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The relationship between one-carbon metabolism and nitric oxide metabolism in pediatric patients with migraine and tension-type headache

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ABSTRACT

Background: Pediatric headache is a common neurological condition that adversely affects quality of life. Vitamin B12 is involved in homocysteine (Hcy) and methylmalonic acid (MMA) metabolism, and nitric oxide (NO) plays a role in neurovascular signaling. This study evaluated vitamin B12–related biomarkers and NO in pediatric migraine and tension-type headache (TTH).

Methods: The study included 39 migraine, 42 TTH and 29 control. Vitamin B12 and folic acid were measured using the Roche Cobas e601, Hcy were measured with the Shimadzu LC-20 HPLC, MMA were measured by a modified LC-MS/MS method, NO were measured using the Griess method.

Results: Hcy levels differed significantly between the migraine and control, as well as between the TTH and control ($p<0.05$). For MMA, a significant difference was observed between the TTH and control groups ($p<0.05$). Total nitrite and nitrate concentrations were significantly higher in patients than in controls($p<0.05$).

Conclusions: Although serum vitamin B12 concentrations did not show a significant difference, median MMA values were elevated in the patient groups. MMA is recognized as a biomarker of subclinical vitamin B12 deficiency, and because oxidative stress may impair the cellular function of vitamin B12, MMA measurement provides valuable information even when serum B12 levels remain within the normal range. Furthermore, endothelial damage induced by Hcy, together with chronic oxidative stress associated with the Hcy–NO pathway, may contribute to the pathogenesis of pediatric migraine and TTH. Consistent with this, the significantly higher levels of NO metabolites in patients compared with controls suggest that NO may play a role in the development of migraine and TTH in the pediatric population.

Keywords: methylmalonic acid, migraine, nitric oxide, pediatrics, tension-type headache

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INTRODUCTION

Headache is a neurological condition with diverse etiologies that adversely affects quality of life and represents a common health problem in children and adolescents. Migraine, an episodic headache that may be unilateral or bilateral, is aggravated by physical activity, persists for hours or even days, and is characterized by intense throbbing or pulsating sensations. Tension-type headache (TTH), in contrast, is typically a mild to moderate, non-throbbing, bilateral headache lasting from 30 minutes to seven days. The diagnosis of migraine and TTH cannot be established through laboratory testing; instead, the diagnostic criteria of the International Headache Society (IHS) are applied [1].

Vitamin B12 serves as a coenzyme for methionine synthase (MS), an enzyme involved in one-carbon metabolism that converts homocysteine (Hcy) to methionine. It is also an essential coenzyme for methylmalonyl-CoA mutase (MCM), which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, a product of amino acid and fatty acid metabolism. In cases of vitamin B12 deficiency, both enzymatic pathways are impaired, leading to increased levels of Hcy and methylmalonic acid (MMA) and their accumulation in tissues. Serum vitamin B12 levels reflect the circulating concentration of the vitamin but do not provide information about its tissue concentration. In the absence of adequate vitamin B12, MMA accumulates in tissues and serves as a functional

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biomarker of vitamin B12 status [2]. MMA is a low molecular weight (118 g/mol), saturated, highly polar dicarboxylic acid that does not bind to plasma proteins, and has been reported to exert neurotoxic effects [3].

Homocysteine is an end product of methionine metabolism. It is a sulfur-containing amino acid produced by the demethylation of methionine [4]. Elevated Hcy levels are associated with endothelial dysfunction, which may contribute to the development and progression of migraine. Hcy concentration is also considered a sensitive marker for assessing vitamin B12 status, as its remethylation to methionine requires methylcobalamin. However, Hcy is not a specific indicator of vitamin B12 deficiency, as its levels also increase in folic acid (FA) and vitamin B6 deficiencies. FA plays an essential role in a variety of biochemical reactions, ranging from nucleotide biosynthesis to the remethylation of Hcy, and it functions as a critical coenzyme in single-carbon transfer processes. FA deficiency impairs the methylation mechanisms within one-carbon metabolism [5].

Nitric oxide (NO) is a free radical composed of one nitrogen and one oxygen atom and is considered a neurotransmitter. NO has been reported to be a key mediator of meningeal vasodilation and a potential nociceptive agent [6]. By inducing vasodilation, NO is thought to play a role in triggering both TTH and migraine attacks [7]. Previous research has demonstrated the involvement of NO in the pathophysiology of migraine [8] as well as TTH [9].

The aim of the present study was to measure serum levels of vitamin B12, FA, Hcy, MMA, and NO in pediatric patients diagnosed with migraine [migraine with aura (MA) and migraine without aura (MO)] and TTH, according to the IHS classification and diagnostic criteria, and to investigate their associations with these disorders. Specifically, we sought to examine the prevalence of vitamin B12 and FA deficiencies in pediatric migraine and TTH, to evaluate the functional markers MMA and Hcy, and to explore the relationship between NO and vitamin B12 metabolism. The potential roles of Hcy, MMA, and NO in the pathophysiology of these disorders were also assessed. To our knowledge, to date, no studies in pediatric populations have simultaneously evaluated one-carbon and NO metabolism. Therefore, this study is of particular importance as it is the first to investigate vitamin B12 and FA, their functional markers (Hcy and MMA), and NO metabolites together in pediatric patients with migraine and TTH.

METHODS

This study was conducted in collaboration with the Department of Medical Biochemistry and the Department of Pediatric Neurology, University of Health Sciences, Ankara Training and Research Hospital. The study protocol was designed in accordance with the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the University of Health Sciences, Ankara Training and Research Hospital (Decision No: 93/2019; 19.12.2019). Written informed consent was obtained from the parents of all participants.

The study included patients diagnosed with migraine (n=39) and TTH (n=42) according to IHS classification and diagnostic criteria. The migraine group was further divided into MO (n=23) and MA (n=16). At the time of blood sampling, all patients with MA, MO, and TTH were in the interictal period. The age- and sex-matched control group (n=29) comprised healthy children with no neurological disorders confirmed after clinical examination. Exclusion criteria included the presence of chronic conditions (kidney, liver, thyroid, or diabetes), malignancies, metabolic, hematological, autoimmune, infectious/inflammatory, or parasitic diseases, as well as the use of vitamin supplements.

Sample characteristics

Blood samples from patients and controls were collected in Vacutainer SST tubes with gel separators (Becton Dickinson) and K3EDTA tubes (Becton Dickinson) in the morning following a 10–12 hour overnight fast. Samples were centrifuged at 1300 × g for 10 minutes. Glucose, creatinine, urea, AST, ALT, total cholesterol, triglycerides, and HDL-C levels were measured using a Cobas 8000 analyser (Roche Diagnostics, Indianapolis, IN, USA). Serum vitamin B12 and FA concentrations were determined with a Cobas e601 immunoassay analyser (Roche Diagnostics, Indianapolis, IN, USA), using reagent kits from the same manufacturer (vitamin B12: REF 07212771 190; FA: REF 07559992 190). Complete blood counts were obtained using Sysmex XN-3000 analysers (Sysmex Co., Kobe, Japan). HbA1c levels were assessed by ion-exchange chromatography on a Tosoh G8 HPLC analyser (Tosoh Bioscience, Inc., South San Francisco, CA, USA). Homocysteine levels were analyzed using the LC-20 HPLC system (Shimadzu Corporation, Tokyo, Japan) with a commercial kit from Immuchrom GmbH (Lot No. IC2801, Heppenheim, Germany). All analyses were performed on the same day as blood collection, except for MMA and NO, for which samples were stored at –80 °C until analysis.

Methylmalonic acid measurements

MMA concentrations were determined by electrospray tandem mass spectrometry (ESI LC-MS/MS) using a modified version of the method described by Kushnir et al. [10]. Methylmalonic acid (METHYL-D3, d3MMA isotope; METHYL-D3, 98%, DLM-387-0.25) with the chemical formula $CD3CH(COOH)_2$, a molecular weight of 121.11, and 98% purity, was used as the internal standard (Cambridge Isotope Laboratories, Inc.). Mass spec-

trometric analyses were conducted with a Shimadzu LC-20-AD chromatography system (Kyoto, Japan) coupled to an ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode. Chromatographic separation was achieved using a Phenomenex C18 reverse-phase column (50 mm × 4.6 mm). The mobile phases consisted of (A) 0.005 mol/L ammonium formate (Sigma 156264) and (B) methanol containing 0.005 mol/L ammonium formate (Sigma 156264), delivered by gradient elution. The total run time for the method was 5 minutes. Data acquisition and processing were performed with Analyst Software Wizard 1.6.1 (ABSCIEX). Calibration curves were generated by plotting the analyte-to-internal standard (d3-MMA) peak area ratio against the nominal analyte concentration. The internal standard was added to all serum samples prior to sample preparation and throughout the analytical procedure. This approach allowed correction for quantitative variability during extraction and analysis by normalization to the internal standard.

Linear regression analysis yielded an r^2 value of 0.9987. The limit of detection (LoD) was established at 0.078 µmol/L. At a concentration of 0.156 µmol/L, intra-run, intra-day, and inter-day precision studies demonstrated coefficients of variation (CV%) of 3.6, 5.2, and 8.1, respectively, and this value was accepted as the limit of quantification (LoQ). Recovery rates were 98.7%, 101.8%, and 103.9% at concentration levels of 1.25 µmol/L, 2.5 µmol/L, and 5 µmol/L, respectively.

NO measurements

Because NO has a very short half-life, its metabolites nitrite and nitrate are commonly measured as indicators of endogenous NO production. Although nitrite and nitrate themselves lack biological activity, their quantification in biological samples provides indirect information on NO levels. Serum nitrite and nitrate levels were measured using an enzymatic reduction method modified from Smarason, followed by the Griess reaction [11]. Nitrate was enzymatically reduced to nitrite by *Aspergillus* nitrate reductase (Sigma-Aldrich, N-7265) in phosphate buffer containing NADPH (Fluka-BioChemika, 93270) and FAD (Sigma, F-6625). Samples were deproteinized using zinc sulfate and sodium hydroxide, and total nitrite was quantified colorimetrically using sulfanilic acid (Griess I) and N-(1-naphthyl)ethylenediamine (Griess II, Sigma, N-9125). At low concentrations, intra-run, intra-day, and inter-day precision studies for total nitrite yielded CV% values of 4.2, 4.5, and 5.8, respectively, while at high concentrations the CV% values were 4.8, 4.9, and 6.0, respectively.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics included mean (x), standard deviation (s), median (m), interquartile range (IQR), and minimum–maximum values. The distribution of data was assessed using skewness, kurtosis, Kolmogorov–Smirnov (with Lilliefors significance correction), Shapiro–Wilk tests, and histogram plots. For descriptive statistics, normally distributed parameters were expressed as mean ± standard deviation, whereas non-normally distributed parameters were presented as median and interquartile range. For group comparisons, normally distributed data were analysed using one-way ANOVA for multiple groups and the Student's t-test for two independent groups. Non-normally distributed data were analysed using the Kruskal–Wallis test for multiple groups and the Mann–Whitney U test for two independent groups. Categorical variables were compared using the chi-square test. A *p*-value < 0.05 was considered statistically significant. Since comparisons were made across three groups, Bonferroni correction was applied, and a *p*-value threshold of < 0.017 (0.05/3) was considered significant. Correlations between variables were evaluated using Pearson's correlation coefficient for normally distributed parameters and Spearman's correlation coefficient for non-normally distributed parameters. Intra-day and inter-day precision analyses were performed using the Analyse-it software package (Analyse-it Software, Ltd., Leeds, UK).

RESULTS

The demographic characteristics of the patients and controls are presented in Table 1. No significant differences in age were observed between the control and patient groups. While no significant sex differences were detected among the control, MA, and MO groups, a signifi-

Table 1. Demographic characteristics of the patient and control groups

	Migraine without aura (n=23)	Migraine with aura (n=16)	Tension type headache (n=42)	Control group (n=29)
Age (years)	15 ± 2	15 ± 2	15 ± 2	14 ± 3
Female, n (%)	12 (52.1%)	9 (56.2 %)	35 (83.3 %)	15 (51.7 %)
Time since onset of symptoms (months)	12 [12-24]	12 [5-24]	12 [5-24]	N / A
Frequency of pain (days per month)	4 [3-8]	4 [3-11]	30 [15-30]	N / A

Results are presented either as mean ± standard deviation or as median with [interquartile range].

cant difference was found between the control and TTH groups ($p < 0.05$). The pain duration did not differ significantly between MA, MO, and TTH groups. Regarding pain frequency, no significant difference was observed between MA and MO groups; however, significant differences were found between MA and TTH, as well as between MO and TTH ($p < 0.05$).

Descriptive statistics (mean, standard deviation, median, and interquartile range) of the measured parameters in pediatric migraine and TTH patients and controls are presented in Table 2.

Comparisons of measured parameters among migraine (MO + MA), migraine with aura, migraine without aura, TTH, and control groups are illustrated in Figures 1 and 2.

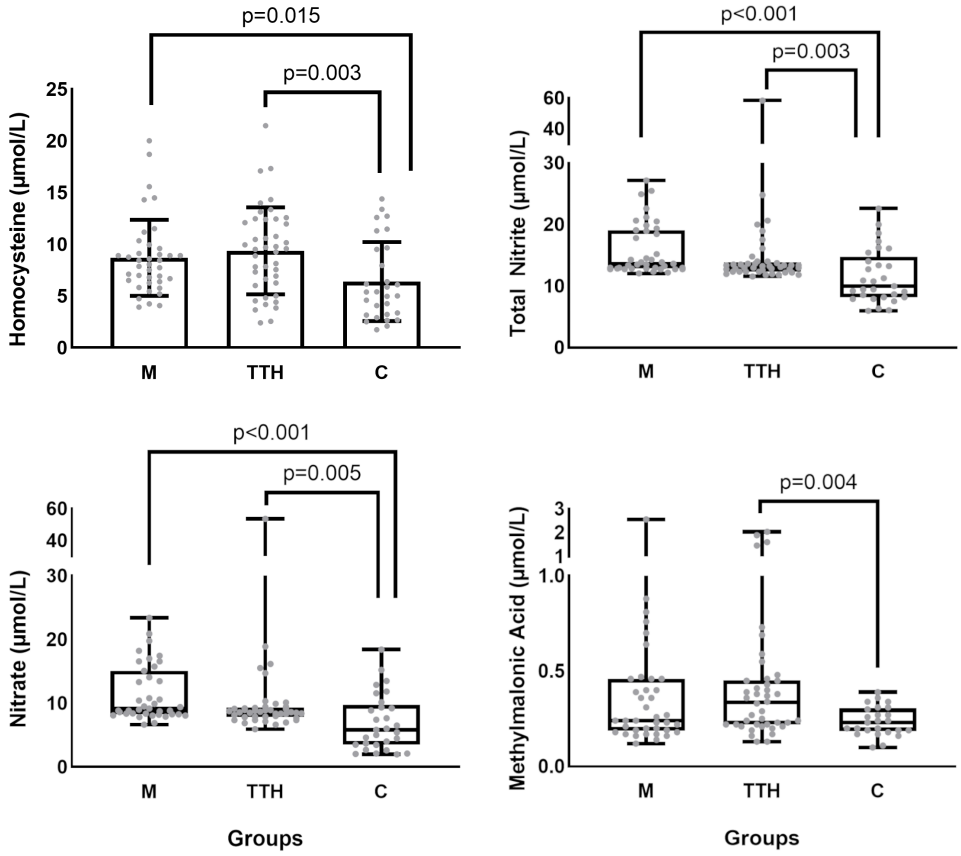

Figure 1. Comparison of homocysteine, total nitrite, nitrate and MMA levels in migraine, TTH and control groups
Group abbreviations: M – migraine patients, TTH – tension-type headache patients, C – control group.

Table 2. Parameters measured in patient and control groups

	Migraine without aura (n=23)	Migraine with aura (n=16)	Tension type headache (n=42)	Control group (n=29)
Glucose (mg/dL)	91 ± 11	89 ± 10	88 ± 8	90 ± 7
Creatinine (mg/dL)	0.64 [0.55-0.68]	0.60 [0.56-0.75]	0.58 [0.53-0.69]	0.64 [0.59-0.76]
AST (U/L)	18 [16-21]	19 [15-22]	15 [14-19]	20 [16-23]
ALT (U/L)	12 [11-14]	11 [9-18]	11 [10-13]	14 [10-20]
HDL-C (mg/dL)	53.6 ± 10.8	50.6 ± 10.3	52.3 ± 10.5	48.3 ± 8.6
LDL-C (mg/dL)	84 [74-98]	73 [59-80]	85 [73-104]	74 [56-86]
Triglycerides (mg/dL)	109 [68-146]	68 [57-101]	85 [55-131]	76 [59-116]
Cholesterol (mg/dL)	154 [137-160]	136 [120-146]	151 [134-172]	135 [112-150]
Vitamin B12 (ng/L)	301 [230-405]	293 [266-357]	278 [212-468]	309 [266-341]
Folic Acid (µg/L)	6.6 ± 2.2	7.2 ± 1.8	7.0 ± 3.4	7.8 ± 3.3
Hcy (µmol/L)	8.7 ± 3.6	8.6 ± 3.9	9.4 ±4.2	6.4 ± 3.8
MMA (µmol/L)	0.238 [0.202-0.459]	0.259 [0.176-0.455]	0.336 [0.218-0.449]	0.230 [0.182-0.306]
Total nitrite (µmol/L)	13.3 [12.7-14.3]	19 [13.1-22.1]	13.2 [12.4-13.8]	10.0 [8.2-14.8]
Nitrite (µmol/L)	4.4 [4.2-4.7]	4.4 [4.0-4.9]	4.6 [4.4-4.9]	4.5 [4.1-5.1]
Nitrate (µmol/L)	8.8 [8.4-10.4]	14.6 [8.1-18.0]	8.6 [7.9-9.3]	5.8 [3.5-9.8]

Results are presented either as mean ± standard deviation or as median with [interquartile range]. Abbreviations: ALT – alanine aminotransferase, AST – aspartate aminotransferase, Hcy – homocysteine, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, MMA – methylmalonic acid.

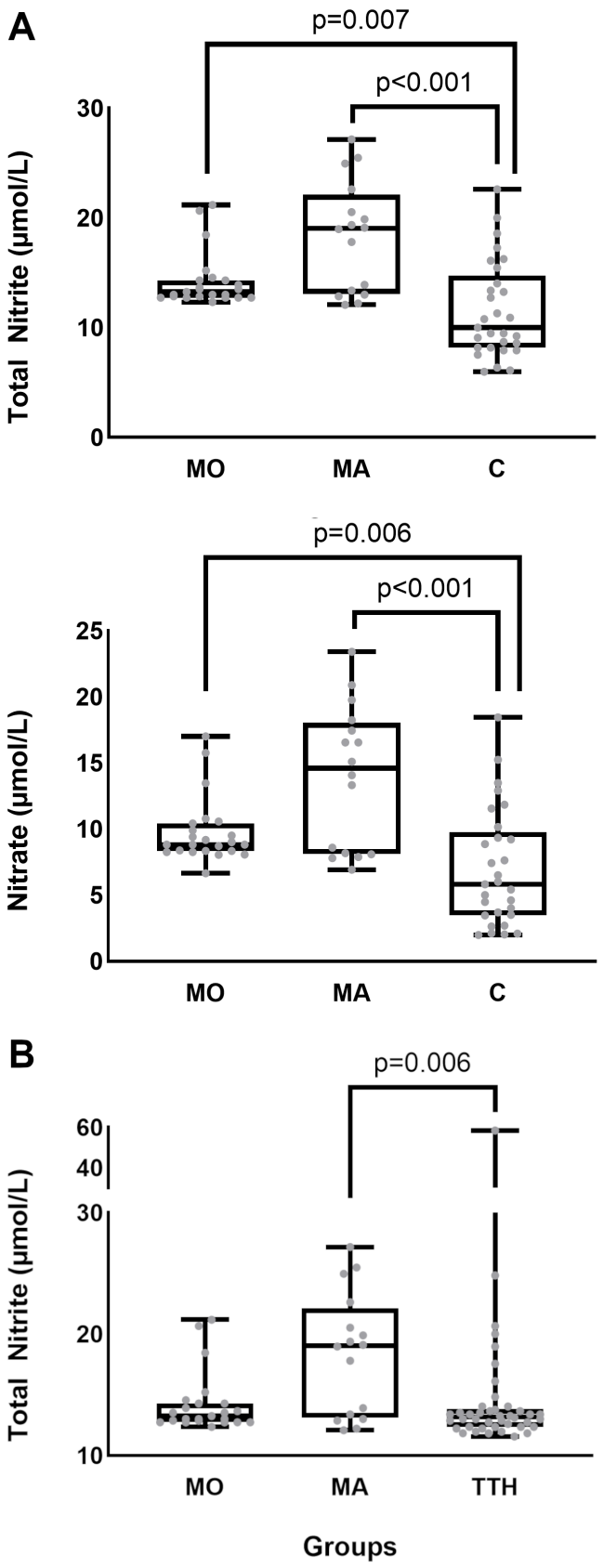

Figure 2. Nitrite and nitrate comparison between groups

(A) Comparison of total nitrite and nitrate levels between migraine subgroups (MA – with aura, MO – without aura) and the control group; (B) Comparison of total nitrite levels between migraine subgroups and the tension-type headache (TTH) group.

Hcy, total nitrite, and nitrate levels were significantly higher in the migraine group (MO + MA) compared with controls (p = 0.015, p < 0.001, and p < 0.001, respectively). In the TTH group, Hcy, total nitrite, nitrate, and MMA levels were significantly higher compared with controls (p = 0.003, p = 0.003, p = 0.005, and p = 0.004, respectively). Total nitrite and nitrate levels were also significantly higher in both MA and MO groups compared with controls; in the MA group, both parameters showed p < 0.001, while in the MO group, total nitrite and nitrate levels showed p = 0.007 and p = 0.006, respectively. Moreover, total nitrite concentrations were significantly higher in MA compared with TTH (p = 0.006). No significant differences were observed for other measured parameters.

DISCUSSION

This study evaluated serum levels of vitamin B12, FA, Hcy, MMA, and NO in pediatric patients with migraine (MA/MO) and TTH. The results demonstrated significantly higher Hcy, nitrite, and nitrate levels in both migraine (MO + MA) and TTH groups compared to controls. Although median MMA levels were higher in patient groups than in controls, this difference reached statistical significance only in the TTH group. To our knowledge, no prior studies have assessed both one-carbon and NO metabolic pathways simultaneously in pediatric migraine and TTH. This research, therefore, provides novel insights into the potential contributions of vitamin B12, FA, and their functional markers (Hcy, MMA) in conjunction with NO metabolites to the pathophysiology of these disorders.

Vitamin B12 and FA are essential cofactors for MS and methylenetetrahydrofolate reductase (MTHFR) in homocysteine metabolism. Previous studies have reported conflicting results regarding vitamin levels in migraine and TTH patients [12-15]. In our study, no significant differences were found between patients and controls. This may in part be explained by the limitations of immunochemical assays for vitamin B12, which measure total vitamin B12 (after conversion to cyanocobalamin (CNCbl)) and may not accurately reflect intracellular status. Methodological challenges, including wide reference ranges and low specificity and sensitivity, may contribute to inconsistent findings across studies.

In our study, both migraine and TTH groups had significantly higher Hcy compared with controls. Elevated Hcy levels and some genetic enzyme variants involved in FA metabolism have been associated with migraine [16-18]. The enzyme MTHFR, encoded by the *MTHFR* gene, plays a critical role in the methionine-folate cycle by catalyzing the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF) [19]. Literature suggests that *MTHFR* C677T polymorphism reduces enzymatic activity and contributes to hyperhomocysteinemia

(HHcy) [20-22]. A study comparing serum folate and Hcy levels among migraine patients with different *MTHFR* genotypes demonstrated that individuals with slow and intermediate metabolic types had significantly lower folate concentrations than fast metabolizers. Furthermore, vitamin supplementation in migraine patients has been shown to reduce Hcy levels and alleviate headache severity effectively [23]. Such genetic factors may help explain elevated Hcy in migraine patients.

A number of population studies have reported an association between plasma Hcy levels and the development of migraine, particularly migraine with aura [16, 18, 24]. In animal models, HHcy has been shown to increase neuronal excitability in the cerebral cortex and enhance the sensitivity of peripheral trigeminal afferents innervating the dura mater. Elevated Hcy levels have thus been proposed as both a potential risk factor for migraine and a prognostic marker for attack severity. Consequently, lowering homocysteine levels may reduce the frequency and severity of migraine attacks [25]. Hcy has been implicated in endothelial dysfunction in migraine [18]. This effect is thought to occur through nitric oxide (NO)-mediated vascular damage.

There is an association between reduced vitamin B12 levels and elevated Hcy levels, which may subsequently precipitate migraine [26]. Elevated homocysteine levels have been associated with deficiencies of vitamin B12, folate, and, to a lesser extent, vitamin B6 [27]. These vitamins play a crucial role in the prevention of HHcy. Accumulating evidence supports the role of B-vitamin supplementation in migraine management. In pediatric patients, six months of vitamin B-complex administration significantly reduced serum Hcy levels [28]. Similarly, clinical trials have demonstrated that B-vitamin supplementation lowers plasma Hcy concentrations and is associated with a reduction in migraine attack frequency [29, 30]. Dietary folate has also been implicated in migraine pathophysiology, with recent findings suggesting its potential role in the prevention of severe headache [31]. In line with this, folic acid supplementation in children with migraine, HHcy, and *MTHFR* polymorphism was shown to decrease headache frequency and improve metabolic abnormalities, leading to recommendations for its use in pediatric migraine prophylaxis [32].

MMA is another functional marker of B12 deficiency. Two studies in adults reported higher MMA in migraine patients [33, 34]. In our study, MMA was significantly higher in pediatric TTH compared with controls, but not in pediatric migraine. This discrepancy may be due to the relatively small sample size in migraine subgroups.

It was especially remarkable that the headache frequency was significantly higher in the TTH group than in the migrain groups. High levels of MMA can be caused by defective or inhibited MCM or by insufficient amounts of vitamin B12. The MCM cofactor is adenosylcobalamin (AdoCbl). Accordingly, an increase in MMA may indicate AdoCbl deficiency rather than deficiency of other vitamin B12 groups [such as CNCbl, hydroxycobalamin (OHB12), or methylcobalamin (MeCbl)]. ATP: cobalamin adenosyltransferase transfers adenosine from ATP to cobalamin to form AdoCbl in mitochondria. Studies show that mitochondrial energy metabolism is impaired in migraines [35]. A similar disorder is also valid for TTH [36]. Insufficient ATP synthesis in both disease groups may reduce MCM activity by inhibiting AdoCbl formation. Thus, a decrease in MCM activity may lead to an increase in MMA again. Increased intracellular MMA levels dose-dependently inhibit the enzyme succinate dehydrogenase, disrupting the Krebs cycle and the function of the mitochondrial electron transport chain. In studies on rats, injection of MMA into the basal ganglia triggered cell death in a dose-dependent manner [37]. High serum levels of MMA detected in our study may be related to mitochondrial energy metabolism dysfunction in these patients.

A potent vasodilator and short-life messenger molecule, NO, is thought to play a role in migraine pathogenesis. Studies have found highly increased NO levels in the attack and inter-attack period compared to controls [38, 39]. NO is also thought to have an essential role in the pathophysiology of TTH. The decrease in pain intensity and muscle tightness with the use of NO synthase inhibitors in treating TTH compared to the placebo group confirms this theory [40]. The NO half-life is very short. Therefore, efforts are made to assess levels of NO metabolites, specifically nitrate and nitrite, which serve as biomarkers due to their lack of direct biological activity. Similar to the literature, NO metabolites were significantly higher in our pediatric patient groups than in the control group. These findings suggest that this molecule may also be involved in the pathophysiology of migraine and TTH in the pediatric population.

A particularly notable finding was the positive correlation between total nitrite and MMA in migraine with aura patients ($r = 0.529$, $p = 0.043$) in our study. In other patient groups, the association was positive, but not significant. Previous studies have shown that NO can inhibit methylmalonyl-CoA mutase (MCM), an enzyme required for MMA metabolism, reducing its activity by up to 50% [41]. The significant positive correlation between MMA and total nitrite, especially in migraine with aura patients, suggests that high levels of NO may inhibit the mitochondrial enzyme MCM and cause elevated MMA.

Limitations of our study are the low number of patients included in the study, especially in pediatric migraine subgroups, not including periods of pain attacks in patient groups in the study, and MTHFR gene mutations, which are known to cause an increase in Hcy concentrations and are thought to be associated with headache syndromes, were not evaluated in the patient and control groups.

CONCLUSIONS

In conclusion, this study is the first to evaluate vitamin B12, FA, their functional markers (Hcy and MMA), and NO metabolites together in pediatric migraine and TTH. Elevated Hcy and NO metabolites in both disorders suggest that endothelial dysfunction and oxidative stress may contribute to pathogenesis. The significant elevation of MMA in the TTH group further supports a potential role of mitochondrial dysfunction. Consistent with the literature, the significantly higher NO metabolite levels observed in patient groups compared with controls suggest that NO may play an important role in the pathophysiology of pediatric headache disorders. The positive correlation between MMA and nitrite in migraine with aura raises the possibility that NO-mediated inhibition of mitochondrial enzymes may contribute to disease mechanisms. These findings highlight the importance of metabolic pathways in pediatric migraine and TTH and underscore the need for larger studies, including genetic analyses (e.g., MTHFR polymorphisms), and evaluations during headache attacks as well as interictal periods.

ABBREVIATIONS

AdoCbl – adenosylcobalamin

CNCbl – cyanocobalamin

CV – coefficients of variation

FA – folic acid

HCY – homocysteine

HHcy – hyperhomocysteinemia

IHS – International Headache Society

LoD – limit of detection

LoQ – limit of quantification

MA – migraine with aura

MCM – methylmalonyl-CoA mutase

MeCbl – methylcobalamin

MMA – methylmalonic acid

MO – migraine without aura

MS – methionine synthase

5-MTHF – 5-methyltetrahydrofolate

MTHFR – methylenetetrahydrofolate reductase

NO – nitric oxide

OHB12 – hydroxycobalamin

TTH – tension-type headache

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None.

AUTHORS' CONTRIBUTION

AMA: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, writing – review and editing, visualization.

MŞ: conceptualization, methodology, validation, writing – original draft preparation, writing – review and editing, supervision, project administration.

AY: conceptualization, validation, writing – review and editing, supervision, project administration.

SA: conceptualization, methodology, validation, writing – review and editing, supervision, project administration.

AÜ: conceptualization, methodology, validation, writing – review and editing, supervision, project administration.

DY: conceptualization, methodology, validation, writing – original draft preparation, writing – review and editing, supervision, project administration.

All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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