BMC Neurology

RESEARCH

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Evaluation of zonulin levels in patients with migraine

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Abstract

Background Zonulin regulates permeability in blood–brain and intestinal barriers. The pathophysiology of migraine is based on the effect of neurogenic inflammation. The aim of the current investigation was to examine the serum zonulin level in individuals suffering from migraine.

Methods The sample comprised 40 individuals who had migraine and 40 controls. Disease duration, attack duration, attack frequency, Visual Analog Scale (VAS) scores, and comorbidities were available for the migraine group. Serum zonulin levels were evaluated by using the ELISA method.

Results There were no statistically significant differences between the two groups concerning age or gender ($p > 0.05$). The zonulin value of patients with migraine was higher when compared to the controls, indicating a significant difference ($p = 0.037$; $p < 0.05$). The zonulin level did not correlate with disease duration, attack duration, VAS score, or attack frequency ($p > 0.05$). The receiver operating characteristic curve analysis of zonulin revealed a cut-off value of 30.58 and above, at which it had 52.50% sensitivity, 77.5% specificity, 70% positive predictive value, and 62% a negative predictive value. The area under the curve was 63.6%, and the standard error value was 6.3%. The analysis also showed a statistically significant correlation between migraine diagnosis and a zonulin level of 30.58 ($p = 0.006$; $p < 0.01$).

Conclusions Elevated zonulin levels in patients with migraine support the disruption of the intestinal barrier and neuroinflammation in these patients. The zonulin level may be a predictive biomarker of migraine. Multicenter, randomized trials are needed to evaluate treatments for intestinal permeability and zonulin levels in migraine patients.

Keywords Zonulin, Migraine, Headache, Gut-brain axis, Intestinal permeability

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Introduction

It has been found that the intestinal epithelium and the brain are bidirectionally correlated, and this correlation has started to be considered in connection with psychiatric and neurological diseases [1–3]. The complex structure of the intestinal epithelium enables it to fulfill digestive, absorptive, neuroendocrine, and immunologic functions. Protein complexes forming tight junctions between enterocytes are crucial for the control of intestinal permeability. Effectively functioning tight junctions and a robust immune system manage the passage of food, bacterial antigens, and toxins. Maintaining a healthy gastrointestinal microbiota, both in composition and quantity, is essential for preserving this balance. The disruption of this barrier causes intestinal microbiota and liposaccharide- like exotoxin molecules to migrate into the intraperitoneal space [4]. Alterations in the intestinal microbiota may affect neurotransmitter levels, having a direct impact on the vagus nerve and consequently the central nervous system, especially on serotonin and gamma-aminobutyric acid [5]. Gastrointestinal symptoms are common in individuals who have migraine, and the frequency of migraine has been found to be higher in those with gastrointestinal conditions, including inflammatory bowel disease, celiac disease (CD), and irritable bowel syndrome (IBS) [6, 7]. For these diseases, there is increased permeability in the barrier of the intestine, which may cause bacterial components such as liposaccharides and incompletely digested food particles to be absorbed into the bloodstream and act as exotoxins [8–10]. It is considered that proinflammatory chemicals may cross the intestinal barrier and enter the trigeminovascular system, which might result in a migraine attack. The intestines contain 95% of serotonin in the body, and research has demonstrated a connection between gastrointestinal effects of migraine and serotonergic pathways [11, 12].

Zonulin is the first protein shown to have a regulatory effect on human tight junctions and is a key biomarker of elevated intestinal permeability. Zonulin results in the activation of EGFR via PAR2, leading to phosphorylation in tight junction proteins, rearrangement among actin filaments, and relaxation of tight junction proteins, thereby contributing to elevated intestinal permeability [13]. By facilitating antigen transport via the intestinal epithelium's paracellular route, elevated zonulin activity may lead to an aberrant immune response and a reduction in immunological tolerance. It has been demonstrated that zonulin directly contributes to the pathogenesis of various autoimmune conditions. It is also linked to diseases with elevated intestinal permeability [13, 14]. For patients with migraine, positive results have been reported in studies conducted with probiotic and symbiotic replacement to regulate the intestinal microbiota and improve intestinal permeability [15, 16]. However, within the context of migraine, zonulin, a significant biomarker of intestinal permeability, has only been analyzed in pediatric patients with migraine [17]. Thus, this investigation was conducted in order to investigate zonulin levels among individuals with migraine during the interictal period and determine their correlation with clinical parameters.

Materials and methods

Sample collection

Ethical approval was secured from the Clinical Trials Review Board of Istanbul Medipol University (number: E-10840098-772.02-1675, date: March 6, 2023). The sample comprised 40 individuals diagnosed with episodic migraine based on the criteria specified in the International Classification of Headache Disorders, third edition [18], who were admitted to the hospital between March 2023 and February 2024, as well as 40 healthy adults constituting the control group. Specific information about the individuals in the migraine group was recorded, which included disease duration, attack duration, attack frequency, pain intensity as evaluated by Visual Analog Scale (VAS) scores, comorbidities, and a history of prophylactic therapy.

The criteria for inclusion in the study were receiving a diagnosis of episodic migraine and being in the age range of 18–65 years. The exclusion criteria were the use of probiotics and prebiotics within the last three months, not giving consent, and exposure to diseases, such as malignancy, hematologic diseases, inflammatory bowel disease and demyelinating disorders of the central nervous system in both of case and control groups.

Analytical method

For the zonulin test, 10 ml of blood was obtained from the individuals with migraine and controls after 12 h of fasting and transferred to gel activator vacuum tubes. After allowing coagulation to occur for 1 h at room temperature, serum separation was achieved by centrifuging it at 1000 g for 20 min. The serum, once separated, was kept at -80 °C until it was ready for analysis. A human zonulin enzyme-linked immunosorbent assay kit (Elabscience) based on the sandwich-ELISA principle was utilized (detection range: 0.78-50 ng/mL, sensitivity: 0.47 ng/mL). The intra-assay and inter-assay coefficient of variation values were both < 10%.

Statistical analysis

A power analysis was conducted using the G*Power (v3.1.9.2) program to ascertain the appropriate sample size. The study's power is represented as 1-β, where β refers to the probability of type II error, with a typical requirement for studies to achieve 80% power. In zonulin measurements for migraine, the effect size was found

Table 1 Participants' descriptive data

		***n* (%)**
Gender*(n = 80)*	Male	19 (23.8)
	Female	61 (76.3)
Age*(n = 80)*	*Mean ± SD*	32.06 ± 6.99
	Median (Min-Max)	32 (20–46)
Zonulin (ng/mL)*(n = 80)*	*Mean ± SD*	27.47 ± 7.57
	Median (Min-Max)	28.6 (6.04–39.5)
Disease duration (years)*(n = 40)*	*Mean ± SD*	7.90 ± 5.56
	Median (Min-Max)	6 (1–20)
Attack frequency (/month) *(n = 40)*	*Mean ± SD*	5.57 ± 5.28
	Median (Min-Max)	3 (1–20)
Attack duration (hours)*(n = 40)*	*Mean ± SD*	25.42 ± 25.12
	Median (Min-Max)	24 (1–96)
VAS score*(n = 40)*	*Mean ± SD*	8.30 ± 1.60
	Median (Min-Max)	8 (4–10)
Comorbidity*(n = 40)*		14 (35.0)
Prophylactic treatment*(n = 40)*		4 (10.0)

to be d = 0.666, with a mean difference of at least 3 units and a standard deviation of 4.5 for a clinical difference between groups. Accordingly, it was determined that to attain 80% power at an α level of 0.05, the minimum number of subjects required for each group was 37. Considering possible losses, 40 individuals were included in each group. The post hoc power (1-β err prob) were performed too, and the result was 51%.

When analyzing the data obtained, the NCSS 2020 (LLC, Kaysville, Utah, USA) statistical software was utilized. Quantitative variables were represented by mean ± standard deviation or median (minimum–maximum) values, and qualitative variables were represented by descriptive statistics (numbers and percentages). The Shapiro- Wilk test and box plots were employed to assess whether the data conformed to a normal distribution. Student's t-test was conducted to for the assessment of two groups in terms of quantitative data with a normal distribution, and the Mann-Whitney U test was employed to assess quantitative data without a normal distribution. On the other hand, Pearson's chi-square test was performed to compare qualitative data. Spearman's correlation analysis was undertaken for the assessment of intervariable correlations. The logistic regression analysis was applied in multivariate evaluations. The results were assessed using a 95% confidence interval and a significance threshold set at $p < 0.05$.

Results

This study was conducted at Medipol Mega University Hospital with a total of 80 individuals (females: 76.3%, $n = 61$; males: 23.8%, $n = 19$). The participants' ages varied between 20 and 46 years, and the mean age value was 32.06 ± 6.99 years. The distribution of descriptive characteristics is shown in Table 1.

Table 2 Comparison of descriptive characteristics between the study groups

		Group		***p***
		Migraine (*n* = 40)	**Control (*n* = 40)**	
Gender	**Male**	8 (20.0)	11 (27.5)	[a]***0.431***
	Female	32 (80.0)	29 (72.5)	
Age	*Mean ± SD*	31.68 ± 7.85	32.45 ± 6.10	[b]***0.624***
	Median (Min-Max)	29 (21–46)	33.5 (20–40)	
Zonulin (ng/mL)	*Mean ± SD*	29.13 ± 7.63	25.81 ± 7.24	[b]***0.037****
	Median (Min-Max)	30.7 (10.5–39.4)	26.9 (6.0-37.7)	

[a]Pearson chi-square test, [b]Student's t-test. *$p < 0.05$

The migraine and control groups did not significantly differ in relation to the age and sex distributions ($p > 0.05$). The mean zonulin level was higher in the migraine group when compared to the control group (Table 2; Fig. 1) ($p = 0.037$). Table 2 shows the comparison of the two groups in terms of descriptive data. When it was evaluated the effects of Zonulin levels, age and gender variables on migraine with logistic regression analysis; it was observed that the model was found to be significant (F = 31.988; $p = 0.001$) and the explanatory coefficient of the model's ratio was 73.8%.The results of logistic regression analysis were shown in Table 3.

Zonulin levels did not have any significant correlation with disease duration, attack frequency, attack duration, or Visual Analog Scale scores. Furthermore, the zonulin levels of the patients did not significantly differ when analyzed based on comorbidities or a history of prophylactic treatment (Table 4).

The receiver operating characteristic analysis revealed a cut-off value of 30.58 and above for zonulin in the differentiation of the migraine and control groups. At this cut-off value, the sensitivity, specificity, positive predictive value, and negative predictive value of zonulin were 52.50%, 77.5%, 70%, and 62%, respectively. The area under the curve (AUC) was calculated to be 63.6%, and the standard error value was 6.3%. The analysis also showed a statistically significant relationship between a migraine diagnosis and zonulin at a threshold level of 30.58 ($p = 0.006$; $p < 0.01$) (Table 5).

The ROC curves of the zonulin measurements of the groups are shown in Fig. 2.

Discussion

This study found that the serum zonulin level measured in the interictal period was higher among individuals with episodic migraine than in healthy controls. Serum zonulin also showed diagnostic accuracy for migraine

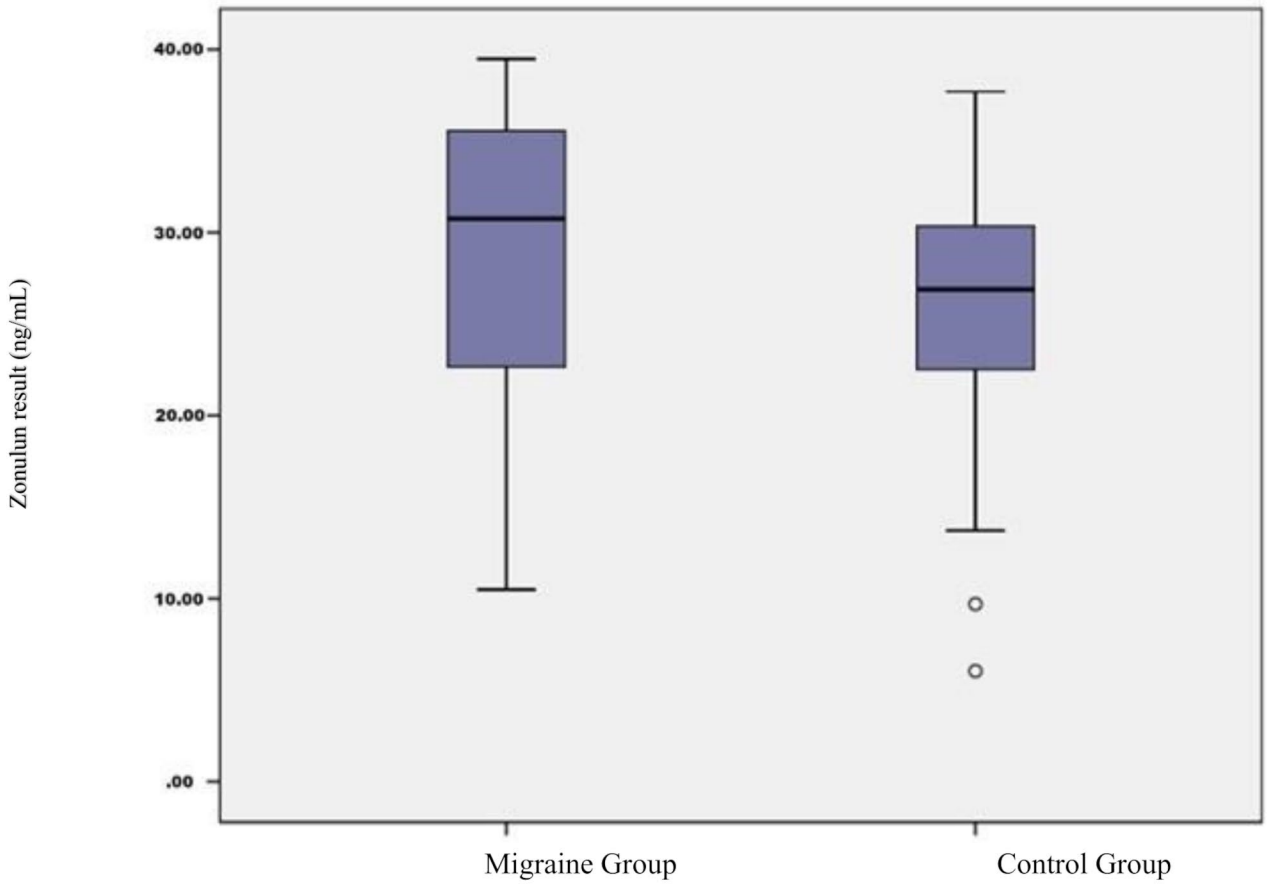

Fig. 1 Distribution of Zonulin Levels by Group

Table 3 The logistic regression analysis of risk factors on migraine

	B	*p*	ODDS	%95 CI	
				Lower	Upper
Zonulin *(≥ 30.58)*	2.021	0.003**	7.549	2.012	28.319
Age	-0.033	0.434	0.968	0.891	1.051
Gender	0.518	0.475	1.679	0.405	6.952

**$p < 0.01$

Table 4 Assessment of zonulin levels in the migraine group according to clinical variables

(n = 40)	**Zonulin (ng/mL)**	
	r	***p***
Disease duration (years)	-0.023	***0.887***
Attack frequency (/month)	-0.094	***0.562***
Attack duration (hours)	-0.158	***0.331***
VAS	0.009	***0.955***
Comorbidity	***Mean ± SD***	
Present	28.54 ± 8.16 (29.5)	[b]***0.705***
Absent	30.24 ± 6.64 (32.2)	
Prophylactic treatment		
Present	29.62 ± 7.52 (31.4)	[b]***0.207***
Absent	24.76 ± 8.15 (23.9)	

r: Spearman's Correlation Coefficient, [b]Mann-Whitney U test

(AUC = 0.63). This study is one of the few studies in the existing literature investigating zonulin levels in migraine patients. The higher zonulin level in patients with migraine suggests that the permeability of the intestinal wall is altered in this patient population. The observed association between zonulin and migraine may be attributed to the role of zonulin as a marker of gut inflammation. Alterations in the zonulin pathway are linked to the "leaky gut" phenomenon, which increases intestinal permeability and allows pro-inflammatory substances to enter the bloodstream, potentially exacerbating migraine. Additionally, zonulin release may contribute to vagal system dysfunction, a known factor in migraine pathophysiology [19]. It is thought that endogenous or dietary histamine in the intestinal mucosa increases intestinal permeability triggered by mast cell degranulation and inflammation. Calcitonin gene-related peptide (CGRP), which is known to be important in the pathophysiology of migraine, also has an important role in the physiology of the gastrointestinal tract, and the system where intestine is most commonly found outside the central nerveous system. Elevated levels of histamine in the gut are thought to be the cause of increased release of CGRP

Table 5 Results of the diagnostic screening test and ROC curve analysis for zonulin

	Diagnostic screening test					ROC curve		*p*
	Cut-off	Sensitivite	Specificity	Positive Predictive Value	Negative predictive value	Area	95% confidence interval	
Zonulin	***≥ 30.58***	52.50	77.50	70.00	62.00	**0.636**	0.513–0.759	***0.006****

*$p < 0.05$

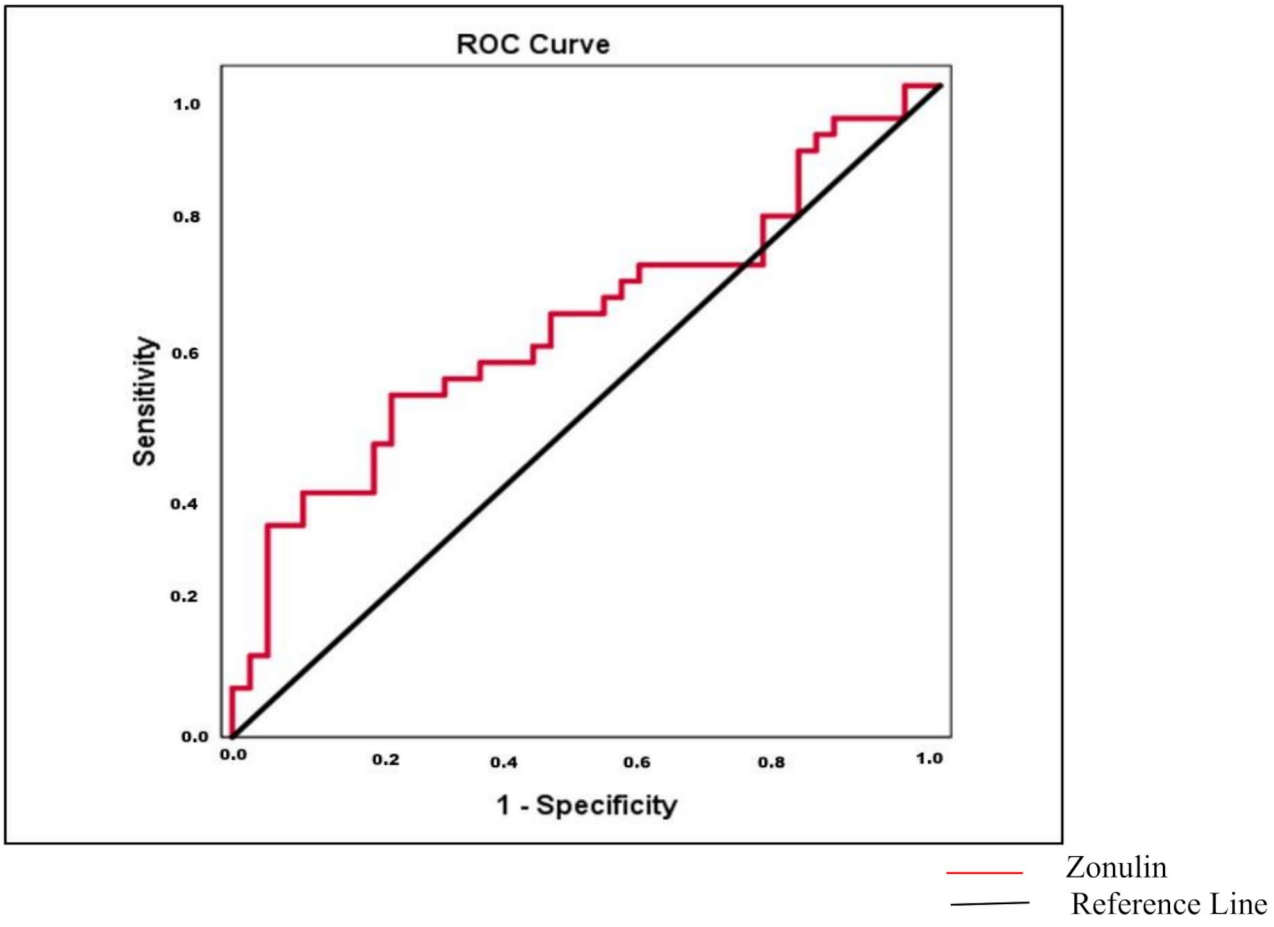

Fig. 2 ROC curve analysis for zonulin

in the gut and elevated levels of CGRP in the peripheral blood. CGRP activates intracranial nociceptive afferents and neurogenic inflammation. Thus, the increasing zonulin level, which is an indicator of intestinal permeability, can be explained by this factor in migraine [20]. However, our results revealed that the zonulin level did not significantly correlate with headache attack frequency, attack duration, or disease duration. The lack of correlation between zonulin levels and headache characteristics could be due to the limited number of subjects recruited. Further prospective large studies, particularly in patients with and without gastrointestinal symptoms, are needed to elucidate the underlying mechanisms.

The intestinal epithelial barrier and the blood-brain barrier control the migration of ions, nutrients, and soluble substances. Additionally, this epithelial barrier prevents gut microbiota migration, while the blood-brain barrier controls immune system trafficking. Tight junctions hold both barriers together, but they may be disrupted by inflammatory factors such as lipopolysaccharide and cytokines [21–23].

The term "gut-brain axis" refers to a reciprocal correlation between the gastrointestinal system and the central nervous system. Many studies indicate that an association between migraine and certain gastrointestinal conditions, including infections caused by *Helicobacter pylori*, IBS, and CD. In cases of intestinal barrier disruptions, the contents of the intestinal lumen run through the mucosal layer and impact the enteric ganglia. They subsequently enter the systemic circulation and affect the central nervous system [13]. Studies indicate that this interaction is affected by many factors, including inflammatory

mediators (interleukin-1β, -6, and − 8, and tumor necrosis factor alpha), gut microbiota profile, neuropeptide and serotonin pathways, levels of stress hormones, and nutrients [24].

Zonulin controls the permeability of both the blood-brain barrier and intestinal epithelial barrier [25]. Consequently, zonulin has been investigated in various neuroinflammatory diseases. A study undertaken by Usta et al. showed higher zonulin values in healthy individuals [26]. These results expand the current knowledge on the dysregulation of intestinal permeability, particularly of zonulin and the blood-brain barrier. In another study, Camara-Lemarroy et al. revealed that zonulin concentrations significantly increased in those with multiple sclerosis and confirmed this elevation through simultaneous magnetic resonance imaging, which showed the disruption of the blood-brain barrier [27]. Zonulin could play a role in disrupting both the intestinal epithelial barrier and the blood-brain barrier during gut dysbiosis by affecting tight junctions. This mechanism may shed light on how the gut-brain axis influences neuroinflammation associated with MS.

Tunçer et al., evaluating pediatric patients with migraine, reported that the zonulin levels did not significantly differ between individuals with migraine and healthy controls [17]. Our study, on the other hand, demonstrated a higher zonulin value in patients with migraine, and the difference was statistically significant. These contrasting results may result from various factors, including the place of residence, disparities in age groups, dietary habits, and individual and genetic characteristics. In addition, there may be a more significant correlation between fecal zonulin levels and intestinal permeability [28], but we did not obtain fecal samples from our patients.

Studies have been conducted to assess the importance of intestinal permeability regulation as the importance of the intestinal brain axis in migraine has become clear. Ghavami et al. assessed the outcomes of 12-week symbiotic treatment in individuals with migraine and reported a significant decline in attack frequency and the number of analgesics used, as well as a significant decrease in serum zonulin levels, an indicator of intestinal permeability, after symbiotic treatment [15]. Several studies have also indicated a decrease in migraine attack severity through probiotic replacement alone [16, 29]. A randomized controlled trial by De Roos et al. in probiotic and placebo groups indicated significantly lower migraine-associated disability and headache-associated disability scores in the probiotic group, despite the absence of significant differences according to the rates of reduction in the number of painful days and the number of painful days longer than 2 days. In addition, the authors found that the zonulin level and inflammatory parameters did were not significantly different between probiotic-treated patients and controls [30]. However, the study only assessed the zonulin level measured in blood, which was higher in the migraine group at a statistically significant level, and found no significant relationship between the level of this parameter and pain intensity or duration. Another study conducted by Vurallı et al. determined that intestinal permeability was higher among individuals with chronic migraine and those suffering from medication overuse headaches than in both episodic migraine and control groups [31]. Our sample did not include any patients diagnosed with medication overuse headache.

The main limitations of this study include the lack of data on dietary habits, food intolerances, non-steroidal anti-inflammatory drug use, and additional tests such as blood lipopolysaccharide measurements for assessing intestinal permeability. It is another limitation that the study is underpowered despite the sample size calculation [32]. The ELISA method used for zonulin measurement have complex workflows that include several time-intensive wash steps. The complexity of ELISA protocol is another limitation for the study.

In conclusion, zonulin, a key marker of intestinal permeability, was found to be elevated in migraine patients. This study further underscores the significance of the gut-brain axis in the pathophysiology of migraine. Larger multicenter, randomized controlled trials are needed to evaluate treatments for regulating intestinal permeability in migraine patients, as well as to assess ictal and interictal zonulin levels in both blood and stool.

Abbreviations

AUC	Area under the curve
CD	Celiac disease
CGRP	Calcitonin gene-related peptide
IBS	Irritable bowel syndrome
ROC	Receiver operating characteristic
VAS	Visual Analog Scale

Acknowledgements

Not Applicable.

Author contributions

All authors reviewed the manuscript.

Funding

There was no funding.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The approval of the Ethics Committee at İstanbul Medipol University was secured before starting the study (approval number: E-10840098-772.02-1675, date: March 6, 2023) Informed consent was obtained from all patients at the time of enrollment. This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Received: 2 December 2024 / Accepted: 28 January 2025
Published online: 04 February 2025

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