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EVALUATION OF NEW INFLAMMATORY PARAMETERS IN PATIENTS WITH CHRONIC SPONTANEOUS URTICARIA: A NEW PREDICTOR OF REFRACTORINESS TO ANTIHISTAMINE TREATMENT

Kronik Spontan Ürtikerli Hastalarda Yeni İnflamatuar Parametrelerin Değerlendirilmesi: Antihistaminik Tedaviye Refrakterliğin Yeni Bir Öngördürücüsü

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This study was approved by Sakarya University Education and Research Hospital Ethics Committee under the number 050.01.04-57.

Abstract

Aim: Antihistamines is used as the first-line treatment in chronic spontaneous urticaria (CSU). We investigated whether complet blood count parameters might be used as a response factor in patients under antihistamine treatment for CSU. We also compared the NLR (neutrophil lymphocyte ratio), PLR (platelet lymphocyte ratio, MPV (mean platelet volume) and RDW (erythrocyte distribution width) levels of CSU patients with healthy controls.

Materials and Methods: This study was conducted on a total of 150 cases, a hundred of which had CSU and fifty healthy control group.

Results: NLR and RDW were significantly higher and MPV was significantly lower in the CSU group. NLR and PLR levels were found significantly lower in patients who responded to antihistamine treatment within 4 weeks. The ROC analysis showed that NLR predicted responders with a sensitivity of 92% and with a specificity of 84%, using a cut-off value of 1,98 (p<.001). The multivariate regression analysis showed that only NLR was found as the predictor of responders.

Conclusion: NLR may be considered as a simple and cost-effective tool to determine which patients can respond to antihistamines. It may be a guide for non-responsive patients to switch to other treatment options in the early period.

Keywords: Urticaria, neutrophil to lymphocyte ratio, antihistamines.

Öz

Amaç: Antihistaminikler kronik spontan ürtikerde (KSU) ilk basamak tedavi olarak kullanılır. Çalışmada KSU için antihistaminik tedavi gören hastalarda tam kan sayımı parametrelerinin yanıt faktörü olarak kullanılıp kullanılamayacağını araştırdık. Ayrıca KSU hastalarının NLR (nötrofil lenfosit oranı), PLR (trombosit lenfosit oranı), MPV (ortalama trombosit hacmi) ve RDW (eritrosit dağılım genişliği) düzeylerini sağlıklı kontroller ile karşılaştırdık.

Materyal ve Metot: Bu çalışma, yüz KSU ve elli sağlıklı kontrol grubuna sahip toplam 150 vaka üzerinde gerçekleştirilmiştir.

Bulgular: KSU grubunda NLR ve RDW anlamlı olarak yüksek ve MPV anlamlı olarak düşüktü. Antihistaminik tedaviye 4 hafta içinde yanıt veren hastalarda NLR ve PLR düzeyleri anlamlı olarak düşük bulundu. ROC analizi NLR'nin, 1,98 kesme değeri kullanılarak, yanıt verenleri % 92 hassasiyet ve % 84 özgüllük ile tahmin ettiğini göstermiştir (p <.001). Çok değişkenli regresyon analizi, sadece NLR'nin yanıt öngördürücüsü olduğunu göstermiştir.

Sonuç: NLR hangi hastaların antihistaminiklere yanıt verebileceğini belirlemek için basit ve uygun maliyetli bir araç olarak düşünülebilir. NLR, yanıt vermeyecek hastaların erken dönemde diğer tedavi seçeneklerine geçmeleri için rehberlik edebilir.

Anahtar Kelimeler: Ürtiker, nötrofil / lenfosit oranı, antihistaminikler.

INTRODUCTION

Chronic urticaria (CU) is a common skin disorder defined by recurrent wheals and pruritus of at least a 6-week duration [1]. Chronic spontaneous urticaria (CSU) is the most common type of CU, comprising up to 90% of all cases of CU [2]. CSU usually lasts from 1 to 5 years, but approximately 14% of patients may have a course of more than 5 years [3]. It has been estimated that chronic idiopathic urticaria will affect 0.6 to 5% of people during their lifetime [4,5]. While the pathogenesis of CSU is not completely

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understood, in most patients, histological examination of CSU patients has revealed perivascular infiltration of CD4+ and CD8+ T lymphocytes, eosinophils, basophils, mast cells, and neutrophils [6]. Accordingly, studies have deemed CSU to be associated with an altered immune response related to chronic systemic inflammation [7,8]. In CSU patients, increased IL-6 and C-reactive protein levels have been found to be significantly associated with urticaria severity [9]. The current guideline on urticaria recommends that second generation H1 antihistamines should be used as the first line treatment of CSU. If necessary, the daily dose can be increased to four fold to provide disease control. However, there is no effective method to predict whether an antihistamine will have a beneficial clinical effect or not. A recent study showed that measurement of the histamine-induced wheal can predict which patients will have a strong clinical response to antihistamines but has limited utility for identifying nonresponders [10].

Neutrophil lymphocyte ratio (NLR) is considered as an inflammatory marker, because of its availability and cost-effectiveness. In the recent studies, NLR is said to be a marker of systemic inflammatory condition in many diseases such as atherosclerosis, myocardial infarction (MI), diabetes mellitus, ulcerative colitis, malignancies [11-14]. Activation of coagulation cascade has been reported in patients with CSU, and a close relationship was found between coagulation and inflammation [15,16]. NLR, Platelet-lymphocyteratio (PLR), Erythrocyte distribution width (RDW) have been studied in many dermatological diseases as various parameters indicating inflammation, and found to be associated with disease activity, prognosis and spread of disease [17,18].

The aim of this study was to evaluate the effectiveness of these parameters in patients with chronic urticaria by comparing them with healthy controls, and to evaluate the correlation of these parameters with the severity of the disease (UAS7). We also investigated whether these parameters could be used as a response factor in patients under antihistamine treatment for CSU.

MATERIAL AND METHODS

Study Design and Population

This was a retrospective, observational, single-center study. The current study included a hundred consecutive patients (70 females, 30 males) who were diagnosed with chronic urticaria and followed by dermatology outpatient clinic in Sakarya University Education and Research Hospital, Sakarya, Turkey. Fifty healthy individuals (29 females, 21 males) without any systemic disease was also included in the study as control group. The normal controls had no previous history of inflammatory and allergic diseases or urticaria. In CSU group, all patients were receiving antihistamine treatment for CSU. The patients who had an another inflammatory, autoimmune or active infectious diseases, malignancy, any systemic diseases or pregnancy, history of immunosuppressive medication, and children who were under the age of 18 were excluded from the study. Complete blood count parameters (CBC) [platelet, neutrophil, lymphocyte counts, red cell distribution width (RDW), mean platelet volume (MPV)] and CRP levels were recorded retrospectively. NLR (was calculated as the neutrophil count divided by the lymphocyte count) and PLR (was calculated as the platelet count divided by the lymhocyte count) were obtained from the CBC results. This study was approved by Sakarya University Education and Research Hospital Ethics Committee under the number 050.01.04-57.

Assessment of Urticaria Activity Score

In spontaneous urticaria, disease activity is assessed by a robust and simple scoring system named as Urticaria Activity Score (UAS7). UAS7 is a weekly composite sum of the pruritus and number of hives score, for measuring the disease activity [1]. The resultant UAS7 score is the sum scores of seven consecutive days (0–42), which determines both the disease activity and efficacy of the ongoing interventions of CSU [19]. Currently, two versions of the daily UAS7 exist; one that assesses the number of hives and the intensity of itch twice daily (every 12 h), and one that assesses hive number and itch intensity once daily (every 24 h) [20]. We used once daily version in the present study. The response status of CSU patients was evaluated at 4 weeks. During this period, the antihistamine doses of the patients who did not respond to antihistamine treatment were increased up to the 4th week. The non-responders were defined as patients whose symptoms could not be controlled by increasing doses of antihistamines (up to fourfold), and responders were defined as those who responded well to antihistamine treatment within 4 weeks.

Statistical analysis

For the statistical analysis, the Statistical Package for the Social Sciences (SPSS), version 16.0 for Windows (SPSS Inc., Chicago, IL) was used. Continuous data were expressed as mean ± standard deviation, and the categorical data were expressed as percentages. The normal distribution of the data was assessed by the Kolmogorov–Smirnov test. Comparisons between groups were performed using a chi-square or Fisher's exact tests for qualitative variables, as appropriate. An independent t-test was used for normally distributed continuous variables, and the Mann–Whitney U test was conducted for non-normally distributed continuous variables, as appropriate. A receiver operating characteristic curve (ROC) was plotted in the CSU population to analyse the predictive capacity of NLR and PLR to determine optimal cut-off points for treatment response. In addition, to determine the impact of the factors on response to antihistamines treatment, multivariate logistic regression analysis was performed. The regression model included age, gender, UAS, CRP, PLR, NLR, MPV, and RDW. A P-value of <0.05 was considered significant.

RESULTS

A total of 100 CSU and 50 control patients were included in the present study. The baseline demographics are depicted in Table 1. The gender and age did not differ in patients with CSU (70% women, mean age 37.0±13.0 years), and healthy controls (58% women, mean age 38.8±12.1 years) (p> .05). The mean disease duration in patients with CSU was 28.3±60.4 months. There was no correlation with the disease duration and complete blood count parameters, UAS and CRP in patients with CSU (p> .05).

When CSU patients were compared with healthy group; NLR, RDW were found significantly higher and MPV was significantly lower in CSU group. There was no significant difference in terms of PLR levels between two groups (p> .05). The comparison of RDW, NLR, PLR and MPV values in CSU patients and healthy controls were summarized in Table 2.

The UAS7 values with CBC parameters and CRP showed no significant statistical association in the correlation analysis (p> .05). On the other hand, when the patient group with chronic urticaria was

divided into two subgroups as mild-moderate disease or severe disease and compared in terms of NLR, RDW, PLR, MPV; there was no significant difference between groups according to these parameters (p> .05).

Table 1. Baseline demographics and clinical characteristics of groups.

Variables	CSU (n=100)	Healthy Controls (n=50)	*p-value*
Gender(female), n (%)	70 (70.0)	29(58.0)	.145
Age, mean ± SD (years)	37.0 ± 13.1	38.8 ± 12.1	.428
Disease duration, (mean±SD) (month)	28.3 ± 60.4		
Urticaria severity, n(%)			
Mild-moderate	35 (35.0)		
Severe	65 (65.0)		

Data presented as mean ± standard deviation or number (%).
CSU; chronic spontaneous urticaria.

Table 2. The comparison of RDW, NLR, PLR and MPV values in CSU patients and healthy controls.

Variables	CSU (n=100)	Healthy Controls (n=50)	*p-value*
RDW	16.1 ± 2.09	15.3 ± 2.66	*.045*
NLR	2.20 ± 2.23	1.56 ± 0.41	*.007*
PLR	133.24 ± 116.6	111.34 ± 34.6	.196
MPV	7.78 ± 1.37	8.82 ± 0.95	*<.001*

Data presented as mean ± standard deviation.
CSU, chronic spontaneous urticaria; RDW, red cell distribution width; NLR, Neutrophil (mm3) /Lymphocyte (mm3); PLR, Platelet count / Lymphocyte (/mm3); MPV, mean platelet volüme

The comparison of RDW, NLR, PLR and MPV values in patients who responder and nonresponder to antihistamine treatment at 4 weeks is shown in Table 3. Patients who responded to antihistamine treatment within 4 weeks had significantly lower NLR (p: .002) and PLR (p: .019) levels. Other parameters were not different between these two groups (p>.05). The ROC analysis showed that NLR predicted responders with a sensitivity of 92% and with a specificity of 84%, by using a cut-off value of 1,98. The AUC (area under the curve) for the NLR was found 0.92 (95% CI, 0.86-0.98; p<.001) (Figure 1). Moreover, the ROC analysis revealed that PLR predicted responders with a sensitivity of 84% and with a specificity of 65%, using a cut-off value of 108,9 and the AUC was found 0.78 (95% CI, 0.68-0.87; p<.001) (Figure 2). The multivariate regression analysis (reported as HR [95% CI]), showed that only NLR 1.25 (95% CI, 1.15-1.36) was found as the predictor of responders (p <.001).

Table 3. Comparison of RDW, NLR, PLR and MPV values in responders or nonresponders to antihistamines treatment at 4 weeks.

	CSU Patients (n = 100)	Responders (n = 62)	Nonresponders (n = 38)	*p-value*
RDW	16.1 ± 2.09	15.8 ± 1.64	16.6 ± 2.61	.104
NLR	2.20 ± 2.23	1.52 ± 0.42	3.31 ± 3.31	*.002*
PLR	133.24 ± 116,6	106.25 ± 36.89	177.28 ± 175.85	*.019*
MPV	7.78 ± 1.37	7.79 ± 1.13	7.75 ± 1.72	.889

Data presented as mean ± standard deviation.
CSU, chronic spontaneous urticaria; RDW, red cell distribution width; NLR, Neutrophil / Lymphocyte; PLR, Platelet count / Lymphocyte ; MPV, mean platelet volume.

Figure 1. Sensitivity and specificity of NLR for predicting response to antihistamines.

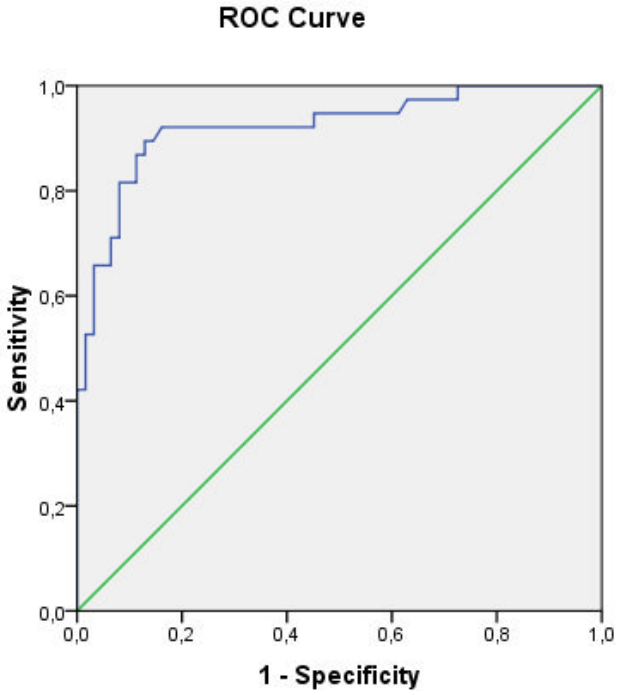
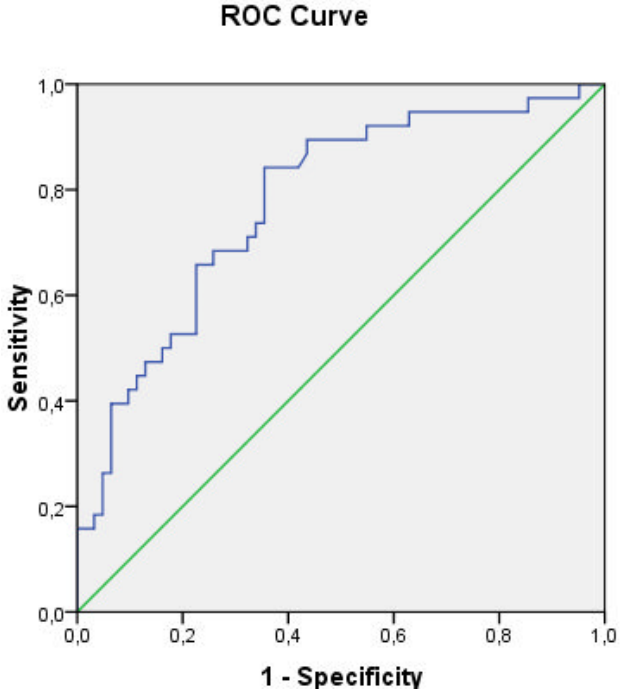


Figure 2. Sensitivity and specificity of PLR for predicting response to antihistamines.



DISCUSSION

The present study consisted of patients admitted to the dermatology outpatient clinic, who used antihistamine treatment, and had a diagnosis of CSU. This study demonstrate that CSU patients had higher NLR, RDW and lower MPV levels than the control group. Moreover, there was no significant correlation between disease activity (UAS7) and CBC parameters. The results also revealed that NLR is a significant indicator for antihistamine treatment response in patients with CSU.

The pathogenesis of CSU is complex and has not been fully clarified. The different mechanisms such as autoimmunity, inflammation and coagulation are thought to be played a role in the pathogenesis of

CSU [16]. The evalution of disease activity and the severity of CSU are important processes in CSU management. The guidelines, for assessing CSU disease activity recommends UAS7, a scoring system based on urticaria symptoms [1]. Although various treatments can be used, second generation H1 antihistamines are used as first-line therapy in the CSU.

NLR calculated by dividing neutrophil count by lymphocyte count, and PLR obtained by dividing platelet count by lymphocyte count. CBC parameters have been evaluated and concluded to be a cheap, easily accessible and reliable parameter and used in inflammatory diseases. NLR and PLR have been studied on rheumatic diseases, inflammatory diseases and coronary diseases and found to be high in almost all cases [21,22]. RDW and MPV have also been considered an inflammatory parameters in many diseases[23].

CBC parameters have been previously studied in different skin disease. Karabay et al found NLR levels higher in CSU patients than in healthy controls, and found positive correlation between the serum CRP and NLR levels. In addition, there was no statistically significant difference in NLR values according to the clinical disease activity as our study [24]. There was no correlation between the serum CRP and NLR levels in our study. Moreover, in the present study, no correlation was observed between the UAS scores and CBC parameters with disease duration, unlike Karabay et al.'s study [24]. Ertas et al showed that patients with severe CSU had higher NLR levels than in healthy controls, and revealed significant decrease in the NLR during omalizumab treatment [25]. In the present study, NLR values were significantly higher in CSU patients compared with healthy controls. Similar to our study, Sarac et al determined NLR and RDW value to be higher in the CSU group and no difference in PLR values between the group of patients with urticaria and the control group [26]. In contrast, the previous reports showed that MPV is increased [25,27] or decreased [28,29] in CSU patients. In our study, MPV was found significantly lower in CSU patients. It has been reported that antihistamines-resistant CSU was characterized by higher MPV [27]. However, it has not been demonstrated in our study. The differences in all these studies may be due to different types of CSU populations, as our study that included patients only on antihistamines treatment.

Ertas et al.showed that MPV and PDW did not increase in the nonresponder group during omalizumab treatment. Our findings also showed that increasing levels of NLR and PLR are linked to the CSU response at week 4 of antihistamines treatment. To our knowledge, in the literature, this result was shown for the first time in this study. The multivariate regression analysis demonstrated that only NLR was found as the predictor of responders. In the present study, NLR predicted responders with a sensitivity of 92% and with a specificity of 84%. Nonresponse to antihistamine treatment may be explained by the presence of more severe inflammation associated with higher NLR levels in these patients. Further more comprehensive prospective studies are needed to support this results.

CONCLUSIONS

We found that NLR levels were higher in CSU patients compared with healthy controls. Accordingly, we propose that high levels of NLR, as a simple, and cost-effective tool, could be a potential predictor of refractoriness to antihistamine treatment in CSU patients. Thus, in patients who are expected to be refractoriness to antihistamine treatment, other treatment alternatives may be used earlier period.

Further studies are needed on the role of NLR, which has proven to be an inflammatory marker, in CSU patients.

Limitations

This study has several limitations. Retrospective design is the main limitation of this study. Moreover, this study is based on a single-center experience and the sample size was relatively small. Furthermore, we did not measure other inflammatory markers including TNF-α, high-sensitivity CRP, interleukins, and chemokines in this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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1. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al: The EAACI/ GA (2) LEN/EDF/WAO guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. Allergy 2014; 69:868-887.
2. Champion RH, Roberts SO, Carpenter RG, Roger JH. Urticaria and angio-oedema. A review of 554 patients. Br J Dermatol. 1969 Aug;81(8):588–97.
3. Toubi E, Kessel A, Avshovich N, Bamberger E, Sabo E, Nusem D, et al. Clinical and laboratory parameters in predicting chronic urticaria duration: a prospective study of 139 patients. Allergy. 2004 Aug;59(8):869–73.
4. Gaig P, Olona M, Muñoz Lejarazu D, Caballero MT, Domínguez FJ, Echechipia S, et al. Epidemiology of urticaria in Spain. J Investig Allergol Clin Immunol. 2004;14(3):214–20.
5. Jiamton S, Swad-Ampiraks P, Kulthanan K, Suthipinittharm P. Urticaria and angioedema in Siriraj medical students. J Med Assoc Thai. 2003 Jan;86(1):74–81.
6. Toubi E, Adir-Shani A, Kessel A, Shmuel Z, Sabo E, Hacham H: Immune aberrations in B and T lymphocytes derived from chronic urticaria patients. J Clin Immunol 2000; 20:371-378.
7. Grzanka A, Machura E, Misiolek M, Polaniak R, Kasperski J, Kasperska-Zajac A: Systemic inflammatory response and calcification markers in patients with long lasting moderate-severe chronic spontaneous urticaria. Eur J Dermatol 2015; 25:26-28.
8. Kasperska-Zajac A: Acute-phase response in chronic urticaria. J Eur Acad Dermatol 2012; 26:665-672.
9. Kasperska-Zajac A, Sztylc J, Machura E, Jop G: Plasma IL-6 concentration correlates with clinical disease activity and serum C-reactive protein concentration in chronic urticaria patients. Clin Exp Allergy 2011; 41:1386-1391.
10. Guillén-Aguinaga S, Jáuregui Presa I, Aguinaga-Ontoso E, Guillén-Grima F, Ferrer M. Updosing nonsedating antihistamines in patients with chronic spontaneous urticaria: a systematic review and meta-analysis. Br J Dermatol. 2016; 175(6):1.153-1.165.
11. Balta S, Celik T, Mikhailidis DP, Ozturk C, Demirkol S, Aparci M, et al. The relation between atherosclerosis and the neutrophil lymphocyte ratio. Clin Appl Thromb Hemost 2016 Jul;22(5):405-11.
12. Sen BB, Rifaioglu EN, Ekiz O, Inan MU, Sen T, Sen N. Neutrophil to lymphocyte ratio as a measure of systemic inflammation in psoriasis. Cutan Ocul Toxicol 2014;33: 223-7.
13. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 2014; 106: dju124.
14. Torun S, Tunc BD, Suvak B, Yildiz H, Tas A, Sayilir A, et al. Assessment of neutrophil-lymphocyte ratio in ulcerative colitis: a promising marker in predicting disease severity. Clin Res Hepatol Gastroenterol 2012; 36: 491-7.
15. Göncü EK, Aktan Ş, Atakan N, Başkan EB, Erdem T, Koca R, et al. The Turkish guideline for the diagnosis and management of urticaria-2016. Turkderm 2016; 50.
16. Asero R, Tedeschi A, Marzano AV, Cugno M. Chronic urticaria: a focus on pathogenesis. F1000Res 2017; 6: 1095.
17. Balkarli A, Kucuk A, Babur H, Erbasan F. Neutrophil/lymphocyte ratio and mean platelet volume in Behcet's disease. Eur Rev Med Pharmacol Sci 2016; 20:3045-50.
18. Ma J, Kuzman J, Ray A, Lawson BO, Khong B, Xuan S, et al. Neutrophil-to-lymphocyte Ratio (NLR) as a predictor for recurrence in patients with stage III melanoma. Sci Rep 2018; 8:4044.
19. Ye YM, Jin HJ, Hwang EK, Nam YH, Kim JH, Shin YS, Park HS: Co-existence of chronic urticaria and metabolic syndrome: clinical implications. Acta Derm Venereol 2013; 93:156-160.
20. Mathias SD, Dreskin SC, Kaplan A, Saini SS, Spector S, Rosen KE. Development of a daily diary for patients with chronic idiopathic urticaria. Ann Allergy Asthma Immunol. 2010;105(2):142–148.
21. Haybar H, Pezeshki SMS, Saki N. Evaluation of complete blood count parameters in cardiovascular diseases: An early indicator of prognosis? Exp Mol Pathol. 2019 Jun 11; 110:104267.
22. Asahina A, Kubo N, Umezawa Y, Honda H, Yanaba K, Nakagawa H. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and mean platelet volume in Japanese patients with psoriasis and psoriatic arthritis: Response to therapy with biologics. J Dermatol. 2017 Oct;44(10):1112-1121

23. Song GG, Lee YH. Red cell distribution width, platelet-to-lymphocyte ratio, and mean platelet volume in ankylosing spondylitis and their correlations with inflammation: A meta-analysis. Mod Rheumatol. 2019 Aug 1:1-6.
24. Karabay EA, Çerman AA, Altunay İK. Serum C-reactive protein, neutrophil-lymphocyte ratio and uric acid levels in chronic spontaneous urticaria. Turkiye Klin J Dermatology 2016; 26: 125-131.
25. Ertaş R, Özyurt K, Karakükçü Ç, Akkuş MR, Özlü E, Avcı A, et al. Evaluation of platelet parameters and neutrophil/lymphocyte ratio during omalizumab treatment in patients with severe chronic spontaneous urticaria. Turk J Med Sci. 2018 Dec 12;48(6):1255-1262.
26. Sarac G, Mantar I, Sener S, Cenk H, Kapicioglu Y. Assessment of change in neutrophil-lymphocyte ratio, platelet-lymphocyteratio in patients with acute and chronic urticaria. Annals of Medical Research. 2018;25(4)719-22.
27. Magen E, Mishal J, Zeldin Y, Schlesinger M. Clinical and laboratory features of antihistamine-resistant chronic idiopathic urticaria. Allergy and Asthma Proceedings. 2011;32(6):460–466.
28. Isiksacan N, Koser M, Cemsitoglu F, Kucuksezer UC, Gurdol F. Platelet and other hemostatic characteristics in patients with chronic urticaria. Angiology.2015 Apr;66(4):387-91.
29. Akelma AZ, Mete E, Cizmeci MN, Kanburoglu MK, Malli DD, Bozkaya D. The role of mean platelet volume as an inflammatory marker in children with chronic spontaneous urticaria. Allergol Immunopathol. 2015 Jan-Feb;43(1):10-3.

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