CLINICAL STUDY

PRO-GASTRIN RELEASING PEPTIDE AND NEUTROPHIL-LYMPHOCYTE RATIO IN LARYNGEAL CANCER

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SUMMARY

Objective: Pro-gastrin-secreting peptide (pro-GRP) has been identified as a growth factor expressed in many cancer types. The neutrophil-lymphocyte ratio (NLR) has also been used as a biomarker in patients with squamous cell cancer (SCC). The aim of this prospective, controlled study was to investigate the relationship between tumor stage and pro-GRP and NLR levels in patients diagnosed with laryngeal SCC.

Methods: This study has been conducted on a total of 93 people consisting of 31 healthy individuals, and 62 patients (30 patients malign; 32 patients benign) with a laryngeal mass who applied to a tertiary referral center and had surgery. Groups were compared in terms of serum pro-GRP and NLR.

Results: The serum pro-GRP values of the malignant, benign and control groups were 17.31±3.30; 15:51±2.87; 14.07±2.43, while the NLR value was 2.99±1.65; 2.14±0.94; 2.05±0.64. Pro-GRP levels of the malignant group were significantly higher than the benign and control groups (p=0.043; p<0.001), and NLR level was significant (p=0.047; p=0.016). Pro-GRP levels of early (15.92±3.60) and advanced stage (18.53±2.54) tumors were found to be statistically significant (p=0.028), but no statistically significant difference was found between NLR levels (p=0.598).

Conclusion: Serum pro-GRP level can serve as a cheap, repeatable, and easily accessible marker in order to distinguish patients with laryngeal cancer from the bening and control groups. Unlike NLR, pro-GRP values differ between early and advanced stage tumors. In future studies pro-GRP can be used as a biomarker in laryngeal cancer patients.

Keywords: Laryngeal Neoplasms; Gastrin-Releasing Peptide; Serum

LARİNKS KANSERİNDE PRO-GASTRİN SALGILAYAN PEPTİD VE NÖTROFİL-LENFOSİT ORANI

ÖZET

Amaç: Pro-gastrin salgılayan peptit (pro-GRP), birçok kanser türünde eksprese edilen bir büyüme faktörü olarak tanımlanmıştır. Nötrofil-lenfosit oranı (NLR) da skuamöz hücreli kanserli (SCC) hastalarda bir biyobelirteç olarak kullanılmıştır. Bu prospektif, kontrollü çalışmanın amacı, larinks SCC tanısı alan hastalarda tümor evresi ile pro-GRP ve NLR düzeyleri arasındaki ilişkiyi araştırmaktır.

Yöntem ve Gereçler: Çalışmaya merkezimizde larinks tümörü nedeniyle opere edilen 62 hasta (30 hasta malign; 32 hasta benign) ile 31 sağlıklı birey dahil edildi. Gruplar arasında serum pro-GRP ve NLR açısından karşılaştırma yapıldı.

Bulgular: Malign, benign ve kontrol gruplarının serum pro-GRP değerleri sırasıyla 17.31±3.30; 15.51±2.87; 14.07±2.43'iken, NLR değeri sırasıyla 2.99 ± 1.65; 2.14 ± 0.94; 2.05 ± 0.64'idi. Malign grubun pro-GRP düzeyi benign ve kontrol gruplarına göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0.043; p<0.001), NLR düzeyi de anlamlı bulundu (p=0.047; p=0.016). Erken (15.92±3.60) ve ileri evre (18.53±2.54) tümörlü hastaların pro-GRP düzeyleri arasında istatistiksel olarak anlamlı fark bulundu (p=0.028), NLR düzeyleri arasında istatistiksel olarak anlamlı fark bulunmadı (p=0.598).

Sonuç: Serum pro-GRP düzeyi larinks kanserli hastaları benign larinks tümörü olan ve sağlıklı bireylerden ayırmak için kullanılabilecek ucuz, tekrarlanabilir ve kolay erişilebilir bir belirteçtir. NLR değerinden farklı olarak pro-GRP değeri erken ve ileri evre tümörlerde farklılık göstermektedir. Gelecekteki çalışmalarda pro-GRP larinks kanserli hastalarda biyobelirteç olarak kullanılabilir.

Anahtar Sözcükler: Laringeal Neoplazmalar; Gastrin Salgılayan Peptid; Serum

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INTRODUCTION

Approximately 90% of the head and neck cancers are squamous cell carcinoma[1]. Squamous cell carcinoma of the head and neck is the sixth most common cancer worldwide[2]. The most significant risk factors in cancer formation are smoking and alcohol. The five-year-survival rate for primary head and neck squamous cell carcinoma is approximately 60%[1,3]. Although the etiology of head and neck squamous cell carcinoma is not known exactly, in the literature

research studies, the epidermal growth factor (EGF) is associated with initiation and development of head and neck squamous cell carcinoma[4].

Gastrin releasing peptide (GRP) is a neuropeptide. It stimulates the gastrin secretion from G cells in the stomach. It manages the functions of the gastrointestinal and central nervous system. The release of gastrointestinal hormones provides smooth muscle cell concentration and epithelial cell proliferation[5,6]. Pro-GRP is the precursor of the gastrin releasing peptide. It is indicated that pro-GRP plays a role in lung, colon, gastric, pancreatic, breast, esophagus, thyroid, and prostate cancer[7,8]. While GRP receptor (GRPR) overexpresses in head and neck squamous cell cancer patients, it is observed that an increased GRPR level causes decreased survival rate in patients with cancer. It is thought that GRPR level inhibition can contribute to resistance to EGF receptor inhibitor in head and neck squamous cell carcinoma [9,10].

Many studies have shown that there is a correlation with systemic inflammatory response in various tumors. Neutrophil-lymphocyte ratio (NLR) is a biomarker for systemic inflammation. The published data suggest that high preoperative NLR may be associated with increased risk of recurrence, tumor aggression, worse prognosis, metastasis tendency and death in various malignancies[11]. NLR was found to be higher in patients with squamous cell cancer than in patients with healthy and benign larynx mass[11].

For our research, patients that applied to our clinic with laryngeal tumor were classified according to the pathology of tumor (benign, malign) and stage of malign tumor (early, advanced). We investigated whether pro-GRP and NLR are biomarkers in laryngeal cancers and compared these biomarker values with each other. To our knowledge, pro-GRP value in laryngeal cancer patients is the first in the literature.

MATERIAL and METHODS

Local ethical committee approval was received for prospective case control research (E-17-1546). Signed informed consent was obtained from all patients who agreed to participate in the study. Venous blood was drawn from preoperative patients with a mass in their larynx that applied to our clinic. Follow up was done on postoperative pathology results of the patients that were included in the research between November 2017 and May 2018. Patients were divided into groups based on their pathology results. The patients with benign pathology result formed the first group and the ones with malignant pathology results formed the second group. Healthy volunteers who applied to our clinic for check-ups and did not have any disease formed the third group as the control group. Patients with malignant pathology results were staged according to TNM classification in accordance with 2018 NCCN Guidelines. The patients with malignant group were divided into two groups as early (stage 1-2) and advanced (stage 3-4) stage. Routine treatment and examination of the patients were not changed. Voluntary patients aged between 18 and 80 who did not have additional disease, history of permanent medication, active infection, and had not received chemotherapy or radiotherapy were included in the research. Complete blood count and pro-GRP values were determined from venous blood of all individuals included in the research. The statistical analysis of these values was performed between groups.

Laboratory examination

One tube of gel-free BD Vacutainer® (Becton, NJ, USA) blood was drawn, then after two hours, serum pro-GRP was measured, using Chemiluminescent Micro particle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. Firstly, sample, assay diluent and anti-ProGRP coated paramagnetic microparticles were combined. Pro-GRP in the sample bound to the anti-ProGRP-coated microparticles. After washing, anti-ProGRP-acridinium-labeled conjugate was added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions were added to the reaction mixture. A direct relationship exists between the amount of pro-GRP in the sample and the relative light units (RLUs) detected by the Architect System Optics (Abbott, Germany). 1P45 Architect ProGRP Reagent Kit (Abbott, Germany) was used for pro-GRP measurement.

The Architect ProGRP assay is designed to have a functional sensitivity of ≤3pg/mL at a total concentration volume (CV) of 20% (Abbott, Germany).

Statistical analysis

Statistical analyses were performed using commercially available software (SPSS Statistics 21; SPSS Inc, an IBM Company, Chicago, Illinois). The Chi-square test was used to compare the categorical variables. The Fisher's exact test was used when Chi-square test did not meet the conditions. Differences between the groups were analyzed by one-way analysis of variance (for multiple comparisons, the Tukey honestly significant difference [HSD] test was used). P values <.05 were considered statistically significant.

RESULTS

The first group consisted of a total of 32 persons with benign laryngeal tumor, 20 (62.5%) of which were male and 12 (37.5%) of which were female. In the second group there were 30 patients with malignant laryngeal tumor diagnosis and 19(63.3%) of them were male and 11(36.7%) of them were female. There were 31 patients in the third group (control group), and 15 (48.4%) of them were male and 16 (51.6%) of them were female. Average age in malignant, benign, and control groups were 46.67, 41.09 and 40.32 years respectively.

Staging of the tumors in the malignant patient group were as follows: 10 (33.33%) patients had T1, 6 (20%) patients had T2, 8 (26.67%) patients had T3 and 6 (20%) patients had T4 tumor.14 (46.67%) patients had early stage and 16 (53.33%) patients had advanced stage cancer.

Pro-GRP values of malign, benign and control groups were 17.31±3.30; 15.51±2.87; 14.07±2.43 respectively. In dual comparisons pro-GRP was found to be higher in the malignant group than in the benign and control groups ($p=0.043$, $p<0.001$ respectively). No significant difference between the benign and control groups was found in terms of pro-GRP ($p=0.121$) (Table 1).

Neutrophil-lymphocyte ratio values of malignant laryngeal tumor, benign laryngeal tumor, and healthy control group were 2.99±1.65; 2.14±0.94; 2.05±0.64 respectively and NLR level of malignant group was found out to be significantly statistically different compared to the benign and control groups ($p=0.047$; $p=0.016$). No significant difference between the benign and control groups was found in terms of NLR ($p=0.871$)

When patients with malignant laryngeal tumor were evaluated by stage, statistically significant difference was found between patients with early stage tumor (15.92±3.60) and patients with advanced stage tumor (18.53±2.54) in terms of pro-GRP ($p=0.028$).

No statistically significant difference was found between patients with early stage (2.82±1.80) and advanced stage tumor (3.14±1.56) in terms of NLR ($p=0.598$).

Table-I: The Demographic Characteristics and the Variables Studied in Groups

	[1]Malign (n=30) Mean ± SD	**[2]Benign (n=32) Mean ± SD**	**[3]Control (n=31) Mean ± SD**	**P**
Age (years)	46.67±7.71	41.09±12.06	40.32±13.27	0.068
Gender				
Female	11 (36.7%)	12 (37.5%)	15 (48.4%)	0.578
Male	19 (63.3%)	20 (62.5%)	16 (51.6%)	
pro-GRP (pg/mL)	17.31±3.30	15.51±2.87	14.07±2.43	***[1-2]p= 0.043*** ***[1-3]p <0.001*** [2-3]p =0.121
NLR	2.99±1.65	2.14±0.94	2.05±0.64	***[1-2]p= 0.047*** ***[1-3]p= 0.016*** [2-3]p =0.871
WBC (10^3/U)	8.20±2.43	7.98±2.62	7.49±2.59	0.691
Platelet (10^3/U)	243.69±52.59	250.72±56.96	268.61±55.53	0.278
Neutrophil (10^3/U)	5.83±2.29	4.85±2.28	4.71±1.79	0.647
Lymphocyte (10^3/U)	2.06±0.71	2.25±0.61	2.33±0.54	0.496

Malign group	**Early Stage (n=14) Mean ± SD**	**Advanced Stage (n=16) Mean ± SD**	**P**
pro-GRP (pg/mL)	15.92±3.60	18.53±2.54	**0.028****
NLR	2.82±1.80	3.14±1.56	0.598

SD, standard deviation; *Pro-GRP, precursor gastrin releasing peptide; NLR, Neutrophil Lymphocyte Ratio; WBC, white blood cell*

DISCUSSION

Inflammation can be caused by infection, autoimmune diseases, malignant and benign tumors and other pathologies. Thus causing infiltration of inflammatory cells in certain regions of the body. Inflammation is thought to contribute to the development and progression of various cancers and inflammation in the body can be detected in peripheral blood. Recent studies have confirmed that there is a link between the tumor's inflammatory microenvironment and the systemic responses induced by the tumor[11]. The increase in neutrophil count and / or decreased lymphocyte counts may suppress lymphocyte-activated killer

cells. These may be possible mechanisms for reducing the survival rate in cancer patients[12]. NLR is a biomarker that can be used to differentiate patients with laryngeal cancer from non-cancer patients[11]. In our study, NLR value can be used to differentiate laryngeal cancer patients from benign larynx patients and healthy individuals. However, there was no statistically significant difference between the NLR values of advanced and early stage laryngeal cancer patients.

Pro-GRP level stend to increase by age in healthy adult subjects, a bit higher concentration is observed in females compared to males[13]. In our research, no statistically significant difference was observed in terms of age between the control group and the studied group. Therefore, age-related deviation in pro-GRP value is prevented.

High serum pro-GRP levels have been observed because pro-GRP is metabolized mainly by the kidneys, especially in chronic renal failure. Therefore, it is recommended that creatinine determinations are made simultaneously with pro-GRP determinations[14]. While drawing blood for serum pro-GRP, blood was drawn from patients for other examinations too. Other blood tests were examined, and in our research, we included patients with normal renal function test results. This way we aimed to eliminate mistakes that might occur in serum pro-GRP values of patients.

There are three techniques used for measuring pro-GRP in serum: time resolved-immuno fluoro metric assay (TR-IFMA) using Auto DELFIA instrument, chemi luminescence assay CMIA) using Architect analyzer and electro chemi luminescence assay (ECLIA) using cobas analyzer[15]. We used CMIA technique in our research.

Six times higher GRP level has been observed in patients with cerebral metastasis squamous cell lung cancer (SCLC)[14]. Apart from this, high GRP has been observed in 62% of patients with colon cancer, 59% of patients with pancreatic adenocarcinoma, 60% of patients with prostate cancer, 39% of patients with breast cancer, 74% of SCLC patients and 42% of patients with lung cancer[16,17]. In another research study, pro-GRP value of patients with medullary thyroid cancer was studied and it has been reported that pro-GRP increases according to medullary thyroid cancer stage. It has been reported that the pro-GRP value according to medullary thyroid cancer stage increases more than carcinoembryonic antigen but less than calcitonin[8].

In another research study, the diagnostic sensitivity of pro-GRP in SCLC was found out to be 84%. When patients with only SCLC were compared to patients with other lung cancer pathology diagnosis, pro-GRP sensitivity of SCLC patients was reported to be higher. In these research studies, similar diagnostic benefit of pro-GRP for neuroendocrine-specific tumors is indicated. Pro-GRP ratio of large cell neuroendocrine carcinoma (LCNEC) patients with high pro-GRP level is reported to be 36.4%. Pro-GRP ratio of non-small cell neuroendocrine lung cancer patients with high pro-GRP level is reported to be 28.6%.

The diagnostic utility of pro-GRP in patients with SCLC is summarized in a meta-analysis research, depending on the results of research studies presented in twenty-one publications. Diagnostic sensitivity of pro-GRP ranges from 54% to 78%, and diagnostic specificity ranges from 72%to99%[18-20].

However, pro-GRP research, for example, in patients with medullary thyroid cancer (MTC), brings out certain challenges in the literature review. MTC has complicated pro-GRP research because of its low prevalence and early diagnosis difficulties. Despite that, it is indicated that calcitonin value that is used in the follow-up of patients with MTC correlates with pro-GRP value which also can be determined with a fast and solid analytical method[8].

The relationship between GRP and treatment of tyrosine kinase inhibitors in SCLC patients is indicated in the research studies conducted. These research studies suggest pro-GRP may be used as a marker of drug resistance[21,22].

As a conclusion of our research, in laryngeal cancers, pro-GRP value in malignant cancer patients is found to be statistically higher compared to benign patients and the healthy

control group. Also, in malignant laryngeal cancer patients, pro-GRP increases according to stage of tumor, and this increase is found to be statistically significant. Although we found the NLR rate to be high in laryngeal cancer patients, we did not find a statistically significant difference between the NLR values in laryngeal cancer staging. Pro-GRP can give us an idea of the possibility of malignancy of the laryngeal mass. We believe that the important missing study in this area is to investigate whether levels of serum pro-GRP have an effect on mortality in laryngeal cancer patients. This research should be performed on more patients and multicentric.

CONCLUSION

Consequently, in our research, pro-GRP level in patients with laryngeal cancer was found out to be higher compared to others individuals. The level of pro-GRP was significanlty associated with the stage of malign tumor. We observed that Pro-GRP was a more sensitive biomarker in laryngeal cancer patients compared to NLR. In future studies pro-GRP can be used as a biomarker in head and neck cancer patients.

Compliance with Ethical Standards

All procedures involving human participants in this study were conducted in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and subsequent amendments or comparable ethical standards.

Disclosures

The authors state that they have no funding, financial relationships, or conflicts of interest.

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