Vitamin B12 and Folic Acid Levels in Patients with Breath Holding Spells

Katılma Nöbeti Olan Hastalarda B12 Vitamini ve Folik Asit Düzeyleri

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ABSTRACT

Objective: Breath holding spells are a benign, non-paroxysmal disorder observed in childhood. The pathogenesis of breath holding spells is still unclear. Dysfunction of the autonomic nervous system, iron deficiency, genetic predisposition, interleukin 1, nitric oxide and cerebral erythropoietin have been implicated in the pathogenesis. The purpose of this study was to compare vitamin B12 and folic acid levels between patients diagnosed with breath holding spells and a healthy group.

Material and Methods: Fifty-one cases diagnosed with breath holding spells in the pediatric neurology clinic were included in the study group (Group 1), and 78 healthy patients presenting to the general clinic in the control group (Group 2). Hemoglobin, hematocrit, mean erythrocyte volume, vitamin B12, folic acid, ferritin, iron, and iron binding capacity levels were compared between the study and the control groups.

Results: Mean folate levels were 18.92±12.83 (5.27-91) ng/mL in the study group and 9.95±8.92 (2-37) ng/mL in the control group. Mean B12 levels were 274.13±214.37 (75-1201) pg/mL in the study group and 408.82±194.18 (120-959) pg/mL in the control group. Statistically significant differences in both vitamin B12 and folic acid levels were observed between the groups (p=0.000 and p=0.002, respectively). No significant difference was determined between the attack groups' (<15/month, >15/month) vitamin B12 (p=0.570) or folic acid (p=0.643) levels.

Conclusion: Based on this study, vitamin B12 and folic acid levels should be routinely evaluated in patients diagnosed with breath holding spells.

Key Words: Child, Folic acid, Treatment, Vitamin B12

ÖZ

Amaç: Katılma nöbetleri, çocukluk çağında görülen iyi huylu, paroksismal olmayan bir hastalıktır. Katılma nöbetlerinin patogenezi çok açık değildir. Patogenezde otonom sinir sistemi disfonksiyonu, demir eksikliği, genetik yatkınlık, interlökin 1, nitrik oksit ve serebral eritropoietinin sorumlu olabileceği ileri sürülmektedir. Bu çalışmada katılma nöbeti olan hastalar ile sağlıklı grup arasında B12 vitamini ve folik asit düzeylerinin karşılaştırılması amaçlandı.

Gereç ve Yöntemler: Çocuk nörolojisi kliniğinde katılma nöbeti tanısı konulan 51 olgu çalışma grubuna (Grup 1), genel pediatri kliniğine başvuran 78 sağlıklı hasta kontrol grubuna (Grup 2) alındı. Çalışma ve kontrol gruplarında hemoglobin, hematokrit, ortalama eritrosit hacmi, vitamin B12, folik asit, ferritin, demir ve demir bağlama kapasiteleri karşılaştırıldı.

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Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by the Institutional Ethics Committee of Adiyaman University, Faculty of Medicine, and ethical approvals were obtained prior to initiation of the research work (22.09.2020-2020/8-18).

Contribution of the Authors / Yazarların katkısı*: *AYDIN H: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***BUCAK IH:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***ERDOGAN S:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Aydin H, Bucak IH and Erdogan S. Vitamin B12 and Folic Acid Levels in Patients with Breath Holding Spells. Turkish J Pediatr Dis 2023;17:113-117.

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Received / Geliş tarihi : 27.03.2022
Accepted / Kabul tarihi : 24.06.2022
Online published : 06.09.2022
Elektronik yayın tarihi
DOI: 10.12956/tchd.1094163

Bulgular: Çalışma grubunda ortalama folat düzeyi 18.92±12.83 (5.27-91) ng/mL, kontrol grubunda ortalama folat düzeyi 9.95±8.92 (2-37) ng/mL, ortalama B12 düzeyi çalışma grubu 274.13±214.37 (75-1201) pg/mL, kontrol grubunda ortalama B12 düzeyi 408.82±194.18 (120-959) pg/mL'di. Gruplar arasında B12 vitamini ve folik asit düzeylerinde istatistiksel olarak anlamlı fark bulundu (p=0.000, p=0.002). Atak gruplarının (<15/ay, >15/ay) B12 vitamini (p=0.570) ve folik asit (p=0.643) düzeyleri arasında anlamlı fark saptanmadı.

Sonuç: Bu çalışma sonucunda katılma nöbeti olan hastalarda B12 vitamini ve folik asit düzeylerinin rutin olarak değerlendirilmesi gerektiği kanaatine varıldı.

Anahtar Sözcükler: Çocuk, Folik asit, Tedavi, B12 Vitamini

INTRODUCTION

Breath holding spells are a benign, non-paroxysmal disorder usually observed in childhood. These may occur in children between the ages of six months and six years. Approximately 0.1-4.6% of children younger than two years have been reported to experience one or more breath holding spells (1,2). Such spells are defined as short, involuntary respiratory arrest in children in response to stimuli such as anger, disappointment, fear or injury, although the pathogenesis is not completely understood (3). Factors that precipitate breath holding spells can be prevented by distracting or keeping the child away from those triggers. The attacks usually end within one minute, and no postictal period is observed. Breath holding spells are divided into three types - pale, cyanotic, and mixed, depending on the color changes at the time of attack. Attacks arise from an imbalance between sympathetic and parasympathetic activity (1). Pale-type attacks result from cardiac inhibition mediated by the parasympathetic system, and cyanotic-type attacks mostly develop as a result of respiratory inhibition through the sympathetic system (4,5). The cyanotic type is the most common form, representing 52% of cases (6). Although attacks are frequently observed between the ages of six months and six years, the average reported age at onset is under 18 months (7).

Several studies investigating why and how breath holding spells occur have implicated dysfunction of the autonomic nervous system, iron deficiency, genetics, interleukin 1, nitric oxide, and cerebral erythropoietin (8,9).

The purpose of the present study was to evaluate the role of vitamin B12 and folic acid in the etiopathogenesis of breath holding spells by comparing these between patients diagnosed with breath holding spells and a healthy control group.

MATERIAL and METHODS

Fifty-one patients diagnosed with breath holding spells in the pediatric neurology polyclinic in the Adıyaman University Faculty of Medicine, Turkey, between October 2017 and June 2019 were analyzed retrospectively. Diagnosis was based on the crying period, followed by breathlessness with open mouth, and a history of attack with color change in the face and/or body, loss of consciousness, and change in tone. All diagnosed patients were invited to attend pediatric neurology clinic check-ups at two-month intervals. The study was approved by the Institutional Ethics Committee of Adiyaman University, Faculty of Medicine, and ethical approvals were obtained prior to initiation of the research work (22.09.2020-2020/8-18).

Patients with concomitant neurological diseases such as epilepsy, mental retardation, cerebral palsy or a chronic systemic disease, history of seizures, any neurological disorder, and patients taking medications such as vitamin B12, folic acid, and iron were not included in the study. Patients who presented to the general pediatric clinic during the study period, who were aged between six and 72 months, and for whom the code Z00.1: routine child health examination was entered were included as the control group.

The cases' demographic characteristics (age, gender), attack types, attack frequencies, personal and family histories, hemoglobin (Hb), hematocrit (Hct), mean erythrocyte volume (MCV), iron (Fe), iron binding capacities (IBC), and hormonal parameters (ferritin, vitamin B12, folic acid) were recorded. Permission for the study was obtained from the local ethics committee (decision no. 2020/8-19).

Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 23.0 software was used for statistical analysis of the study data. The Independent Sample T test was used for parameters with normal distribution and Fischer's Exact test was applied to evaluate categorical variables. The variables were expressed as mean ± standard deviation, number (n), and percentage (%). Equality of variance in the groups was evaluated using Levene's test. p <0.050 value was assumed for statistical significance.

RESULTS

The study group consisted of 51 patients, 32 (62.7%) male and 19 (37.3%) female, and the control group 78 patients, 51 (65.4%) male and 27 (34.6%) female. Mean ages were 3.32 ±1.01 (1-6) years in the study group and 3.04 ±1.19 (1-6) years

Table I: Demographic features of the study and control groups.

	Study group n (%)	Control group n (%)	p
Male	32 (62.7)	51 (65.4)	0.186
Female	19 (37.3)	27 (34.6)	0.186
Age (years), mean±SD	3.32±1.11	3.04±1.19	0.174

Table II: Laboratory parameters in the study and control groups.

	Study group mean±SD	Control group mean±SD	p
Hb (g/dL)	11.19±1.11	11.85±1.47	0.008
Hct (%)	34.87±3.95	36.04±3.87	0.097
MCV (fL)	69.06±13.57	74.60±6.83	0.003
Ferritin (ng/mL)	16.94±14.52	17.63±12.25	0.808
Iron (ug/dL)	47.43±21.67	55.54±34.07	0.161
Iron binding capacity (ug/dL)	290.39±71.73	295.77±83.30	0.734
Folate (ng/mL)	9.95±8.92	18.92±12.83	0.001
Vitamin B12 (pg/mL)	274.13±214.37	408.82±194.18	0.002

Hb: *Hemoglobin,* ***Hct:*** *Hematocrit,* ***MCV:*** *Mean corpuscular volume,* ***SD:*** *Standard deviation*

Table III: A comparison of the attack groups' vitamin B12 and folic acid levels.

Attack frequency	<15/month mean±SD	>15/month mean±SD	p
Folate (ng/mL)	18.13±7.5	23.69±30.03	0.643
Vitamin B12 (pg/mL)	249.6±206.13	406±223.08	0.570

in the control group. No significant difference was observed between the groups in terms of gender (p=0.186) or age (p=0.174) (Table I). Age at diagnosis in the study group was 1.57±0.76 (0.5-4) years. Examination of the distribution of the different types of breath holding spell revealed pale type in six (11.8%) cases, cyanotic type in 29 (56.9%), and mixed type in 16 (31.4%). A history of breath holding spells was present in the families of 16 (31.4%) patients. The frequency of attacks was <15 attacks/month in 43 (84.3%) cases and >15 attacks/month in eight (15.7%). The mean attack duration was 1.60 ±1.98 (0.15-10) minutes.

Mean Hb levels were 11.19±1.11 (8.80-13.60) g/dL in the study group and 11.85±1.47 (8.72-16.70) g/dL (p=0.008) in the control group, while mean MCV values were 69.06±13.57 (60-88) fL in the study group and 74.60±6.83 (55.11-96.97) fL in the control group (p=0.003). Mean Hct values were 34.87±3.95 (28-51.5) in the study group and 36.04±3.87 (27.97-51.1) (p=0.097) in the control group, while mean ferritin levels were 16.94±14.52 (1.6-75) ng/mL in the study group and 17.63±12.25 (4-66) ng/mL in the control group (p=0.808). Mean iron levels were 47.43±21.67 (20-126) ug/dL in the study group and 55.54±34.07 (5-140) ug/dL in the control group (p=0.161), while mean iron binding capacity (IBC) values were 290.39±71.73 (123-446) ug/dL and 295.77±83.30 (110-499) ug/dL, respectively (p=0.734). No significant difference was observed between the groups' mean Hct, ferritin, Fe, or IBC values.

Mean folate levels were 9.95±8.92 (2-37) ng/mL in the study group and 18.92±12.83 (5.27-91) ng/mL in the control group (p=0.001), while mean B12 levels were 274.13±214.37 (75-1201) pg/mL and 408.82±194.18, respectively (120-959) pg/mL (p=0.002), indicating low statistical significance (Table II).

No significant difference was determined between the attack groups' (<15/month, >15/month) vitamin B12 (p=0.570) or folic acid (p=0.643) levels (Table III).

DISCUSSION

The study data were consistent with the previous literature, with the cyanotic type emerging as the most common form (56%), and a mean age at diagnosis of 18 months.

The pathophysiology of breath holding spells is unclear. Current studies on the subject report an association between iron deficiency (with or without anemia) and breath holding spells, especially since iron supplementation is effective in reducing breath holding spell attacks. This hypothesis focuses on the link between iron deficiency anemia and breath holding spells, cerebral erythropoietin content, nitric oxide production, and interleukin 1. Iron plays a vital role in the formation of the tyrosine hydroxylase enzyme required for the synthesis of catecholamine, as well as for the functioning of enzymes and neurotransmitters in the central nervous system (CNS) (10,11). Iron deficiency causes an increase in serotonin activity and/or a decrease in reductases, and thus an increase in sympathomimetic neurotransmitters (12,13). In their meta-analysis of observational studies, Hetch et al. reported that the use of iron supplements reduced the frequency of attack episodes in patients with iron deficiency together with breath holding spells, with approximately 84% of children experiencing a >50% reduction in attack frequency (14). Anemia causes hypoxia in vital organs including the heart and nervous system, which play an important role in changing the autonomic balance (15). Recurrent breath holding spells cause episodes of hypoxia, including cerebral anoxia, which may in turn give rise

to short-term tonic-clonic seizures in the extremities. Another protective response to cerebral anoxia is increased brain EPO production. A relative depletion of iron occurs since this is consumed in erythropoiesis. The child may gradually become anemic, which may result in more breath holding spells as a result of behavioral 'agitation' (10). A few recent studies have shown that dysfunction of the autonomic nervous system plays a primary role in the occurrence of breath holding spells (16,17). Another study suggested that breath holding spells may be associated with delayed maturation in brain-stem myelination in children (18). While a statistically significant difference was found between the groups in terms of mean Hb and MCV levels in the present study, significant difference was observed in terms of mean iron, iron binding capacity, Hct, or ferritin levels.

Vitamin B12 and folic acid are members of the vitamin B family. Both play a role in various pathways (19). Depending on the neuroanatomical distribution of vitamins in the mammalian brain, it has been suggested that these compounds may have more important activities than their better-known metabolic functions (20,21). Deficiency in vitamin B12 and folic acid may manifest in the form of neurological features or megaloblastic anemia. Physicians should look for signs of dorsal column involvement (such as loss of sense of position and vibration and ataxia), lateral column involvement (including spasticity, hyperreflexia, and Babinski sign positivity), and spinothalamic tract involvement (at the sensory level) in cases of vitamin B12 deficiency (22). Areas of demyelination at cerebral MRI have been reported in patients with vitamin B12 deficiency of with diseases capable affecting the metabolism thereof (23-25). The neurological manifestations of folate deficiency are similar to those of vitamin B12 deficiency, such as cognitive impairment, dementia, depression, and, less frequently, peripheral neuropathy and subacute combined spinal cord degeneration (26). The functional and specific functions of folic acid in the child brainstem are currently unknown. Duque-Díaz and Coveñas described the presence and distribution of cell bodies (fusiform, small/medium, with one short dendrite) containing folic acid in child brainstem. It appears that the number of cell bodies diminishes in an age-dependent manner (27).

Vitamin B12 and folic acid levels play an important role in homocysteine metabolism, and hyperhomocysteinemia is observed in case of folic acid and vitamin B12 deficiency (28). Hyperhomocysteinemia leads to the production of hydrogen peroxide and superoxide free radicals. This condition causes oxidative damage in vascular endothelial cells. Nitric oxide (NO) release by endothelial cells has been shown to decrease with high homocysteine levels (29). NO consists of the semi-essential amino acid L arginine created by the endothelial nitric oxide synthetase enzyme, on condition that vitamin B6, folic acid and vitamin B12 are present at sufficient levels (30). NO is also a powerful endogenous vasodilator (31). Studies have shown that vitamin B12 and folic acid deficiency cause various hematological, neurological, and psychiatric disorders (19). Hyperhomocysteinemia is observed in case of deficiency of both vitamin B1 and folic acid, resulting in decreased NO release from the endothelium and diminished NO synthesis. Vasoconstriction occurs a result of this decrease in NO, and hypoxic attacks may occur. A limited number of studies have investigated vitamin B12 levels in patients with breath holding spells. Arslan et al. (32) compared vitamin B12 levels between 30 patients with breath holding spells and a healthy group, with no significant difference being found between the two. In the present study, vitamin B12 and folic acid levels were significantly lower in the study group compared to the control group. However, no significant difference was observed between the attack groups in terms of vitamin B12 or folic acid levels. This study is the first to examine the folic acid level in breath holding spells, and also the first to examine the relationship between vitamin B12 and folic acid in breath holding spells.

The main limitation of this study is that homocysteine level were not examined among the other laboratory parameters related to vitamin B12.

CONCLUSION

The study data indicate that vitamin B12 and folic acid levels should be routinely evaluated in children diagnosed with breath holding spells. Further prospective studies with larger numbers of participants are now required.

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