ASSESSMENT OF SERUM MMP-9, TIMP-1 LEVELS AND MMP-9/TIMP-1 RATIO IN MIGRAINE PATIENTS WITH AND WITHOUT AURA

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Background and purpose – Matrix metalloproteinases (MMP) are the enzymes responsible for proteolytic activity of extracellular matrix proteins. Tissue inhibitors of metalloproteinases (TIMPs) are their endogenous inhibitors. MMP-9 acts on the basal membrane of cerebellar epithelium and is antagonized by TIMP-1. MMP-9/TIMP-1 ratio exhibits the net activity of MMP-9. These enzymes are thought to have a role in migraine physiopathogenesis.
Methods – Total of 50 treatment-naive migraine patients (25 with aura and 25 without aura) with no other diseases, were included. 25 healthy control subjects of corresponding age and gender were enrolled. For MMP-9 and TIMP-1 analysis, one serum sample from control group and two samples from patients were collected (during headache and headache-free periods). The enzyme levels were quantitatively analyzed by competitive ELISA method. Duration and severity of the pain and duration of the disease were recorded.
Results – There was no significant difference in MMP-9 levels between patient and control groups during headache and headache-free periods (p: 0,746, p: 0,243). TIMP-1 levels were significantly lower and MMP-9/TIMP ratios were higher comparing with the control group (p: 0.001). Positive correlation was obtained between the duration of pain and MMP-9 levels in the headache-free period for both patient groups ($p<0.05$).

A SZÉRUM MMP-9- ÉS TIMP-1-SZINTJEI, VALAMINT AZ MMP-9/TIMP-1 ARÁNY AURÁVAL VAGY A NÉLKÜL MIGRÉNBEN SZENVEDŐ BETEGEK ESETÉN

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Háttér és cél – A mátrix metalloproteinázok (MMP) felelősek az extracelluláris mátrix fehérjéinek proteolyticus aktivitásáért. Az MMP-k endogén inhibitorai a szöveti metalloproteináz-inhibitorok (TIMP). Az MMP-9 a cerebellaris epithelium bazális membránjára hat, és a TIMP-1 antagonizálja. Az MMP-9/TIMP-1 arány az MMP-9 nettó aktivitását mutatja. A jelenlegi elképzelés szerint ezeknek az enzimeknek szerepük van a migrén patogenezisében.
Módszerek – Összesen 50, korábban nem kezelt, egyéb betegségben nem szenvedő migrénbeteget (25 fő aurával, 25 aura nélkül) vontunk be vizsgálatunkba. A kontrollcsoportot 25, életkorban és nemben illeszkedő egészséges személy képezte. Az MMP-9- és TIMP-1-szintek elemzéséhez a kontrollcsoport tagjaitól egy, a betegektől két (fejfájásos, illetve fejfájásmentes időszakban) szérummintát gyűjtöttünk. Az enzimek mennyiségét kompetitív ELISA módszerrel elemeztük. Rögzítettük a fájdalom időtartamát és erősségét, valamint a betegség fennállásának időtartamát.
Eredmények – Nem volt szignifikáns különbség a betegek (fejfájásos, illetve fejfájásmentes időszakban mérve), valamint a kontrollszemélyek között az MMP-9-szintekben (p: 0,746, p: 0,243). A betegek TIMP-1-szintje szignifikánsan alacsonyabb, MMP-9/TIMP-1 aránya magasabb volt, mint a kontrollszemélyeké (p: 0,001). Pozitív korrelációt találtunk mindkét betegcsoport fejfájásmentes periódus-

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There was also a positive correlation between MMP-9/TIMP-1 ratio and severity of pain ($p<0.05$).
Conclusion – In our study, low TIMP-1 levels of patients in both headache and headache-free periods suggest that disturbance of proteolytic protection has a role in neuro-inflammation and pain in migraine. Therefore, these enzymes could be potential targets in migraine therapies.

Keywords: *matrix metalloproteinases, MMP-9, TIMP-1, migraine*

ban mért MMP-9-szintje és a fájdalom időtartama között $p < 0,05$). Szintén pozitív korrelációt találtunk az MMP-9/TIMP-1 arány és a fájdalom erőssége között ($p < 0,05$).
Következtetés – Vizsgálatunk során a betegek fejfájásos, illetve fejfájásmentes időszakaiban mért alacsony TIMP-1-szint arra utal, hogy a proteolyticus védelem zavarai szerepet játszanak a migrén kapcsán jelentkező neuroinflammációban és fájdalomban. Ebből következően, ezek az enzimek a jövőbeli migrénellenes gyógyszerek támadáspontjaiként szerepelhetnek.

Kulcsszavak: *mátrix metalloproteinázok, MMP-9, TIMP-1, migrén*

Migraine is an episodic primary headache disorder accompanied by neurological, gastrointestinal and autonomic symptoms[1]. Effective treatment of migraine not only improves the quality of life of the patient but also has a benefit for individual and social labor and national economy.

Neural depolarization and vascular events that are triggered by internal and external effects have roles in physio-pathogenesis of migraine along with the presence of easily inducible cerebellar cortex[2]. Cortical depression is the first step of chain reactions. This will cause activation of trigemino-vascular system and neurogenic inflammation[3]. As a result of neurogenic inflammation, dura mater and vasodilatation of blood vessels increase in blood stream velocity and protein extravasation occur[1, 4].

Physiopathologies of migraine with and without aura are shown to be different in some aspects. There are evidences that show genetic factors play a more prominent role in migraine with aura comparing to migraine without aura, however responsible gene or genes and their loci have not been fully demonstrated yet[5]. Cortical spreading depression is known to be the principal underlying mechanism of aura. Apparent symptoms of aura, which can confirm the clinical diagnosis of cortical spreading depression (CSD), are missing in migraine without aura. Cortical hypo-perfusion, which has been demonstrated in studies carried out in patients with migraine without aura, suggests the presence of CSD, however without a clinical outcome[6].

Matrix metalloproteinases (MMP) are the enzymes catalyzing the degrading of extracellular matrix components. They are synthesized by several cell groups, mainly macrophages and lymphocytes[7]. Their functions and roles in the pathophysiology of many disorders, such as cancers, arthritis, infections, cardiovascular and neurological diseases have been studied since the 1960s[8].

They cause disturbance of blood-brain barrier (BBB), leukocyte infiltration, demyelination, axonal injury and astrogliosis. MMPs are thought to contribute the neurological inflammation through destruction of BBB. These effects are balanced by their specific endogenous inhibitors, which are called tissue inhibitors of metalloproteinases (TIMPs). TIMPs have a preventive effect against neurogenic inflammation that is originated by MMPs. This protective mechanism has a major role in the stabilization of physiopathological events[9, 10].

One of these MMPs, of which serum levels were investigated during migraine attacks, was MMP-9 and it was found to have pro-inflammatory and proteolytic effects. MMP-9 causes destruction of the basal membrane components, collagen type 4-5 and fibronectin through tumor necrosis factor-alpha (TNF-alpha) and therefore contributes to development of neurogenic inflammation by affecting BBB permeability. TIMPs inhibit the activation of the latent enzyme form and sustain the catalytic activity by binding MMPs with an irreversible and non-covalent way. Thus, TIMPs has the strict control of MMP enzyme activity and MMP/TIMP ratio[11, 12].

In this study, we aimed to make a comparison between the healthy control group subjects and migraine patients with and without aura both during headaches and headache-free periods in terms of serum MMP-9 and its tissue inhibitor TIMP-1 levels. In addition, we aimed to determine if MMP-9, TIMP-1 and MMP-9/TIMP-1 ratios were different between patients, suffering from migraine with or without aura. We also aimed to investigate serum MMP-9, TIMP-1 levels in migraine patients with and without aura during headache and headache-free periods and to evaluate MMP-9/TIMP-1 ratio. Therefore, the study results may provide insight for the development of migraine treatment options.

Materials and methods

Twenty five patients without aura (F/M: 21/4) and 25 patients with aura (F/M: 22/3) were included in the study from the patients applied to our outpatient clinics, who were treatment naive and at least 18 years old. Patients were not included due their genders but due to eligibility and consent for enrollment. Ethical approval for the study was obtained from the Ethics Committee of Bakirkoy Prof. Dr. Mazhar Osman Training and Research Hospital for Psychiatry, Neurology and Neurosurgery (Istanbul, Turkey; Issue date: 31.3.2009; Project no: 2009/10).

Medical history, personal and familial history of the patients was obtained. Physical and neurological examinations were performed. To exclude other central nervous system pathologies, computerized tomography and/or magnetic resonance imaging evaluations of patients were also performed. Patients, diagnosed with migraine with and without aura according to the International Classification of Headache Disorders (ICHD) -3[13] were included in the study.

None of the subjects was on prophylactic treatment in the previous 3 months. Criteria of exclusion were autoimmune diseases, collagen diseases, statin intake, pregnancy and smoking. Patients were instructed not to take symptomatic treatment before blood extraction. Pain characteristics, duration of the disease, sociodemographic characteristics of patients were recorded to related forms. Pain level was evaluated on visual analogue scale (VAS). For headache-free period, the patients were invited back for blood sampling after at least seven days of pain-free span.

Control group consisted of total 25 subjects (F/M: 18/7), who met the inclusion criteria of no headache or other neurological diseases, no systemic disorders and who were at least 18 years old. Informed consent forms were obtained after verbal and written information was provided to patients and control group subjects.

Two blood samples from each patient (one for attack and one for headache-free periods) and one blood sample from each control group subject were collected. Blood serum was obtained after clotting the blood for 20 minutes in Vacuette jelly tube with clot activator and by centrifugation for 10 minutes at 4000 g. Serum samples were stored at -30 ºC until the time of analysis.

Serum MMP-9 levels were quantitatively analyzed by using competitive ELISA method (Human MMP-9 ELISA; Floor No: ELH-MMP9-001), serum TIMP-1 levels were quantitatively analyzed by using competitive enzyme immunoassay method (ELISA Human TIMP -1; Floor No. : ELH-TIMP-001) in Haseki Training and Research Hospital, Biochemistry Laboratory. The serum samples were added to (ELISA) wells with MMP-9 antibodies. Biotinylated anti-human MMP-9 antibodies were added on to the MMP-9 enzyme-antibody complexes formed in the wells. After that, HRP-conjugated streptavidin was added to this mix and the intensity of color occurred in reaction to tetramethylbenzidine (TMB) substrate, reflected the level of MMP-9. In order to determine serum TIMP-1 levels, serum samples were added to the wells that contained TIMP-1 antibodies and biotinylated anti-Human TIMP-1 antibodies were later added to this mix. HRP-conjugated streptavidin was added and the intensity of color occurred in reaction to TMB substrate, reflected the level of TIMP-1.These analyzes were performed by ELX 800 Biotek microplate ELISA device at 450 nm. MMP-9 and TIMP-1 concentrations were calculated with Gen software program of ELISA device.

STATISTICAL ANALYSIS

SPSS (Statistical Package for Social Sciences) for Windows 15.0 program was used for the statistical analysis of study results. One-way ANOVA test for the comparison of normally distributed parameters' quantitative data and Tukey's Honest Significant Difference test for the determination of differentiation group were used, in addition to descriptive statistical methods (mean, standard deviation). Mann Whitney U test was used for the comparison of parameters, which were not normally distributed, between two groups. Paired sample t test was used for intergroup analysis of normally distributed parameters. Chi-square test and Fisher's Exact Chi-square tests were used for the comparison of qualitative data. Pearson and Spearman's correlation analysis was used for the evaluation of inter-parameter correlations. Significance was considered at $p < 0.05$ level.

Results

Patient demographics are summarized in **Table 1**.

No statistically significant difference was found between groups in terms of age and gender (p: 0.351, p: 0.319).

Blood samples were collected from migraine patients without aura and with aura after 8.20±6.39 hours and 11.0±8.10 hours of onset of the headache, respectively. No statistically significant difference was found between the groups in terms of the time of blood collection (p: 0.626).

Table 1. *Evaluation of age and gender according to study groups*

		Migraine without aura (n=25) Mean±SS (Median)	Migraine with aura (n=25) Mean±SS (Median)	Control (n=25) Mean±SS (Median)	Total (n=75) Mean±SS (Median)	*p*
+Age		33.84±2.68 (35)	36.40±8.01 (35)	36.20±7.37 (35)	35.36±7.92 (35)	*0.351*
		n (%)	n (%)	n (%)	n (%)	
Gender	Female	21 (84%)	22 (88%)	18 (72%)	61 (81.3%)	*0.319*
	Male	4 (16%)	3 (12%)	7 (28%)	14 (18.7%)	

One way ANOVA test was used.
+Chi-Square test was used.
Abbreviations: n: Number, SD: Standard Deviation

Table 2. *Comparison of MMP-9 and TIMP-1 serum levels and calculated MMP-9/TIMP-1 ratios during headache and headache-free periods in the migraine patients and the control groups*

		Migraine patients (n=50)	Control (n=25)	*P*
Headache period	MMP-9	115.80±55.62	118.49±12.78	*0.746*
	TIMP-1	348.47±47.04	559.20±118.53	*0.001***
	MMP-9/TIMP-1	0.33±0.15	0.22±0.04	*0.001***
Headache-free period	MMP-9	109.28±52.20	118.49±12.78	*0.243*
	TIMP-1	348.38±34.01	559.20±118.5	*0.001***
	MMP-9/TIMP-1	0.33±0.17	0.22±0.04	*0.001***

Student t test was used. **p<0.01
Abbreviations: MMP-9: Matrix metalloproteinase-9, n: Number, SD: Standard Deviation, TIMP-1: Tissue inhibitor of metalloproteinase-1

No statistically significant difference was found between MMP-9 levels of control group and patients in both attack and headache-free periods (p: 0.746, p: 0.243). Serum TIMP-1 levels of all patients were found significantly lower in attack period comparing with the control group (p: 0.001). MMP-9/TIMP-1 ratio of migraine patients were determined as significantly higher comparing with the control group (p: 0.001). Data summarized in **Table 2**.

No statistically significant difference was found between MMP-9 levels of control group and migraine patients with and without aura in both attack and headache-free periods (p: 0.161, p: 0.194). TIMP-1 levels were significantly lower (p: 0.001, p: 0.001) and MMP-9/TIMP-1 ratio was significantly higher (p: 0.002, p: 0.002) in migraine patients with and without aura in the comparison to control group **(Table 3.)**.

MMP-9 levels of migraine patients without aura was found higher, but it was not statistically significant (p>0.05).

No statistically significant difference was found between the patient groups both during attack and headache-free periods in the comparison of MMP-9, TIMP-1 levels and MMP-9/TIMP-1 ratio (p = 0.542, p = 0.177, p = 0.886, p = 0.739, p = 0.751, p = 0.705).

Evaluation of pain characteristics related to the MMP-9, TIMP-1 levels and MMP-9/TIMP-1 ratio revealed a positive correlation with the duration of pain and serum MMP-9 levels in headache-free period for migraine patients both with and without aura [41.5% and 39%, respectively (p<0.05)]. In the headache period, a statistically significant negative correlation was found as 41.6% between TIMP-1 levels and the duration of disease (p<0.05).

Furthermore, no statistically significant correlation was found between TIMP-1 level and the severity of pain (p>0.05); a positive 43.3% correlation was found between MMP-9 levels and the severity of pain; a 41.8% statistically significant positive correlation was found between MMP-9/TIMP-1 ratio and the severity of pain (p<0.05) in migraine patients with aura during headache period **(Table 4)**.

Gender differences were not included since this was out of the scope of this study.

Our results also showed a slightly positive correlation between MMP-9 levels, MMP-9/TIMP-1 ratio and time of blood sampling during headache

Table 3. *Distribution of parameters in headache and headache-free periods according to study groups*

		Without aura (n=25) Mean±SS	With aura (n=25) Mean±SS	Control* (n=25) Mean±SS	*p*
Headache period	MMP-9	128.12±63.95	103.48±43.72	118.49±12.78	*0.161***
	TIMP-1	355.45±52.41	341.50±40.86	559.20±118.53	*0.001***
	MMP-9/TIMP-1	0.35±0.16	0.31±0.13	0.22±0.04	*0.001***
Headache-free period	MMP-9	119.04±64.66	99.55±34.41	118.49±12.78	*0.194***
	TIMP-1	340.16±36.54	344.60±36.54	559.20±118.5	*0.001***
	MMP-9/TIMP-1	0.36±0.20	0.29±0.11	0.22±0.04	*0.002***

One way ANOVA test was used.
*Blood was drawn one time from the control group since they do not have headache episodes. **p<0.01
Abbreviations: MMP-9: Matrix metalloproteinase-9, n: Number, SD: Standard Deviation, TIMP-1: Tissue inhibitor of metalloproteinase-1

Table 4. *Distribution of parameters in headache and headache-free periods according to study groups*

		Headache period Mean±SS	Headache-free period Mean±SS	*P*
Migraine without aura (n=25)	MMP- 9	128.12±63.95	119.04±64.66	*0.542*
	TIMP- 1	355.45±52.41	340.16±36.54	*0.177*
	MMP -9/TIMP- 1	0.35±0.16	0.36±0.20	*0.886*
Migraine with aura (n=25)	MMP- 9	103.48±43.72	99.55±34.41	*0.739*
	TIMP- 1	341.50±40.86	344.60±36.54	*0.751*
	MMP -9/TIMP- 1	0.31±0.13	0.29±0.11	*0.705*

Paired samples t-test was used.
Abbreviations: MMP-9: Matrix metalloproteinase-9, n: Number, SD: Standard Deviation, TIMP-1: Tissue inhibitor of metalloproteinase-1

attacks **(Figure 1)**. However, this was not statistically significant.

Discussion

MMPs take part in many processes in the brain and BBB, so they have been extensively studied in neurological diseases. These studies pointed out that MMPs disrupt myelin-based protein, resulting in demyelination and therefore can take part in multiple sclerosis pathophysiology and disease progression. Several MMP subtypes including MMPs-2, -3, -7, -9, -12,-13,-28 and more were found to be elevated in different studies. Some leukocytes can release MMP-2 and -9 that open endothelial tight junctions and damage basal membrane anchor proteins and further disrupt BBB integrity[14, 15]. MS studies showed their potential use as biomarkers in disease onset, progress and follow up[16].

Bruno et al. found[17] that levels of plasmin, MMP-2, MMP-9, and MMP-2/MMP-9 proteolytic activity were elevated in the cerebral aneurysmal wall in comparison to normal arteries. Therefore neuroinflammation can take role in formation of aneurysms and can also be a potential target for therapy. MMP-2 and MMP-9 were also found to be elevated in stroke patients and reported to be involved in recovery mechanisims with hazardous consequences in acute phase and restorative effects in poststroke phase[18, 19]. *Zhong* et al. reported that after ischemic stroke higher MMP-9 and also TIMP-1 levels in serum predicted higher mortality and major disability[20, 21].

Neuroinflammatory and neurodegenerative effects of MMPs (especially MMP-2 and -9) are also studied in epilepsy, neurodegenerative dementias, Parkinson's disease and migraine and showed promising associations[14, 22–24]. Higher levels of TIMP-1 in patients with Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis were also demonstrated[25]. Due to their close relationship with the central nervous sytem and its pathologies MMPs and their inhibitors attract rightful attention everday as possible disease markers and/or therapeutic targets[8, 14].

CSD and further activation of the trigemino-vascular system are known to have a role in migraine. Neurogenic inflammation in the lining of blood vessels, which was triggered by vasoactive neuro-

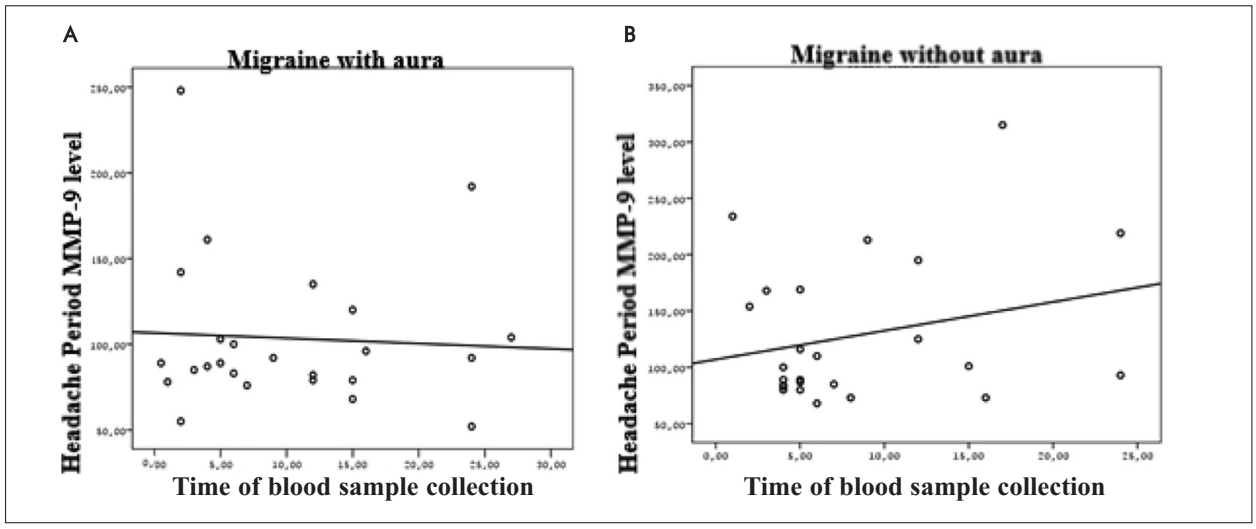

Figure 1. *Relationship with MMP-9 serum levels during headache and time of blood sample collection from patients* **A.** *with migraine with aura and* **B.** *with migraine without aura*

transmitters, released from trigeminal nerve endings that are induced by trigemino-vascular reflex, and disruption of BBB are important steps in the pathogenesis of migraine[26, 27].

In the animal studies investigating the correlation between MMPs and CSD and the BBB, CSD was found to activate brain MMPs and altered the permeability of the BBB, and the MMP inhibitor suppressed this reaction. In addition, this activity and upregulation of CSD was found to start a process: disturbing BBB with an unknown mechanism. Indeed, MMP-9 level in the cerebral cortex ipsilateral to the pain was shown to elevate 3-6 hours after the initiation of CSD and reached its maximum value within 24 hours and was maintained for 48 hours[28, 29].

It has been thought, that CSD activates MMP-9 in addition to the other mediators, causes local gaps in BBB, and changes the concentration of ions and transmitters in extracellular regions in order to generate pain response by affecting dural nociceptors. MMP-9 is found to be especially responsible for lysis of collagen type 4, which is one of the basal membrane components of cerebellar epithelium and responsible for maintaining the integrity of BBB. Several studies showed that these enzymes contribute to development of neurogenic inflammation by affecting the permeability of BBB[30–33].

In a mice study, *Lambert* et al. showed that expression and activity of MMP-2, MMP-3 and MMP-9 caused vasogenic edema through destruction of BBB in an early period and though this primary destruction of BBB was temporary, after 24-48 hours with further MMP-3 and MMP-9 expression and activation main intensive destruction was produced[34].

Several studies, evaluating the relationship between BBB and MMPs during migraine attack found that MMPs cause BBB damage[35]. *Imamura* et al. obtained serum MMP-9 levels of migraine patients with and without aura and tension type headache (TTH) patients, then compared them with serum MMP-9 levels of healthy control group subjects[36]. Serum MMP-9 levels showed no significant difference between patients with tension type headaches and control group. However, serum MMP-9 levels of migraine patients were higher than both TTH patients and the control group. MMP-9 levels were not significantly different between migraine patients either with or without aura. In our study, although there was no statistically significant difference, MMP-9 levels of migraine patients without aura were found to be higher during headache period. This may suggest the presence of silent CSD in migraine without aura. In the light of these findings, MMP-9 can be evaluated as a molecular marker for neurogenic inflammation process and for BBB damage[37].

MMP-9 levels were also found to be higher in headache-free periods in comparison to headache periods, but this difference was not statistically significant. Although it was also not statistically significant, our results showed a slightly positive correlation between MMP-9 levels, MMP-9/TIMP-1 ratio and time of blood sampling during attacks. In several studies, MMP-9 levels were reported to ele-

vate within 3-6 hours after the onset of pain and reached their maximum level in 24 hours. Average time of blood sample collection in our study was 9 hours and this timeline was almost the start point for the MMP-9 elevation. As leaving the patients without medication for 24 hours would not be ethical, this period was used for blood sampling. However, the slightly positive relationship between MMP-9 levels and the time of blood sampling may reflect the MMP-9 activity during attacks.

There was a statistically significant positive correlation between serum MMP-9 levels and the duration of pain in migraine patients either with or without aura in both periods, while this relationship was not detected for TIMP-1. It is known that neuroinflammation process is prolonged as the duration of pain is prolonged, so MMP-9 levels are also expected to elevate respectively. In these periods, MMP-9/TIMP-1 ratio elevated because of the increased levels of MMP-9 with increased pain intensity, but TIMP-1 levels remained almost at the same level. This suggested and supported previous findings that MMP dependent inflammation was increased and TIMP's protective effect was relatively decreased[12].

Our results on duration and severity of the pain show that prevention of neuro-inflammation in early period is crucial. This suggests that just like triptans, which are known to have a role in neuroinflammation, MMP inhibitors may be useful in early periods of headache and may become a treatment option[38].

Some investigators suggest that MMP-9/TIMP-1 ratio reflects the net MMP-9 activity rather than the absolute MMP-9 levels[39]. In their study, *Martins-Oliveria* et al. found increased MMP-9/TIMP-1 ratio in migraine patients without aura and stated that elevation of net MMP-9 activity is important in migraine[23]. As being one of the most important MMP-9 inhibitors, the level of TIMP-1 was found higher in migraine patients with aura, so MMP-9/TIMP-1 rate in these patients was low. High MMP-9/TIMP-1 ratio in patients with migraine without aura, and low MMP-9 / TIMP-1 ratio in patients with migraine with aura suggested different MMP profiles and the difference in their pathophysiology in migraine patients either with or without aura.

Similar to the aforementioned study, we found higher serum MMP-9 levels in migraine patients without aura compared to the control group and lower serum MMP-9 levels in migraine patients with aura compared to the control group in both headache and headache-free periods, though this difference was not statistically significant. This suggests not only that the migraine with aura and without aura are different disorders, but also different MMPs can be responsible for these two disorders.

One study analyzed different haplotypes of MMP-9 gene and revealed that patients with certain haplotypes had higher MMP-9 plasma concentrations. This suggests that migraine pathogenesis is even more complex than we presumed. Genetic background of individuals may have an effect on disease physiopathogenesis and the patients can have different outcomes with the same therapies[24]. Further studies on individualized therapies are needed.

In our study, low serum levels of TIMP-1 in migraine patients with and without aura indicate an impaired anti-proteolytic effect. Similar TIMP-1 levels in both headache and headache-free periods may show a completely disrupted process and also the presence of an underlying genetic mechanism. MMP-9/TIMP-1 ratio, which is thought to reflect the net MMP-9 activity, has been found significantly higher in migraine patients either with or without aura comparing with the control group. This result of our study suggests that the main factor for BBB impairment is due to the increase in net MMP-9 activity depending on the decreased protective effect of TIMP-1.

Conclusions

There are no current established biomarkers for migraine diagnosis and treatment process. However, it is clear that MMP enzymes and their endogenous tissue inhibitors TIMPs have a role in migraine physiopathogenesis. In our study, MMP-9 serum levels in different periods (headache and headache-free) showed that this enzyme could play a role in the pathophysiology of migraine. However, the most interesting point of our study is low levels of TIMP-1, causing increases in MMP-9/TIMP-1 ratio, which suggests that TIMP-1 deficiency in this enzyme complex plays a role especially in headache period and its protective effect is impaired. This might open a new path for research regarding treatment options targeting this enzyme.

CONFLICT OF INTERESTS
Authors declare no conflict of interests.

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