

1 The Implication of the Preoperative Neutrophil to Lymphocyte Ratio in the Diagnosis of 2 Laryngeal Squamous Cell Carcinoma: a Retrospective Analysis

3 **ABSTRACT:**

4 **Aims:** There is growing evidence that the neutrophil to lymphocyte ratio (NLR) may be used as a prognostic
5 factor in many head and neck cancer types. We aimed to investigate the diagnostic implication of the NLR as a
6 biological marker in predicting the malignancy of laryngeal lesions.

7 **Study Design:** This is a retrospective study.

8 **Methodology:** The medical records of the patients who underwent an operation for a laryngeal pathology
9 between September 2010 and January 2015 were retrospectively reviewed. For the control group, 50 age and sex
10 matched patients were selected among the patients who underwent septoplasty. Preoperative neutrophil to
11 lymphocyte ratio was calculated for each subject in the malignant, benign and control groups.

12 **Results:** Comparison of the three groups yielded a statistically significant difference between the groups
13 ($p < 0.05$, $p = 0.025$). Comparison of the malignant group with the healthy control group showed that the median
14 NLR was higher in the malignant group ($p < 0.05$, $p = 0.011$).

15 **Conclusions:** Preoperative NLR was significantly elevated in laryngeal cancer patients in our study. We are of
16 the opinion that, this cheap and easily accessible hematological parameter may have a potential role in predicting
17 the malignancy of a space occupying laryngeal lesion. Further clinical studies should be conducted to clarify this
18 association.

19 **Keywords:** laryngeal carcinoma; neutrophil to lymphocyte ratio; differential diagnosis; hematological
20 parameters; predictive role.

21 **1. Introduction:**

22 Neutrophils are considered to be the first line of defense against various infections and they constitute
23 50-70 % of all polymorphonuclear leukocytes in the human circulation [1]. Despite their well-established role in
24 the host defense system against microorganisms, relatively little is known regarding the functions of neutrophils
25 in cancers involving humans [2]. There appears to be an increased level of circulating neutrophils in patients
26 with advanced stage cancers probably due to secretion of certain cytokines by the tumor tissue. These cytokines,

among others, GM-CSF, G-CSF, IL-1, IL-6 and vascular endothelial growth factor were found to be responsible for the presence of neutrophilia in the bloodstream [3-5].

However, neutrophils were proposed to demonstrate conflicting functions in cancer and reported to have both anti-tumor and pro-tumor subpopulations. Neutrophils infiltrating the tumor were found to release various mediators that increase, rather than decrease, tumor angiogenesis and malignant cell intravasation [1,6,7]. Furthermore, it was pointed out that neutrophils from head and neck squamous cell carcinoma patients were different from their counterparts in the healthy subjects both in terms of functionality and cell counts [8,9].

There is growing evidence that the neutrophil to lymphocyte ratio (NLR), a routinely available biological marker of systemic inflammation, may be used as a prognostic factor in many head and neck cancer types. Increased NLR was shown to be an indicator of poor prognosis in oropharyngeal carcinoma, nasopharyngeal carcinoma, and thyroid carcinoma patients [8,10,11]. Elevated NLR was also proposed to be a useful inflammatory marker to differentiate lung cancer patients from healthy control patients in a recent study [12].

Literature review revealed that, there are only a few studies regarding the role of NLR as either a predictive marker for prognosis or a diagnosis of malignancy in patients with a laryngeal pathology. To the best of our knowledge, only in one study, NLRs of the patients with pre-cancerous and malignant lesions were compared with NLRs of the patients with benign laryngeal lesions and NLRs of the former group were found to be significantly higher [13]. Therefore, in this study, we aimed to investigate the diagnostic implication of NLR as a biological marker in predicting the malignancy of laryngeal lesions

2. Methodology:

The medical records of the patients who underwent an operation for a laryngeal pathology between September 2010 and January 2015 in a tertiary referral center were retrospectively reviewed. Patients who had a complete blood count analysis done before the laryngeal biopsy procedure were enrolled into the study. The patients were allocated into two groups according to their definitive pathology report, either benign or malignant. Patients with a history of diabetes mellitus, chronic hypertension, chronic obstructive pulmonary disease and other chronic inflammatory conditions were also excluded. For the control group, 50 age and sex matched patients were selected among the patients who underwent septoplasty within the same period of time.

54 Preoperative white blood cell, neutrophil and lymphocyte counts were investigated and neutrophil to lymphocyte
55 ratio was calculated for each subject.

56 Statistical Package for Social Sciences (SPSS) for Windows version 17 (Illinois, Chicago) was used for
57 statistical analysis. Variables were tested for normality using Kalmogorov-Smirnov test. The descriptive data
58 were presented as median (minimum-maximum). For the comparison of the quantitative data that did not
59 display a normal distribution among 3 groups, Kruskal Wallis test was used. For 2 group comparisons, Mann
60 Whitney U test was used. In all tests, $p < 0.05$ was accepted to be statistically significant.

61 **3. Results and Discussion:**

62 Between September 2010 and January 2015 a total of 118 patients were found to be operated for a
63 laryngeal pathology. 21 patients were excluded from the study due to their co-existing chronic inflammatory
64 conditions. A total of 97 patients were investigated. The patients were divided into two groups according to their
65 histo-pathological diagnosis. 53 patients were found to have laryngeal squamous cell carcinoma and 44 patients
66 were found to have a benign laryngeal lesion (e.g. laryngeal nodule or polyp). The NLRs of the patients in
67 malignant, benign and control groups were calculated. Comparison of the three groups using Kruskal Wallis test
68 yielded a statistically significant difference among the groups ($p < 0.05$, $p = 0.025$) (Table 1).

69 Comparison of the malignant group with the healthy control group showed that the median NLR was
70 higher in the malignant group. The difference was statistically significant ($p < 0.05$, $p = 0.011$). Comparison of the
71 malignant group with the benign group showed that the median NLR was also higher in the former group. This
72 difference, however, did not have a statistical significance ($p < 0.05$, $p = 0.314$). The median NLR was found to be
73 higher in the benign group in comparison to the control group but this difference was not statistically significant
74 ($p = 0.060$, $p > 0.05$).

75 In this study, we aimed to assess the NLRs of the patients with benign or malignant laryngeal lesions
76 and compare them with the NLRs of the healthy control subjects. Our results revealed a statistically significant
77 difference between these three groups. The median NLR was lower in healthy controls than the other groups.
78 The difference between the healthy group and the malignant group was statistically significant. These findings
79 were consistent with the previous studies indicating the relationship between increased NLR and various cancer
80 types in humans [13,14].

81 The implication of the NLR as a biological marker for predicting the survival was extensively
82 investigated in the literature for various types of cancers. The NLR was suggested to be superior to other
83 hematological parameters in predicting the short or long term mortality in breast cancer patients. Individual
84 parameters were proven to be altered by physical, physiological and pathological factors but the NLR was found
85 to be relatively more stable even under the influence of such factors. It was also proposed that the NLR might
86 represent both the inflammatory and immune pathways which are opposing in nature but exist together in cancer
87 patients [15]. An elevated NLR was reported to be an independent factor for poor prognosis in patients with
88 colorectal, lung, and renal cancers [16-18].

89 As far as head and neck malignancies are concerned, the NLR was also found to be increased in patients
90 with certain tumors. A high preoperative NLR was reported to be associated with poor survival in patients with
91 hypopharyngeal squamous cell carcinoma [19]. The NLR was also accepted to be a 'prognosticator' in patients
92 with metastatic nasopharyngeal carcinoma [20]. The association between the NLR and disease-free survival in
93 papillary thyroid carcinoma patients was investigated in one study and they concluded that, elevated NLR could
94 be a negative prognostic factor for disease free survival [21]. In a previous study, Duzlu et al. attempted to
95 identify the potential prognostic role of the NLR in patients with laryngeal cancer and they found that the NLR
96 was increased especially in the presence of regional lymph node metastasis and advanced stage disease [22].

97 Besides its prognostic role in various cancers, the role of the NLR in the diagnosis of many clinical
98 conditions was also extensively studied in the literature [23, 24]. In addition, the implication of the elevated NLR
99 in the diagnosis of malignancy was also investigated by researchers. Elevated NLR was proposed to be a useful
100 marker in diagnosis lung cancer [12]. Literature review revealed that, the diagnostic role of the NLR in laryngeal
101 carcinoma was not extensively investigated. Only in one study by Kum et. al., the NLRs of patients with benign,
102 precancerous and malignant laryngeal lesions were compared and diagnostic value of the NLR in laryngeal
103 lesions were analyzed. It was concluded that, the NLR could be a useful inflammatory marker to differentiate
104 laryngeal squamous cell carcinoma from benign and precancerous laryngeal lesions [13].

105 In the present study, the NLRs of the healthy control subjects were also investigated and compared with
106 NLRs of the patients with either a benign or malignant laryngeal lesion. The findings indicated that the
107 inflammation elicited by either benign or malignant lesions in the laryngeal region could be successfully
108 reflected by increased NLR levels. NLR was increased significantly in cancer patients in comparison to healthy
109 subjects. This increase was in concordance with the previously reported studies pointing out the role of the NLR
110 as a reliable inflammatory or immune marker in human cancer [6, 13, 15].

4. Conclusion:

In conclusion, preoperative NLR was significantly elevated in laryngeal cancer patients in our study. Laryngeal biopsy and imaging modalities are the golden standards in the diagnosis of squamous carcinoma of the larynx. Although it is not possible to suggest it as a substitute for these golden standards, we are of the opinion that, this cheap and easily accessible hematological parameter may have a potential and adjunctive role in the diagnosis of malignant laryngeal lesions. Further clinical studies should be conducted to clarify this association and determine a cut-off value for the NLR.

Ethical statement: All procedures performed in studies involving human participants were in accordance with the 1964 Declaration of Helsinki and the 2013 National Code on Clinical Researches. According to the National Code, for retrieving the data retrospectively, approval of the ethics committee is not necessary.

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Table Legends:

Table 1 Comparison of the groups in terms of the median NLR values

^a: The difference between the three groups in terms of the median NLR was statistically significant

^b: The difference between the malignant group and healthy control group was statistically significant
(p<0.05, p=0.011)

Figure Legends:

Figure 1 The distribution of the NLR values among the groups.

Variable	Groups	Median (min-max)	p value
Neutrophil to Lymphocyte Ratio (NLR)	Malignant	1.9455 (0.62-6.01)	0.025 ^a
	Benign	1.7745 (1.08-3.94)	
	Healthy Control	1.5404 (0.79-6.49) ^b	

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($p < 0.05$, $p = 0.011$)

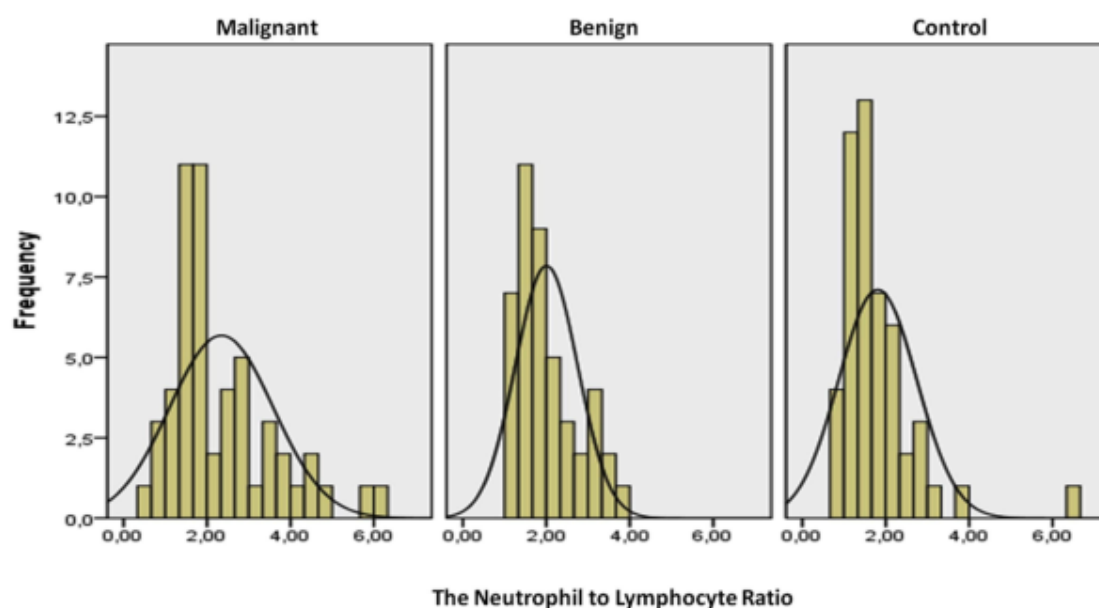


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