RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2025.62593
J Ankara Univ Fac Med 2025;78(1):13-19

MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Blood Leukocyte Ratios: Equivocal Parameters for Allergic Diseases

Kan Lökosit Oranları: Alerjik Hastalıklar için Şüpheli Parametreler

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Abstract

Objectives: Leukocytes play crucial roles in allergic and immunologic diseases. We compared neutrophil-lymphocyte ratio (NLR), basophil-lymphocyte ratio (BLR), eosinophil-neutrophil ratio (ENR), and eosinophil-lymphocyte ratio (ELR) in 100 drug-induced urticaria-angioedema, 100 drug/venom-triggered anaphylaxis cases, and 100 healthy controls to assess their association with the severity of the allergic reaction.

Materials and Methods: Retrospective analysis of blood leukocyte counts and ratios was performed.

Results: Median NLR was 1.975 in patients (2.09 in urticaria-angioedema, 1.905 in anaphylaxis) and 1.815 in controls. NLR was significantly higher in patients (p=0.038) and notably elevated in urticaria-angioedema (p=0.008). No significant differences in NLR were found between the urticaria-angioedema and anaphylaxis groups (p=0.09) or between the anaphylaxis and control groups (p=0.342). Other leukocyte ratios showed no significant changes between patient and control groups (p>0.05).

Conclusion: NLR may indicate allergic responses but doesn't have predictive value for reaction type or severity.

Keywords: Anaphylaxis, angioedema, inflammation, leukocyte, urticaria

Öz

Amaç: Lökositler, alerjik ve immünolojik hastalıklarda önemli roller oynar. Nötrofil-lenfosit oranı (NLR), bazofil-lenfosit oranı (BLR), eozinofil-nötrofil oranı (ENR) ve eozinofil-lenfosit oranı (ELR) ölçümlerini, 100 ilaçla tetiklenen ürtiker-anjioödem, 100 ilaç/venom ile tetiklenen anafilaksi olgusu ve 100 sağlıklı kontrol olgusunda karşılaştırarak bu oranların alerjik reaksiyonun şiddeti ile ilişkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Kan lökosit sayıları ve oranlarının retrospektif analizi gerçekleştirildi.

Bulgular: Hastalarda median NLR değeri 1,975 idi (ürtiker-anjioödem grubunda 2,09, anafilaksi grubunda 1,905), kontrol grubunda ise 1,815 olarak belirlendi. NLR, hasta grubunda anlamlı derecede yüksekti (p=0,038) ve özellikle ürtiker-anjioödem grubunda belirgin şekilde yükselmişti (p=0,008). Ürtiker-anjioödem ve anafilaksi grupları arasında (p=0,09) ve anafilaksi ile kontrol grupları arasında (p=0.342) NLR değerlerinde anlamlı fark bulunmadı. Diğer lökosit oranları, hasta ve kontrol grupları arasında anlamlı değişiklikler göstermedi (p>0,05).

Sonuç: NLR, alerjik reaksiyonlar için bir belirteç olabilir, ancak reaksiyon türü veya şiddeti için öngörü değeri bulunmamaktadır.

Anahtar Kelimeler: Anafilaksi, anjioödem, enflamasyon, lökosit, ürtiker

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Received/Geliş Tarihi: 18.01.2024 **Accepted/Kabul Tarihi:** 09.01.2025 **Publication Date/Yayınlanma Tarihi:** 21.03.2025

Cite this article as/Atıf: Ekinci A, Altıner S. Blood leukocyte ratios: equivocal parameters for allergic diseases. J Ankara Univ Fac Med. 2025;78(1):13-19

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Introduction

Urticaria, angioedema, and anaphylaxis share a common pathogenesis but exhibit varying levels of severity within the spectrum of immunoglobulin E (IgE)-mediated type I hypersensitivity (1,2). Anaphylaxis often presents with skin manifestations, including flushing, urticarial plaques, and angioedema affecting the tongue, lips, eyelids, and uvula, progressing rapidly to systemic involvement in cardiovascular, respiratory, gastrointestinal, and neural systems (3).

In individuals sensitized to a specific trigger, agent-specific IgE binds to its receptor on mast cell surfaces (4). Upon re-exposure to the triggering factor, mast cells release a diverse array of mediators (4). Furthermore, these mediators from mast cells contribute to the activation of other leukocytes, such as neutrophils, eosinophils, basophils, and lymphocytes (5).

Several studies have demonstrated variations in leukocyte ratios across various conditions, including inflammatory, allergic, infectious, malignant, and cardiovascular diseases, compared to healthy individuals. It has been emphasized that these ratios serve as useful parameters for predicting disease presence, severity, or prognosis (6-8).

In this study, our aim was to evaluate the neutrophil-lymphocyte ratio (NLR), basophil-lymphocyte ratio (BLR), eosinophil-neutrophil ratio (ENR), and eosinophil-lymphocyte ratio (ELR) in cases of drug-induced urticaria-angioedema and drug- or venom-triggered anaphylaxis admitted to our clinic. We sought to elucidate the potential of these markers in identifying individuals who may exhibit mild reactions to specific triggers via type I hypersensitivity or progress to a more severe form of trigger-induced allergic reaction, namely anaphylaxis.

Materials and Methods

Patient Selection and Ethical Issues

In our retrospective study, we analyzed data from 13,753 patients aged 18 and above who visited our clinic between January 2020 and October 2023. Of these, 1,873 had urticaria and/or angioedema, and 176 had anaphylaxis based on International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-10) during their first visit, confirmed by World Allergy Organization criteria (9). Among those with urticaria and/or angioedema, we identified 482 cases with coinciding drug reactions, encoded with the ICD code of Y57. Excluded were 96 with chronic urticaria-angioedema and 1,295 with urticaria lacking the ICD code of Y57. From cases with possible drug reactions and urticaria and/or angioedema, 382 were excluded due to missing data or exclusion criteria.

Exclusion criteria included additional allergic diseases, immune deficiency, chronic inflammatory diseases, infectious diseases, use of anti-inflammatory or immunomodulator drugs, liver or renal diseases, malignancy, hematological disease, and pregnancy. Cases with documented urticaria and/or angioedema at admission were also excluded.

Seventy-six cases diagnosed with anaphylaxis were excluded due to undetermined triggers or meeting exclusion criteria. In total, 200 patients, comprising 100 drug-related urticaria-angioedema cases and 100 anaphylaxis cases with known triggers, were included in the study.

A study, investigating the predictive effects of BLR and ELR parameters on anaphylaxis risk, was used as a reference for the power analysis of this research (10). The study comparing anaphylaxis, urticaria/angioedema, and a healthy control group by One-Way analysis of variance (ANOVA) estimated an effect size of 0.21, a type 1 error rate of 5%, and a power of 90%. The power analysis determined a total estimated sample size of 291 for the study, with 100 patients allocated to each group to ensure equal distribution.

As healthy controls, 100 age- and sex-matched participants were selected from those admitted to our institution's general internal medicine outpatient clinic between January 2020 and October 2023 for routine health check-ups or regular occupational evaluation, encoded with the ICD code of Z00 for an encounter for a general examination. The flowchart for creating patient groups is presented in Figure 1.

Prior to commencing the research, necessary approval was obtained from the Human Research Ethics Committee of Ankara University (approval number: i09-647-23, date: 02.11.2023). Informed consent has been obtained from the participants. The investigation adhered to the Helsinki Declaration in its conduct (11).

Evaluation of Data

Age, gender, comorbidities, and a detailed history regarding the drug associated with urticaria and/or angioedema or triggering factor associated with the hypersensitivity reaction diagnosed as anaphylaxis were reviewed. The leukocyte counts and leukocyte ratios were obtained from the complete blood count test performed during the routine evaluation of urticaria-angioedema, anaphylaxis, and healthy control participants. The complete blood count test was conducted during outpatient follow-ups of patients in inactive periods where disease symptoms were not present; the aim was to evaluate whether leukocyte ratios can be used to predict the risk and severity of allergic reactions in individuals without symptoms and signs of allergy.

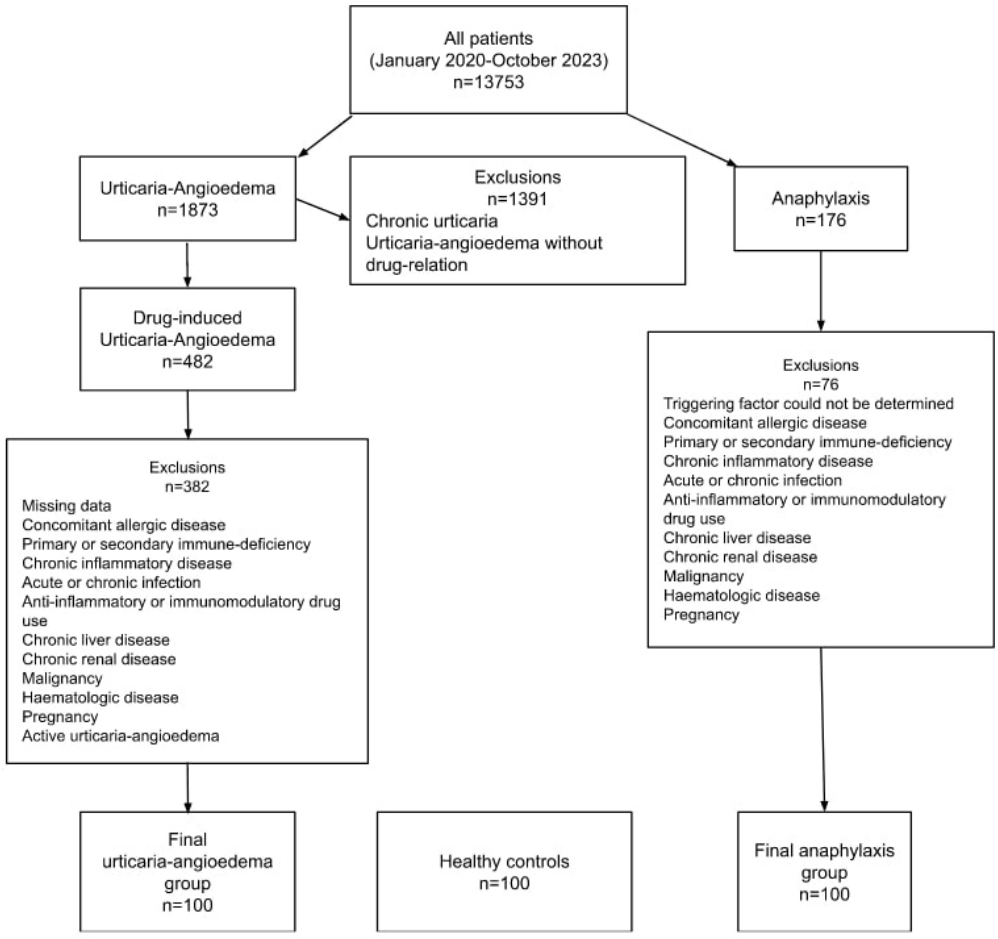

Figure 1: Flowchart for designation of patient and control groups

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 27 software. Descriptive analyses were presented as frequency and percentage for categorical variables and as median (minimum-maximum) or mean ± standard deviation for continuous variables. Independent group comparisons in categorical variables were made using chi-square tests. The suitability of continuous variables to a normal distribution was examined visually (histograms and probability graphics) and using analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Two independent groups were analyzed with the Student's t-test or Mann-Whitney U test, as appropriate. For comparisons of more than two independent groups, Kruskal-Wallis test or One-Way ANOVA was used. The prognostic properties of leukocyte parameters in predicting allergic reactions were examined by receiver operating characteristics (ROC) analysis. In the presence of a significant cut-off value, the sensitivity and specificity of the test were calculated. For statistical significance, the type-1 error level was set at 5%.

Results

The percentages of female and male cases were provided, with 66% females and 34% males for 100 urticaria-angioedema subjects, 61% females and 39% males for 100 anaphylaxis subjects, and 66% females and 34% males for 100 healthy control subjects. Additionally, the mean age and age range for each group were specified, with the mean age of 40.19±13.35 years for urticaria-angioedema, 40.06±12.02 years for anaphylaxis, and 38±11.58 years for healthy control subjects. There was no significant difference between the patient and control groups in terms of gender and age (p=0.738 and p=0.445, respectively).

In the urticaria-angioedema group, the prevalence of comorbidities was as follows: hypertension in 13 cases (13%), diabetes in 12 cases (12%), hypothyroidism in 6 cases (6%), and multiple comorbidities in 12 cases (12%). Similarly, in the anaphylaxis group, the respective numbers were 10 cases (10%) for hypertension, 9 cases (9%) for diabetes, 6 cases (6%) for hypothyroidism, and 11 cases (11%) for multiple comorbidities. Statistical analysis indicated no significant difference in

comorbidity distribution between the urticaria-angioedema and anaphylaxis groups (p=0.506, p=0.489, p=1.00, p=0.825, respectively).

In the anaphylaxis group, the reaction-triggering factors were venom exposure in 54 cases (54%) and medication in 46 cases (46%). Demographic data for the patient and control groups are presented in Table 1.

The absolute neutrophil counts in the patient group were significantly higher than in the control group (p=0.006). Within the patient group, urticaria-angioedema cases exhibited significantly higher absolute neutrophil counts compared to the control group (p=0.004). No significant difference was found in absolute neutrophil counts between the anaphylaxis group and the control group or between the urticaria-angioedema group and the anaphylaxis group (p=0.055 and p=0.338, respectively).

There was a significant difference in the absolute eosinophil counts between the patient group and the control group (p=0.041). Within the patient group, absolute eosinophil counts were significantly higher in anaphylaxis cases compared to the control group (p=0.011). No significant differences were detected in absolute eosinophil counts between the urticaria-angioedema group and the control group or between the urticaria-angioedema group and the anaphylaxis group (p=0.326 and p=0.118, respectively).

On the other hand, there were no significant differences in absolute basophil and lymphocyte counts between the patient and control groups (p=0.138 and p=0.093, respectively). A comparison of the absolute leukocyte counts for the patient and control groups is presented in Table 2.

Table 1: Demographic and clinical parameters of the patient and control groups

Demographic and clinical parameters	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3
Age (years) mean ± SD (range)	40.19±13.35 (18-72)	40.6±12.02 (18-67)	38±11.58 (19-59)	0.380	0.491	0.388
Gender (F/M) (n)	66/34	61/39	66/34	1.000	0.463	0.463
Comorbidities (n)	22	22	-	-	-	1.000
Hypertension (n)	13	10	-	-	-	0.506
Diabetes (n)	12	9	-	-	-	0.489
Hypothyroidism (n)	6	6	-	-	-	1.000
Multiple comorbidities (n)	12	11	-	-	-	0.825
Reaction triggers						
Venom (n)	-	54	-	-	-	-
Penicillin group antibiotic (n)	31	24	-	-	-	0.268
Non-penicillin antibiotic (n)	16	11	-	-	-	0.301
Analgesic (n)	47	36	-	-	-	0.114
Antibiotic and analgesic (n)	11	9	-	-	-	0.637
Other drugs (n)	21	11	-	-	-	0.053

p^1: Comparison between urticaria-angioedema and healthy control groups, p^2: Comparison between anaphylaxis and healthy control groups, p^3: Comparison between urticaria-angioedema and anaphylaxis groups
F: Female, M: Male, SD: Standard deviation

Table 2: Absolute leukocyte counts in patient and control groups

Leukocyte counts (median)	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3	p^4
Neutrophil ($x10^9$/L) (range)	4.445 (2.1-9.37)	4.2 (2.08-8.15)	3.955 (1.89-10.8)	**0.006**	**0.004**	0.055	0.338
Basophil ($x10^9$/L) (range)	0.04 (0-0.11)	0.04 (0-0.1)	0.05 (0.01-0.16)	0.138	0.136	0.296	0.848
Eosinophil ($x10^9$/L) (range)	0.12 (0-0.8)	0.145 (0.01-1.18)	0.12 (0.01-0.58)	**0.041**	0.326	**0.011**	0.118
Lymphocyte ($x10^9$/L) (range)	2.095 (1.06-4.55)	2.22 (1.15-4.46)	2.13 (1.29-4.87)	0.093	0.935	0.213	0.205

Bold value: The absolute neutrophil counts in the patient group were significantly higher than in the control group (p=0.006). Within the patient group, urticaria-angioedema cases exhibited significantly higher absolute neutrophil counts compared to the control group (p=0.004). There was a significant difference in the absolute eosinophil counts between the patient group and the control group (p=0.041). Within the patient group, absolute eosinophil counts were significantly higher in anaphylaxis cases compared to the control group (p=0.011). The bold values represent statistical significance. p^1: comparison between patient and healthy control groups, p^2: comparison between urticaria-angioedema and healthy control groups, p^3: comparison between anaphylaxis and healthy control groups, p^4: comparison between urticaria-angioedema and anaphylaxis groups

Table 3: Leukocyte ratios in patient and control groups

Leukocyte ratios (median)	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3	p^4
NLR (range)	2.09 (0.64-5.03)	1.905 (0.87-4.3)	1.815 (0.5-6.47)	**0.038**	**0.008**	0.342	0.090
BLR (range)	0.018 (0-0.05)	0.017 (0-0.064)	0.021 (0.002-0.096)	0.092	0.676	0.484	0.800
ENR (range)	0.0305 (0-0.143)	0.037 (0.003-0,27)	0.031 (0.001-0.259)	0.511	0.890	0.619	0.524
ELR (range)	0.058 (0-0.519)	0.062 (0.005-0.421)	0.056 (0.005-0.212)	0.093	0.782	0.480	0.656

Bold value: In the patient group, NLR values were significantly higher compared to the control group (p=0.038). Within the patient group, NLR values were significantly higher in urticaria-angioedema cases compared to the control group (p=0.008). The bold values represent statistical significance. p^1: Comparison between patient and healthy control groups, p^2: Comparison between urticaria-angioedema and healthy control groups, p^3: Comparison between anaphylaxis and healthy control groups, p^4: Comparison between urticaria-angioedema and anaphylaxis groups
NLR: Neutrophil-lymphocyte ratio, BLR: Basophil-lymphocyte ratio, ENR: Eosinophil-neutrophil ratio, ELR: Eosinophil-lymphocyte ratio

The NLR median values were 2.09 (0.64-5.03), 1.905 (0.87-4.3), and 1.815 (0.5-6.47) in the urticaria-angioedema, anaphylaxis, and control groups, respectively. In the patient group, NLR values were significantly higher compared to the control group (p=0.038). Specifically, within the patient group, NLR values were significantly higher in urticaria-angioedema cases compared to the control group (p=0.008). No significant difference was found in NLR values between urticaria-angioedema cases and anaphylaxis cases (p=0.09), and no significant difference was detected in NLR values between anaphylaxis cases and the control group (p=0.342).

However, there was no significant difference between the patient and control groups in terms of BLR, ENR, and ELR values (p=0.092, p=0.511, and p=0.093, respectively). A comparison of the leukocyte ratios of patients and control groups is presented in Table 3.

According to ROC analysis, NLR was identified as having a statistically significant but limited predictive feature for the development of allergic reactions, with an area under the curve of 0.573 (95% confidence interval: 0.506-0.641, p=0.038). When the threshold value for NLR was set at 1,885, the sensitivity of the test was 55.5%, and the specificity was 55%. The ROC analysis regarding NLR is visually presented in Figure 2.

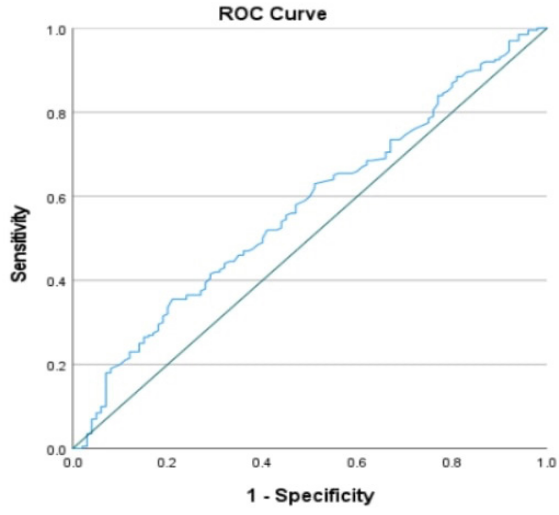

Figure 2: ROC curve of NLR
ROC: Receiver operating characteristics, NLR: Neutrophil-lymphocyte ratio

Discussion

Although urticaria and angioedema are relatively common diseases in the population, the pathogenesis of these diseases has not yet been clearly elucidated (2). Dermal mast cells secrete a wide variety of cytokines, activating various inflammatory cells such as eosinophils, neutrophils, and T lymphocytes, which contribute to disease pathogenesis (2).

In our study, we observed higher NLR values in the patient group compared to the control group; however, subgroup analyses revealed that the difference in NLR values was associated with the urticaria-angioedema group rather than the anaphylaxis group.

Several studies present inconclusive results regarding the association between NLR and various type I hypersensitivity disorders.

In a meta-analysis conducted on the relationship between NLR and asthma, NLR was found to be significantly higher in asthma cases compared to healthy controls, and it was also found to be higher in cases with asthma exacerbations compared to stable asthma cases (12). However, in a different study, no significant difference was found in NLR between asthma cases and healthy controls. Researchers attributed this discrepancy to the fact that the asthma cases included in the study had not experienced a recent attack (13).

It has been stated that in cases of allergic rhinitis, NLR was determined to be higher compared to the healthy control group, and it was correlated with the severity of the disease (14,15). However, in a different study conducted on allergic rhinitis, NLR was found to be lower in allergic rhinitis cases compared to healthy controls. Additionally, it was found to

be lower in persistent allergic rhinitis cases compared with intermittent allergic rhinitis cases (16). This difference was interpreted as indicating that the NLR could be considered as a fragile parameter since the NLR value may vary depending on additional clinical conditions, including infectious diseases that might be present in the examined patient groups that may coexist with allergic rhinitis (16).

To the best of our knowledge, our study is the first to evaluate blood leukocyte parameters in drug-related acute urticaria-angioedema cases and anaphylaxis cases with known trigger factors by comparing them with the healthy control group. However, there is also a study in which blood leukocyte rates were evaluated in anaphylaxis cases and chronic spontaneous urticaria cases by comparing them with the healthy control group, and another study evaluated NLR in anaphylaxis cases that were and were not resistant to epinephrine treatment (10,17). In the first of these studies, no significant difference was found in NLR values between anaphylaxis, chronic spontaneous urticaria, and healthy control groups. However, BLR and ELR values were found to be significantly higher in anaphylaxis cases compared with chronic spontaneous urticaria and healthy control groups (10). In the second study, NLR was found to be significantly lower in anaphylaxis cases that were resistant to epinephrine treatment compared to non-resistant anaphylaxis cases (17). However, we detected no significant relationships regarding BLR, ENR, and ELR parameters in our study.

There are several possible reasons for the differences between the data we obtained from our study and the data obtained in other studies in the literature. The presence of confounding factors that may cause subclinical inflammation, and that could not be evaluated in the participants included in our retrospectively designed study may have affected our results. Another possibility is that, while the urticaria-angioedema group in our study consisted only of drug-triggered patients, the anaphylaxis group included cases triggered by venom as well. Different triggering factors may have affected the absolute leukocyte counts and leukocyte ratios to a different extent. Last but not least, although the normal value ranges for BLR, ENR, and ELR are not defined in the literature, the normal NLR value range in healthy adult individuals has been reported as 0.78-3.53 (18). In our study, the NLR median values and the median values of absolute leukocyte counts in the patient and control groups were within the normal range defined in the literature. Thus, despite the findings obtained in our study, NLR values may not actually hold prognostic significance.

In our study, ROC analysis suggests that NLR has a statistically significant but relatively modest predictive capability for identifying individuals with allergic reactions .

Study Limitations

The main limitations of our study include being a retrospective study conducted in a single center and the relatively small number of cases included. Additionally, the lack of classification regarding the severity of the clinical picture, other than the diagnosis of urticaria-angioedema and anaphylaxis cases, is one of the limiting factors in our study. Another limitation of the present study is the lack of data regarding urticaria activity score at the time of blood sampling. It is possible to obtain more enlightening results with the help of further prospective and multicenter prospective studies.

Conclusion

There is contradictory data regarding blood leukocyte parameters in various allergic diseases in the literature. In our study, although the NLR value was higher in the patient group with a history of allergic reactions compared to the healthy control group, it was determined that this difference was not predictive of the severity of the reaction, or progression to anaphylaxis. Identifying individuals at high risk of developing type-I hypersensitivity against culprit agents is a complex task, often requiring a multi-faceted approach involving various biomarkers and clinical parameters.

Ethics

Ethics Committee Approval: Prior to commencing the research, necessary approval was obtained from the Human Research Ethics Committee of Ankara University (approval number: i09-647-23, date: 02.11.2023).

Informed Consent: Informed consent has been obtained from the participants. The investigation adhered to the Helsinki Declaration in its conduct.

Acknowledgments

This study was presented as a poster presentation in XXIX. National Allergy and Clinical Immunology Congress (XXIX. Ulusal Allerji ve Klinik İmmünoloji Kongresi), 29.11.2023-03.12.2023, Antalya, Türkiye.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.E., S.A., Concept: A.E., S.A., Design: A.E., S.A., Data Collection and/or Processing: A.E., Analysis and/or Interpretation: A.E., S.A., Literature Search: A.E., Writing: A.E., S.A.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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