



Evaluation of inflammatory markers in patients with migraine

Migren hastalarında enflamatuvar belirteçlerin değerlendirilmesi

Aysel Tekeşin¹, Abdülkadir Tunç²

Abstract

Aim: The aim of this study was to evaluate erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), neutrophil-to-lymphocyte ratio (NLR), monocyte to HDL-C ratio (MHR), and bilirubin levels in patients with migraine and compare with levels measured in healthy subjects.

Methods: This retrospective case-control study included 136 migraine patients and 80 healthy controls who were admitted to our neurology outpatient clinic or emergency between June 2017 and June 2018. Laboratory examinations, disease characteristics, presence of aura, and the presence of white matter hyperintensities (WMHs) were recorded and demographic features and laboratory data were compared between two groups.

Results: The mean age of the case group was 36.9 ± 10.6 years and the control group was 37 ± 12 years. In the patient group, total bilirubin, direct bilirubin, indirect bilirubin, and lymphocyte values were significantly lower than the control group. NLR, hsCRP, and ESR levels were significantly higher in the patient group than the control group. The patient and control groups did not show a significant difference according to the presence of WMHs. In the group with aura, monocyte and MHR values were higher than those without aura. The hsCRP values in the group with aura were significantly lower than the group without aura.

Conclusion: This study showed that low bilirubin and high NLR, MHR, hsCRP, and ESR values might be potential inflammatory markers in migraine patients. These markers may contribute to our understanding of the pathophysiology of migraine. Since the cost of these tests is cheap and they are widely used, it is possible to believe that they may be useful for protecting migraine patients against long-term comorbidities.

Keywords: Migraine, inflammation, laboratories, biomarker

Öz

Amaç: Bu çalışmanın amacı migren hastalarında eritrosit sedimentasyon hızı (ESR), yüksek duyarlılıklı C-reaktif protein (hsCRP), nötrofil-lenfosit oranı (NLO), monosit-HDL-C oranı (MHO) ve bilirubin düzeylerinin değerlendirilmesi ve sağlıklı bireylerde ölçülen seviyelerle karşılaştırılmasıdır.

Yöntemler: Bu retrospektif vaka-kontrol çalışmasına Haziran 2017-Haziran 2018 tarihleri arasında hastanemiz nöroloji polikliniğine veya acil servisine başvuran 136 migren hastası ve 80 sağlıklı kontrol dahil edildi. Tüm katılımcıların laboratuvar tetkikleri, hastalık özellikleri, aura varlığı ve beyaz cevher lezyonlarının varlığı kaydedildi ve demografik özellikler ve laboratuvar verileri iki grup arasında karşılaştırıldı.

Bulgular: Vaka grubunun yaş ortalaması 36.9 ± 10.6 yıl ve kontrol grubunun 37 ± 12 yıl idi. Hasta ve kontrol grupları arasında yaş ve cinsiyet açısından anlamlı fark saptanmadı. Vaka grubunda total bilirubin, direkt bilirubin, indirekt bilirubin, lenfosit değerleri kontrol grubuna göre anlamlı derecede düşüktü. Hasta grubunda NLO, hsCRP, ESR düzeyleri kontrol grubuna göre anlamlı yüksekti. Vaka ve kontrol grupları beyaz cevher lezyonları varlığı açısından anlamlı farklılık göstermedi. Aurası olan ve olmayan migren hastalarında yaş, cinsiyet, total bilirubin, direkt bilirubin ve indirekt bilirubin değerleri, NLO, ürik asit, lenfosit ve ESR düzeyleri açısından anlamlı fark saptanmadı. Auralı grupta monosit ve MHO değerleri aurasız gruba göre yüksekti. Auralı grupta hsCRP değeri aurasız gruba göre istatistiksel olarak anlamlı düşüktü.

Sonuç: Bu çalışma, migren hastalarında düşük bilirubin ve yüksek NLO, CRP ve ESR değerlerinin potansiyel enflamatuvar marker olabileceğini göstermiştir. Bu markerlar hastalığın patofizyolojisini anlamamıza katkıda bulunabilir. Ayrıca söz konusu testlerin maliyeti ucuz ve yaygın kullanılan tetkikler olduğundan özellikle migren hastalarını olası uzun dönem komorbiditelerden korumak açısından faydalı olabileceği görülmüştür.

Anahtar Kelimeler: Migren, enflamasyon, laboratuvar, biyomarker

¹ Health Sciences University, Istanbul Training and Research Hospital, Clinic of Neurology, Istanbul, Turkey.

² Sakarya University, Sakarya Training and Research Hospital, Clinic of Neurology, Sakarya, Turkey.

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Sorumlu yazar / Corresponding author:

Abdülkadir TUNÇ

Adres/Address: Sakarya University, Sakarya Training and Research Hospital, Clinic of Neurology, Sakarya, Turkey

e-posta: drkadirunc@hotmail.com

Tel/Phone: (+90) 5545144524

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Introduction

Migraine is a common, multifactorial primary headache disorder. It is characterized by recurrent throbbing headache, nausea, and sensitivity to stimulants such as sound, light, and movement [1]. The prevalence ranges from 5% to 20% in the general population [2]. Although the pathophysiology is still debatable, it's thought that some peptides (e.g., substance P, calcitonin gene-related peptide, and neurokinin A) are released from the perivascular trigeminal regions after the activation of the brain tissue; that caused inflammation and dilation of the extraparenchymal vessels [3].

Erythrocyte sedimentation rate (ESR) and high sensitivity C-reactive protein (hsCRP) are the most widely used inflammatory markers in current clinical practice [4, 5]. The neutrophil-to-lymphocyte ratio (NLR) was reported to be novel inflammatory biomarkers in patients with Guillain-Barré syndrome and venous thrombosis [6, 7]. Increased monocyte counts and decreased HDL-cholesterol (HDL-C) levels were also found to be associated with inflammation, and monocyte to HDL-C ratio (MHR) was suggested to be used as a potential inflammatory biomarker [8, 9]. A significant relationship was shown between high bilirubin levels and antiinflammation in various diseases [10].

Therefore, in this study we aimed to evaluate ESR, hsCRP, NLR, MHR, and bilirubin levels in patients with migraine and compare with levels measured in healthy subjects. Secondly, their relationship with the presence of aura and cranial magnetic resonance imaging (MRI) findings were evaluated.

Material and methods

This retrospective case-control study included 136 migraine patients and 80 healthy controls aged between 16 and 66 years, who were admitted to our neurology outpatient clinic or emergency between June 2017 and June 2018.

It was approved by our local ethics committee. An informed written consent was taken from all the participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The inclusion criterion for migraine patients was the diagnosis of migraine according to the International Classification of Headache Disorders III (beta version) (ICHD-III) [11]. Exclusion criterion were as follows: other ICHD-III diagnosis (e.g., tension type headache, cluster headache etc.); pregnancy/puerperium, any systemic diseases, neurological disorders, inflammatory diseases or any other condition that can affect the inflammatory biomarkers, and the patients under medical treatment with any antiinflammatory medications.

The control group was selected among the relatives of the migraine patients, hospital employees, or the general population. Written informed consent was obtained from them for voluntary participation in the study. The inclusion criteria for the control group were as follows: absence of migraine, the maximum frequency of any headache less than 1 episode per month (e.g., tension type headache, or any other type of chronic headache). Exclusion criteria for the healthy controls were the same as those for the migraine patients.

The blood samples were obtained by careful venipuncture during emergency or outpatient clinic admission before medical treatments. Samples for hematological tests were collected in EDTA tubes, and in dry tubes for biochemical tests. The laboratory investigations included serum hemogram, full

biochemistry profiles including HDL, bilirubins, hsCRP, and ESR, which were measured using automated standard laboratory methods. Automated hematology analyzer XE-1200 (Sysmex, Kobe, Japan) was used for complete blood count's measurements. A molecular analyzer (Roche Diagnostics, Mannheim, Germany) was used for the biochemical measurements. Hs-CRP measurement was done using an automatized analyser (Beckman Coulterw IMMAGE) using nephelometric measurement.

Demographic features, laboratory examinations, disease characteristics, presence of aura, brain MRI findings were recorded. A computerized database was used to collect the laboratory data. NLR and MHR ratios were calculated and compared between the patients and healthy subjects.

Statistical Analysis

The data were transferred into the IBM SPSS Statistics 22.0 program and the analyses were completed. Mean, standard deviation, median, lowest- highest, frequency and ratio were included in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov-Smirnov test. Independent sample t test, and Mann-Whitney U test were used in the analysis of quantitative independent data. A chi-square test was applied to analyze the significance of the multiple comparison of relative frequencies among the groups, and Fischer test was used when chi-square test did not meet the conditions. Spearman correlation analysis was used for correlation analysis. The limit for statistical significance was accepted as $p < 0.05$.

Results

The mean age of the case group was 36.9 ± 10.6 years and the control group was 37 ± 12 years. No significant difference was found between the patient and control groups in terms of age and gender. In the patient group, total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB) and lymphocyte values were significantly lower than the control group ($p = 0.001$, $p = 0.005$, $p = 0.001$, and $p = 0.001$, respectively). NLR, hsCRP, and ESR levels were significantly higher in the patient group ($p = 0.001$, $p = 0.001$, and $p = 0.001$, respectively).

The patient and control groups did not show a significant difference according to the presence of white matter hyperintensities (WMHs) ($p = 0.320$) (Table 1). There was no significant difference in terms of age, gender, total bilirubin, direct bilirubin, and indirect bilirubin values, NLR, uric acid, lymphocyte, and ESR levels in patients with and without aura ($p > 0.05$). In group with aura (MwA), monocyte and MHR values were higher than those migraine patients without aura (MwoA) ($p = 0.002$, $p = 0.013$). The hsCRP values in the MwA group were significantly lower than the MwoA group ($p = 0.026$) (Table 2).

Discussion

Our study revealed that inflammation may be connected with the pathogenesis of migraine. ESR, hsCRP, and NLR values were significantly higher and bilirubin, and lymphocyte values were significantly lower in migraine patients compared with the healthy subjects in this study. Monocyte and MHR values were higher in MwA patients.

It has been revealed that migraine pathogenesis is strongly related to the neurogenic inflammation [12]. In the literature, it has also been demonstrated that a significant association is detected between migraine and cardiovascular

Table 1. Evaluation of demographic and laboratory findings in migraine patients and healthy controls.

	Controls (n=80)	Patients(n=136)	P
Age (year) ^β	37±12	36.9±10.6	0.823
Gender [‡]			0.140
Female	70 (87.5)	127 (93.4)	
Male	10 (12.5)	9 (6.6)	
Smoking [‡]			0.453
No	69 (86.3)	112 (82.4)	
Yes	11 (13.8)	24 (17.6)	
Alcohol [‡]			0.531
No	80 (100)	134 (98.5)	
Yes	0 (0)	2 (1.5)	
Presence of WMHs [‡]			0.320
No	73 (91.3)	118 (86.8)	
Yes	7 (8.8)	18 (13.2)	
Lesion count (n) ^β	5.86±5.08	4.39±3.93	0.409
Uric Acid (mg/dL) ^β	3.87±0.79	4.05±1.15	0.151
Total Bilirubin (mg/dL) ^β	0.89±0.36	0.60±0.34	0.001
Direct Bilirubin (mg/dL) ^β	0.16±0.07	0.14±0.09	0.005
Indirect Bilirubin (mg/dL) ^β	0.74±0.31	0.46±0.27	0.001
Neutrophil count (×10 ³ /μl) ^β	4.12±1.30	4.27±1.46	0.697
Lymphocyte count (×10 ³ /μl) ^β	2.66±0.71	2.26±0.67	0.001
Monocyte count (×10 ³ /μl) ^β	0.52±0.16	0.50±0.18	0.252
Neutrophil/Lymphocyte ^β	0.61±0.69	2.16±2.15	0.001
Monocyte/HDL ^β	0.011±0.004	0.01±0.004	0.080
hsCRP (mg/L) ^β	0.34±0.23	0.58±0.41	0.001
HDL(mg/L) ^β	50.5±12.7	53.1±11.6	0.072
ESR (mm/h) ^β	14.0±8.2	20.5±10.2	0.001

[‡]: n (%), ^β: Mean±standard deviation

ESR: Erythrocyte sedimentation rate; HDL: High density lipoprotein; hsCRP: High sensitivity C-reactive protein; WMHs: White matter hyperintensities.

disease, stroke, and atherosclerosis, and a causal link has been put forward between migraine attacks and inflammatory arteriopathy of the brain vessels [13, 14]. Emerging evidences about the migraine pathogenesis showed that several cytokines mainly IL-6, TNF, and IL-10 are involved, wherein sensitization of nerve endings can be promoted by these inflammatory cytokines during recurrent migraine attacks [15, 16]. Moreover, increased CRP values have been demonstrated to be related to migraine, and have been considered to be an inflammatory marker in migraine patients [17]. Consistent with these studies, our study revealed an increased serum concentration of ESR and hsCRP levels in patients with migraine compared with healthy individuals.

Interestingly, lower levels of TB, DB, and IB were found in migraine patients, and a negative correlation between CRP and bilirubin levels was detected in a study of Peng et al [18]. In another earlier trial, serum bilirubin was exhibited an antiinflammatory property and was shown to be stronger than other antioxidants [19]. In support of this, our study observed lower bilirubin levels in migraine patients. An overconsumption of bilirubin by neurogenic inflammation may be responsible from these physiological phenomenon.

The NLR is a simply calculated biomarker from the lymphocyte and neutrophil counts in the venous blood samples. It was investigated in several studies and elevated levels were shown to be important biomarker in coronary artery diseases [20, 21]. The role of MHR in predicting inflammation has been demonstrated by limited number of studies [22, 23]. It has been shown to be an independent predictor of cardiovascular events in chronic renal failure patients and poor prognosis for cardiovascular mortality [9]. Our study showed higher levels of NLR in migraine patients. On the other hand, monocyte and MHR values were significantly higher in MWA patients. In the literature, some inflammatory biomarkers such as high leptin, Soluble urokinase plasminogen activator receptor, apolipoprotein E, and CRP levels were found to be increased in MWA patients supporting the inflammatory hypothesis [24-27]. Further studies

with serum markers and inflammatory cytokines are needed to evaluate the possible relationship.

Table 2. Evaluation of demographic and laboratory findings of the MWA and MwoA patients.

	Aura (-) (n=95)	Aura (+) (n=41)	P
Age(mean±sd)	36.4±10.2	38.1±11.5	0.413
Gender [‡]			0.198
Female	87 (91.6)	40 (97.6)	
Male	8 (8.4)	1 (2.4)	
Smoking [‡]			0.908
No	78 (82.1)	34 (82.9)	
Yes	17 (17.9)	7 (17.1)	
Alcohol [‡]			1.000
No	93 (97.9)	41 (100)	
Yes	2 (2.1)	0 (0)	
Presence of WMHs [‡]			0.356
No	84 (88.4)	34 (82.9)	
Yes	11 (11.6)	7 (17.1)	
Lesion count ^β	4.45±4.13	4.29±3.90	0.963
Right Localisation [‡]	32 (33.7)	16 (39)	
Left localisation [‡]	21 (22.1)	7 (17.1)	0.747
Bilateral localisation [‡]	42 (44.2)	18 (43.9)	
Migraine duration, (year) ^β	7.3±4.8	6.8±6.6	0.161
Attack frequency, (month) ^β	3.6±2.5	4.1±3.4	0.814
Attack duration, (hour) ^β	14.6±15.3	28.0±22.6	0.002
Uric Acid (mg/dL) ^β	4.00±1.04	4.15±1.36	0.699
Total Bilirubin (mg/dL) ^β	0.60±0.34	0.58±0.33	0.368
Direct Bilirubin (mg/dL) ^β	0.15±0.10	0.12±0.06	0.089
Indirect Bilirubin (mg/dL) ^β	0.46±0.27	0.46±0.27	0.608
Neutrophil count (×10 ³ /μl) ^β	4.19±1.52	4.46±1.30	0.111
Lymphocyte count (×10 ³ /μl) ^β	2.22±0.68	2.36±0.62	0.279
Monocyte count (×10 ³ /μl) ^β	0.47±0.14	0.58±0.21	0.002
Neutrophil/Lymphocyte ^β	2.21±2.51	2.03±0.88	0.676
Monocyte/HDL ^β	0.009±0.004	0.012±0.005	0.013
hsCRP (mg/L) ^β	0.63±0.41	0.48±0.40	0.026
HDL (mg/L) ^β	53.0±11.2	53.4±12.7	0.917
ESR (mm/h) ^β	21.6±10.6	18.0±9.0	0.064

[‡]: n (%), ^β: Mean±standard deviation

ESR: Erythrocyte sedimentation rate; HDL: High density lipoprotein; hsCRP: High sensitivity C-reactive protein; WMHs: White matter hyperintensities.

An association between MWA and WMHs was shown in most of the previous studies [28, 29]. Contrary, there was no significant differences in terms of presence and number of WMHs between migraine patients and healthy controls. That might be due to low number of patients in our study.

Our study had some limitations. First, the number of patients were relatively low. Second, the correlation between evaluated serum markers and inflammatory cytokines such as IL-6, IL-10, and TNF were not analyzed. It was unclear whether these biomarkers and systemic inflammation had a causative relationship. Third, body mass index and disease duration were not evaluated, and finally, evaluation of oxidative stress biomarkers would be more precious.

This study showed that low bilirubin and high NLR, MHR, hsCRP, and ESR values might be potential inflammatory markers in migraine patients. These markers may contribute to our understanding of the pathophysiology of migraine. In addition, since the cost of these tests is cheap and they are widely used, it is possible to believe that they may be useful for protecting migraine patients against long-term comorbidities.

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