Increased Hemoglobin and Plateletcrit Levels Indicating Hemoconcentration in Pediatric Patients with Migraine

Sevim Şahin, Betül Diler Durgut¹, Beril Dilber, Elif Acar Arslan, Tülüy Kaşak, Ali Cansu

Division of Pediatric Neurology, Department of Pediatrics, School of Medicine, Karadeniz Technical University, Trabzon, Turkey and ¹Division of Pediatric Neurology, Maternity and Children Diseases Education and Research Hospital, Giresun University, Giresun, Turkey

Introduction: Fluid intake was reported to reduce migraine attacks. This may be due to its effect on hemoconcentration. Hemoconcentration may manifest itself by increasing in the hemoglobin and platelet-related values. This study aimed to reveal hemoconcentration by evaluating complete blood cell counts in attack-free periods of pediatric patients with migraine.

Materials and Methods: Consecutive children with migraine (n = 70) and tension-type headache (TTH) (n = 65) were compared with the control groups. Control 1 (n = 70) and control 2 (n = 60) groups consisted of age- and gender-matched patients, respectively. Control 2 group patients had gastrointestinal symptoms leading to fluid loss, which may have caused hemoconcentration. To evaluate hemoglobin and platelets together, the M1-value was created by multiplying hemoglobin level by plateletcrit.

Results: The M1-value was higher in the migraine group than in control 1 and TTH groups (P = 0.017 and 0.034) and the hemoglobin and hematocrit levels were also higher in the migraine group than in control 2 group (P = 0.013 and 0.012). Female patients with migraine had higher hemoglobin levels as compared to the female patients in control group 1 (P = 0.041). Male patients with migraine had higher M1-values than the male patients in control group 1 (P = 0.034). In the subgroup of migraine with aura (n = 10), folic acid was significantly lower than the other patients with migraine (P = 0.02).

Conclusion: This study suggests that migraine may be accompanied with hemoconcentration in children.

Keywords: Child, hemoconcentration, hemoglobin, migraine, plateletcrit

Introduction

Migraine is the third most common disease in both males and females.¹,² It is a polygenic and multifactorial disease.³ In recent years, the sensitivity of pain pathways and the possibility of attacks originating from central nervous system have been mostly considered in migraine etiopathogenesis.⁴ Messenger molecules such as nitric oxide (NO), 5-hydroxytryptamine, and calcitonin gene-related peptide are involved in the migraine pathophysiology.⁵ There is no report found in the literature indicating the presence or absence of hemoconcentration in patients with migraine. However, it has been reported that supplemental fluid consumption reduced the frequency and duration of migraine episodes, supporting the presence of hemoconcentration in patients.⁶

Hemoconcentration manifests itself with an increase in hemoglobin and hematocrit levels.⁷ It may also lead to an increase in platelet counts.⁷ The plateletcrit level has been proposed as an effective option in detecting the quantitative abnormalities of platelets.⁸ Concentrations of blood cellular elements (i.e., hematocrit) are closely related to blood viscosity.⁹,¹⁰ Thus, hemoconcentration affects the degree of...
disturbance of the flow streamlines.\textsuperscript{[9]} In a previous study, migraine in children with sickle cell anemia has been reported to be associated with lower hemoglobin levels.\textsuperscript{[11]} However, hemoglobin or plateletcrit levels in children with migraine have not been evaluated. This study aimed to evaluate the hemoglobin and plateletcrit levels to provide clues about hemoconcentration in children with migraine. In addition, the ferritin, vitamin B12, and folic-acid levels were investigated considering their effects on hemoglobin levels.

**Materials and Methods**

A total of 70 patients with migraine and 65 patients with tension-type headache (TTH) who were consecutively referred to the pediatric neurology outpatient clinic were included in the two study groups. The International Classification of Headache Disorders 3-beta was used to reevaluate the diagnoses of the participants. Those who suffered from probable migraine were excluded from the study sample. The medical records of the participants were evaluated retrospectively to detect their complete blood counts (CBCs) taken during attack-free periods. Control group 1 consisted of 70 healthy children, who matched to the migraine group in terms of age and gender. Control group 2 consisted of 60 patients whose CBCs were measured after gastrointestinal complaints such as nausea, vomiting, and diarrhea, which might cause fluid loss and hemoconcentration. Their age and gender distribution were similar to the migraine group. The age of the participants at the time of the laboratory examinations was taken into consideration.

The hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration, red blood cell (RBC) distribution width, erythrocyte and thrombocyte counts, mean platelet volume, platelet distribution width, and plateletcrit levels in the participants were recorded. To obtain clues about the hemoconcentration, M1-value was also created by multiplying hemoglobin level by plateletcrit.

The vitamin B12 levels of the participants in the last 3 years were reviewed retrospectively. Vitamin B12 levels <200 pg/mL were considered deficient. The participants, who had a vitamin B12 deficiency for the last 3 years, were accepted as having a history of vitamin B12 deficiency. The vitamin B12, folic acid and ferritin levels, a history of vitamin B12 deficiency and a family history for headaches in the migraine and TTH groups, and attack frequency in the migraine group only were also recorded.

The migraine subgroups divided according to the attack frequency included participants with four or more episodes per month (n = 21) and fewer than four episodes per month (n = 31). The other subgroups were formed based on gender, presence of the aura, history of vitamin B12 deficiency, and family history for headaches.

**Statistical analysis**

The data in the migraine group were compared with the TTH and control groups. The migraine subgroups were also compared with each other. The Kolmogorov–Smirnov test was used to determine whether the distribution of the data was normal. The Student’s t test was used when the distribution was normal, whereas the Mann–Whitney U test was used when it was otherwise. Pearson’s chi-square and Fisher’s exact tests were used in the comparison of the groups in terms of gender distribution, history of vitamin B12 deficiency and a family history for headaches. The statistical significance level was determined as $P < 0.05$.

**Results**

The characteristics of the patients and control groups are presented in Table 1. In the migraine group, the M1-value (Hb level $\times$ plateletcrit level) was significantly higher as compared to the TTH group ($P = 0.034$) [Table 2]. A family history of headaches was also significantly more common in the migraine group ($P = 0.012$) [Table 1]. A history of vitamin B12

| Table 1: The general characteristics of the patients |
|---------------------------------------------------|
|                                               |
| **Migraine** group ($n = 70$) | **TTH** ($n = 65$) | **Control 1** ($n = 70$) | **Control 2** ($n = 60$) | **Comparison of the groups $P$ value** |
| Age, mean ± SD, years | $12.7 ± 3$ | $12.1 ± 3$ | $12.8 ± 2.9$ | $11.9 ± 2.5$ | $>0.05$* |
| Female/male ratio | 1.26 | 1.32 | 1.26 | 0.88 | $>0.05$ |
| History of vitamin B12 deficiency (%) | 44.3% | 43.1% | – | – | $>0.05$ |
| Family history of headache (%) | 44.8% | 23.8% | – | – | **0.012** |

SD = standard deviation, TTH = tension-type headache
Statistically significant $P$ values are marked in bold characters

*Student’s $t$ test was used, and other statistical analyses were performed with Pearson’s chi-square test
deficiency was similar between the migraine and TTH groups [Table 1].

When compared to control group 1, the M1-value was higher in the migraine group ($P = 0.017$) [Table 2]. In the comparison of the migraine group and control group 2, the hemoglobin and hematocrit levels were higher in the migraine group ($P = 0.013$ and $P = 0.012$). These levels were not statistically different between the control groups. In the comparison of the TTH and both control groups, the M1-values were not found to be different ($P > 0.05$) [Table 2]. In the TTH group, the MCV and MCH levels were higher, as compared to control group 2 ($P = 0.03$, Student’s $t$ test and $P = 0.019$, Mann–Whitney $U$ test, respectively).

In the migraine group, the erythrocyte count and hemoglobin and hematocrit levels were lower in female participants than in males ($P = 0.000$, 0.004, and 0.002, respectively) [Table 3]. In control group 1, the erythrocyte count, and hemoglobin and hematocrit levels were also lower in female participants ($P = 0.000$, 0.001, and 0.000, respectively) [Table 3]. The hemoglobin levels were higher in females with migraine as compared to the female participants in control group 1 ($P = 0.041$, Student’s $t$ test). However, the M1-value did not show any statistical difference. The M1-values were significantly higher in male participants with migraine as compared to males in control group 1 ($P = 0.034$, Student’s $t$ test).

In the migraine group, migraine with aura was present in 10 (14.7%) patients. In these patients, the folate levels were significantly lower as compared to the other patients with migraine ($P = 0.02$) [Table 3]. In the subgroups divided according to the attack frequency, these levels were not statistically different ($P > 0.05$).

**DISCUSSION**

This study revealed various clues about the presence of hemoconcentration in children with migraine. The M1-value ([hemoglobin level] × [plateletcrit level]) created to reflect the changes in at least two blood cell lines was significantly higher in the patients with migraine, as compared to both TTH and control 1 groups. In the migraine group, the hemoglobin and hematocrit levels were also significantly higher as compared to those in control group 2 who had various symptoms that could lead to hemoconcentration. The hemoconcentration in the patients with migraine has not been emphasized in previous reports. However, several studies have underlined the relationship between increased hemoglobin and headache.[12] In a population study conducted on female patients, the likelihood of migraine was reported to decrease

| Table 2: Comparison of the data in migraine and other groups |
|------------------|----------------|----------------|----------------|----------------|----------------|
| Parameters       | Migraine group| TTH group      | Control 1      | Control 2      | Comparison of |
|                  |                |                |                |                | migraine and |
|                  |                |                |                |                | TTH $p_1$    |
|                  |                |                |                |                | Comparison of |
|                  |                |                |                |                | migraine and |
|                  |                |                |                |                | control 1 $p_2$|
|                  |                |                |                |                | Comparison of |
|                  |                |                |                |                | migraine and |
|                  |                |                |                |                | control 2 $p_3$|
| Hb (g/dL)        | 13.39 ± 1.08   | 13.16 ± 1.06   | 13.06 ± 1.07   | 12.95 ± 0.87   | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    | 0.013
|                  |                |                |                |                | $P > 0.05$    | 0.012
| Hct (%)          | 39.86 ± 3.21   | 39.15 ± 2.96   | 39.05 ± 2.98   | 38.58 ± 2.39   | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| Erythrocyte count ($\times 10^3 /µL$) | 4.79 ± 0.4 | 4.69 ± 0.32 | 4.71 ± 0.4 | 4.74 ± 0.35 | $P > 0.05$ |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| MCV (fL)         | 82.9 ± 5.5     | 83.5 ± 5.1     | 82.9 ± 5.2     | 81.6 ± 4.9     | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| MCH (pg)         | 27.9 ± 2.2     | 28.1 ± 2       | 27.9 ± 2.1     | 27.4 ± 1.9     | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| MCHC (g/dL)      | 33.7 ± 0.8     | 33.6 ± 1       | 33.7 ± 1.4     | 33.6 ± 1       | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| RDW (%)          | 13.4 ± 0.9     | 13.6 ± 1.7     | 13.6 ± 1       | 13.5 ± 0.9     | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| Platelet count ($\times 10^9 /µL$) | 314 ± 7.7 | 294 ± 7.5 | 291 ± 6.6 | 290 ± 6.5 | $P > 0.05$ |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| MPV (fL)         | 8.6 ± 1.2      | 8.6 ± 1.2      | 8.6 ± 1.1      | 8.7 ± 1.1      | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| PDW (%)          | 16 ± 1.1       | 16.3 ± 0.8     | 15.2 ± 2.6     | 15.2 ± 2.6     | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| Plateletcrit (%) | 0.27 ± 0.06    | 0.25 ± 0.06    | 0.25 ± 0.05    | 0.25 ± 0.07    | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
|                  |                |                |                |                | $P > 0.05$    |
| M1-value**       | 3.55 ± 0.83    | 3.26 ± 0.73    | 3.23 ± 0.73    | 3.27 ± 0.89    | 0.034         |
|                  |                |                |                |                | 0.017         |
|                  |                |                |                |                | $P > 0.05$    |
| Vit. B12 (pg/mL)| 235 ± 118      | 236 ± 118      | –              | –              | $P > 0.05$    |
|                  |                |                |                |                | –             |
| Folate (ng/mL)   | 7.43 ± 2.24    | 8.2 ± 2.5      | –              | –              | $P > 0.05$    |
|                  |                |                |                |                | –             |
| Ferritin (ng/mL) | 30.1 ± 1.9     | 29.3 ± 1.3     | –              | –              | $P > 0.05$    |
|                  |                |                |                |                | –             |

$Hb$ = hemoglobin, $Hct$ = hematocrit, $MCH$ = mean corpuscular hemoglobin, $MCHC$ = mean corpuscular hemoglobin concentration, $MCV$ = mean corpuscular volume, $MPV$ = mean platelet volume, $PDW$ = platelet distribution width, $RDW$ = red blood cell distribution width, $TTH$ = tension-type headache

Statistically significant $P$ values are marked in bold characters

*Mann–Whitney $U$ test was used. Other analyses were performed with Student’s $t$ test

**Hemoglobin level × plateletcrit
in patients with a hemoglobin level of <11.5 g/dL.\textsuperscript{[13]} Headaches associated with chronic altitude sickness and polycythemia have also been associated with higher hemoglobin levels.\textsuperscript{[13,14]} On the contrary, in this study, the hemoglobin levels were evaluated in children with migraine for the first time.

There are various speculations about the effect of increased hemoglobin levels on headache. Various observations have indicated that this may be due to an increase in blood viscosity. There is also evidence that erythrocyte is a modulator in the metabolism of NO, a potent vasodilator agent.\textsuperscript{[12]} The increased frequency of migraine in essential thrombocythemia supports the fact that the increased platelets can also lead to headache. Migraine is the most common vaso-occlusive symptom in essential thrombocythemia.\textsuperscript{[1,15]} Platelet activation and the formation of platelet-leukocyte aggregates increase the release of various pro-inflammatory cytokines, and contribute to the local sterile inflammation.\textsuperscript{[1,16]}

An acute increase in hematocrit may be the result of a relative increase in the RBC mass due to a decrease in intravascular volume. The primary causes of volume depletion may be the fluid loss from various pathways such as from the gastrointestinal tract. Another cause of hemoconcentration is the constriction of the circulatory system, which causes a shift in the balance of forces governing the fluid exchange at tissue level.\textsuperscript{[9]} The volume reduction in the circulatory system due to catecholamine discharge under stressful conditions is a good example for the latter one. This catecholamine discharge may also increase the RBC mass circulating within the vascular system by the introduction of reserve RBCs from the splenic region into the circulation.\textsuperscript{[9]} In this study, it was suggested that hemoconcentration was not related to the stress in the patients with migraine, considering the fact that their attack frequencies did not show any effect on hemoconcentration related results.

The anatomical regions involved in migraine pathophysiology may give various clues about the cause of hemoconcentration in patients with migraine. At the earliest stage in migraine, activation occurs in the posterior and lateral regions of the hypothalamus and at the midbrain ventral tegmentum junction. The periaqueductal gray matter and dorsal pons, noradrenergic locus coeruleus, and serotonergic dorsal raphe nucleus are other regions of the selective activation at this stage.\textsuperscript{[17,18]} The orexinergic system is established in the nuclei of lateral and posterior regions of the hypothalamus.\textsuperscript{[19]} The orexin-containing neurons affect not only the pain modulation, but also the monoaminergic activity of the sleep cycle.\textsuperscript{[19,20]} We think that sleep disorders frequently seen in patients with migraine may predispose to hemoconcentration. Sleeping difficulties were reported in approximately half of the patients with migraine,\textsuperscript{[20,21]} and renal conservation of electrolytes and water physiologically occurred during sleep.\textsuperscript{[22]}

In this study, patients with TTH had significantly increased MCV and MCH levels as compared to those in control group 2. This increase may be due to the fact that vitamin B12 deficiency is more common in children with TTH.\textsuperscript{[23]} In this study, in a 3-year period, the cumulative incidences of vitamin B12 deficiency were found to be 43% and 44% in the patients with migraine and TTH, respectively. Therefore, vitamin

### Table 3: Comparison of the migraine subgroups

| Groups and parameters | $n$ | Hemoglobin (g/dL) | Hematocrit (%) | Erythrocyte count ($\times 10^{6}/\mu L$) | Platelet count ($\times 10^{3}/\mu L$) | Plateletcrit (%) | M1-value* | Folate (ng/mL) |
|-----------------------|-----|------------------|----------------|----------------------------------------|-------------------------------------|----------------|------------|---------------|
| **Migraine group**    |     |                  |                |                                        |                                     |                |            |               |
| Gender                |     |                  |                |                                        |                                     |                |            |               |
| Female                | 39  | 13.05 ± 0.81     | 38.8 ± 2.3     | 4.63 ± 0.38                            | 317 ± 81.4                          | 0.27 ± 0.06    | 3.5 ± 0.85 | 7.5 ± 2.3    |
| Male                  | 31  | 13.82 ± 1.22     | 41.2 ± 3.7     | 4.99 ± 0.34                            | 309 ± 72.1                          | 0.26 ± 0.05    | 3.6 ± 0.81 | 7.3 ± 2.1    |
| $P$ Value             |     |                  |                |                                        |                                     |                |            |               |
| **Aura**              |     |                  |                |                                        |                                     |                |            |               |
| Yes                   | 10  | 14 ± 1.2         | 41.5 ± 3.4     | 4.86 ± 0.4                             | 327 ± 94                            | 0.27 ± 0.07    | 3.8 ± 1.1 | 5.8 ± 1.1    |
| No                    | 58  | 13.3 ± 1         | 39.7 ± 3.1     | 4.79 ± 0.4                             | 310 ± 75                            | 0.26 ± 0.06    | 3.5 ± 0.8 | 7.7 ± 2.3    |
| $P$ Value             |     |                  |                |                                        |                                     |                |            |               |
| **Control group 1**   |     |                  |                |                                        |                                     |                |            |               |
| Gender                |     |                  |                |                                        |                                     |                |            |               |
| Female                | 39  | 12.67 ± 0.79     | 37.9 ± 2.4     | 4.55 ± 0.36                            | 300 ± 71                            | 0.26 ± 0.06    | 3.26 ± 0.82 | –             |
| Male                  | 31  | 13.53 ± 1.18     | 40.5 ± 3.3     | 4.91 ± 0.35                            | 280 ± 59                            | 0.24 ± 0.05    | 3.2 ± 0.61 | –             |
| $P$ Value             |     |                  |                |                                        |                                     |                |            |               |

Statistically significant $P$ values are marked in bold characters. Statistical comparisons were made by Student’s $t$ test. *Hemoglobin level $\times$ plateletcrit.
B12 deficiency should be considered in patients with headaches.

The folic-acid levels were significantly lower in patients with migraine with aura as compared to other patients with migraine in this study. No reports comparing the folic-acid levels in the migraine subgroups were found in the literature. However, the supplementation of folic acid has been reported to reduce homocysteine levels and migraine disability in patients with migraine with aura.\[24]\n
The family history for headache in the patients with migraine (45%) was significantly higher as compared to the TTH group (24%). This ratio varies between 34% and 90% in other studies.\[8]\n
The presence of migraine in the first-degree relatives has been reported to increase two–four-fold the risk of migraine.\[25,26]\n
There were various limiting factors in this study. One of them was its retrospective nature. For this reason, the two control groups were included. The first control group consisted of age and gender-matched patients. The second group consisted of patients with gastrointestinal symptoms, which could lead to dehydration resulting in hemoconcentration. Another limitation was the small number of participants who had migraine with aura. Although the results obtained in this group supported previous studies, it is more convenient to confirm these results in larger groups.

In conclusion, this study revealed various results indicating the hemoconcentration in pediatric patients with migraine. We suggest that hemoconcentration might be related to the sleep disturbances reported in previous studies in patients with migraine.\[20]\n
Even if hemoconcentration is a pathophysiological secondary event, it may lead to an increase in blood viscosity leading increased migraine severity. Therefore, consideration of hemoconcentration may be beneficial in the management of patients with migraine. In this study, cumulative incidences of vitamin B12 deficiency were high in both patients with migraine and TTH. In addition, in patients with migraine with aura, folic-acid levels were lower. These results suggest that deficiencies of these vitamins should be considered in patients with migraine.

Ethical policy and institutional review board statement
The authors confirm that this retrospective study was conducted in conformity with the ethical principles of international medical research institutions.

Financial support and sponsorship
Nil.

Conflicts of interests
There are no conflicts of interest.

REFERENCES
1. Danese E, Montagnana M, Lippi G. Platelets and migraine. Thromb Res 2014;134:17-22.
2. Steiner TJ, Stovner LJ, Birbeck GL. Migraine: the seventh disabler. Headache 2013;53:227-9.
3. Ducros A, Tournier-Lasserve E, Bousser MG. The genetics of migraine. Lancet Neurol 2002;1:285-93.
4. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia 2013;33:629-808.
5. Spigt MG, Kuijper EC, Schayck CP, Troost J, Knipschild PG, Linsen VM, et al. Increasing the daily water intake for the prophylactic treatment of headache: a pilot trial. Eur J Neurol 2005;12:715-8.
6. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37:247-8.
7. Andrews NP, Gralnick HR, Merryman P, Vail M, Quyyumi AA. Mechanisms underlying the morning increase in platelet aggregation: a flow cytometry study. J Am Coll Cardiol 1996;28:1789-95.
8. Chandrashekar V. Plateletcrit as a screening tool for detection of platelet quantitative disorders. J Hematol 2013;2:22-6.
9. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost 2003;29:435-50.
10. Cokellet GR. Rheology and tube flow of blood. In: Skalak R, Chien S, editors. Handbook of engineering. New York, NY: McGraw-Hill; 1987. pp. 14.1-17.
11. Dowling MM, Noetzel MJ, Rodeghier MJ, Quinn CT, Hirtz DG, Ichord RN, et al. Headache and migraine in children with sickle cell disease are associated with lower hemoglobin and higher pain event rates but not silent cerebral infarction. J Pediatr 2014;164:1175-1180.e1.
12. Lippi G, Cervellin G, Mattiuzzi C. Migraine and erythrocyte biology: a review. Int J Lab Hematol 2014;36:591-7.
13. Aamodt AH, Borch-Johnsen B, Hagen K, Stovner LJ, Asberg A, Zwart JA. Headache prevalence related to haemoglobin and ferritin. The HUNT study. Cephalalgia 2004;24:758-62.
14. Arregui A, León-Velarde F, Cabrera J, Paredes S, Vizcarra D, Umeres H. Migraine, polycythemia and chronic mountain sickness. Cephalalgia 1994;14:339-41.
15. McIntyre KJ, Hoagland HC, Silverstein MN, Petitt RM. Essential thrombocytopenia in young adults. Mayo Clin Proc 1991;66:149-54.
16. Burstein R, Jakubowski M, Rauch SD. The science of migraine. J Vestib Res 2011;21:305-14.
17. Dodick DW. Migraine. Lancet 2018;6736:1-16.
18. Maniyar FH, Sprenger T, Monteiith T, Schankin C, Goadsby PJ. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. Brain 2014;137:232-41.
19. Holland PR. Headache and sleep: shared pathophysiological mechanisms. Cephalalgia 2014;34:725-44.
20. Lin YK, Lin GY, Lee JT, Lee MS, Tsai CK, Hsu YW, et al. Associations between sleep quality and migraine frequency: a cross-sectional case-control study. Medicine (Baltimore) 2016;95:e3554.
21. Vgontzas A, Cui L, Merikangas KR. Are sleep difficulties associated with migraine attributable to anxiety and depression? Headache 2008;48:1451-9.

22. Rubin RT, Poland RE, Gouin PR, Tower BB. Secretion of hormones influencing water and electrolyte balance (antidiuretic hormone, aldosterone, prolactin) during sleep in normal adult men. Psychosom Med 1978;40:44-59.

23. Calik M, Aktas MS, Cecen E, Piskin IE, Ayaydın H, Ornek Z, et al. The association between serum vitamin B12 deficiency and tension-type headache in Turkish children. Neurol Sci 2018;39:1009-14.

24. Lea R, Colson N, Quinlan S, Macmillan J, Griffiths L. The effects of vitamin supplementation and MTHFR (C677T) genotype on homocysteine-lowering and migraine disability. Pharmacogenet Genomics 2009;19:422-8.

25. Eidlitz-Markus T, Haimi-Cohen Y, Zeharia A. Association of age at onset of migraine with family history of migraine in children attending a pediatric headache clinic: a retrospective cohort study. Cephalalgia 2015;35:722-7.

26. Lemos C, Castro MJ, Barros I, Sequeiros J, Pereira-Monteiro I, Mendonça D, et al. Familial clustering of migraine: further evidence from a Portuguese study. Headache 2009;49:404-11.