

# Neutrophil-lymphocyte Ratio and Serum Ferritin, Folate, Vitamin B12 and 25-hydroxyvitamin D Levels in Children and Adolescents with Primary Headaches

*Primer Baş Ağrısı Olan Çocuk ve Adölesanlarda Nötrofil-lenfosit Oranı, Serum Ferritin, Folik Asit, B12 Vitamini ve 25-hidroksivitamin D Düzeyleri*

Elif Çelik<sup>1</sup>, Ayşe Anık<sup>2</sup>, Müge Ayanoğlu<sup>3</sup>

<sup>1</sup>Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatrics, Aydın, Turkey

<sup>2</sup>Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatrics, Division of Neonatology, Aydın, Turkey

<sup>3</sup>Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Aydın, Turkey



## Keywords

Headache, complete blood count, 25-hydroxyvitamin D, ferritin, vitamin B12, folic acid

## Anahtar Kelimeler

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## Address for Correspondence/Yazışma Adresi:

Elif Çelik MD,  
Aydın Adnan Menderes University Faculty of  
Medicine, Department of Pediatrics, Aydın,  
Turkey

Phone : +90 505 478 55 18

E-mail : gencelif80@yahoo.com

ORCID ID: orcid.org/0000-0002-0298-4088

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## Abstract

**Objective:** Headache is one of the most common complaints in general pediatric outpatient clinics. We aimed to investigate the complete blood count (CBC) parameters and serum levels of 25-hydroxyvitamin D [25(OH)D], ferritin, vitamin B12 and folic acid in children and adolescents with primary headaches.

**Materials and Methods:** In this study, 149 patients with primary headache [migraine, tension-type headaches (TTH) and unclassified headache] and 104 healthy controls, aged 5 to 18 years, were evaluated retrospectively. Age, gender, physical examination findings and laboratory parameters [CBC, 25(OH)D, vitamin B12, folic acid and ferritin] were also recorded for both groups.

**Results:** The headache types in the primary headache group were TTH (48.1%), migraine (28.2%) and unclassified headache (23.7%). The primary headache group had lower median levels of ferritin and serum vitamin B12 than healthy controls ( $p=0.028$  and  $p=0.014$ , respectively). The neutrophil-lymphocyte ratio (NLR) was significantly higher in the primary headache group ( $p=0.033$ ). A binary logistic regression analysis model showed low ferritin levels as an independent risk factor for primary headaches (odds ratio =1.018, 95% confidence interval =1.003-1.032).

**Conclusion:** The study results demonstrated lower serum vitamin B12 and ferritin and a higher NLR in children with primary headaches when compared to healthy children. We suggest that these findings require support from larger prospective studies.

## Öz

**Amaç:** Baş ağrısı, genel çocuk polikliniklerinde en sık görülen şikayetlerden biridir. Bu çalışma ile birincil baş ağrısı olan çocuk ve ergenlerde, tam kan sayımı (TKS) parametreleri, 25-hidroksivitamin D [25(OH)D], ferritin, vitamin B12 ve folik asit düzeylerini araştırmayı amaçladık.

**Gereç ve Yöntemler:** Bu çalışmada 5-18 yaş arasındaki primer baş ağrısı olan 149 hasta [migren, gerilim tipi baş ağrısı (GTBA), sınıflandırılmamış baş ağrısı] ve 104 sağlıklı kontrol retrospektif olarak değerlendirildi. Her iki grup için de yaş, cinsiyet, fizik muayene bulguları ve laboratuvar parametreleri [TKS, 25(OH)D, vitamin B12, folik asit, ferritin] kaydedildi.

**Bulgular:** Birincil baş ağrısı grubundaki baş ağrısı tipleri GTBA (%48,1), migren (%28,2) ve sınıflandırılmayan baş ağrısı (%23,7) idi. Birincil baş ağrısı grubunda ortalama ferritin ve serum B12 vitamini seviyeleri sağlıklı kontrollere göre anlamlı olarak daha düşüktü (sırasıyla  $p=0,028$ ,  $p=0,014$ ). Nötrofil lenfosit oranı (NLO), birincil baş ağrısı grubunda anlamlı olarak daha yüksekti ( $p=0,033$ ). Lojistik regresyon analizi, düşük ferritin seviyelerinin birincil baş ağrıları için bağımsız bir risk faktörü olduğunu göstermiştir (olasılık oranı =1,018, %95 güven aralığı =1,003–1,032).

**Sonuç:** Bu çalışmada birincil baş ağrısı olan çocuklarda sağlıklı çocuklara göre serum vitamin B12 ve ferritin düzeyleri daha düşük, NLO daha yüksek olarak bulunmuştur. Bu sonucun daha fazla sayıda hastayı içeren prospektif çalışmalarla desteklenmesi gerektiğini düşünmekteyiz.

## Introduction

Headache is one of the most common neurological symptoms in children and adolescents and affects approximately 88% of this age group (1). Headaches are important health problems due to their effects on academic achievements, physical, and mental condition. Also, headaches constitute the majority of admission to pediatric outpatient clinics and the most prevalent types of primary headaches are migraine and tension-type headaches (TTH). In recent years, the prevalence of childhood migraine and TTH has increased due to the lifestyle changes of children (2).

One of the most widely accepted hypotheses in the pathogenesis of migraine is neurogenic inflammation. It is thought that some external factors (hormones, stress, and diet) initiate attacks in genetically susceptible individuals, stimulate noradrenergic and serotonergic nerve fibers, causing vasodilation of intracranial vessels, the release of inflammatory neuropeptides (substance P, neurokinin, calcitonin gene-related protein) by stimulating the trigeminal nerve, and ultimately causing vasodilation, inflammation, and pain (3). Apart from these, there are a few studies indicate that primary headaches may be associated with platelet count, and levels of hemoglobin, vitamin D, ferritin, folate, and vitamin B12 (4-7).

Iron is the most commonly consumed nutrient in the human diet. Studies have shown that iron plays a crucial role in the production of serotonin, dopamine, and norepinephrine (8). Many studies exist investigating the neurological effects of iron deficiency. It has been reported that children with iron deficiency had more anxiety, depression, social and attention deficit problems (9,10). Additionally, an association between serum ferritin level and depression was reported in adults (10). Also, low serotonin levels between the attacks and increased levels during attacks were reported in patients with headaches (11).

The *methylenetetrahydrofolate reductase (MTHFR)* gene allows the remethylation of homocysteine to methionine. It is claimed that decreased MTHFR activity predisposes to migraine (12). There are some studies show that supplementation with folate and vitamin B12 ameliorate the attacks of migraines, especially in patients with certain genetics variants of enzymes involved in homocysteine metabolism (13). There are few studies investigating serum vitamin B12 levels in children with primary headaches (4,6).

Evidence shows that homocysteine decreases noradrenaline and serotonin synthesis (14). Folic acid, vitamin B6, and vitamin B12 have been shown to lower serum homocysteine levels and reduce migraine symptoms (15).

Neutrophils and lymphocytes have important roles in the inflammatory response. Since the number of neutrophils increases and the number of lymphocytes decreases as a response to stress, the neutrophil-lymphocyte ratio (NLR) is used as an indicator of inflammation (16). Several studies exist concerning the relationship between hematological parameters including NLR and headaches. The results of these previous studies are inconsistent (5,17,18).

The relationship between vitamin D and primary headaches is not well elucidated. Some studies reported a possible relationship between vitamin D and migraine. Although the relation between vitamin D and primary headaches is not clear, calcium, which plays a role in the contraction of smooth muscle cells may have a role. Also, the widespread distribution of vitamin D receptors in human brain tissue suggests that vitamin D may have autocrine/paracrine properties in the brain (19).

A limited number of studies have evaluated the relationship between complete blood count (CBC) parameters, serum levels of 25-hydroxyvitamin D [25(OH)D], ferritin, vitamin B12, folic acid levels, and primary headaches in pediatric patients. Thus, we aimed to investigate the CBC, serum levels of 25(OH)

D, ferritin, vitamin B12, and folic acid in children and adolescents with primary headaches.

## Materials and Methods

This retrospective study included children and adolescents aged 5-18 years who were admitted to the general pediatric outpatient clinic with headaches for at least 6 months between June 2020 and November 2020. Exclusion criteria were as follows; children with chronic diseases (autoimmune diseases, infections, neuro-metabolic diseases, mental retardation, endocrinological diseases, psychiatric disorders), acute infections, secondary headaches, children who were taking any medication or vitamin supplements. The diagnosis of primary headaches (migraine, TTH, unclassified headache) was made by a pediatric neurologist according to the International Classification of Headache Disorders (ICDH III-beta) criteria (20). The control group included healthy children without a history of headache or any diseases who were admitted to the general pediatric outpatient clinic for evaluation before sportive activities. Age, gender, physical examination findings, and laboratory parameters [CBC, 25(OH)D, vitamin B12, folic acid, ferritin] were also recorded for both groups.

We have obtained the approval of the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 11, date: 25.02.2021) outlined in the Second Declaration of Helsinki. No informed consent has been collected since it is a retrospective study.

### Statistical Analysis

Statistical analyses were performed using the SPSS software version 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics (kurtosis and skewness), visual methods (histogram), and analytical tests (Kolmogorov-Smirnov test) were used to determine the normal distribution of numerical variables. Categorical data were presented with n and %, and numerical data with mean  $\pm$  standard deviation (SD) if normally distributed, and median (IQR) if non-normally distributed. In the comparison of two groups, student t-test was used if the data was normally distributed, and Mann-Whitney U test was used if the data were non-normally distributed. Chi-square test was used for comparison of categorical data. Possible factors determined by the prior analysis were analyzed

by binary logistic regression analysis to determine the risk factors. A p-value  $<0.05$  was accepted as statistically significant.

## Results

### a) Patient Characteristics

A total of 149 children with a headache and 104 healthy control subjects were included in the study. Fourteen children were excluded due to a secondary headache (eight hypertension, four sinusitis, two refractive errors, one allergic rhinoconjunctivitis). There were no significant differences between the groups in terms of age, SD scores (SDS) for weight, height, and body mass index ( $p>0.05$ ). The headache types in the study group were TTH (48.1%), migraine (28.2%), and unclassified headache (23.7%). Thirty-eight percent of the patients had a positive family history of a primary headache.

### b) Laboratory Characteristics

White blood cell, platelet counts, hemoglobin, hematocrit, serum 25(OH)D, and folic acid levels were not different between the groups. The median levels of ferritin and serum vitamin B12 were significantly lower in the primary headache group than those of healthy controls ( $p=0.028$ ,  $p=0.014$ , respectively). The NLR was significantly higher in the primary headache group ( $p=0.033$ ) (Table 1). Based on binary logistic regression analysis including NLR, serum ferritin, and B12 levels, every 1-unit decrease in serum ferritin constitutes a 1.018 (95% confidence interval =1.003-1.032) fold risk for primary headache (Table 2).

## Discussion

In the present study, serum levels of ferritin and vitamin B12 were lower and NLR was higher in children with a primary headache than those of control subjects. We also found low ferritin levels as an independent risk factor for primary headaches.

Migraine is the most common type of primary headache in young children (21,22). However, there are studies also reporting different results. In a Korean study, the prevalence of TTH was reported to be higher than migraine in children both aged below 7-years and those aged above 7-years (23). According to a study conducted in school-age children in Turkey, the estimated prevalence rates of unclassified headache, migraine, and TTH were 4.6%, 7.2%, and

**Table 1. The comparison of demographic features and laboratory results between the groups**

	Primary headache group (n=135)	Control group (n=104)	p
Age (years) <sup>†</sup>	13 (5.80)	12.95 (6.15)	0.177
Female	77 (57%)	50 (48%)	0.169
Male	58 (43%)	54 (52%)	
Weight-SDS <sup>†</sup>	-0.13 (2.10)	-0.21 (2.40)	0.611
Height-SDS*	0.05±0.98	0.13±1.35	0.143
BMI-SDS*	-0.01±1.46	0.28±1.57	0.713
Hemoglobin (mg/dL) <sup>†</sup>	13.20 (1.60)	13.30 (1.58)	0.234
Hematocrit (%) <sup>†</sup>	39.50 (4.10)	40.60 (4.88)	0.186
Platelet (x10 <sup>3</sup> ) <sup>†</sup>	288.50 (85.00)	293.00 (113.50)	0.455
WBC <sup>†</sup>	7650 (2720)	7025 (2417)	0.480
NLR <sup>†</sup>	1.50 (0.92)	1.24 (0.78)	0.033
Vitamin B12 (pg/mL) <sup>†</sup>	273.00 (137.00)	303.00 (160.25)	0.028
Folic acid (ng/mL) <sup>†</sup>	7.70 (3.90)	7.40 (3.33)	0.323
Ferritin (mg/dL) <sup>†</sup>	21.38 (20.52)	27.28 (22.41)	0.014
25-hydroxyvitamin D (ng/mL) <sup>†</sup>	20.20 (12.50)	21.05 (11.30)	0.148

SDS: Standard deviation score, BMI: Body mass index, WBC: White blood cell, NLR: Neutrophil-lymphocyte ratio, \*mean ± standard deviation, †median (interquartile range)

**Table 2. Binary logistic regression analysis for prediction of independent risk factors for primary headaches**

	β	Odds ratio	95% CI for OR	Upper limit	p
			Lower limit		
Ferritin	0.017	1.018	1.003	1.032	0.015
Vitamin B12	0.001	1.001	1.000	1.003	0.129
NLR	-0.170	0.843	0.600	1.185	0.329
Constant	-0.964	0.382	-	-	0.051

CI: Confidence interval, OR: Odds ratio, NLR: Neutrophil-lymphocyte ratio

7.8%, respectively (24). Just et al. (25) suggested that ethnic and geographic differences might have a role in the prevalence of headache type. Methodological differences might also have a contribution to the different results. Consequently, the prevalence of primary headache types can vary from country to country, and also region to region (25). TTH was the most frequent type of primary headache in the present study.

Iron deficiency (with or without anemia) may lead to different symptoms, such as angular cheilitis, koilonychia, hair loss, loss of appetite, fatigue, sleep disorders, restless leg syndrome, breath-holding

spell, dry and rough skin (26). Few studies exist investigating the association between headaches and serum ferritin levels. Aydin et al. (4) reported that levels of serum ferritin, folate, vitamin B12, and 25(OH)D were significantly lower in patients with migraines than those of the control group. In a case-control study, the levels of hemoglobin and serum ferritin were significantly lower in females with migraines than those of the control group (7). Similarly, in the present study, serum ferritin levels were significantly lower in the primary headache group. Moreover, we found every 1-unit decrease in serum ferritin constitutes a 1.018 fold risk for primary

headache. There are studies regarding the association between anxiety, depression, and primary headaches. In a study by Rousseau-Salvador et al. (27), children with chronic daily headaches had higher depression scores than the standardized reference population. Romano et al. (28) found a significant relationship between anxiety, depression, and primary headaches. In a case-control study, 67 females with depression were compared with 125 healthy subjects and serum ferritin levels were significantly lower in the depression group. Therefore, it is suggested that there was a probable association between depression and decreased ferritin levels before the occurrence of anemia (10). The association between migraine and serotonin is a controversial issue for decades. Curran et al. (29) showed that the urinary excretion of the 5-hydroxyindoleacetic acid, which was the main metabolite of serotonin, was increased. Hoyer et al. (11) reported a low serotonin content between migraine attacks and increasing levels during attacks. Therefore, these results gave rise to the thought of migraine was associated with chronically low serotonin levels with transient increases during attacks. Kaladhar and Narasinga Rao (30) performed a study in rats to investigate the effects of iron deficiency on serotonin uptake and showed decreased serotonin uptake. The results of the present study can be explained by the studies which were mentioned above concerning the relationship between iron deficiency and serotonin uptake.

We found lower vitamin B12 levels in children with primary headaches than control subjects. Vitamin B12 deficiency may lead to various neurological disorders such as motor delay, apathy, seizures, and involuntary developmental disorders (31). There are few studies concerning the association between migraine, TTH, and vitamin B12 levels (4,6,32). In a case-control study (6), it is showed that serum vitamin B12 levels were significantly lower in pediatric patients with TTH than those of the control. Togha et al. (32) showed that serum vitamin B12 levels were significantly lower in adults with migraines. Similarly, serum vitamin B12 levels were significantly lower in the primary headache group than those of the control group in the present study. As we browse through the probable explanations of the relationship between serum vitamin B12 levels and pain; animal studies showed that vitamin B12 may have both central

and peripheral cyclooxygenase (COX) inhibitory features. The possible relation between the pain and vitamin B12 could be partially explained by the inhibitory action of vitamin B12 on both central and peripheral COX enzymes that demonstrated in animal studies. Additionally, a probable role to control COX levels during the inflammatory process was also proposed (33,34). Furthermore, vitamin B12 has a neurotransmitter-moderated antinociceptive effect by lowering homocysteine levels which decreases serotonin synthesis (14).

Folate is a water-soluble synthetic form of folic acid which is also necessary for homocysteine metabolism (35,36). Studies exist concerning the relationship between folate and headaches (4,37). There are reports suggesting dietary supplementations with folate and B vitamin complexes in patients with primary headaches (38). In the present study, no significant differences were found between the case and control groups, in terms of folate levels.

NLR is associated with peripheral inflammation and oxidative stress in chronic neurological diseases. Additionally, it is used as a predictor of cardiovascular disease, stroke, and cancer prognosis (39). In the present study, the median NLR value was significantly higher in the primary headache group than in the control group. The results of the previous studies regarding the relationship between hematological parameters and headaches are inconsistent. In a case-control study, NLR was significantly higher in adults with acute migraine attacks than those of the control group (5). In another study, there were no significant differences between the patients with migraine, and the control group, in terms of NLR values (40). A case-control study performed in adults with migraine showed no significant differences between the three groups (migraine with aura, migraine without aura, and healthy control), in terms of NLR (17). However, the levels of C-reactive protein, platelet to lymphocyte ratio, and neutrophil to monocyte ratio were higher in the migraine group than those of the healthy subjects. In a study by Turan et al. (18), higher levels of procalcitonin during migraine attacks were found. In a study by Domingues et al. (41), the levels of chemokines were examined in adults with TTH. According to the results, interleukin 8 levels were higher in the TTH group than in the control group. Moreover, the patients with a headache at the time



of blood sampling had higher levels of monocyte chemoattractant protein-1 than the patients without a headache at the time of blood sampling. Based on these previous study results, it was suggested that proinflammatory mechanisms might have a role in the pathogenesis of TTH and migraine (5,17,18,41). The results of the present study were consistent with the most of the previous studies concerning the association between primary headaches and inflammation.

Vitamin D levels are associated with bone mineralization, non-specific pain, and inflammatory skeletal myopathy. Also, there are studies regarding the relationship between vitamin D deficiency and headache. In a cross-sectional study, a significantly low level of serum 25(OH)D in non-migraine headaches was reported (42). Prakash et al. (43) proposed that headache symptoms were also benefitted from calcium and vitamin D therapies in patients with chronic tension headache and osteomalacia. Gungor et al. (44) found that the severity of headache was related to low serum 25(OH)D levels in children with migraines. In the present study, there were no significant differences between primary headache and healthy control groups, in terms of 25(OH)D levels.

There are some limitations in our study. Firstly, the study was performed in a single-center with relatively small sample size. Secondly, it was a retrospective study which might have a risk of bias.

## Conclusion

The present study showed lower levels of vitamin B12 and ferritin, and higher NLR in children with primary headaches. Furthermore, serum ferritin levels were found as an independent risk factor for primary headaches. Hence, we suggest that the CBC, iron, and vitamin B12 status of the children and adolescents with primary headaches should be evaluated.

## Ethics

**Ethics Committee Approval:** We have obtained the approval of the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (decision no: 11, date: 25.02.2021) outlined in the Second Declaration of Helsinki.

**Informed Consent:** No informed consent has been collected since it is a retrospective study.

**Peer-review:** Externally and internally peer-reviewed.

## Authorship Contributions

Concept: E.Ç., Design: E.Ç., M.A., Supervision: E.Ç., Materials: E.Ç., Data Collection or Processing: A.A., M.A., Analysis or Interpretation: A.A., M.A., Literature Search: A.A., M.A., Writing: A.A., M.A., Critical Review: A.A., M.A., E.Ç.

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