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Investigation of Vitamin D and Vitamin B12 Levels in Children Diagnosed with Attention Deficit Hyperactivity Disorder

Dikkat Eksikliği ve Hiperaktivite Bozukluğu Tanılı Çocuklarda D Vitamini ve B12 Vitamini Düzeylerinin İncelenmesi

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ABSTRACT

OBJECTIVE: Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood. In this context, studies showing the possible roles of vitamin B12 and vitamin D in the pathophysiology of ADHD are noteworthy. In this study, we aimed to investigate the levels of vitamin B12 and vitamin D in children diagnosed with ADHD.

MATERIALS and METHODS: A total of 96 children who applied to the Child Mental Health and Diseases Polyclinic of Uşak University Training and Research Hospital were included in this retrospective study. Two groups were formed in the study: an ADHD group and a control group. Demographic data such as age and gender, and vitamin B12 and vitamin D levels of individuals in the ADHD group and the healthy group were retrospectively examined through the hospital's electronic system. Statistical analyses; The Kolmogorov-Smirnov test, Student t-test, and Mann Whitney-U test for numerical data not showing normal distribution were included.

RESULTS: There was no statistically significant difference in the mean age between the ADHD group and the control group. Vitamin B12 and vitamin D levels were found to be higher on average in the control group, and the difference between the groups was statistically highly significant ($p<0.0001$).

CONCLUSION: Although studies examining the relationship between ADHD and vitamin B12 and vitamin D levels are increasing in the current literature, the results show some inconsistencies. In this study, the significantly lower levels of vitamin D and vitamin B12 in the ADHD group suggest that deficiencies in these vitamins may be related to the pathophysiology of ADHD.

KEYWORDS: ADHD, vitamin B12, vitamin D

ÖZ

AMAÇ: Dikkat eksikliği ve hiperaktivite bozukluğu (DEHB), çocukluk çağının en yaygın nörogelişimsel bozukluklarından biridir. Bu bağlamda, B12 vitamini ve D vitamininin DEHB patofizyolojisindeki olası rollerinin olabileceğini gösteren çalışmalar dikkat çekmektedir. Bu çalışmada DEHB tanısı almış çocuklarda B12 vitamini ve D vitamini düzeylerini araştırmayı amaçladık.

GEREÇ ve YÖNTEM: : Retrospektif olan bu çalışmaya Uşak Üniversitesi Eğitim ve Araştırma Hastanesi Çocuk Ruh Sağlığı ve Hastalıkları Polikliniği'ne başvuran toplamda 96 çocuk dahil edildi. Çalışmada DEHB grubu ve kontrol grubu olmak üzere iki grup oluşturuldu. DEHB grubu ve sağlıklı grubunu oluşturan bireylere ait yaş, cinsiyet gibi demografik veriler ve B12 vitamini, D vitamini düzeyleri hastanenin elektronik sistemi üzerinden retrospektif olarak incelendi. İstatistiksel analizler; Kolmogorov-Smirnov testi, Student t-testi, normal dağılım göstermeyen numerik verilerde Mann Whitney-U testini içeriyordu.

BULGULAR: DEHB grubu ile kontrol grubunun yaş ortalaması arasında istatistiksel anlamlılık yoktu. B12 vitamini ve D vitamini düzeyi kontrol grubunda ortalama olarak daha yüksek olduğu tespit edildi, gruplar arasındaki fark istatistiksel olarak ileri düzeyde anlamlıydı ($p<0,0001$).

SONUÇ: Mevcut literatürde DEHB ile B12 vitamini ve D vitamini düzeyleri arasındaki ilişkiyi inceleyen çalışmalar giderek artmakla birlikte, sonuçlar bazı tutarsızlıklar göstermektedir. Bu çalışmada DEHB grubunda D vitamini ve B12 vitamini düzeylerinin anlamlı derecede düşük olması, bu vitaminlerin eksikliğinin DEHB fizyopatolojisiyle ilişkili olabileceğini düşündürmektedir.

ANAHTAR KELiMELER: DEHB, B12 vitamini, D vitamini

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INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of childhood and is characterized by inattention, hyperactivity, and impulsivity (1). Its prevalence among school-aged children worldwide ranges from 5% to 7%, and it is diagnosed 2 to 2.5 times more frequently in boys than in girls (2). ADHD is a chronic disorder that profoundly affects academic achievement, social relationships, and quality of life.

Although the etiology and pathogenesis of ADHD have not been fully elucidated, the disorder is believed to result from a complex interaction among genetic predisposition, perinatal risk factors, environmental toxins, and neurobiological changes (3). Recent research has focused on the effects of dietary habits and micronutrients, including vitamins and minerals, on brain development, myelination, and neurotransmitter metabolism. Neuronal energy metabolism, neurotransmitter synthesis (dopamine, serotonin, and norepinephrine), and synaptic plasticity depend directly on adequate levels of essential vitamins (4).

Vitamin D, traditionally associated with calcium-phosphate metabolism and bone health, has also been shown to play critical roles in the central nervous system (CNS). Vitamin D receptors (VDRs) and the enzyme 1-alpha-hydroxylase are widely expressed in brain regions such as the hippocampus, prefrontal cortex, cerebellum, and striatum (5). The presence of these receptors suggests that vitamin D has a direct regulatory role in processes such as neurogenesis, neuronal differentiation, neurotransmitter release, and neuroprotection (6). Low vitamin D levels have been suggested to exacerbate ADHD symptoms by contributing to dysfunction of the dopaminergic and serotonergic systems (7). A recent case-control study reported significantly lower serum vitamin D levels in children with ADHD than in healthy controls (8).

Vitamin B12 (cobalamin) is an essential cofactor for the synthesis and integrity of the myelin sheath. It contributes to homocysteine metabolism through the activity of methionine synthase and methylmalonyl-CoA mutase. Hyperhomocysteinemia can lead to neuronal apoptosis and oxidative stress through neurotoxic mechanisms (9, 10). Vitamin B12 deficiency is closely associated with cognitive decline, depression, anxiety, and attention disorders (11). Recent systematic reviews have shown that serum vitamin B12 levels are significantly lower in children with ADHD (12). In a study by Lukovac et al. (13), vitamin B12 levels were low and homocysteine levels were significantly elevated in boys with ADHD, suggesting that these parameters may serve as potential biomarkers of ADHD.

In light of current evidence, our study aimed to investigate the potential relationship between vitamin D and vitamin B12 deficiencies and the pathophysiology of ADHD by comparing serum vitamin D and vitamin B12 levels in children aged 6-10 years diagnosed with ADHD to a healthy control group.

MATERIALS AND METHODS

Study Design and Population

This retrospective case-control study included children who presented to the Child and Adolescent Psychiatry Outpatient Clinic at Uşak University Training and Research Hospital between January 1, 2020, and August 31, 2021. The study group comprised children aged 6-10 years who had been diagnosed with ADHD according to DSM diagnostic criteria documented in the medical records, had no comorbid psychiatric disorders (anxiety, depression, autism spectrum disorder, etc.), had no acute or chronic physical illness, and were not receiving medication or vitamin supplements at the time of blood collection. The ADHD group comprised 48 children (37 males and 11 females). The control group comprised 48 healthy children (35 males and 13 females) in the same age range who had no psychiatric diagnosis or systemic illness and were not receiving medication.

The study protocol was approved by the Ethics Committee for Non-Interventional Clinical Research at Uşak University Faculty of Medicine on April 20, 2022 (Decision No. 80-80-13). All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Data Collection

Demographic data, including age and sex, and routine biochemical test results were retrospectively retrieved from the hospital's electronic database (Hospital Information Management System). Serum 25-hydroxyvitamin D and vitamin B12 (cobalamin) levels were measured in the hospital's central laboratory using the electrochemiluminescence immunoassay

(ECLIA) method on a Roche Cobas analyzer. Vitamin D levels<20 ng/mL were classified as deficient, levels from 20 to<30 ng/mL as insufficient, and levels ≥30 ng/mL as sufficient.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Kolmogorov-Smirnov test. For normally distributed data (age and vitamin D), the independent-samples t test was used for between-group comparisons; for non-normally distributed data (vitamin B12), the Mann-Whitney U test was used. Sex distributions were compared using the chi-square test. Continuous variables are presented as mean±standard deviation (SD) for normally distributed data and as median (interquartile range [IQR]) for non-normally distributed data. Statistical significance was set at $p<0.05$.

RESULTS

A total of 96 children were included in the study: 48 controls (35 boys and 13 girls) and 48 children diagnosed with ADHD (37 boys and 11 girls). The mean age was 7.813±1.249 years in the ADHD group and 8.146±1.304 years in the control group; the difference between the groups was not statistically significant (Table 1). When vitamin D and vitamin B12 levels were compared between the groups, both were significantly lower in the ADHD group (Table 1).

Table 1. *Sociodemographic characteristics and vitamin B12 and vitamin D levels of the study groups*

	ADHD (n=48)	Control (n=48)	p-value
Age (years), mean±SD	7.81±1.24	8.14±1.30	0.200 (t test)
Sex (M/F)	37 / 11	35 / 13	0.624 (chi-square test)
Vitamin B12 (pg/mL), median (IQR)	282 (155.8)	515.5 (82.7)	<0.0001 (Mann-Whitney U)
Vitamin D (ng/mL), mean±SD	13.90±5.12	27.29±10.16	<0.0001 (t test)

ADHD: Attention-deficit/hyperactivity disorder, SD: Standard deviation, IQR: Interquartile range. Statistical significance was set at $p<0.05$.
M: Male, F: Female

The mean vitamin D level was 13.90±5.12 ng/mL in the ADHD group, which was below the 20 ng/mL threshold used to define vitamin D deficiency in this study. In the control group, the mean level was 27.29±10.16 ng/mL, and the difference between the groups was highly statistically significant ($p<0.0001$). The median serum vitamin B12 level was 282 pg/mL (IQR, 155.8) in children diagnosed with ADHD and 515.5 pg/mL (IQR, 82.7) in the control group. The difference between the groups was highly statistically significant ($p<0.0001$).

DISCUSSION

This retrospective case-control study demonstrated that serum vitamin B12 and vitamin D levels were significantly lower in children aged 6-10 years diagnosed with ADHD than in healthy controls. These findings are consistent with a growing number of national and international studies published over the past five years that highlight the potential role of micronutrient deficiencies in the etiopathogenesis of ADHD. In our study, the male-to-female ratio of 3.36:1 in the ADHD group was consistent with established epidemiological data indicating that ADHD is diagnosed two to three times more frequently in boys than in girls. This difference has been attributed to the greater prevalence of the inattentive subtype and more prominent internalizing symptoms among girls, which may contribute to underdiagnosis. The similar mean ages of the two groups indicate that the observed differences in vitamin levels were unlikely to be attributable to age.

In our study, the mean vitamin D level in the ADHD group was below the 20 ng/mL threshold used to define vitamin D deficiency in the study. A statistically significant difference was found between the ADHD and control groups, and this result is consistent with many studies in the literature.

In a case-control study conducted in 2023 by Massoodi et al. (8) on 45 children with ADHD and 45 healthy children aged 6-12 years, it was reported that the mean serum vitamin D levels

were lower in the ADHD group compared to the control group, and the difference was statistically significant. In the same study, an inverse correlation was observed between vitamin D levels and ADHD severity; however, this relationship was not statistically significant. In another study conducted in 2024 by Elshahawi et al. (14) on 108 children (54 with ADHD and 54 in the control group), it was reported that the mean weekly vitamin D intake was significantly lower in the ADHD group compared to the control group, and that vitamin D levels were an independent predictor of ADHD susceptibility.

A large-scale study published in Frontiers in Psychiatry in 2025, involving 657 children aged 4-10 years, showed that serum vitamin D levels were significantly lower in the ADHD group compared to the control group (15). The study also examined fat-soluble vitamins A, D, and E among ADHD symptom subtypes, suggesting that vitamin profiles may differ between subtypes. Numerous scientific studies are increasingly understanding the mechanisms underlying the role of vitamin D in the pathophysiology of ADHD. Vitamin D receptors (VDRs) are highly expressed in brain regions associated with attention and executive function, such as the hippocampus, prefrontal cortex, and striatum. Vitamin D regulates dopamine and norepinephrine synthesis by modulating tyrosine hydroxylase activity; it also supports neuronal survival by increasing the expression of neurotrophic factors (NGF and GDNF) and reducing oxidative stress. Yang et al. (16) A study conducted in 2023 suggested that although serum 25(OH) D levels below normal are not a primary pathogenic factor in ADHD, they may act as a significant factor contributing to the exacerbation of ADHD symptoms in some school-aged children.

A 2025 study by Zhang et al. (17) found that vitamin D deficiency was associated with longer sleep duration and sleep-related breathing problems in children aged 6-14 years diagnosed with ADHD; however, no direct difference was observed in ADHD symptoms or functional impairment. These findings suggest that vitamin D status may affect the clinical burden of ADHD indirectly through sleep-related pathways rather than through a proven direct effect on core symptoms. Furthermore, the number of randomized controlled trials investigating the effects of vitamin D supplementation on ADHD symptoms has increased in recent years. In a double-blind randomized controlled trial published in 2024 by Mirhosseini et al. (18), children diagnosed with ADHD received 50,000 IU of vitamin D3 weekly for 2 months in conjunction with neurofeedback therapy. At the end of the study, significant increases in 25(OH)D levels and serum calcium levels were reported in the treatment group. In addition, it was emphasized that in the treatment group, significant reductions were observed in relative theta power and theta/beta and theta/alpha power ratios under both eye conditions, and significant correlations were found between Conners scores and some brainwave improvements (18). These findings suggest that vitamin D supplementation may have beneficial effects on neurophysiological parameters and behavioral outcomes in children with ADHD.

A review published in 2025 by Urbaniak et al. (19) concluded that vitamin D supplementation may be a beneficial adjunctive therapy to alleviate ADHD symptoms. A review by Kitaneh et al. (20) also comprehensively addressed the role of vitamin D in the pathophysiology and management of ADHD.

Studies conducted in Türkiye have also presented findings parallel to our study, supporting an association between ADHD and vitamin D deficiency. A study conducted in 2014 by Goksugur et al. (21), involving 60 children with ADHD and 30 healthy controls, showed that serum 25-hydroxyvitamin D levels were significantly lower in children with ADHD compared to healthy controls; this was one of the first studies in Türkiye to investigate the relationship between ADHD and vitamin D. Şahin et al. (22) In a 2018 study by Bala et al. (23) involving 40 children with ADHD and 40 healthy controls aged 6-12 years, both serum vitamin D and vitamin D receptor (VDR) levels were found to be significantly lower in the ADHD group; this was the first study to examine VDR levels in ADHD. In a 2016 study by Bala et al. (23) involving 34 children with ADHD, 16 children with autism spectrum disorder (ASD), and 27 healthy controls, vitamin D levels were found to be significantly lower in the ADHD group compared to the healthy control group, highlighting the importance of vitamin B12 and vitamin D supplementation for both ADHD and ASD groups.

Another study conducted in Türkiye showed that ADHD symptoms in both childhood and adulthood were more pronounced in women with vitamin D deficiency (24). However, there are also some scientific studies reporting conflicting results. A recent Turkish study on the subject reported no significant difference in mean serum vitamin D levels between children with ADHD and healthy controls (25).

These conflicting findings highlight the numerous confounding variables affecting vitamin D levels in ADHD (including dietary habits, sun exposure, seasonal variation, genetic factors, and environmental influences) and the need for larger prospective studies. Furthermore, Türkiye's location in the northern hemisphere and the fact that sunlight does not directly reach the earth's surface for most of the day suggest that vitamin D deficiency may be a significant cause in this country.

In our study, the median vitamin B12 level in the ADHD group was found to be significantly lower than in the control group, and the difference between the groups was statistically highly significant. These findings are in strong agreement with recently published meta-analyses and systematic reviews. Razavinia et al. (12) A systematic review and meta-analysis published by analyzed six studies involving 982 children in 2024 and showed that vitamin B12 levels were significantly lower in children with ADHD compared to healthy controls. After a sensitivity analysis conducted because of high heterogeneity (I^2 = 93%), heterogeneity decreased to I^2 = 21%, while the between-group difference remained significant .This meta-analysis supports a potential association between vitamin B12 status and ADHD (12).

In a 2024 study by Lukovac et al. (13) involving 133 boys aged 6.5–12.5 years (67 with ADHD and 66 in a healthy control group), homocysteine levels were significantly higher and vitamin B12 levels were significantly lower in the ADHD group. Multivariate logistic regression analysis showed that homocysteine could be a potential prognostic marker for ADHD. The researchers suggested that high homocysteine and low vitamin B12 levels could be used as biomarkers for the diagnosis and prognosis of ADHD.

A systematic review and meta-analysis by Tan et al. (26) in 2023, involving 56 studies and 37,932 participants, showed that vitamin B12 levels were significantly lower in individuals with ADHD compared to the control group. The same study found that higher vitamin B12 intake was associated with a reduced risk of depression and behavioral problems. Researchers have highlighted that vitamin B12 deficiency may be a reversible cause of some mental health disorders in children and adolescents. In a study by Adıgüzel Akman and Sarıgedik (27) in 2025, they found that vitamin B12 and vitamin D levels in the group with ADHD and cognitive dissociation syndrome (CDS) were significantly lower than in the groups with only ADHD and control groups. This finding suggests that vitamin deficiencies may contribute to different ADHD phenotypes to varying degrees and may be more pronounced in comorbid conditions such as CDS.

Interestingly, a study conducted in 2025 by Kabukçu Başay et al. (28), involving 42 children with ADHD and 36 healthy control subjects, reported that vitamin D levels were higher in the ADHD group, while vitamin B12 and folic acid levels showed no significant difference between the groups. In the same study, total oxidant status (TOS), oxidative stress index (OSI), and homocysteine levels were shown to be significantly higher in the ADHD group. These findings differ from the general trend in the literature and highlight the complexity of the underlying biological processes of ADHD and the potential importance of adaptive cellular factors. The role of vitamin B12 in the pathophysiology of ADHD has been elucidated through various mechanisms, particularly homocysteine metabolism. Vitamin B12 acts as a cofactor for methionine synthase in the conversion of homocysteine to methionine; this reaction is central to the methionine cycle, which is critical for neurodevelopment and neuronal maintenance (29). In vitamin B12 deficiency, homocysteine accumulation can lead to neuronal damage by causing excessive NMDA receptor stimulation and excitotoxicity (30). Homocysteine also acts as an NMDA receptor agonist and exerts neurotoxic effects, particularly on dopaminergic neurons. Vitamin B12 can alleviate neuroinflammation by scavenging reactive species, limiting the harmful effects of excess homocysteine, and reducing NMDA receptor-mediated excitotoxicity. Furthermore, decreased S-adenosylmethionine (SAM) synthesis impairs its methylation and thus the synthesis of amine neurotransmitters such as dopamine, serotonin, and norepinephrine. Folic acid and vitamin B12 deficiencies negatively affect neurotransmitter production by impairing methylation capacity in the brain (31). Studies in children with ADHD have shown significant associations between high homocysteine and low vitamin B12 levels (13); This neurotransmitter imbalance has been associated with the core symptoms of ADHD, namely inattention, impulsivity, and hyperactivity. Genetic variants in folic acid metabolism and mild hyperhomocysteinemia may also affect behavioral problems associated with ADHD (31).

Study Limitations

The principal limitations of this study are its retrospective design, which precludes causal inference, and the inability to control for confounding factors such as dietary habits, sun exposure, physical activity, seasonal variation in vitamin D levels, and body mass index. In addition, the associations between symptom severity and vitamin levels were not examined, and the sample size was relatively small. Nevertheless, our findings are consistent with the evidence currently available in the literature and make an important contribution to the field.

CONCLUSION

In this study, serum vitamin B12 and vitamin D levels were found to be significantly lower in children diagnosed with attention deficit/hyperactivity disorder (ADHD) compared to healthy controls. Our study suggests that these lower values may contribute to the onset of the disorder through mechanisms involving neurotransmitter metabolism, myelination, and neuroprotection. Our findings indicate that nutritional status and vitamin levels should be assessed as part of a multidisciplinary evaluation of children diagnosed with ADHD. Future placebo-controlled, double-blind, prospective randomized controlled trials should investigate the effects of vitamin supplementation, optimal dosage regimens, and long-term safety profiles across ADHD subtypes.

Ethics: This retrospective study was conducted through a review of medical records and Hospital Information Management System (HBYS) databases at Uşak University Training and Research Hospital. The study protocol was approved by the local ethics committee (approval number: 80-80-13), and the study was conducted in accordance with the Declaration of Helsinki.

Etik: Bu retrospektif çalışma, Uşak Eğitim ve Araştırma Hastanesi'ndeki tıbbi kayıtlar ve Hastane Bilgi Yönetim Sistemi (HBYS) veri tabanları incelenerek gerçekleştirildi. Çalışma protokolü yerel etik kurul tarafından onaylandı (onay numarası: 80-80-13) ve çalışma Helsinki Bildirgesi'ne uygun olarak yürütüldü.

Author contribution status; Concept: AŞB, AA. study design: AŞB, AA. literature review: BU, AŞB. data collection and processing: AŞB, AA. writing: AŞB, SD.

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