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Comparison of Neutrophil Lymphocyte Ratio in Benign, Premalignant and Malignant Laryngeal Lesions

Benign, Premalign ve Malign Laringeal Lezyonlarda Nötrofil Lenfosit Oranının Karşılaştırılması

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ABSTRACT Objective: Benign (BG), premalignant (PG) and malignant (MG) lesions can be seen in the laryngeal mucosa. Histopathological examination is absolutely necessary to differentiate these lesions. The ability to differentiate these lesions with inexpensive, easily applicable biomarkers before biopsy can contribute to the planning of the patient's treatment. The aim of this study is to examine whether neutrophil lymphocyte ratio (NLR) can be used as a biomarker to differentiate MG laryngeal lesions from BG laryngeal lesion and PG laryngeal lesion. **Material and Methods:** This retrospective study was performed on 72 patients who underwent biopsy to determine their histopathological diagnosis for laryngeal lesions. We analyzed the patient files for clinical, histopathological and laboratory data. The patients were divided into three groups as BG, PG and MG groups according to their histopathological findings. Groups were compared in terms of NLR and other laboratory data. **Results:** The mean NLR was 2.22±0.61, 2.57±0.8, 3.14±0.91 in the BG, PG and MG, respectively (p=0.001). NLR was higher in MG than BG (p<0.001). NLR values between BG-PG and PG-MG were similar. **Conclusion:** NLR is higher in MG laryngeal lesions than BG laryngeal lesions. However, NLR is not different from BG and MG lesions in PG laryngeal lesions.

Keywords: Laryngeal carcinoma; benign laryngeal lesions; premalignant laryngeal lesions; neutrophil lymphocyte ratio

ÖZET Amaç: Laringeal mukozada benign (BG), premalign (PG) ve malign (MG) lezyonlar görülebilir. Bu lezyonların ayrımında mutlaka histopatolojik inceleme gereklidir. Biyopsi öncesinde bu lezyonların ucuz, kolay uygulanabilir biyobelirteçlerle ayırt edilebilmesi, hastanın tedavisinin planlanmasına katkıda bulunabilir. Bu çalışmanın amacı, nötrofil lenfosit oranının (NLO) MG laringeal lezyonları, BG laringeal lezyonlardan ve PG laringeal lezyonlardan ayırt etmek için bir biyobelirteç olarak kullanılıp kullanılamayacağını incelemektir. **Gereç ve Yöntemler:** Bu retrospektif çalışma, larenks lezyonlarının histopatolojik tanısını belirlemek için biyopsi yapılan 72 hasta üzerinde gerçekleştirildi. Hasta dosyaları klinik, histopatolojik ve laboratuvar verileri için analiz edildi. Hastalar histopatolojik bulgularına göre BG, PG ve MG olarak 3 gruba ayrıldı. Gruplar NLO ve diğer laboratuvar verileri açısından karşılaştırıldı. **Bulgular:** Ortalama NLO, BG, PG ve MG'de sırasıyla 2,22±0,61, 2,57±0,8, 3,14±0,91 idi (p=0,001). MG'de NLO, BG'den daha yüksekti (p<0,001). BG-PG ve PG-MG arasındaki NLO değerleri benzerdi. **Sonuç:** NLO, MG laringeal lezyonlarda BG laringeal lezyonlardan daha yüksektir. Ancak NLO, PG laringeal lezyonlarda BG ve MG lezyonlardan farklı değildir.

Anahtar Kelimeler: Larinks kanseri; benign larinks lezyonları; premalign larinks lezyonları; nötrofil lenfosit oranı

Benign (BG), premalignant (PG) and malignant (MG) lesions can be seen in the laryngeal mucosa as a result of exposure to physical, chemical and biological agents. BG lesions are nodules, polyps or cysts, often caused by excessive use of the voice or vocal cord trauma.[1] Laryngeal MG lesions are most frequently seen due to tobacco use. The most common histopathological type is squamous cell carcinoma and it is the most common tumor in the head and neck region. According to the data of the United States of America for 2017, there were 13,360 new cases and 3,660 deaths due to laryngeal cancer.[2,3]

While MG transformation is not observed in BG lesions of the larynx, the development of laryngeal

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carcinoma occurs as a result of a series of cellular morphological changes. The process that starts with squamous hyperplasia is followed by the development of mild/moderate dysplasia, then severe dysplasia/carcinoma *in situ* and finally it is completed with invasive carcinoma.[4]

Although endoscopic imaging methods have an important place in the definition of laryngeal lesions, histopathological examination is absolutely necessary for definitive diagnosis and distinction. Narrow band laryngeal endoscopy is more successful in predicting the characteristic of the lesion than classical endoscopic examination performed under white light.[5] However, for clinics that do not have this technological infrastructure, there is still a need for inexpensive, easy-to-access biomarkers that can provide an idea to the clinician and predict the behavior of the lesion.

It is known that inflammation plays an important role in the formation and proliferation of cancer cells. Chronic inflammation prevents apoptosis, increases angiogenesis, and can lead to cancer development by causing DNA damage.[6] Neutrophils and lymphocytes, which are among the most important cells of chronic inflammation, are the first and regulatory cells of inflammation. The neutrophil lymphocyte ratio (NLR) calculated by a simple mathematical operation (neutrophil count/lymphocyte count) is used to determine the prognosis and severity of inflammation.[7] Although it has been shown in previous studies that high NLR is associated with poor prognosis, tumor recurrence, metastasis ability, and death, the utility of NLR in laryngeal tumors in the differentiation of BG-MG, PG-MG or BG-PG is not clear.[8-11]

The present study aimed to show whether the NLR values in BG, PG and MG tumors of the larynx are statistically different.

MATERIAL AND METHODS

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, after obtaining approval from the Ankara City Hospital No. 1 Clinical Research Ethics Committee (Date: 31.03.2021, Number: 1703). Because the study was retrospective, informed consent was not obtained.

The records of all patients who underwent direct laryngoscopy for laryngeal lesions between March 2019 and March 2021 were retrospectively reviewed for clinical, laboratory and histopathological data. Demographic characteristics, histopathological diagnosis, pre-treatment neutrophil count, white blood cell count, lymphocyte count and NLR (neutrophil count/lymphocyte count) of each patient were analyzed. According to the histopathological diagnoses of the patients, 3 groups were formed as BG, PG and MG. Patients with autoimmune disease, presence of a known BG or MG tumor anywhere other than the larynx, presence of acute or chronic infection, hematological and endocrinological disorders, history of hormone replacement therapy including corticosteroids, and chronic renal failure were excluded from the study. All laboratory examinations were performed within 1 week before the biopsy procedure. An automatic blood cell counter (Siemens ADVIA 2120i hematology analyzer with auto slide, Erlangen, Germany) was used for complete blood count (CBC). CBC analyzes were performed within a few hours after blood sampling. The groups were compared for leukocyte, neutrophil, lymphocyte counts and NLR.

STATISTICAL ANALYSIS

All statistical analyses were performed using the SPSS 21.0 software package for Windows (SPSS inc., Chicago, IL, USA). Mean and standard deviation values for variables were examined. Groups were analyzed by Kolmogorov-Smirnov or Shapiro-Wilk test to determine whether the distribution was normal. Kruskal-Wallis test was used for abnormally distributed data and $p<0.05$ was considered to be significant statistically.

RESULTS

There were a total of 72 patients, 58 (80.6%) women and 14 (19.4%) men who met the inclusion criteria. The mean age of all patients was 55.4±11.4 years. According to the histopathological diagnosis of the lesion in the larynx, 22 patients were in BG, 20 patients were in PG, and 30 patients were in MG (Table 1).

Of the 22 patients in the BG, 3 were diagnosed with Reinke's edema, 11 with vocal polyp, 4 with

vocal cord nodules, and 4 with chronic inflammation. Twelve of 20 patients in the PG had mild-moderate dysplasia, 8 of them severe dysplasia/carcinoma *in situ*; 30 patients in the MG were diagnosed with squamous cell carcinoma (Table 1).

The mean leukocyte count was 6.88±2 10^3/U, 7.32±1.56 10^3/U, 7.17±1.5 10^3/U in the BG, PG and MG, respectively. The difference was not statistically significant. The mean neutrophil count was 4.28±1.07 10^3/U, 4.37±1.35 10^3/U, 4.74±0.97 10^3/U in the BG, PG and MG, respectively. The difference was not statistically significant. Mean lymphocyte count was 1.98±0.45 10^3/U, 1.8±0.57 10^3/U, 1.59±0.46 10^3/U in the BG, PG and MG, respectively (p=0.016). It was observed that the number of lymphocytes was significantly decreased in MG compared to BG (p=0.012) (Table 2).

The mean NLR was 2.22±0.61, 2.57±0.8, 3.14±0.91 in the BG, PG and MG, respectively (p=0.001). NLR was higher in MG than BG (p<0.001). NLR values between BG-PG and PG-MG were similar (Table 2, Figure 1).

DISCUSSION

BG, PG and MG lesions can be seen in the larynx. Today, histopathological examination is still required for the definitive diagnosis of these lesions. The availability of easy-to-apply and inexpensive markers to predict the behavior of laryngeal lesions before biopsy can enable earlier planning of the patient's treatment process, which may help improve the survival of patients with MG lesions.

The effect of inflammation on cancer development and prognosis is now well known. A number of chemoattractants secreted from tumor cells attract a variety of leukocytes, including neutrophils, dendritic cells, macrophages, eosinophils, and lymphocytes, into the tumor microenvironment.[12,13] The fact that these neutrophils migrating to the tumor microenvironment have a short half-life of 7-10 hours may suggest that they may not have an effect on tumor progression. However, studies in recent years have shown that the increase in the number of tumor-associated neutrophils correlates with poor prognosis.[14-17]

TABLE 1: The descriptive characteristics of the groups.

		BG (n=22)	PG (n=20)	MG (n=30)
Histopathological diagnosis		Reinke's edema (3) (14%) Vocal polyp (11) (50%) Vocal cord nodule (4) (18%) Chronic inflammation (4) (18%)	Mild/moderete dysplasia (12) (60%) Severe dysplasia/carsinoma in situ (8) (40%)	Squamous cell carcinoma (30) (100%)
Gender	Female	(1) (4%)	(4) (20%)	(9) (30%)
	Male	(21) (96%)	(16) (80%)	(21) (70%)
Age*		48.6±12.8	59.4±10	57.7±9.3

*Mean±standard deviation; BG: Benign group; PG: Premalignant group; MG: Malignant group.

TABLE 2: The comparison of the leukocyte, neutrophil, lymphocyte count and neutrophil lymphocyte ratio between the groups.

	BG	PG	MG	P*	P1**	P2**	P3**
Leukocyte (10^3/U)***	6.88±2	7.32±1.56	7.17±1.5	0.92	0.81	0.92	0.98
Neutrophil (10^3/U)***	4.28±1.07	4.37±1.35	4.74±0.97	0.18	0.99	0.31	0.64
Lymphocyte (10^3/U)***	1.98±0.45	1.8±0.57	1.59±0.46	0.016	0.62	0.012	0.44
NLR***	2.22±0.61	2.57±0.8	3.14±0.91	0.001	0.34	<0.001	0.07

*Kruskal-Wallis test; **Post hoc Tamhane test; ***Mean±standard deviation; P1: BG vs PG; P2: BG vs MG; P3: PG vs MG; BG: Benign group; PG: Premalignant group; MG: Malignant group; NLR: Neutrphil lymphocyte ratio.

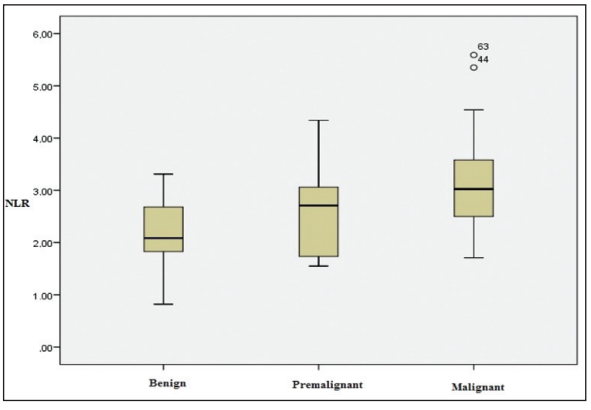

FIGURE 1: Mean neutrophil-to-lymphocyte ratio values in benign, premalignant and malignant laryngeal lesions. NLR: Neutrphil lymphocyte ratio.

In another study, it has been shown that the anti-tumoral activities of lymphocytes and natural killer cells can be inhibited by neutrophils and this is correlated with neutrophil count.[18] However, the present study could not find a relationship between BG, PG and MG lesions of the larynx in terms of neutrophil count.

A healthy immune response needs healthy lymphocytes. They are absolutely essential for the cellular anti-tumoral immunity of the host. In comparison with neutrophils, lymphocyte infiltration into the tumor microenvironment is associated with a good prognosis because lymphocytes protect the host by preventing the proliferation of tumor cells.[19] The present study showed that the lymphocyte count is significantly reduced in MG laryngeal tumors compared to BG laryngeal tumors.

NLR is an inexpensive, easily measurable indicator of inflammation that does not require any extra cost other than CBC. High NLR caused by an increase in neutrophils and/or a decrease in lymphocytes is considered a weakening of the anti-tumor activity of the body. In the past, the negative effect of high NLR on the prognosis of head and neck cancers has been demonstrated by meta-analysis.[20,21] However, there are a few studies with different results evaluating the usability of NLR for the differentiation of BG, PG and MG in laryngeal lesions.[10,11,22-25]

In the present study, it was found that NLR in MG laryngeal lesions was statistically higher than BG laryngeal lesions. However, in PG lesions, NLR was not different from BG and MG groups. In a similar study by Eskiizmir et al., the authors found no difference between NLR values in BG, PG and MG lesions. However, they reported that the prognosis worsens as the NLR increases in patients with MG tumors.[23] Yılmaz et al. in their study, in which they did not include patients with PG laryngeal lesions, encountered higher NLR values in patients with MG laryngeal lesions than in patients with BG laryngeal lesions.[26] Düzlü et al. showed that patients with MG laryngeal tumors had higher NLR values compared to the control group in their study, which included patients who underwent septorhinoplasty as a control group and did not include patients with PG laryngeal lesions.[24]

Kum et al. conducted the first study designed to demonstrate the usability of NLR in the differentiation of BG, PG and MG laryngeal lesions. In their study, pre-treatment NLR values of 80 patients with BG laryngeal lesions, 63 patients with PG laryngeal lesions and 66 patients with MG laryngeal lesions were compared. At the end of the study, the researchers found higher NLR values in the MG group compared to the BG group and in the PG group compared to the BG group, but they did not find a difference between the PG group and the MG group.[11] Bulgurcu et al. did not include patients with BG laryngeal lesions in their study, they only compared pre-treatment NLR values in patients with PG and MG laryngeal lesions. Researchers reported higher NLR in MG group compared to PG group unlike our study.[10] In another study by Kara et al., groups were divided into the BG, PG, T1 MG and gross tumor (T2, T3, T4) and pre-treatment NLR values were compared between groups. While patients with gross tumor had higher NLR values compared to the other groups, there was no difference between BG, PG and T1 MG groups.[25]

The most important limitation of our study may be the small sample size. However, the fact that some different results were obtained in the studies mentioned above with larger patient groups may alleviate this handicap.

CONCLUSION

In conclusion, while NLR can differentiate MG laryngeal lesions from BG laryngeal lesions, it cannot differentiate PG-MG and BG-PG laryngeal lesions according to our study. More studies and meta-analyses are needed in the future in order for the clinical use of NLR to become widespread.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

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: *Aykut İkincioğulları, Mustafa Çolak;* ***Design:*** *Hacı Hüseyin Dere;* ***Control/Supervision:*** *Hacı Hüseyin Dere;* ***Data Collection and/or Processing:*** *Ali Rıza Yağmur, Şeyda Akbal Çufalı;* ***Analysis and/or Interpretation:*** *Nagihan Gülhan Yaşar;* ***Literature Review:*** *Mustafa Çolak, Nagihan Gülhan Yaşar;* ***Writing the Article:*** *Mustafa Çolak;* ***Critical Review:*** *Ali Rıza Yağmur;* ***References and Fundings:*** *Şeyda Akbal Çufalı;* ***Materials:*** *Mustafa Çolak.*

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