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The Evaluation of Neutrophil-to-Lymphocyte Ratio as a Novel Marker in Patients with Pterygium

Pterjiumlu Hastalarda Bir Belirteç Olarak Nötrofil/Lenfosit Oranının Değerlendirilmesi

Cemile ÜÇGÜL ATILGAN[a], Pınar KÖSEKAHYA[a], Kemal TEKİN[a], Selam Yekta ŞENDÜL[c], Mehtap ÇAĞLAYAN[b], Yasin Şakir GÖKER[a], Mustafa KOÇ[a]

[a]Ankara Ulucanlar Eye Training and Research Hospital, Clinic of Ophthalmology, Ankara, TURKEY
[b]Şişli Hamidiye Etfal Training and Research Hospital, Clinic of Ophthalmology, İstanbul, TURKEY
[c]Gazi Yaşargil Training and Research Hospital, Clinic of Ophthalmology, Diyarbakır, TURKEY

ABSTRACT Objective: To evaluate the neutrophil-to-lymphocyte ratio (NLR) as an indicator of inflammation in patients with pterygium and to compare data with those of healthy subjects. **Material and Methods:** Two hundred patients who underwent pterygium surgery (study group) and 200 age and sex matched subjects who underwent strabismus surgery in our clinic (control group) were included in this study. Patients with any ocular and systemic infection history and previously undergoing surgery for pterygia and recurrent pterygia were excluded from the study. Neutrophil and lymphocyte counts of all participants were recorded from all blood samples taken before surgery retrospectively. The NLR was calculated by dividing the neutrophil counts by the lymphocyte counts and compared between groups. Normality of data was analyzed with the Kolmogorov-Smirnov test. An independent t-test was used to compare the variables between the study and control groups. **Results:** The mean age was 52.61±13.35 and 51.86±14.42 years in the study and control groups, respectively (p=0.58). The neutrophil count was 4.18±1.28 in study group and 4.17±1.37 in control group. The lymphocyte count was 2.11±0.55 in study group and 2.17±0.72 in control group. There was no significant difference between groups in terms of neutrophil, lymphocyte counts and NLR (p=0.91, p=0.36, p=0.54 respectively). **Conclusion:** In our study, the NLR seems not to be an independent predictor of pterygium disease.

Keywords: Biomarkers; lymphocyte, neutrophil; pterygium

ÖZET Amaç: Pterjiumlu hastalarda inflamasyonun bir belirteci olarak Nötrofil-Lenfosit oranını değerlendirmek ve sağlıklı bireylerin nötrofil-lenfosit oranlarıyla karşılaştırmak. **Gereç ve Yöntemler:** Kliniğimizde primer pterjium cerrahisi geçiren 200 hasta (çalışma grubu) ile yaş ve cinsiyet açısından çalışma grubu ile uyumlu şaşılık cerrahisi geçiren 200 hasta (kontrol grubu) çalışmaya dahil edildi. Herhangi bir oküler ve sistemik enfeksiyonu olan, daha önce pterjium cerrahisi geçiren ya da nüks pterjiumu olan hastalar çalışma dışı bırakıldı. Bütün katılımcıların nötrofil ve lenfosit değerleri cerrahi öncesi alınan rutin kan örneklerinden retrospektif olarak kaydedildi. Nötrofil-lenfosit oranı, nötrofil sayısının lenfosit sayısına bölünmesi ile hesaplandı. Tüm değerler çalışma ve kontrol grubu arasında kıyaslandı. Çalışmada hastaların dağılımının normal olup olmadığının değerlendirilmesi için Kolmogorov-Smirnov testi, gruplar arası değişkenleri değerlendirmek için ise bağımsız t-test kullanıldı. **Bulgular:** Ortalama yaş, çalışma ve kontrol grubunda sırasıyla 52,61±13,35 ve 51,86±14,42 yıldı (p=0,58). Nötrofil sayısı çalışma grubunda 4,18±1,28 ve kontrol grubunda 4,17±1,37 idi. Lenfosit sayısı ise çalışma grubunda 2,11±0,55 ve kontrol grubunda 2,17±0,72 olarak saptandı. Nötrofil, lenfosit sayısı ve nötrofil-lenfosit oranı açısından çalışma ve kontrol grubu arasında anlamlı fark yoktu (sırasıyla p=0,91, p=0,36, ve p=0,54). **Sonuç:** Çalışmamızda, nötrofil-lenfosit oranının pterjium hastalığının bağımsız bir belirteci olmadığı gösterilmiştir.

Anahtar Kelimeler: Belirteç; lenfosit; nötrofil; pterjium

Pterygium has been described by Duke-Elder as a triangular-shaped degenerative and hyperplastic ocular surface lesion that advances gradually and extends from the conjunctiva to the cornea limitlessly.[1] It is thought that the most important cause of the development of pterygium is the destruction in limbal stem cells due to chronic ultra violet (UV) exposure for a long time.[2,3] Focal limbal damage due to UV radiation triggers migration-altered limbal stem cells towards the cornea. Although it is a common disease

Correspondence: Cemile ÜÇGÜL ATILGAN
Ankara Ulucanlar Eye Training and Research Hospital, Clinic of Ophthalmology, Ankara, TURKEY/TÜRKİYE
E-mail: cemileucgul@gmail.com

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in the world, that it occurs mostly within peri-equatorial latitudes supports the exposure of chronic UV light as an important factor triggering the development of pterygium.[4] Although pterygium was defined more as a degenerative disease previously, nowadays it is also regarded as a proliferative, inflammatory process together with angiogenesis and tissue remodeling. Pterygium tissue is frequently clinically inflammated and proinflammatory cytokines such as interleukin (IL)1, IL6, IL8, tumor necrosis factor alpha (TNF-alpha) and growth factors such as epidermal growth factor (EGF), transforming growth factor (TGF), platelet derived growth factor (PDGF) and matrix metalloproteinase (MMP) such as MMP-1, MMP-3 due to cumulative UV radiation are contributory to extensive cellular proliferation, angiogenesis, connective tissue remodeling and inflammation.[5] In a study by Chui et al., the histopathologic features of pterygium were classified as common, uncommon and atypical.[6] They evaluated intravascular, subepithelial and intraepithelial leukocyte infiltrations as common histopathologic features. As shown in the previous studies, they demonstrated that these leukocyte infiltrations are composed of neutrophils, T cells, plasma cells, macrophages and mast cells.[7,8]

As known, neutrophils are the active components of inflammation while lymphocytes are its regulatory and protective components.[9] Recently, neutrophil-to-lymphocyte ratio (NLR), which is calculated by dividing the neutrophil count to lymphocyte count in a peripheral blood sample, has been frequently encountered as a simple and inexpensive marker that can be used in the evaluation of inflammatory responses of the various systemic diseases such as diabetes, Alzheimer disease, lung cancer and colorectal cancer.[10] Although ocular diseases often cause local inflammation compared to a systemic inflammatory response, the NLR was investigated in various ocular disorders like age-related macular degeneration; retinal vein occlusion; non-arteritic anterior ischemic optic neuropathy; keratoconus; vernal keratoconjonctivitis; dry eye and primary open-angle glaucoma.[11-17] NLR should change systemic blood parameters to be an important factor in local inflammatory diseases. Although pterygium is thought to have originated from the UV-altered limbal stem cells, recently there have also appeared some hypotheses related to the origin of bone marrow pregnitor cells (BM-PC).[18,19] Lee et al. claimed that there is evidence that suggests mobilization of bone marrow-derived endothelial cells (BM-EC) in pterygium.[20] The fact that BM-PCs are important in the pathogenesis of the pterygium convinced us that even if it is a local inflammatory disease, it can also change systemic blood parameters.

We have hypothesized that high pre-operative NLR will guide us in the evaluation of the pterygium tissue before surgery, in determining the surgical option such as pterygium surgery with autograft and/or mitomycin-C and in preferring local anti-inflammatory drugs such as cyclosporine in post-operative follow-up period.

So, in this study, we aimed to evaluate NLR values in patients with pterygium as a marker of inflammatory response and compared data with those of healthy subjects.

MATERIAL AND METHODS

This retrospective study was conducted by reviewing the medical records of 200 patients with pterygium (study group) and 200 age-matched patients who under went strabismus surgery in our clinic (control group).

Following the approval by the Non-Drug Clinical Research Ethics Advisory Committee of the Ankara NumuneTraining and Research Hospital, the patients were requested to sign a standard informed consent form. (ethics committee approval number dated January 25, 2017 was 1121). The study was carried out in accordance with the Declaration of Helsinki. Patients with active infection, diabetes mellitus, systemic hypertension, cardiovascular disease, hematological malignancy or disorder, autoimmune disease, steroid use; any ophthalmic disease such as glaucoma, keratoconus, optic neuropathy; previously undergoing surgery for pterygia; and recurrent pterygia were excluded from the study. Blood samples were taken from the antecubital vein before surgeries and drawn into dipotassium ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Blood samples were measured with an automated blood cell

counter (Beckman Coulter, Fullerton, California, USA) within 2 hours after blood collection. Levels of neutrophil and lymphocyte were recorded. NLR, which is the count of neutrophil divided by the count of lymphocyte, was calculated for each participant.

STATISTICAL ANALYSIS

Statistical analysis was performed with Statistical Package for the Social Science software version 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Kolmogorov-Smirnov test was used for evaluating the normality of data. Descriptive statistics were performed as mean ± standard deviation (SD) and an independent t-test was used to compare variables between the study and control groups. A p value of less than 0.05 was considered statistically significant.

RESULTS

The mean age was 52.61±13.35 and 51.86±14.42 years in the study and control groups, respectively (p=0.58). As shown in Table 1, the mean values of neutrophil and lymphocyte count were similar between the groups. The mean NLR was 2.1±0.89 in the study group and 2.05±0.80 in the control group. There was no significant difference between groups in terms of NLR (p=0.542) (Table 1).

DISCUSSION

Pterygium is a common ocular surface disease affecting only humans and it is characterized by proliferation, inflammation, neovascularization, extracelluler matrix remodeling linked to proinflammatory cytokines such as IL1, IL6, IL8, TNF-alpha, growth factors such as EGF, TGF, PDGF, and matrix metalloproteinases such as MMP-1, MMP-3.[5,21] Major cause in the pathogenesis of pterygium is the chronic UV exposure.[2,3] Oxidative stress may play a role in the pathogenesis of pterygium.[22] Moreover, UV-induced nuclear factor-KB (NF-KB) may induce the secretion of IL-1, IL-6, TNF-alpha, VEGF from skin and IL-1, IL-6, TNF-alpha from corneal epithelial cells and contribute to development of pterygium.[23,24] Hereditary predisposition is also thought to contribute to the development of pterygium.[25] As the cornea is a tissue with its own stem cells, it is believed that pterygium originates from UV-altered limbal stem cells due to its ability to regenerate ocular surface damage. Coroneo et al. suggested two-stage hypothesis for the pathogenesis of pterygia: 1) The initial deterioration of the limbal corneal-conjunctival barrier. 2) The conjunctivalization of the cornea.[26] Actually the exact pathogenesis of pterygium is still unknown. Of all the topics in the pathogenesis of pterygium, the cell origins and the nature of initial trigger take the lead in terms of their controversial aspect. Although limbal stem cells have a major role in the development of pterygium, there is increasing evidence that bone marrow-stem cells (BM-SCs) and BM-PCs are responsible for the pathogenesis of pterygium.[18,19] BM-SCs undergo an intermediate phase before their differentiation. The cells in this intermediate stage are termed "precursor or progenitor cells". Progenitor cells in the adult tissue are semi-differentiated and can divide into mature cells. For example, myeloid and lymphoid progenitor cells are derived from hematopoietic stem cells. In circulation, while lymphoid progenitor cells turn into natural killers, T and B lymphocytes, myeloid progenitor cells turn into neutrophils, basophils, eosinophils, monocytes, platelets and red blood cells.[27] In a study conducted by Lee et al. , the authors found that endothelial progenitor cells (EPCs), which are important in vasculogenesis, were higher in both circulation and pterygium tissue and have also been found to increase their chemotactic factors in both tear and circulation.[20] Moreover, they determined that EPCs and their chemoattractants were higher in the recurrent pterygium than in the primary pterygium. They also thought that hypoxia, which was detected by anterior segment flourescein angiography, was a triggering cause of EPCs.

As in most other eye diseases, pterygium is a local inflammatory and degenerative disease rather

TABLE 1: Complete blood count characteristics of all participants.

	Study group	Control group	p value*
Neutrophils, 10^9/L	4.18±1.28	4.17±1.37	0.919
Lymphocytes, 10^9/L	2.11±0.55	2.17±0.72	0.366
NLR	2.10±0.89	2.05±0.80	0.542

*Independent samples t test. NLR: Neutrophile-to-Lymphocyte ratio.

than systemic disease. Among the potential causes of inflammation and sublethal hypoxia, which consecutively prompt proinflammatory signals to bone marrow, are the environmental factors, such as UV, heat and wind. Again in the study by Lee et al., increased leucocyte and erythrocyte sedimentation rate levels in the primary and recurrent pterygium groups supported chronic inflammation in the ocular surface of pterygium patients.[20]

Platelets, lymphocytes and neutrophils are the three main parameters of complete blood count (CBC). It was shown in a variety of former studies that thrombocytosis, peripheral lymphopenia and neutrophilia reflect the whole inflammatory status of the body well.[28,29] NLR is simply obtained from CBC measurements by dividing the count of neutrophils by the count of lymphocytes at no extra cost unlike other inflammation biomarkers such as TNF-alpha, IL-6, IL-1 alpha. As it is more stable and less likely to be influenced by physiological and physical factors, it has been found to be more beneficial than the other white blood cell parameters.

The use of NLR as an indicator and prognostic marker for many systemic inflammatory diseases such as coronary artery disease, myocardial infarction, diabetes mellitus and cancer has led to the idea that NLR can be used as a prognostic factor in inflammatory-related eye diseases. The fact that NLR was found high in the patients with age-related macular degeneration, diabetic retinopathy, keratoconus, dry eye, glaucoma, non-arteritic anterior ischemic optic neuropathy, retinal vein occlusion and recurrent optic neuritis supports that NLR can be used as a prognostic marker even in the ophthalmological diseases, which have a localized balance with little systemic involvement.[11-17] Although the above-mentioned studies have found that the NLR is high in many eye diseases, there is still no clinical use in ophthalmologic practice as far as we know, because it is a more recently defined entity.

The fact that BM-PCs are involved in the pathogenesis of pterygium indicates that pterygium originates from more general cellular response rather than local cellular response. In parallel to our belief that peripheric blood parameters might change in patients with pterygium, we hypothesized this study, but we did not find significantly high NLR values in our patients. However, we evaluated the patients with stage-2 and stage-3 pterygium tissue without a history of long-term use of local anti-inflammatory drugs.

One of the limitation of our study was that we did not stage the pterygium tissue. The other was that we did not evaluate the other CBC values except neutrophils and lymphocytes.

CONCLUSION

Even if we found that, on contrary to our expectation, NLR was not an independent predictor of pterygium in this study, this issue must be researched further, especially in patients with primary and recurrent pterygium by staging pterygium tissue together with preoperative and postoperative CBC values.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Cemile Üçgül Atılgan, Pınar Köşekahya;* ***Design:*** *Mehtap Savar Çağlayan, Yasin Şakir Göker;* ***Control/Supervision:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Kemal Tekin;* ***Data Collection and/or Processing:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Yasin Şakir Göker;* ***Analysis and/or Interpretation:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Kemal Tekin, Mustafa Koç;* ***Literature Review:*** *Selam Yekta Şendül, Mehtap Savar Çağlayan;* ***Writing the Article:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Kemal Tekin;* ***Critical Review:*** *Mustafa Koç, Mehtap Savar Çağlayan;* ***References and Fundings:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Kemal Tekin;* ***Materials:*** *Cemile Üçgül Atılgan, Pınar Kösekahya, Kemal Tekin, Yasin Şakir Göker.*

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