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Vitamin D levels in children and adolescents with autism

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Abstract

Objective: This study aimed to investigate the relationship between autism spectrum disorder (ASD) and vitamin D levels in children and adolescents.

Methods: We measured serum 25-hydroxyvitamin D (25-OHD) levels in 1529 patients with ASD aged 3 to 18 years, without any additional chronic diseases. Levels of 25-OHD were compared according to sex, age ($<$11 or $\geq$11 years), and birth season. Additionally, laboratory parameters (calcium, phosphorus, alkaline phosphatase, and 25-OHD) of 100 selected patients with ASD were compared with those of the healthy control group.

Results: Vitamin D deficiency or insufficiency was found in approximately 95% of all patients. Levels of 25-OHD in adolescent patients with ASD aged 11 to 18 years were significantly lower than those in patients aged younger than 11 years. In the 100 selected patients with ASD, mean serum 25-OHD levels were significantly lower and alkaline phosphatase levels were higher compared with those in healthy children.

Conclusion: Our study suggests a relationship between vitamin D and ASD in children. Monitoring vitamin D levels is crucial in autistic children, especially adolescents, to take protective measures and treat this condition early.

Keywords

Autistic spectrum disorder, children, neurodevelopmental disorders, vitamin D deficiency, adolescent, alkaline phosphatase

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Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders, which are associated with social, communicative, and cognitive developmental delay with onset at the early developmental stage. The main characteristics of ASD include social problems, verbal and non-verbal communication disorders, repetitive behavioral patterns, and low motivation.[1] According to the Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5), which was published as a new classification in 2013, ASD includes autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified, and Asperger's syndrome.[2] Recently, there has been a significant increase in the frequency of ASD.[3] In 2012, Elsabbagh et al. reported that the incidence of autism had increased by 70% since the 1970s.[4] Indeed, the frequency of ASD was 4/10,000 in 1989 and 60 to 70/10,000 in 2005, and its current prevalence is as high as 1/88.[3,5] With this increase in the frequency of ASD, investigation of the factors that can lead to autism became essential. Changes in the diagnostic criteria of ASD through time, broadening of the autism spectrum, and increased awareness of this disease are among factors explaining the increase in frequency of ASD. In modern life, a less active indoor lifestyle and exposure to chemicals or nutrients may affect development of these diseases.[6]

Although the etiology of autism is not fully understood, there is a genetic predisposition, which may be triggered by nutritional and environmental factors, such as infections, immunological problems, endocrine system-disrupting chemicals, heavy metal intoxication, oxidative stress, fetal alcohol syndrome, and vitamin D deficiency.[7–9] Vitamin D is considered to maintain homeostasis of the brain by protecting DNA from oxidative stress.[10,11] Vitamin D has important roles in proliferation and differentiation, calcium signaling, and neurotrophic and neuroprotective actions in the brain, and it may also alter neurotransmission and synaptic plasticity. Many epidemiological studies have reported that vitamin D deficiency is associated with a wide range of neuropsychiatric disorders and neurodegenerative diseases.[12–14] Additionally, a few studies have shown that vitamin D levels are lower in children with autism compared with their peers.[15,16] However, the role of vitamin D in the etiology of ASD is still not understood. Vitamin D insufficiency is considered not only an underlying factor, but also a consequence, of dietary choices and lifestyle in autistic children.

In the present study, we aimed to investigate vitamin D levels in autistic children and to determine the contributing factors involved in the relationship between ASD and vitamin D levels.

Materials and methods

Patients and groups

This study was performed in a group of patients who were diagnosed with ASD according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-4 or -5 at the Child Psychiatry Center. These patients were referred to Istanbul Üniversity-Cerrahpaşa, Cerrahpaşa Medical Faculty Children's Metabolism Clinic between January 2006 and January 2015 to investigate if there was an underlying metabolic disease. This retrospective and cross-sectional study was carried out in two stages. In the first stage, the files of the patients were reviewed and those who met the inclusion criteria were included. The inclusion criteria for the first stage were as follows: (1) diagnosis with autism according to DSM-4 (before 2013)[17] or DSM-5 criteria (after 2013);[18] (2) aged

between 3 and 18 years; (3) no diagnosis of a metabolic disease, or additional neurological (including no history of epilepsy or antiepileptic drugs) and systemic chronic disease; and (4) no intake of vitamin D during the past 6 months before determining vitamin D levels.

Patients with ASD who met these criteria were included in the study as group 1. In all of the patients, to determine vitamin D levels, we measured serum 25-hydroxyvitamin D (25-OHD) levels, which are the major circulatory form with a half-life of 2 to 3 weeks.

Patients were classified into the following four categories: (1) severe vitamin D deficiency, 25-OHD levels <10 ng/mL (24.96 nmol/L); (2) moderate deficiency, 25-OHD levels of 10 to 19 ng/mL (24.96–47.42 nmol/L); (3) mild deficiency, 25-OHD levels of 20 to 29 ng/mL (49.92–72.38 nmol/L); and (4) normal/optimal levels, between 30 (74.88 nmol/L) and 80 ng/mL (199.68 nmol/L).[19,20] According to the recommendations of other studies,[10,11,21] we categorized vitamin D levels as deficient if 25-OHD levels were <20 ng/mL , insufficient if they were between 20 and 29 ng/mL, and sufficient if they were >30 ng/mL.

Age, birth season, and sex of the patients were recorded from the files. Mean serum 25-OHD levels were compared according to the sex and birth season of the children. We planned to investigate whether mean vitamin 25-OHD levels in autistic adolescents are different from those in younger children and the proportion of vitamin D deficiency in autistic adolescents. Therefore, all patients were divided into two groups of <11 years old and ≥ 11 years old, which is regarded as the starting age of adolescence.[22,23] Mean 25-OHD levels in these two groups were compared.

For the second stage, 100 patients with ASD who had full records of some additional biochemical parameters and anthropometric measurements (group 2) were recruited from patients in group 1. Serum calcium, phosphorus, alkaline phosphatase, and vitamin D levels, height, weight, and measurements were recorded. The control group was composed of 100 healthy children who had not been diagnosed with any chronic disease, had not received vitamin D treatment during the past 6 months, and had similar demographic characteristics with group 2. Mean 25-OHD levels in group 2 and the control group were compared according demographic characteristics, anthropometric measurements, and laboratory parameters. The proportion of patients with vitamin D deficiency or vitamin D insufficiency was determined in both groups. Additionally, group 2 patients were classified into same four categories as mentioned above.

Laboratory studies

All laboratory parameters of the patients were examined in the same university hospital laboratory. Serum 25-OHD, which is a vitamin D metabolite, was measured by the enzyme immunoassay method using the LIAISON® device. Additional baseline biochemical parameters were measured from serum, such as calcium, phosphorus, and alkaline phosphatase, on the basis of previous recommendations[19,20] according to standard laboratory procedures.

Ethical approval

This study was approved by the institutional research Ethics Committee of Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa (A-36, 12/03/2014) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki (https://cerrahpasa.istanbulc.edu.tr/tr/_) All patients who agreed to participate in this study provided informed consent before their inclusion in the study.

Statistical analysis

Categorical variables are presented as number and percentage, and continuous variables are shown as the mean and standard deviation. The chi-square and Fisher's exact test were used to compare categorical data. The Student's t-test and one-way ANOVA were performed to compare numerical parameters. Additionally, post-hoc evaluation was carried out following one-way ANOVA, using Tukey's honestly significant difference test. Statistical analysis was performed using IBM SPSS version 19.0 (IBM Corp., Armonk, NY, USA). P values <0.05 were considered significant.

Results

Patients in group 2

A total of 1529 children with ASD were enrolled in the study. The majority (80%; $n = 1222$) of these patients were boys ($P<0.001$). The mean age of the patients was 5.8 ± 3.4 years.

The mean 25-OHD level was 17.87 ± 7.46 ng/mL (44.6 ± 19.62 nmol/L) in all of the patients (Table 1). The mean 25-OHD level was similar between boys and girls. In patients in group 1, mean serum 25-OHD levels were <10 ng/mL in 13% of patients with ASD, 10 to 19 ng/mL in 44.6% of patients with ASD, 20 to 29 ng/mL in 37% of patients with ASD, and ≥ 30 ng/mL in 5.4% of patients with ASD. Therefore, 57.7% of patients had vitamin D deficiency, while 37% showed vitamin D insufficiency (Table 2).

No significant difference was found in 25-OHD levels according to birth seasons between the groups (Table 3). When all patients with ASD were grouped by age (11 years as the margin), the mean 25-OHD level was significantly lower in children aged ≥ 11 years than in younger children aged <11 years ($P = 0.014$) (Table 1).

Patients in group 2

The laboratory results and demographic characteristics were compared between patients in group 2 ($n = 100$) and the control group ($n = 100$). The mean age of the 100 patients with ASD in group 2 was 5.95 ± 3.13 years and the mean age of subjects in the control group was 6.68 ± 3.8 years. A total of 70% were boys and 30% were girls in the control group. There were no significant differences in age and sex between these two groups.

The mean vitamin D level was significantly lower in patients in group 2 than in those in the control group ($P = 0.037$) (Table 1).

Table 1. Mean serum 25-OHD levels in the patients and controls.

Groups	Mean 25-OHD levels (nmol/L)	P
Group 1 (n = 1529)	44.60 ± 18.62	
Girls	45.85 ± 18.55	0.18
Boys	44.28 ± 18.65	
Patients aged ≥11 years	41.41 ± 18.79	0.014
Patients aged <11 years	45.08 ± 18.57	
Group 2 (n = 100)	42.86 ± 19.84	0.037
Control group (n = 100)	48.57 ± 22.36	

Values are ± standard deviation. The Student's t test was used for comparisons. 25-OHD: 25-hydroxyvitamin D.

Table 2. Serum 25-OHD levels in patients and controls.

25-OHD levels	Group 1 (n = 1529)	Group 2* (n = 100)	Control group* (n = 100)
<10 ng/mL (24.96 nmol/L)	199	19	12
10–19 ng/mL (24.96–47.42 nmol/L)	682	46	43
20–29 ng/mL (49.92–72.38 nmol/L)	566	32	39
≥30 ng/mL (74.88 nmol/L)	82	3	6

*P>0.05 (Fisher's exact test). 25-OHD: 25-hydroxyvitamin D.

Table 3. Serum 25-OHD levels in group 1 according to birth seasons.

Birth seasons	25-OHD levels (nmol/L)	P*
Spring (n = 369)	44.93 ± 19.67	0.059
Summer (n = 402)	44.98 ± 18.60	
Autumn (n = 394)	42.53 ± 17.17	
Winter (n = 364)	46.05 ± 19.02	
Total (n = 1529)	44.63 ± 18.65	

Values are ± standard deviation. *Tamhane test-one-way ANOVA. 25-OHD: 25-hydroxyvitamin D.

Examination of laboratory parameters showed that the mean serum calcium level was not significantly different between group 2 and the control group. However, the mean serum alkaline phosphatase level in group 2 was significantly higher than that in the control group (P = 0.011). Similarly, the mean phosphorus level in group 1 was significantly higher than that in the control group (P = 0.015) (Table 4).

Discussion

ASD as a neurodevelopmental disorder is considered a major clinical problem because of its increasing frequency over the years. Determination of the underlying cause of ASD can be promising for its treatment and/or prevention. In addition to genetic factors, vitamin D deficiency has been recently discussed in the etiology of autism as an environmental factor. In our study of a large autistic patient group without any additional chronic diseases living in Istanbul, vitamin D deficiency and insufficiency were detected in almost 95% of the patients. We found that 58% of patients with ASD had vitamin D deficiency and 13% had severe deficiency. Additionally, the mean serum 25-OHD level, which is a measurable indicator of vitamin D, was significantly lower in children and adolescents with autism than in healthy controls. Similarly, recent studies showed that vitamin D levels in children with ASD were significantly lower than in their counterparts.[24–27] By contrast, in a study of a group of Caucasian autistic children, the patients did not have low vitamin D levels.[28] Vitamin D has neuroprotective effects, especially by antioxidant activity, neuronal calcium regulation, and neurotransmitter regulation, and it affects several neurotrophic factors and immunomodulation. Vitamin D deficiency leads to disturbance of these processes. Vitamin D activity begins in the intrauterine period and its strongest effects appear to be on the nervous system.[29] Cannell et al. reported that maternal low 25-OHD levels at 18 weeks of pregnancy were associated with a significantly increased risk of the offspring being diagnosed with autism. Therefore, these authors suggested vitamin treatment for core symptoms of autism.[30] In another recent study, the researchers concluded that administration of high-dose vitamin D was effective in ameliorating the core symptoms of ASD.[31] Some autistic

Table 4. Laboratory values in group 2 and the control group.

Laboratory parameters	Group 2 (n = 100)	Control group (n = 100)	P*
Calcium (nmol/L)	2.56 ± 0.12	2.55 ± 0.10	0.44
Phosphorus (nmol/L)	1.58 ± 0.16	1.52 ± 0.16	0.015
Alkaline phosphatase (U/L)	230 ± 83	204 ± 58	0.011

*Student's t test.

symptoms that diminished after vitamin D administration in rachitic children have been reported.[32]

Because vitamin D can stimulate protection and growth of neuronal cells, it may slow down progression of neurodegenerative diseases. Vitamin D deficiency affects neuronal differentiation, axonal communication, and brain structure. Therefore, the role of vitamin D as the underlying cause of a wide range of neuropsychiatric disorders and neurodegenerative diseases (e.g., autism, depression, multiple sclerosis, Alzheimer's disease, Parkinson's disease, and attention deficit hyperactivity disease.) at all ages, not only in infancy, has been supported by epidemiological studies.[33] Taken together, our study and other studies suggest that there may be an association between low vitamin D levels and ASD. This will be an important direction for future research.[34]

Etiological analysis includes prenatal and postnatal studies for the first 3 years. Older age is associated with factors contributing to the development of vitamin D deficiency, such as living conditions, environmental factors, and nutritional characteristics of autistic children. The reason for vitamin D levels being low in autistic patients is unclear. We cannot claim that vitamin D deficiency was an etiological factor in our autistic children and adolescent aged 3 to 18 years. Vitamin D is an active steroid obtained by dietary uptake or synthesized in human skin after exposure to sunlight. Notably, autistic children tend to benefit from sufficient sunlight, while their parents are inclined to keep them in closed environments because they cannot be left alone to play in open areas like other healthy children. Infants and children with ASD often have food selectivity and restricted diets, which places them at risk for nutritional deficiencies.[35] Consequently, vitamin D synthesis or intake may be reduced in these children. However, vitamin D deficiency/insufficiency is commonly believed to be both a cause and a result of ASD.

Autism occurs more frequently in newborns during winter and spring.[33] In our study, mean 25-OHD levels were not significantly different among the four birth seasons. Similarly, Meguid et al.[36] found that there was no significant effect of birth season in relation to either vitamin D or ASD compared with controls.

Elevated serum alkaline phosphatase levels and low phosphorus levels are essential markers for diagnosis of vitamin D deficiency.[37] İn this study, vitamin D deficiency was supported by increased alkaline phosphatase levels in group 2. Similarly, the mean phosphorus level was also higher, not lower, in group 2 than in the control group. However, sample timing, nutritional status, and the duration of vitamin D deficiency can affect calcium and phosphorus levels.

Levels of 25-OHD in adolescent patients with ASD aged 11 to 18 years were significantly lower than those in younger patients

aged <11 years in our study. This difference is assumed to be related to the increased vitamin D requirements during adolescence. In fact, adolescence is a critical period when restructuring process of bone development occurs. Moreover, adolescence has been reported to be an essential risk factor for vitamin D deficiency.[38]

Remarkably, although the rate of vitamin D deficiency/insufficiency was high in patients with ASD, it was also relatively high in the healthy control group in our study. Relatively high rates of subclinical vitamin D deficiency have been reported in otherwise healthy infants children and adolescents in several countries, including in Turkey.[39,40] Nevertheless, the mean serum 25-OHD level was higher in patients with ASD than in controls in our study.

Limitations of the study

Although our study included a large sample of participants, it has some limitations. Our study was limited by data on children who were kept on avoidance/restriction diets. Additionally, data on the duration of outdoor activity, sun exposure, and detailed life style were lacking.

Conclusion

Our study shows that serum 25-OHD levels in children with ASD are significantly lower than those in healthy controls, especially in the adolescent period. To determine whether vitamin D deficiency is a cause or a consequence of ASD, more detailed multicenter, prospective studies are required considering all risk factors (e.g., pregnancy period). Monitoring vitamin D levels in autistic children, especially adolescents, is required to take protective measures and treat this condition early.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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