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Cystatin C as a potential biomarker to evaluate migraine

Cistatina C como potencial biomarcador para avaliação de enxaqueca

Turan AKDAĞ[1], Ali Ulvi UCA[2]

ABSTRACT

Background: Migraine is a multifactorial neurovascular syndrome and closely associated to inflammation. Cystatin C (Cys C) is a neuroendocrine polypeptide which also plays a role in inflammation. **Objective:** To investigate the levels of Cys C in migraine patients without aura. **Methods:** A total of 80 participants were included in the study; 40 patients and 40 healthy controls. Serum Cys C levels were investigated by using enzyme-linked immunosorbent assay (ELISA). Statistical analysis were performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc, IL, USA). **Results:** Serum Cys C levels were found as 73.88 ng/mL in the patient group and 24.92 ng/mL in the healthy control group, being significantly higher among patients (p=0.000). Serum Cys C levels were significacntly different across age subgroups among patients (p=0.049), but not among controls. However, visual analog scale (VAS) (p=0.707), disease duration time (p=0.725) and body mass index (p=0.136) were not significantly different between the two groups. **Conclusion:** Our findings demonstrate that high serum Cys C levels are independently associated to migraine without aura. To the best of our knowledge, this is the first study to determine the serum levels of Cys C in patients with migraine. Thus, serum Cys C may be a potential biomarker of migraine.

Keywords: Migraine Disorders; Cystatin C; Headache.

RESUMO

Introdução: A enxaqueca é uma síndrome neurovascular multifatorial e está intimamente associada à inflamação. A cistatina C (Cys C) é um polipeptídeo neuroendócrino que também desempenha papel importante na inflamação. **Objetivo:** Investigar os níveis de Cys C em pacientes com enxaqueca sem aura. **Métodos:** Foram incluídos no estudo 80 participantes; 40 pacientes e 40 controles saudáveis. Os níveis séricos de Cys C foram investigados usando o ensaio de imunoabsorção ligado à enzima (*enzyme-linked immunosorbent assay* – ELISA). A análise estatística foi realizada utilizando o *Statistical Package for the Social Sciences* (SPSS), versão 22.0 (SPSS Inc, IL, EUA). **Resultados:** Em nosso estudo, os níveis séricos de Cys C foram encontrados em 73,88 ng/mL no grupo de pacientes e 24,92 no grupo de controle saudável, sendo os níveis significativamente maiores nos pacientes (p=0,000). Os níveis séricos de Cys C foram significativamente diferentes entre faixas etárias no grupo de pacientes (p=0,049). No entanto, a escala visual analógica (EVA) (p=0,707), o tempo de duração da doença (p=0,725) e o índice de massa corporal (p=0,136) não foram significativamente diferentes entre os dois grupos. **Conclusão:** Nossos achados demonstram que altos níveis séricos de Cys C estão independentemente associados à enxaqueca sem aura. Até onde sabemos, este é o primeiro estudo a determinar os níveis séricos de Cys C em pacientes com enxaqueca e os resultados sugerem que o Cys C sérico pode ser um potencial biomarcador nessa condição clínica.

Palavras-chave: Transtornos de Enxaqueca; Cistatina C; Cefaleia.

Migraine is a primary headache accompanied by neurological, gastrointestinal and autonomic disorders that usually appear as severe, throbbing and unilateral. Migraine is characterized by recurrent headache attacks and affects about 10–20% of the world population[1]. It is well known that migraine attacks have a negative impact on social relationship and productivity of the patient, both within the family and in the workplace. Depending on the costs of treatment and loss of labor, migraine brings economic burden to people and society[2].

As a clinical condition, migraine is diagnosed by the International Headache Society (IHS) diagnostic criteria. Common symptoms of migraine without aura are as follows: pain on one side of the head, throbbing pain, sensitivity

[1]Necmettin Erbakan University, Meram Vocational School, Konya, Turkey.

[2]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Neurology, Konya, Turkey.

Turan AKDAĞ https://orcid.org/0000-0003-3175-6751; Ali Ulvi UCA https://orcid.org/0000-0002-5783-8061

Correspondence: Turan Akdağ; E-mail: turanakdag570@gmail.com

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to light, photophobia, sensitivity to sound, phonophobia, pain or discomfort that is made worse by physical activities[3]. One study concluded that about 64% of migraines are classified as migraine without aura type[4]. Vascular, neurogenic and biochemical theories have been proposed in the pathogenesis of migraine attacks[5]. However, the underlying causes of the pathophysiology of migraine remain unclear. Sensitive and predictive biomarkers in migraine are important for early diagnosis, prognosis and treatment.

New investigations suggest that some peptides and cytokines may play a role in the pathogenesis of migraine[6]. A study performed by Fan et al. revealed that calcitonin gene-related peptide (CGRP) level can differentiate migraine from non-migraine headache[7]. Another study concluded that pentraxin 3 (PTX 3) levels were significantly higher in patients with migraine[8]. As seen from the literature, recent studies have focused on the relation between migraine and new peptides.

Cystatin C (formerly named gamma trace, post gamma-globulin) is a peptide, member of the cystatin superfamily from proteinase inhibitors, with a molecular weight of about 13 kDa and composed of 122 amino acids. It is produced by nearly all human cells and excreted into the bloodstream[9]. As a potent inhibitor of lysosomal proteinases and cysteine proteases, cystatin C (Cys C) modulates inflammatory response, extracellular matrix degradation and phagocytic functions. Also, Cys C is a sensitive indicator for various chronic inflammatory diseases linked to oxidative stress and apoptosis[10]. It has been previously shown that inflammation and oxidative stress play a role in the pathophysiology of migraine[11].

To the best of our knowledge, the present study is the first to investigate the relation between the serum Cys C level and migraine. This study was carried out to measure serum Cys C levels in patients migraine without aura and to determine prognostic value of Cys C in migraine.

METHODS

Eighty participants were included into the study as two groups: 40 patients with migraine without aura and 40 healthy controls, who were admitted to the Neurology department in the period April-November, 2018.

Migraine patients without aura were diagnosed by an experienced neurologist according to the International Headache Society (IHS) diagnostic criteria (3rd edition, beta version). The diagnostic criteria for migraine without aura were as follows: 1. At least five headache attacks lasting 4–72 hours under/without treatment; 2. Headache episodes displaying at least two of the following characteristics: a. Pulsating quality, b. Unilateral location, c. Pain to moderate or severe intensity to prevent daily living activities, d. Increased pain with physical activity; 3. At least one of the following symptoms during pain episodes: a. Phonophobia and photophobia, b. Nausea and/or vomiting (3).

The group of healthy controls was composed by healthy volunteers without any headache complaint or history of migraine. All voluntary participants were aged 20 to 59 years; ages were categorized in 10-year intervals. The exclusion criteria of both groups were hypertension, diabetes, psychiatric diseases, endocrinopathies, anti-inflammatory and steroid drugs or analgesic medication for at least two months before the study. The patients and healthy controls had no metabolic diseases before the study. Informations about participants were obtained from self-reported questionnaires. The visual analogue scale (VAS) was applied to migraine patients without aura; the rating pain intensity ranged from 0 (painless) to 10 (the worst painful)[12]. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m), according to the World Health Organization (WHO)[13].

Blood samples (10 mL) taken from each patient were transferred to biochemistry tubes to determine Cys C levels. The samples were separated after coagulation. Within 20 minutes samples were centrifuged at 3000 rpm for 10 minutes and stored in deep freeze at -80°C until assay. After that, the serum levels of Cys C were measured by enzyme linked immunosorbent assay (ELISA). The present study was approved by the Ethics Committee of Necmettin Erbakan University Medicine Faculty, Turkey and conducted based on the principles of the Declaration of Helsinki. A written informed consent was obtained from each participant prior to the study.

Cystatin C measurement

Concentration of serum Cys C levels was measured by using an ELISA kit (Sunred Biological Technology, China, Human (Cys-C) ELISA Kit:). The minimum detectable concentration (sensitivity) was 0.427 ng/mL; assay range was 0.6–100 ng/mL, and intra-assay variation was <8%. We followed the manufacturer's instructions for all the analyses. The absorbances of the samples were measured at 450 nm and recorded by Absorbance Microtiter Plate Reader (ELx800TM, BIO-TEK instruments, USA).

Statistical analysis

The statistical analyses were performed by Statistical Package for the Social Sciences (SPSS) software package Version 22.0 (SPSS Inc, Chicago, IL, USA). Differences between migraine patients without aura and healthy control group were evaluated with the Mann-Whitney U test. Data were expressed as mean ± standard deviation (X±SD). One-way analysis of variance and the Kruskal-Wallis test were used for age categories, VAS score and disease duration time. Results were considered to be statitically significant at $p<0.05$.

RESULTS

In the study, the mean ages of the 40 patients with migraine without aura and of the 40 healthy controls were 37.47 and 38.93 years, respectively. As seen in Table 1, serum levels of Cys C were 73.88 ng/mL in the migraine without aura group and 24.92 ng/mL in the healthy control group (p=0.000). However, BMI and frequency of migraine attacks were not significantly different between the two groups (p=0.136 and p=0.198, respectively). Cys C levels were significantly different among age subgroups within the migraine group (p=0.049), with no significant difference among healthy controls (p=0.728) (Table 2). As shown in Table 3, no significant differences were found in Cys C levels at VAS score and disease duration (p=0.707 and p=0.725, respectively).

Table 1. Comparison of sociodemographics, Cys C levels and some clinical findings between migraine patients without aura and healthy controls.

Parameter		MWA	Healthy control group	p-value
Age (years), mean		37.47	38.93	-
Sex (n)	Male	10 (25%)	10 (25%)	-
	Female	30 (75%)	30 (75%)	-
Serum Cys C (ng/mL)	Mean	73.88	24.92	0.000*
	n	40	40	
	St. Dev	44.01	29.66	
BMI (kg/m^2)		21.64±0.74	22.08±0.82	0.136
Frequency (per month)		6.025	-	0.198

MWA: migraine patients without aura; *p<0.05.

Table 2. Comparison of the levels of Cys C according to age categories in migraine patients without aura and healthy controls.

Age categories		MWA (Cys C)	Healthy group (Cys C)
20–29	Mean	91.87	3.41
	n	9	8
	St. Dev	35.46	4.43
30–39	Mean	47.86	5.82
	n	15	12
	St. Dev	44.13	5.86
40–49	Mean	87.22	3.10
	n	11	13
	St. Dev	36.61	4.31
50+	Mean	90.22	3.97
	n	5	7
	St. Dev	45.99	7.67
p-value		0.049*	0.728

MWA: migraine patients without aura; *p<0.05.

DISCUSSION

Migraine is a highly prevalent neurological disorder and affects approximately 10–20% of the world's population[14]. While migraine reduces the quality of life of individuals, it leads to economic burden on people and society due to treatment costs. Although the etiology of migraine has yet to be fully understood, the pathogenesis of the disorder is considered to be multifactorial. In this sense, vascular, neurogenic and biochemical events play a role in the pathogenesis of migraine[5]. Among the several hypotheses related to migraine, the leading ones are connected to the activation of the trigeminovascular system (TS), the cortical hyperexcitability and the neuronal and glial interactions[15].

Recent studies show that some peptides and cytokines may play a role in the pathogenesis of migraine and neurogenic inflammation, whereas proinflammatory cytokines are associated to pain[15,16]. Neurogenic inflammation (NI) is a locally induced inflammatory response and characterized by vasodilation, increased vascular permeability, mast cell degranulation, and release of neuropeptides, including SP and calcitonin gene-associated peptide (CGRP). NI also plays an important role in the pathogenesis of many diseases, such as migraine, fibromyalgia, dystonia and multiple chemical susceptibility[17].

Cys C is a nonglycosylated cysteine proteinase inhibitor and consists of 122 amino acids with a single polypeptide chain[18]. It is encoded as the "housekeeping type" Cys 3 (CST3) gene, and produced by all nucleated cells at a constant rate. This protein is freely filtered in the renal glomeruli and reabsorbed and catabolised in the proximal tubules[19]. Besides that, Cys C is expressed in virtually all organs of the body, such as adrenal medulla, pancreas, thyroid gland, adenohypophysis and brain cortical neurons[20]. Investigations imply that Cys C is a typical secretory protein and can be found in high concentrations in cerebrospinal fluid, blood, saliva, and semen. Although the primary structure, physicochemical and immunological properties of Cys C have been determined, their biological roles have not yet been fully understood. However, members of the cysteine proteinase family are involved in many cellular events, such as; catabolism of intracellular proteins, proteolytic degradation of prohormones, collagen metabolism and penetration of malignant cells into tissues[21].

Table 3. Comparison of the levels of Cys C according to VAS score and duration of disease in migraine patients without aura.

		value	df	p-value
VAS score	Cys C	133.333	144	0.707
Duration (MWA)	Cys C	378.778	396	0.725
	N	40		

Several studies concluded that Cys C has a potent regulator role in the inflammatory process, defense against viral and bacterial infections[22]. In a previous study, it has been suggested that Cys C modulates leukocyte chemotaxis and phagocytosis and, therefore, plays a regulatory role in the inflammatory process[23]. Moreover, Bäcklund et al. proposed that Cys C have a protective role in chronic inflammatory disorders, such as rheumatoid arthritis and atherosclerosis[24]. Although Cys C antagonizes TGF-beta signal transformation, it is not an acute phase protein and is affected by cellular damage and immune response[25].

Newly investigations emphasize that the levels of Cys C are increased or decreased in many diseases. Xiao et al. reported that higher serum Cys C levels were observed in cerebral stroke patients compared to healthy controls[26]. Interestingly, Cys C levels are increased in the cerebrospinal fluid of patients with Alzheimer's disease and Creutzfeldt-Jakob disease[27,28].

Our study revealed a remarkable increase in serum CysC levels in migraine patients without aura compared to healthy controls (p=0.000). Zao et al. evaluated the relation between Cys C and cerebral infarction and determined that Cys C level was significantly lower in the cerebral infarction group than in the control one ($p<0.01$)[29]. In a recent study, lower serum levels of Cys C were observed in patients with acromegaly in comparison to controls[30]. One previous study showed no association between BMI and Cys C levels[31]. In our study no significant difference was observed between migraine patients and controls in relation to BMI.

Many studies have been conducted on the inflammatory aspects of migraine and associations between various inflammatory biomarkers and migraine have been noted. The level of C-reactive protein (CRP), an inflammatory biomarker, has been associated to coronary heart disease and stroke previously[32], which was also found to be elevated in migraine patients[33]. In addition, a study emphasized that CRP levels are increased in migraine patients without aura[34]. Interestingly, Shlipak et al. concluded that Cys C levels has a significant linear correlation with CRP[35]. This may be an explanation for the increased Cys C levels in our study. Association between inflammation and migraine has been previously observed and serum levels of fibrinogen and D-dimer have been linked to migraine[36].

Recent investigations suggest that Cys C levels also have a diagnostic value in different diseases and metabolic disorders, such as chronic kidney disease[37], breast cancer[38], obesity[39] and multiple sclerosis[40].

Predicting circulating biomarkers may facilitate the diagnosis of migraine and evaluation of prognosis. To the best of our knowledge, we report the first study to determine the serum levels of Cys C in patients with migraine without aura. However, we acknowledge that our study has some limitations, the main one being the small sample size.

Future studies should address whether Cys C will have a diagnostic value in migraine. Serum levels of Cys C may differ during migraine attacks. In this sense, we suggest that serum Cys C levels during migraine attacks shall be investigated.

In summary, high serum levels of Cys C were found in patients with migraine without aura. Cys C levels could be an useful marker to screen patients with the diagnosis of migraine. The determination of Cys C in migraine patients with aura may provide a diagnostic value.

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