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NEÜ YAYINEVİ

Prognostic Importance of Tumor Buddings in Larynx Squamous Cell Carcinomas

Larenksin Skuamöz Hücreli Karsinomlarında Tümör Budding'in Prognostik Önemi

Yasemin Gonul[1], Mithat Aricigil[2], Pembe Oltulu[3], Miyase Orhan[2]

[1]Konya Beyhekim Education and Research Hospital, Otolaryngologist, Otorhinolaryngology Department, Konya, Turkey
[1]Necmettin Erbakan University, Meram Medical Faculty of Medicine, Department of Otorhinolaryngology, Konya, Turkey
[1]Necmettin Erbakan University, Meram Medical Faculty of Medicine, Department of Pathology, Konya, Turkey

Address correspondence to: Miyase Orhan, Necmettin Erbakan University, Meram Faculty of Medicine, Department of Otorhinolaryngology, Konya, Turkey
e-mail: miyaseorhann@gmail.com

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Öz

Amaç: Larenksin skuamöz hücreli karsinomu, güvenilir prognostik belirteçlerin eksikliği nedeniyle yönetimi zor bir hastalıktır. Literatürde, tümör tomurcuklanması (TB) bazı malignitelerde kötü prognozu öngördüğü gösterilmiştir, ancak larengeal kanserde TB'nin prognostik önemi belirsizliğini korumaktadır. Bu çalışmanın amacı, larenksin skuamöz hücreli karsinomlarında TB'nin prognoza etkisini ve diğer prognostik faktörlerle olan ilişkisini değerlendirmektir.
Hastalar ve Yöntem: Kulak Burun Boğaz kliniğinde 2008-2015 yılları arasında larenksin skuamöz hücreli karsinomu tanısı konulan ve cerrahi tedavi veya postoperatif kemoradyoterapi uygulanan 60 olgu incelendi. Olguların yaşları, özgeçmişleri, TNM (tümör, nod, metastaz) sınıflandırmaları, radyolojik görüntülemeleri, uygulanan cerrahi yöntemleri ve patolojik sonuçları dosyalardan elde edildi. Tümörün Hematoksilen&Eozin boyalı preparatlarından immunhistokimyasal PanCK boyası yapılarak tümör tomurcuklanması skorlamaları patoloji bölümünde değerlendirildi. Elde edilen veriler ile klinikopatolojik değişkenler arasındaki ilişki incelendi.
Bulgular: Bu çalışmada, larenksin skuamöz hücreli karsinomunda perinöral infiltrasyon ile tümör tomurcuklanması arasında istatistiksel olarak anlamlı bir ilişki olduğu bulunmuştur (P=0.006). Ayrıca, tümör tomurcuklanması perinöral infiltrasyon ile patolojik lenf nodu tutulumu açısından bağımsız bir risk faktörü olarak görülmüştür (p=0.003). Patolojik lenf nodu tutulumu, lenfovasküler invazyon açısından bağımsız bir risk faktörü olarak belirlenmiştir (p=0.028).
Sonuç: Çalışmamız, larenksin skuamöz hücreli karsinomu için bilinen prognostik faktörler arasında TB ile perinöral infiltrasyon arasında anlamlı bir ilişki olduğunu göstermektedir ve bu nedenle tümörün prognozunu belirlemede önemli bir rol oynayabilir.

Anahtar Kelimeler: Laringeal kanser, prognoz, tümör tomurcaklanması, neoplazi invazivliği

Abstract

Aim: Laryngeal squamous cell carcinoma poses a management challenge due to the lack of reliable prognostic markers. Although tumor budding (TB) has been shown to predict poor prognosis in some malignancies, its prognostic significance in laryngeal cancer remains uncertain in the literature. Therefore, the objective of this study is to evaluate the impact of TB on prognosis and its correlation with other established prognostic factors in laryngeal squamous cell carcinoma.
Patients and Methods: In the department of otolaryngology the files of 60 patients with laryngeal squamous cell carcinoma who underwent surgery, postoperative chemoradiotherapy between 2008 and 2015 were analyzed retrospectively. The patient's history, family history, age, TNM (tumor, node, metastases) classification, radiological imaging, type of surgery performed, and the results of the pathological specimen were evaluated. PanCK immunohistochemical staining was performed on old paraffin block sections containing tumoral tissue, previously stained with Hematoxylin & Eosin. The TB scores were evaluated by the pathology department, and the association between all obtained parameters and clinicopathological variables was analyzed.
Results: Our findings showed a significant association between tumor budding and perineural infiltration, a known prognostic factor for laryngeal carcinoma (P=0.006). TB was found to be an independent risk factor for perineural infiltration and pathological lymph node involvement (p=0.003). Pathological lymph node involvement was also found to be an independent risk factor for lymphovascular invasion (p=0.028).
Conclusion: Our study provides evidence for a significant association between tumor budding and perineural infiltration, which are established prognostic factors in laryngeal carcinoma. This suggests that tumor budding may be an important factor in determining tumor prognosis.

Keywords: Laryngeal cancer, prognosis, tumor budding, neoplasm invasiveness

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INTRODUCTION

Squamous cell carcinomas account for over %95 of all cases of laryngeal cancer, the form with the highest incidence of head and neck cancer (1). The incidence of laryngeal cancers mostly happens during the fifth and seventh decades of life, with males being affected at a ratio of 3.83 times more than females (2). Smoking and alcohol habits are considered as major risk factors the development of laryngeal cancers (3). The presence of cervical metastases is one of the leading factors defining the prognosis of laryngeal cancer. The localization of the primary tumor, tumor size, degree of differentiation, and the time of onset of symptoms are the factors that affect the frequency of cervical metastases (4). It is thought that factors such as TNM (tumor, node, metastasis) classification and histological grading, which are accepted as prognostic factors, are insufficient to determine the prognosis, due to the presence of laryngeal carcinomas with different clinical courses despite similar clinical and histomorphological appearances. Predicting this clinical course difference would be possible with the use of additional prognostic parameters. Therefore, in recent years, studies on many tumor suppressor genes, oncogenes, and proliferation rate determinants have been done for finding new prognostic factors in laryngeal carcinomas (5). Higher rates of a disease-free lifetime, survival, and organ preservation can be achieved in laryngeal cancers by applying the treatment protocol according to these prognostic factors. Tumor budding which is stated as a negative prognostic factor in many cancer types is considered one of the determinants. The presence of small groups of cells or isolated single cells scattered from the invasive tumor area to the stroma is defined as tumor budding which is likely to be a prognostic factor in laryngeal cancers as well (6,7).

The aim of this study is to state the effect of tumor budding on the prognosis of laryngeal carcinoma cases and to analyze its relevance with other well-known prognostic factors.

PATIENTS AND METHODS

This study is approved by the Local Ethics Committee (Approval number: 2015-245). The files of 60 patients who got a diagnosis of laryngeal squamous cell carcinoma in a third-class reference health center between 2008 and 2015 were reviewed retrospectively.

Demographic characteristics of the patients, personal and family history, age, TNM stages, radiological imaging, types of surgery, and specimen findings were evaluated. In order to make the histopathological evaluation of the study and to determine the immunohistochemical antibodies, paraffin-embedded blocks of all cases' pathologies were extracted from the pathology laboratory archive of the hospital. Appropriate cases with pathologies that have clearly observable lower borders and tumor depth were taken in this study. Sections of 4mμm thickness obtained from paraffin-embedded tissues are stained with hematoxylin&eosin. For immunohistochemical analysis, sections were taken to special poly-L-Lysine slides. PanCK (Mouse anti-Cytokeratin Clone AE1/AE3, LOT 51024681, South San Francisco, CA, USA) antibodies were used for the immunohistochemical examination. Ventana Benchmark XT automatic immunostaining device was used for the staining procedure. All prepared and stained materials were evaluated with the Olympus BX41 light microscope.

Evaluation of Tumor Budding

Immunocytochemical staining with PanCK was also used to provide a more reliable evaluation of TB in addition to H&E-stained preparations. Cytoplasmic staining was accepted as a positive reaction for PanCK. All tumoral and epithelial cells show positive reactions with PanCK. Cell groups containing 1-5 tumoral cells at the lower border of the tumor and around large masses close to the border were evaluated as TB, provided that they were in at least 3 areas in immunohistochemical PanCK stained preparations under X400 magnification with Olympus BX41 microscope. Although there are many different classifications for this assessment, the TB classification, which is commonly used in head and neck cancers, was used (8-11).

The TB classification defined by Wang et al. (8) in head and neck cancers was modified by the addition of a non-TB group in our study and graded as follows;
-non-TB group (grade 0): 0 TB
-Low-risk group (grade 1); less than 5 TB
-High-risk group (grade 2); 5 or more TB
These TB groups were classified as low-grade (grade 0 and grade 1) and high-grade (grade 2) (Figure 1).

Statistical Analysis

The data collected from the cases were coded and transferred into a computer program. SPSS (Statistical Package for Social Science, Worldwide Heaquarters SPSS Inc.) 16.0 Windows package program was utilized for statistical evaluation. The results were presented as either mean ± standard

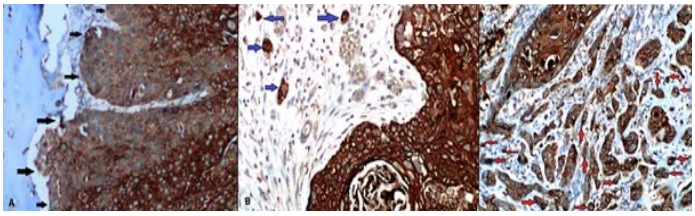

Figure 1. Modified TB Classification for Head and Neck Cancers. (Immunohistochemical PanCK stained preparations under Olympus BX41 microscope at X400 magnification. A: non-TB group (grade 0), B: Low-risk group (grade 1), C: High-risk group (grade 2))

deviation or median (minimum-maximum) related to the nature of the data.

Chi-Square Independence Test (PearsonChi-Square) was used to evaluate if there was an individual association between the dependent variables (TB) and independent risk factors. Also, the related odds ratios and 95% confidence intervals of odds ratios based on these values were obtained with this test. Logistic regression analysis is performed using a forward stepwise method to identify the most significant independent risk factors, and odds ratios were calculated for the significant factors with their corresponding 95% confidence intervals. The statistical significance level is accepted as $p < 0.05$.

RESULTS

58 of the cases (97,7%) are male, and 2 (2,1%) are female; the mean age of cases is 59.81±8.98 years (47-83). Total laryngectomy was performed in 32 cases and partial laryngectomy was performed in 28 cases.

Bilateral functional neck dissection was performed in 51 cases, 4 cases were treated with unilateral radical neck dissection and unilateral functional neck dissection, 4 cases were undergone unilateral modified radical neck dissection and unilateral neck dissection, and 1 case was treated with unilateral functional neck dissection. In the postoperative period, chemoradiotherapy was given to 32 patients, and radiotherapy was given to 6 patients as an additional treatment. In 22 cases, no additional treatment was needed. The mean follow-up time of the cases was 29.65 ± 23.09 months (1-79). Squamous cell carcinoma was present in all 60 cases. In 14 cases (23.3%) good differentiation, in 25 (41.6%) cases moderate differentiation, and in 21 cases (35%) less differentiation was observed. 49 (81.6%) of the tumors were supraglottic, 8 (13.3%) of the cases were glottic, and 3 (5%) were subglottic. In 2 cases (3.3%) T1, in 19 cases (31.6%) T2, in 17 cases (28.39%) T3, in 19 cases (31.6%) T4a and in 3 cases (5%) T4b was observed. Stage 1 was present in 2 cases (3.3%), Stage 2 in 15 cases (25%), stage 3 in 18 cases (30%), stage 4a in 21 cases (35%), and Stage 4b was present in 4 cases (6.6%). Pathological lymph node metastasis was detected in 23 (38.3%) cases. A preoperative nodule was detected in 14 (23.3%) cases.

According to tumor size, there were 11 (18.3%) cases between 0-2 cm, 38 cases (63.3%) between 2-4 cm, and 11 cases (18.3%) larger than 4 cm. According to tumor depth, there were 13 (21.6%) cases under 1 cm, 29 (48.3%) cases between 1-2 cm, and 18 (30%) cases larger than 2 cm. Tumor stroma was mild in 15 (25%) cases, moderate in 23 (38.3%) cases, and severe lymphocyte infiltration in 22 (36.6%) cases. 24 cases (40%) had perineural invasion, and lymphovascular invasion was detected in 13 cases (21.6%).

According to the TB classification (Wang et al.) that we adapted from head and neck cancers for our study, there were 13 cases (21.6%) with no TB at all (8). There were 27 (45%) cases in grade 1, 14 (23.3%) cases in grade 2, and 6 (10%) cases in grade 3.

Throughout the follow-up period of the cases, it was discovered that 10 cases (11.9%) had experienced a recurrence of laryngeal cancer within the local or regional area. The results could not be reached to evaluate the distant metastasis in 2 cases. Distant metastasis was detected in 11 cases of the remaining 58 patients (6.3%). During the follow-up, a secondary primer tumor was diagnosed in 3 cases (5%).

According to our findings, there was a significant

Table 1. Correlation between Tumor Budding and Perineural Infiltration in Patients

Tumor Budding	Perineural Infiltration		N	P
	Positive (n=24)	Negative (n=36)	60 (100%)	
Low Grade	11 (45.80%)	29 (80.60%)	40 (66.70%)	0.006
High Grade	13 (54.20%)	7 (19.40%)	20 (33.30%)	

X^2 (Pearson Chi-Square)

association between tumor budding and perineural infiltration (P=0.006) (Table 1), but no significant correlation was observed between tumor budding and other clinicopathological prognostic factors, except for perineural infiltration (p=0.643, 0.375, 0.776, 0.348, 0.624, 0.576, 0.975; tumor depth, lymphovascular invasion, tumor stage, pathologic lymph node, local recurrence, tumor diameter, distant metastasis respectively). When the risk factors regarding lymphovascular invasion, local recurrence, metastasis, perineural invasion, and pathologic lymph were researched, pathological lymph node involvement for the lymphovascular invasion is an independent risk factor (p=0.028, Odds rate=4.53, %95 confidence interval= 1.181-17.404).

We found that preoperative lymph node involvement was an independent risk factor for local recurrence (p=0.035, Odds ratio=4.66, 95% confidence interval=1.113-19.569). Additionally, tumor budding was identified as an independent risk factor for perineural infiltration (p=0.003, Odds ratio=19.86, 95% confidence interval=2.71-145.11). In addition, preoperative lymph node involvement was found to be an independent risk factor for perineural infiltration (p=0.007, Odds ratio=8.40, 95% confidence interval=1.78-39.47).

Furthermore, we observed that tumor budding was also an independent risk factor for pathological involvement of the lymph nodes (p=0.003, Odds ratio=19.86, 95% confidence interval=2.719-145.112). In summary, our results suggest that tumor budding and perineural infiltration are strongly associated and may serve as important prognostic factors for patients with certain types of cancer.

DISCUSSION

Although the location, size, cervical metastasis, and cellular differentiation of the primary lesion are established as the primary factors influencing the prognosis of laryngeal carcinomas, the need to identify new prognostic factors arises from the observation of different clinical outcomes in cases with similar characteristics. The effect of TB on the prognosis of laryngeal carcinomas and their relation with other known prognostic factors were investigated in our study.

Tumor budding is a histopathological feature seen in the progressive border of neoplasia (12). Today, tumor budding is recognized as a significant histopathological feature associated with lymph node metastasis, recurrence, distant metastasis, and reduced survival rates in different types of cancers, including colorectal cancer, esophageal carcinoma, anal carcinoma, pancreatic carcinoma, lung carcinoma, and ampulla carcinoma (13-16). Moreover, the "International Union Against Cancer" has included tumor budding as an additional prognostic factor (12, 17-21). However, literature has shown that in the studies in which TB was examined, different methods have been utilized for the TB rating, and there is no common rating methodology in this issue. For that reason, in this study, the rating method used by Wang et al. was used to evaluate TB's relationship with other known prognostic factors (8). According to this rating, it was found that perineural infiltration had a meaningful relationship with TB.

This study evaluated tumor budding (TB) as an independent risk factor for both pathological lymph node involvement and perineural invasion. In a previous study by Sarioglu et al., which investigated tumor budding in laryngeal carcinoma, it was reported that tumor budding and pathological lymph node involvement were independent risk factors for distant metastasis, leading to the description of tumor budding as a prognostic factor for laryngeal carcinoma (22).

In our study, we found that tumor budding was an independent risk factor for pathological lymph node involvement, even though it was not found to be a risk factor for metastasis due to the short follow-up period in our cases. However, our study demonstrated that tumor budding is a significant independent risk factor for perineural infiltration, which suggests that tumor budding can be considered a prognostic factor.

In this study, pathological lymph node involvement is an independent risk factor for lymphovascular involvement. The spread of laryngeal carcinomas from the primary site occurs as a result of permeation into vascular, neural, or lymphatic vessels or as a process of embolization (23). Tumor cells passing through the lymphatic vessels reach lymph nodes. Tumor cells may remain in the lymph node due to various factors or may migrate to extracapsular spread and neighboring lymph nodes. Extracellular extension occurs when the tumor cells extravasate out of the lymphatic vessels into soft tissues (23). Taking all of this data into account, pathological lymph node involvement as an independent risk factor for lymphovascular invasion can be considered quite reasonable.

Preoperative lymph node involvement is an independent risk factor for perineural invasion in the study. In the literature, the perineural invasion has

generally been found to be a related factor to loco-regional recurrence (21). Furthermore, it has been reported that perineural invasion affects the prognosis negatively as an effective factor in overall survival and disease-specific survival (24, 25). Therefore, the result obtained in our study would be compatible with the literature. In addition, when the tumor spread pattern described above is considered, it is logical to say that preoperative lymph node involvement for perineural infiltration is an independent risk factor.

In head and neck cancers, one of the most substantial independent prognostic factors is lymph node involvement (26, 27). Lymph node involvement significantly reduces both disease-specific survival and overall survival, especially when combined with vascular invasion in head and neck cancer cases. The existence of metastatic lymph nodes in the cervical region, independent of the primary site, in squamous cell carcinomas of the upper respiratory-digestive tract, reduces the 5-year survival rate by 50%. Therefore, for local recurrence preoperative lymph node involvement is considered as an independent risk factor like the literature.

The tumor stage regarding distant metastasis is an independent risk factor in this study. The T-phase of the primary lesion was reported to affect the risk of cervical metastases (28, 29). In a study, cervical metastasis was reported in 15-40% of tumors in the T1 stage, 35-42% in the T2 stage, 50-65% in the T3 stage, and 65% of tumors in the T4 stage (30). Therefore, we think that tumor staging as an independent risk factor regarding metastasis is consistent with the literature.

Limitations of our study include its retrospective design. Additionally, the sample size was relatively small, which may have limited the statistical power of our findings. Finally, our study was conducted in a single center, which may limit the generalizability of our results to other populations.

CONCLUSION

Our study highlights a significant relationship between tumor budding (TB) and perineural infiltration, both of which are established prognostic factors in laryngeal squamous cell carcinoma. TB also emerges as an independent risk factor for perineural infiltration and lymph node involvement based on pathological analysis. This underscores the importance of TB in determining the prognosis of laryngeal carcinoma and may necessitate changes in postoperative adjuvant therapy. Furthermore, our results confirm the independent risk factors for lymphovascular invasion, perineural invasion, local recurrence, and metastasis as established in previous literature. These findings align with the current understanding of tumor spread patterns and disease progression in head and neck tumors.

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Address correspondence to: *Miyase Orhan, Necmettin Erbakan University, Meram Faculty of Medicine, Department of Otorhinolaryngology, Konya, Turkey*
e-mail: *miyaseorhann@gmail.com*

REFERENCES

1. American Cancer Society. Laryngeal and hypopharyngeal cancer. [Internet]. Atlanta: American Cancer Society; c2021 [cited 2023 May 11]. Available from: https://www.cancer.org/content/dam/CRC/PDF/Public/8664.00.pdf.
2. Marchiano E, Patel DM, Patel TD, et al. Subglottic squamous cell carcinoma: A population-based study of 889 cases. Otolaryngol Head Neck Surg 2016;154(2):315-21.
3. Manjarrez ME, Ocadiz R, Valle L, et al. Detection of human papillomavirus and relevant tumor suppressors and oncoproteins in laryngeal tumors. Clin Cancer Res 2006;12(23):6946-51.
4. Candela FC, Shah J, Jaques DP, et al. Patterns of cervical node metastases from squamous carcinoma of the larynx. Arch Otolaryngol Head Neck Surg 1990;116(4):432-5.
5. Nohata N, Hanazawa T, Kinoshita T, et al. MicroRNAs function as tumor suppressors or oncogenes: Aberrant expression of microRNAs in head and neck squamous cell carcinoma. Auris Nasus Larynx 2013;40(2):143-9.
6. Manjula B, Augustine S, Selvam S, et al. Prognostic and predictive factors in gingivo buccal complex squamous cell carcinoma: Role of tumor budding and pattern of invasion. Indian J Otolaryngol Head Neck Surg 2015;67:98-104.
7. Satoh K, Nimura S, Aoki M, et al. Tumor budding in colorectal carcinoma assessed by cytokeratin immunostaining and budding areas: Possible involvement of c-Met. Cancer Sci 2014;105(11):1487-95.
8. Wang C, Huang H, Huang Z, et al. Tumor budding correlates with poor prognosis and epithelial-mesenchymal transition in tongue squamous cell carcinoma. J Oral Pathol Med 2011;40(7):545-51.
9. Luo WR, Gao F, Li SY, et al. Tumour budding and the expression of cancer stem cell marker aldehyde dehydrogenase 1 in nasopharyngeal carcinoma. Histopathology 2012;61(6):1072-81.
10. Marangon Junior H, Rocha VN, Leite CF, et al. Laminin-5 gamma 2 chain expression is associated with intensity of tumor budding and density of stromal myofibroblasts in oral squamous cell carcinoma. J Oral Pathol Med 2014;43(3):199-204.
11. Almangush A, Bello IO, Keski-Säntti H, et al. Depth of invasion, tumor budding, and worst pattern of invasion: Prognostic indicators in early-stage oral tongue cancer. Head

Neck 2014;36(6):811-8.
12. Ohike N, Coban I, Kim GE, et al. Tumor budding as a strong prognostic indicator in invasive ampullary adenocarcinomas. Am J Surg Pathol 2010;34(10):1417-24.
13. Koike M, Kodera Y, Itoh Y, et al. Multivariate analysis of the pathologic features of esophageal squamous cell cancer: Tumor budding is a significant independent prognostic factor. Ann Surg Oncol 2008;15:1977-82.
14. Moriya Y, Niki T, Yamada T, et al. Increased expression of laminin-5 and its prognostic significance in lung adenocarcinomas of small size: An immunohistochemical analysis of 102 cases. Cancer 2001;91(6):1129-41.
15. Nilsson PJ, Rubio C, Lenander C, et al. Tumour budding detected by laminin-5 γ2-chain immunohistochemistry is of prognostic value in epidermoid anal cancer. Ann Oncol 2005;16(6):893-8.
16. Roh M, Lee J, Choi P. Tumor budding as a useful prognostic marker in esophageal squamous cell carcinoma. Dis Esophagus 2004;17(4):333-7.
17. Miyata H, Yoshioka A, Yamasaki M, et al. Tumor budding in tumor invasive front predicts prognosis and survival of patients with esophageal squamous cell carcinomas receiving neoadjuvant chemotherapy. Cancer 2009;115(14):3324-34.
18. Masuda R, Kijima H, Imamura N, et al. Tumor budding is a significant indicator of a poor prognosis in lung squamous cell carcinoma patients. Mol Med Rep 2012;6(5):937-43.
19. Ohtsuki K, Koyama F, Tamura T, et al. Prognostic value of immunohistochemical analysis of tumor budding in colorectal carcinoma. Anticancer Res 2008;28(3B):1831-6.
20. Karamitopoulou E. Tumor budding cells, cancer stem cells and epithelial-mesenchymal transition-type cells in pancreatic cancer. Front Oncol 2013;2:209.
21. Yilmaz T, Hosal AS, Gedikoğlu G, et al. Prognostic significance of vascular and perineura invasion in cancer of the larynx. Pathol Res Pract 1998;19(2):83-8.
22. Sarioglu S, Acara C, Akman FC, et al. Tumor budding as a prognostic marker in laryngeal carcinoma. Pathol Res Pract 2010;206(2):88-92.
23. Jose J, Moor JW, Coatesworth AP, et al. Soft tissue deposits in neck dissections of patients with head and neck squamous cell carcinoma: Prospective analysis of prevalence, survival, and its implications. Arch Otolaryngol Head Neck Surg 2004;130(2):157-60.
24. Magnano M, Bongioannini G, Lerda W, et al. Lymphnode metastasis in head and neck squamous cells carcinoma: Multivariate analysis of prognostic variables. J Exp Clin Cancer Res 1999;18(1):79-83.
25. Harrison LB, Sessions RB, Hong WK. Head and Neck Cancer: A Multidisciplinary Approach. In: Mendenhal WM, Werning JW. Early-stage cancer of the larynx general principles and management. Lippincott Williams & Wilkins, 2009:339-53.
26. Rodrigo JP, Dominguez F, Suárez V, et al. Focal adhesion kinase and E-cadherin as markers for nodal metastasis in laryngeal cancer. Arch Otolaryngol Head Neck Surg 2007;133(2):145-50.
27. Forastiere A, Koch W, Trotti A, et al. Head and neck cancer. N Engl J Med 2001;345(26):1890-900.
28. Paperella MM, et al. Otolaryngology Head and Neck Surgery. In: Thawley S. Cysts and tumors of the larynx. WB Saunders Company, 1991:32-33.
29. Myers EN, Cancer of the Neck. In: Suen JY. Cancer of the head and neck. New York: Churchill Livingstone,1989:221-54.
30. Dedivitis R, Peretti G, Hanna E, et al. Laryngeal Cancer. In: Kirchner J. Spread and barriers to spread of cancer within the larynx. New York: Thieme Medical Publishers, 1991:7-15.