory assay in clinical practice. ALT activity is not only measured to detect liver disease, but also to monitor overall health. ALT activity is influenced by various factors, including viral hepatitis, alcohol consumption, and medication (Liu, Que, Xu, & Peng, 2014).

Autism is now seen that it affects 1% of the world's population and disproportionately affects men (Loomes, Hull, & Mandy, 2017). Considering the ratio of boys to girls in autistic children, it is seen that boys are dominant (Ibrahim, Voigt, Katusic, Weaver, & Barbaresi, 2009). In a study conducted by Görmez et al. In pediatric adolescent psychiatry, 11.1% of individuals with autism were girls and 88.9% were boys (Görmez, Örengül, Baljinnyam, & Aliyeva, 2017). According to a study by Michael L. et al., More than 73% of the patients with autism were male (Levy, Levy, Hoff, & Conklın, 2010).

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According to the our study, the ratio of boys to girls was 1,5 in children with autism and 0,87 in the control group and consistent with the literature.

In conculusion,we believe that randomized controlled trials using adequate doses of vitamin D3 are necessary in children with autism, and that further studies on nutritional disorders and behavioral problems, metabolic differences, and biomarkers are necessary.

5. Conclusion

In conculusion,we believe that randomized controlled trials using adequate doses of vitamin D3 are necessary in children with autism, and that further studies on nutritional disorders and behavioral problems, metabolic differences, and biomarkers are necessary.

6. Statistical analysis

Data were analyzed using SPSS 25.0 statistical package program. Descriptive statistics (frequency, percentage distribution, mean, median etc.) were used for statistical analysis. Student t-test was used for parametric data and Mann–Whitney U test was used to compare the non-parametric data. Subgroups were formed according to different characteristics and it was examined whether the quantitative variables fit the normal distribution. $p < 0,05$ was considered statistically significant.

Conflicts of interest

Since our study was retrospective, no financial support was required**.**

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