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Original Article

Tryptophan metabolism in children with migraine: The role of kynurenine pathway

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ABSTRACT

Background: Migraine is a common neurological disorder in children, significantly impacting quality of life and academic performance. The kynurenine pathway, a major metabolic route of tryptophan, plays a critical role in neuroinflammation and neurotransmission, yet its involvement in pediatric migraine remains unexplored. This study aims to investigate alterations in kynurenine pathway metabolites in children with migraine and assess their correlation with headache frequency and severity.

Methods: A case-control study was conducted including pediatric patients diagnosed with migraine ($n = 45$) and healthy controls ($n = 48$). Serum levels of tryptophan (TRP) and its kynurenine pathway metabolites—including kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3−HK), and 3-hydroxyanthranilic acid (3-HANA)—were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The Pediatric Migraine Disability Assessment (PedMIDAS) scores were used to evaluate the functional impact of migraine. Statistical analyses included comparisons between groups and correlation assessments between metabolite levels and clinical parameters.

Results: KYN, KYNA, and the KYN/TRP ratio were significantly higher in the migraine group compared to controls ($p < 0.05$). KYNA/3-HK ratios showed a negative correlation with headache frequency and PedMIDAS scores, whereas 3-HK levels were positively correlated with PedMIDAS scores. Receiver operating characteristic curve analysis identified KYN as a potential biomarker for distinguishing migraine patients from controls, with a sensitivity of 86.7 % and specificity of 45.8 % at a cutoff value of 1415.

Conclusion: This study is the first to evaluate kynurenine pathway metabolites in pediatric migraine. The findings suggest that alterations in the tryptophan-kynurenine pathway, particularly increased KYN and KYNA levels, may serve as compensatory mechanisms in migraine pathophysiology. Future studies should explore the therapeutic implications of targeting the kynurenine pathway in pediatric migraine treatment.

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1. Introduction

Migraine is the most common primary headache disorder in childhood and adolescence. Recurrent headaches due to migraines significantly impair the quality of life and school performance of children [1]. Its prevalence ranges between 5 and 40 % among the pediatric population [2]. Migraine is a clinical diagnosis. According to the International Classification of Headache Disorders (ICHD-32018), the diagnosis is made according to the duration and location of headache, the presence of nausea-vomiting, photophobia-phonophobia, and the absence of another underlying disease [3]. Management involves both attack treatment and preventive treatment. Preventive treatment includes lifestyle modification, behavioral treatments, and medications with minimal adverse effect profile, while analgesics and triptans are the backbone of attack treatment [4].

Migraine is a complex disorder with a wide range of symptoms and multifactorial pathogenesis [5]. Four mechanisms, which are not mutually exclusive, play a role in the pathophysiology of migraine: (i) peripheral sensitization of the trigeminovascular system; (ii) central sensitization of the caudal trigeminal nucleus and other central nervous system (CNS) pain processes; (iii) activation of brainstem migraine generators; and (iv) cortical spreading depression (CSD) [6]. Glutamate, the major excitatory neurotransmitter of the CNS, plays a role in all these processes, therefore, studies on migraine have been directed at glutamatergic neurotransmission [7]. In addition to glutamate, serotonin (5-HT) affects nociceptive pain by its vasoconstrictor effects on vessels [8]. The kynurenine pathway, which is the focus of our study, is closely related to glutamatergic neurotransmission and potential neuropeptides affecting these processes [9] [10].

Tryptophan (TRP) is an essential amino acid involved in various metabolic reactions. It is converted into serotonin and melatonin via the serotonin pathway [11]. The kynurenine pathway is responsible for 95 % of TRP metabolism. This pathway is closely related to glutamatergic serotonergic mechanisms, and the catabolites of this pathway have become central to migraine research [12]. In the kynurenine pathway, L-TRP is first metabolized to an unstable form, N-formyl-L-kynurenine (NFK). Kynurenine formamidase enzyme catalyzes the hydrolysis of NFK to kynurenine (KYN). KYN is then converted into kynurenic acid (KYNA) via the kynurenine aminotransferase enzyme or into 3-hydroxykynurenine (3-HK) via kynurenine monooxygenase. 3-HK is the precursor of 3-hydroxyanthranilic acid (3-HANA), quinolinic acid (QUIN), and xanthurenic acid [13] [14]. KYNA is proven to be a competitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor responsible for glutamate-mediated excitotoxicity, which led to the potential of this pathway to be targeted in migraine treatment [9]. Subsequently, studies have gained momentum, and these metabolites have been evaluated in adult patients with chronic and episodic migraine [15] [16].

The tryptophan-kynurenine pathway is a crucial biochemical route that influences various biological processes, and multiple factors regulate its activity. In pathological conditions such as cancer, stress, depression, migraine, infections, and autoimmune diseases, the regulation of this pathway can vary significantly. Diurnal variation and the sleep-wake cycle influence kynurenine metabolism through their connection with melatonin and serotonin production, potentially leading to neuroinflammation and mood alterations [17]. During infections, the immune system is activated, increasing the diversion of TRP into the kynurenine pathway via indoleamine 2,3-dioxygenase (IDO1), which triggers pro-inflammatory responses and exacerbates its effects on the nervous system [18]. The menstrual cycle, due to fluctuations in estrogen levels, affects TRP metabolism and can increase pain sensitivity, particularly in migraine patients [19]. The use of analgesics, especially nonsteroidal anti-inflammatory drugs (NSAIDs), can modulate the kynurenine pathway, thereby influencing pain mechanisms and altering immune responses [20]. Migraine attack periods, on the other hand, may intensify brain inflammation, disrupting the balance between KYNA and QUIN, a key neuromodulatory mechanism impacting migraine severity [21].

To our knowledge, metabolites of the kynurenine pathway have not been studied in pediatric migraine patients. In this study, we aimed to measure the metabolites of kynurenine pathway (TRP, KYN, KYNA, 3-HK, 3-HANA) in children with migraine and compare them with healthy children, and to determine the correlation between the frequency and severity of headache and the levels of these metabolites.

2. Materials and method

2.1. Participants

Our study protocol was designed in accordance with the Declaration of Helsinki and was approved by the Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee with the decision number 2023/0884. All participants and their parents/caregivers provided consent to participate in the study.

Patients who presented to Pediatric Neurology Clinic with headaches and were diagnosed with migraine according to ICHD-3 diagnostic criteria, did not receive migraine treatment, were included in the study. Patients with psychiatric comorbidities, systemic inflammatory or infectious diseases, neurological disorders, sleep disorders, or mental retardation were excluded from the study. Blood samples were collected during the attack-free phase, which was defined as the absence of headache for at least one week. During this period, no analgesic medications were used. Patients did not report insomnia, and sleep-wake cycles were not assessed.

In our study, the control group was composed of children who presented for routine well-child visits, including growth and development monitoring, vaccination follow-up, or school-entry health checkups. These individuals had no chronic illnesses or acute infections, did not report experiencing headaches, were not using any medications, and had normal findings on physical examination. They were selected to match the case group in terms of age and sex. Participants were not asked to restrict any food or liquid intake or follow a specific diet before sample collection. The samples were collected in the morning hours after a minimum of 8 h of fasting.

The medical history and clinical characteristics of the patients followed with a migraine diagnosis were evaluated, and the PEDMIDAS (Pediatric Migraine Disability Assessment) [22] questionnaire was administered to all patients. Furthermore, all patients were evaluated by two pediatric neurologists, ensuring a standardized clinical assessment.

Serum samples were collected into BD Vacutainer SST II Advance serum gel separator tubes, centrifuged at 2000 $\times g$ for 10 min, and subsequently stored at −80 °C until analysis. Serum levels of kynurenine pathway metabolites were quantified through liquid chromatography-tandem mass spectrometry (LC-MS/MS) employing a modified method [23], incorporating protein precipitation with acetonitrile containing 1 % formic acid, and subsequent sample evaporation under nitrogen gas, as outlined in prior studies [24] [25]. The separation of analytes was achieved using a Shimadzu High-Performance Liquid Chromatography (HPLC) system (Kyoto, Japan) equipped with a 50 mm × 4.6 mm Luna C18 reverse-phase column with a 3 μm particle size. The detection of kynurenine pathway metabolites was performed on an ABSciex API 3200 tandem mass spectrometer (Applied Biosystems/MDS Sciex) operating in positive electrospray ionization mode. The coefficients of variation (%CV) for both intra-assay and inter-assay measurements were less than 10 % for all metabolites.

2.2. Statistical analysis

Statistical Package for Social Sciences (SPSS) version 27.0 was used for statistical analyses. Descriptive statistics of the data included mean, standard deviation, median, range (minimum and maximum), frequency, and ratio. The distribution of variables was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. The independent samples

t-test was used in the analysis of quantitative independent data with normal distribution, while Mann-Whitney *U* test was preferred to evaluate quantitative independent data with non-normal distribution. Analysis of qualitative independent data was performed using the chi-square test. Due to the non-normal distribution of the data, Spearman's rank correlation analysis was employed.

The level of effect and cut-off value were investigated using receiver operating characteristic curves. $P \leq 0.05$ was considered significant.

3. Results

Forty-five (48.4 %) patients with a migraine diagnosis and 48 (51.6 %) healthy control subjects were included in our study. Routine laboratory tests of all participants were found to be within normal range. All patients in the migraine group had migraine without aura and were not receiving prophylactic medication for migraine treatment. The mean PedMIDAS score was calculated as 25.9 ± 25.1, while the average headache frequency was determined to be 9.4 ± 5.9 days per month. Demographic characteristics of our patients with migraine are presented in Table 1.

There was no significant difference in terms of age and gender between the control and study groups ($p > 0.05$) (Table 2). KYN (Fig. 1), KYN to TRP ratio, and KYNA were found to be higher in migraine patients compared to the control group. Comparison of laboratory values between the control and study groups is presented in Table 2.

Comparison of monthly frequency of headaches and PedMIDAS scores against levels of TRP metabolites in migraine patients is presented in Table 3. KYNA/3-HK ratios are negatively correlated with frequency of headaches and PedMIDAS scores, while 3-HK levels exhibit a positive correlation with PedMIDAS scores.

KYN level was significant [area under the curve 0.664 (0.553–0.774)] in distinguishing between control and case groups. A cut-off value of 1415 for KYN was found to be significant in distinguishing between control and case groups [area under the curve 0.663 (0.551–0.774)] (Table 4). At this KYN cut-off, the sensitivity in distinguishing between control and case groups was 86.7 %, positive prediction was 60.0 %, specificity was 45.8 %, and negative prediction was 78.6 % (Table 4).

4. Discussion

TRP metabolism plays an important role in host physiology by producing many essential molecules. Many studies on the treatment of neurological, psychiatric, metabolic, infectious, intestinal diseases and cancer by targeting the products and enzymes in this metabolism are ongoing [26]. In our study, we demonstrated the correlation of abnormalities in the TRP metabolism and migraine by determining the levels of serum tryptophan-kynurenine pathway metabolites in pediatric patients for the first time. The structural effect of the kynurenine pathway is limited in the CNS. Peripherally derived KYN and 3-HK can cross the blood-brain barrier and feed the pathway in the brain parenchyma. Therefore, serum levels of KYN metabolites can inform about CNS levels [14].

TRP is an essential amino acid that is used in the synthesis of many proteins, enzymes, and neurotransmitters. Alterations in TRP levels may play a role in the pathophysiology of various neuropsychiatric and neurodegenerative diseases [26] [18]. TRP is also a precursor of various components that play a probable role in migraine pathogenesis (e.g. 5-HT and kynurenines), which may explain the association between TRP and migraine [12]. TRP levels in adult migreuners were controversial. Although Ren found lower serum TRP levels in migraine patients than in the control group in migraine without aura in attack free period, Alam and Curto observed higher TRP levels in migraine patients and reported that this increase was especially pronounced in the aura phase of migraine [15] [27] [28]. In the current study, we found no significant difference in TRP levels between migraine and control groups. All of our patients had migraine without aura and the levels was detected in attack free period. Fila et al. evaluated urinary TRP metabolites in the interictal phase in patients with episodic migraine and, found no significant difference between the control group and the migraine group in urinary TRP levels [29]. Urinary metabolites can be assumed to reflect blood metabolite levels. One study revealed that decrease in TRP levels increases headache, nausea and photophobia in migraine patients [30]. In fact, it has been reported that approximately 1 g of TRP intake halves the risk of migraine [31]. In our study, we found no correlation between TRP levels and headache frequency or PedMIDAS scores. The lack of a significant difference may be attributed to the absence of any regulation in the dietary intake of TRP among the patients.

KYN is the first metabolite of the tryptophan-kynurenine pathway and the main source of other metabolites and can cross the blood-brain barrier [32]. Curto et al. found low KYN levels in chronic migraine patients and Tuka et al. showed low KYN levels in the interictal period of patients with episodic migraine [15] [16]. On the contrary, another study found urinary KYN levels to be elevated in the interictal period in episodic migraine [29]. We observed higher KYN levels in migraine patients compared to the control group. An increase in KYN and KYNA levels has been reported to have a protective effect against migraine attacks [12]. The observed elevation in KYNA levels in our patients may be attributed to their attack-free period. Besides in a study conducted in rats, KYN combined with probenecid was shown to reduce nitroglycerin-induced headache [33]. Another animal study found that female rats have increased CSD during the period with increased estrogen levels. KYN treatment increased cortical KYNA levels leading to significant decrease in CSD frequency [34]. This finding indicates that KYNA is influenced by the female hormonal system [16]. In our study, we considered the possibility that KYN levels might have been influenced by hormonal changes, given that 67 % of the patients were female.

IDO1, which converts TRP to KYN, plays an important role in controlling the kynurenine pathway and can be considered as a measure of the rate of TRP metabolism [35]. It was reported that migraine stimulate the activation of IDO1 which led to increase the levels of KYNA and KYN [27]. Fila et al. confirmed this proposal by detecting an increased KYN/TRP ratio in migraine patients [29]. Consistent with the literature, we also found an increased KYN/TRP ratio in pediatric patients diagnosed with migraine.

Similar to KYN, 3-HK crosses the blood-brain barrier and forms a source for the kynurenine pathway in the brain parenchyma [14]. 3-HK is reported to have neurotoxic effects by increasing oxidative stress [36]. There is also another study suggesting its dual pro- and antioxidant effects [37]. Curto et al. found lower 3-HK levels in patients with cluster headaches and migraine compared to the control group [15] [38]. In our study, no difference was observed in 3-HK levels between migraine patients and the control group. Tuka et al. reported that 3-HK levels increased from the onset of the attack and that this increase was a protective compensatory mechanism [16]. In our study, no correlation was observed between headache frequency and 3-HK levels in migraine patients, but a significant positive correlation was revealed with

Table 1
Demographic characteristics of patients with migraine.

		N	%
Family History of Migraine	(−)	12	26.7 %
	(+)	33	73.3 %
Duration of Headaches	1–6 Months	7	15.6 %
	6–12 Months	6	13.3 %
	1–2 Years	16	35.6 %
	2–4 Years	16	35.6 %
PedMIDAS	Little to None	16	35.6 %
	Mild	16	35.6 %
	Moderate	3	6.7 %
	Severe	10	22.2 %

Table 2
Plasma concentrations of tryptophan metabolites in control and study subjects.

	Control Group (n = 48)				Study Group (n = 45)				p	
	Mean ± sd / n (%)			Median	Mean ± sd / n (%)			Median		
Age	13.560	±	3.033	14.500	13.848	±	2.589	14.250	0.709	m
Sex, female	25 (52 %)				30 (67 %)				0.153	X^2
Tryptophan (ng/ml) (x10^3)	44.307	±	35.664	38.100	50.797	±	31.775	43.500	0.125	m
Kynurenine (ng/ml) (x10^3)	2.216	±	2.203	1.585	2.868	±	1.956	2.530	***0.007***	m
Kynurenine/Tryptophan	0.050	±	0.015	0.047	0.058	±	0.019	0.058	***0.018***	t
3-OH Kynurenine (ng/ml)	2.132	±	2.530	1.265	2.369	±	2.459	1.540	0.526	m
Kynurenic Acid/3-OH Kynurenine	81.559	±	77.039	60.540	96.752	±	88.271	68.850	0.337	m
Kynurenic Acid/Kynurenine	0.048	±	0.022	0.046	0.045	±	0.019	0.040	0.368	m
Kynurenic Acid (ng/ml)	99.671	±	107.989	80.550	126.322	±	108.253	101.000	***0.043***	m
3-OH-Anthranilic Acid (x10^3)(ng/ml)	2.267	±	4.619	0.613	1.250	±	1.774	0.463	0.658	m

[t] Independent samples t-test / [m] Mann-Whitney U test / [X2] Chi-square test

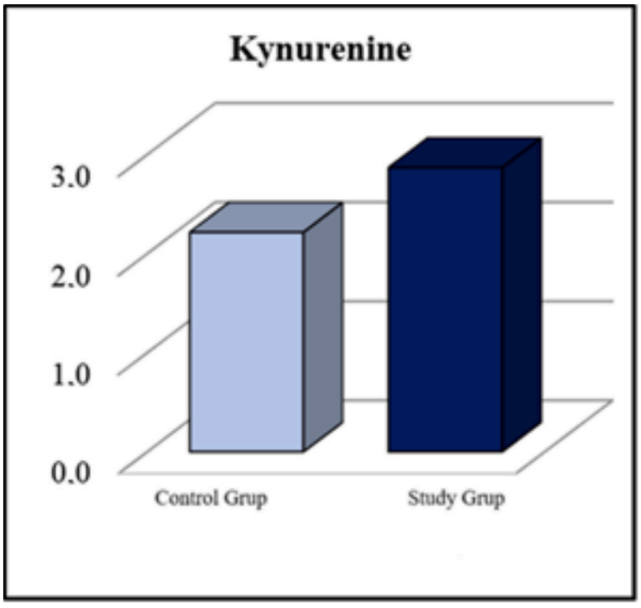

Fig. 1. Comparison of kynurenine levels. Illustrates a significant increase in kynurenine (KYN) levels among children diagnosed with migraine when compared to healthy controls ($p < 0.05$). This elevation suggests a potential dysregulation in the kynurenine pathway, which may contribute to the pathophysiology of pediatric migraine.

Table 3
Correlation of monthly frequency of headaches and PedMIDAS scores with levels of tryptophan metabolites in migraine patients.

		Frequency of Headaches (per Month)	PedMIDAS score
Tryptophan (ng/mL)	R	−0.062	0.011
	P	0.684	0.942
Kynurenine (x10^3) (ng/mL)	R	0.068	−0.009
	P	0.656	0.956
Kynurenine/Tryptophan	R	0.167	0.079
	P	0.272	0.608
3-OH Kynurenine (ng/mL)	R	0.283	0.514
	P	0.059	***0.000***
Kynurenic Acid/3-OH Kynurenine	R	−0.314	−0.537
	P	***0.036***	***0.000***
Kynurenic Acid /Kynurenine	R	−0.128	−0.039
	P	0.404	0.799
Kynurenic Acid (ng/mL)	R	−0.047	0.012
	P	0.757	0.936
3-OH-Anthranilic Acid (ng/mL)	R	−0.013	0.174
	P	0.931	0.254

Spearman correlation (R, Spearman's rank correlation coefficient; P, p-value)

Table 4
Effect of kynurenine levels in distinguishing between migraine patients and control subjects.

		Area Under the Curve		95 % Confidence Interval		P
Kynurenine (x10^3)		0.664		0.553	–0.774	***0.007***
Kynurenine cut-off of 1415		0.663		0.551	–0.774	***0.007***
		Control Group	Study Group			%
Kynurenine	≤1415	22	6	Sensitivity		86.7 %
	>1415	26	39	Positive Prediction		60.0 %
				Specificity		45.8 %
				Negative Prediction		78.6 %

pedMIDAS scores. This also indicates the protective effect of 3-HK.

KYNA is effective on NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate receptors, and G protein-coupled receptor 35 (GPR35), which take part in pain process and neuroinflammation [6]. A new and promising modality in migraine treatment is monoclonal antibodies effective on calcitonin gene-related peptide (CGRP) and its receptors. KYNA and its analogs are considered to function in migraine treatment via CGRP [12]. KYNA inhibits CSD [39], but its inability to cross the blood-brain barrier prevents its therapeutic use [32]. Chauvel et al. administered KYN and KYN combined with probenecid to rats and found an increase in cortical KYNA concentrations in both male and female rats and a decline in CSD frequency in female rats [40]. In their study, Curto et al. observed low KYNA levels in chronic migraine patients [15]. This low level of KYNA, which is a competitive antagonist of NMDA receptors, supports the theory that NMDA receptors are hyperactive in migraine [41]. In our study, we showed higher KYNA levels in migraine patients compared to healthy controls. In concordance with our findings, Tuka et al. also observed elevated interictal KYNA levels [16]. Kiss et al. triggered CSD in rats and observed an increase in KYNA in the same hemisphere, which was shown to reduce malonate toxicity in the cortex [42]. This increased KYNA observed in our study also suggests a protective compensatory effect of KYNA against pain in migraine.

3-HANA is considered a pro-oxidant and pro-inflammatory molecule because it causes neuronal toxicity and neuronal death by producing reactive oxygen species [43]. There are studies showing that 3-HANA, like 3-HK, can have both pro-oxidant and antioxidant effects [44] [45]. Another study has shown the anti-inflammatory and immune/neuroprotective effects of 3-HANA [46]. In their studies on patients with cluster headaches and chronic migraine, Curto et al. showed 3-HANA levels to be lower than the control groups [15] [38]. On the other hand, Tuka et al. found no significant difference in ictal/interictal 3-

HANA levels of migraine patients compared to healthy controls [16]. Similar to this study, we also did not observe a significant difference in 3-HANA levels between migraine patients and control groups in the current study.

Variations in findings across studies can be attributed to methodological differences, population heterogeneity, and biological factors. As in our study, most previous studies collected blood samples in the morning, minimizing circadian rhythm-related variability [47] [15] [38] [16]. However, differences in analytical methods may contribute to discrepancies; while Curto and Tuka employed the LC-MS method, consistent with our study, Fila analyzed metabolites in urine, complicating direct comparisons [15] [38] [29] [16]. Furthermore, Curto [15] [38] and Fila [13] included patients who were undergoing migraine treatment and using analgesics, whereas our study focused on a drug-free population, which may have influenced metabolite levels [48]. Additionally, Curto's [38] study predominantly included male patients, while the majority of participants in other migraine studies were female, suggesting that hormonal variability could impact biomarker levels [49]. Tuka [16] assessed patients in both ictal and interictal phases, whereas Curto [15] and Fila [13] focused solely on the interictal period, potentially leading to metabolic discrepancies [50]. Although sleep disturbances are known to influence kynurenine metabolism, this factor was not systematically evaluated in previous studies [51]. Thus, discrepancies in statistical significance across studies may not only stem from measurement reliability and consistency but also from variations in patient populations, clinical characteristics, and external influences, such as dietary intake, medication use, and hormonal fluctuations. Ultimately, ensuring the stability of measurement systems, minimizing population heterogeneity, and controlling for external confounding variables are critical in elucidating inconsistencies across studies. Therefore, future research should adopt more rigorous methodologies to account for these factors.

The limitations of our study can be outlined as follows: First, the primary limitation is our inability to assess the participants' TRP intake. Second, due to the absence of prior pediatric studies on the migraine-kynurenine pathway, all our comparisons were made with adult studies. Third, while some metabolites of the kynurenine pathway can cross the blood-brain barrier, others cannot. Evaluating the kynurenine pathway solely in plasma rather than in cerebrospinal fluid (CSF) also represents a significant limitation. Additionally, we did not assess the menstrual cycles of the patients, which could be a relevant factor given the hormonal influence on TRP metabolism and its potential impact on kynurenine pathway activity. Future studies should consider controlling for these variables to ensure a more comprehensive understanding of the metabolic changes associated with migraine.

5. Conclusion

Our study is the first to measure the kynurenine pathway metabolites and show the correlation between abnormalities in this pathway and migraine in pediatric patients diagnosed with migraine. We think that KYN, KYNA and 3-HK may act as protective compensatory mechanisms in migraine. Evaluating complex pathways such as the kynurenine pathway in migraine will be guiding both for understanding the pathophysiology and developing new treatments.

Author contribution

All authors meet the ICMJE authorship criteria. All authors have made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, or revising it critically for important intellectual content, and all have approved the final version to be submitted.

Şeyma Sönmez Şahin: Writing-rewiew&editing, Writing original draft, Formal analysis, Conceptualization, Data curation**. Esra Paydaş Hataysal**: Writing-rewiew&editing, Writing original draft, Formal analysis, Data Curation. **Elif Yüksel Karatoprak**: Writing-rewiew&editing, Writing original draft, Formal analysis. **Ferit Durankuş:**Data curation. **Ayşegül Özel**: Data curation. **Hüsemettin Vatansev**: Data curation, Formal analysis. **Fadime Ovalı:** Data curation, Formal analysis.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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