Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2016/351

Original Article

Laryngeal Biopsies with Special Reference to Malignant Tumors: A Histopathological Study

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Abstract

Introduction: Larynx serves us with three functions such as protective, respiratory, and phonatory. There are many risk factors which affect larynx and cause various disorders. Malignancy can develop in any part of the larynx, but the cure rate depends on the location of the tumor. The aim of the present study is to study the spectrum of lesions seen in the laryngeal biopsies received and to study the different histological types of malignant tumors.

Materials and Methods: A total of 95 patients were included in this study. From all the chronic cases, biopsy was obtained to classify the type of the disease histologically and also the aim of biopsy study requested by clinician was to rule to rule out malignancy. Biopsy procedure was done in operation theater by E.N.T surgeon. The biopsy sections were stained with routine hematoxylin and eosin stains and examined.

Results: Out of 95 laryngeal biopsies, 25 biopsies from inflamed larynx, 49 from neoplastic growths, 8 from laryngeal nodules, 2 from laryngoceles, 2 showed no significant lesions, 1 from the infected cyst, and 8 were inadequate biopsies. Out of 41 malignant tumors, 39 were squamous cell carcinoma, basaloid squamous type and adenocarcinoma were one each. A highest number of malignant tumors were seen between 41 and 60 years.

Conclusion: In recent days, pre-operative biopsy procedures give good results because of fiber optic endoscopic assisted biopsies, help to confirm malignancy, and also help to differentiate the tumors. Accurate diagnosis with biopsy on clinical suspicious lesions will help to treat patients early and promptly. Health education in communities in relation to tobacco usage will help to reduce the incidence of malignant tumors.

Key words: Biopsy, Histopathology, Larynx, Malignant tumors

INTRODUCTION

The larynx is a respiratory organ situated in the respiratory tract in between pharynx and trachea. The cavity is lined by mucous membrane; hence, various lesions ranging from acute non-infective laryngitis to various malignancies can be seen. Larynx serves us with three functions such as protective, respiratory, and phonatory.[1,2]

There are many risk factors which affect larynx and cause various disorders. These include vocal abuse or overuse, which may cause vocal cord nodules, polyps, or laryngitis. Smoking and drinking can cause laryngeal cancer. Traumas or neurological conditions may cause vocal cord paralysis as well as swallowing difficulties (dysphagia). Other disorders are contacted ulcers, laryngoceles, and spasmodic dysphonia.

For proper diagnosis of laryngeal lesions, various diagnostic aids such as X-ray examination, computed tomography, magnetic resonance imaging, and ultrasonography have become widely available. These powerful methods have proved useful in supplementing, clinical determination of the size, and extent of laryngeal tumors.

However, to distinguish malignancy from the panoply of disorders that may have a similar gross appearance such as fungal, mycobacterial infections, syphilitic gummas, idiopathic granulomatous disorders, benign neoplasms, a tissue diagnosis must be made. The accepted standard for

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www.ijss-sn.com

Month of Submission : 04-2016
Month of Peer Review : 05-2016
Month of Acceptance : 05-2016
Month of Publishing : 06-2016

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diagnosis is histopathologic examination of tissue obtained at laryngoscopy of biopsy.[3,4]

Hence, the role of biopsy is indispensible in diagnosing malignant tumors, which are more commonly seen and also useful to identify the different types of tumors. Biopsy is also important clinically, which is often neglected; the degree of differentiation is significant.[5]

Squamous cell carcinoma (SCC), the most common malignant tumor about 95% seen in larynx, is habit oriented, preventable, and curable. In diagnosing such tumors, histopathological examination is of immense help. Malignancy can develop in any part of the larynx, but the cure rate depends on the location of the tumor. There is an increase in death toll rate from 76,000 deaths in 1990 to 88,000 deaths in 2012.[6] In the United States, 60% of five survival rates were reported.[7]

The aim of the present study is to study the spectrum of lesions seen in the laryngeal biopsies received and to study the different histological types of malignant tumors.

MATERIALS AND METHODS

The present study, which is prospective, based on study of laryngeal biopsies received in the Department of Pathology, Government Medical College, Ananthapuramu, for 2 years.

A total number of 95 patients were selected and included in this study for laryngeal biopsies. Patients were explained about the procedure and benefit due to biopsy report when they agree for biopsy informed consent has taken. Before doing this study, institutional approval from Ethical Committee was obtained.

All chronic cases presenting with complaints of hoarseness of voice, dyspnea, dysphagia, pain, and stridor irrespective of the lesion present in the larynx were included in this study. There were no specific complaints related to any of the lesions of larynx.

The lesions located in the anterior aspect of epiglottis, oropharynx, and pyriform fossa were excluded in this study.

From all the chronic cases, biopsy was obtained to classify the type of the disease histologically and also aim of biopsy study requested by clinician was to rule to rule out malignancy. Biopsy procedure was done in operation theater by E.N.T surgeon.

The material thus obtained was immediately preserved in 10% neutral formalin and sent to the department of pathology. The tissue obtained was fixed in 10% neutral formalin for 24 h in the department of pathology after verifying the particulars of the patient. After 24 h, the tissue was processed for histopathological study. The tissue sections were processed using histokinette and embedded in paraffin wax. Serial sections were taken from paraffin blocks using rotary microtome of 4-6 μ thick. The sections were stained with routine hematoxylin and eosin stains. Special stain-like Giemsa was used whenever needed. All the results were entered into excel sheet and analyzed.

An attempt was made in the present study to classify the laryngeal diseases, histologically from the biopsy tissue obtained from chronic cases of laryngeal problems.

Statistical analysis was done using danielSoper.com by calculating Chi-square and degree of freedom for analyzing *P* value. The $P < 0.001$ was considered significant.

RESULTS

A total number of surgical biopsies received during 2 years were 6898. Of these, laryngeal biopsies formed were 95 (1.37%). Out of 95 laryngeal biopsies, 25 biopsies from inflamed larynx, 49 from neoplastic growths, 8 from laryngeal nodules, 2 from laryngoceles, 2 showed no significant lesions, 1 from infected cyst, and 8 were inadequate biopsies (Table 1).

Microscopic examination of 23 cases of chronic non-specific laryngitis showed stratified squamous epithelium, showing parakeratosis, acanthosis. Subepithelially congested blood vessels chronic inflammatory cells formed by lymphocytes plasma cells and fibroblasts (Figures 1 and 2).

Table 1: The histological findings of biopsies received from 95 cases

Lesion	Number of biopsies received (%)
Inflammatory lesions	25 (26.3)
Chronic non-specific laryngitis	23 (24.2)
Tuberculosis of larynx	1 (1.1)
Scleroma larynx	1 (1.1)
Benign mucosal disorders	11 (11.6)
Vocal nodules	8 (8.4)
Laryngoceles	2 (2.1)
Non-specific infected cyst	1 (1.1)
Benign tumors	8 (8.4)
Squamous papillomas	7 (7.4)
Hemangioma	1 (1.1)
Malignant tumors	41 (43.2)
Squamous cell carcinoma	39 (41.1)
Basaloid squamous cell carcinoma	1 (1.1)
Adenocarcinoma	1 (1.1)
Normal cord	2 (2.1)
Inadequate biopsies	8 (8.4)
Total	95 (100)

Microscopy of tuberculosis (TB) of the larynx showed stratified squamous epithelium. Subepithelially well-defined granulomas were seen granulomas were made up of central caseous necrosis surrounded by epithelial cells, Langhans type of giant cells, and peripheral mantle of lymphocytes and fibroblasts.

Microscopy of scleroma larynx showed stratified squamous epithelium. Subepithelially Mikulicz cells, plasma cells, and Russell bodies were seen. Giemsa demonstrated *Klebsiella rhinoscleromatis* organisms within the Mikulicz cells.

Laryngeal nodules histologically showed stratified squamous epithelium. Subepithelially dilated vascular channels, along with chronic inflammatory cell infiltrate was seen.

Laryngocele histologically showed a cyst wall lined by pseudostratified columnar epithelium. Subepithelially inflammatory cell infiltrate.

Squamous papilloma showed stratified squamous epithelium with acanthosis, papillomatosis of varying degree and a fibrovascular core (Figure 3).

Hemangioma histologically showed stratified squamous epithelium. Subepithelially vascular channels of various sizes filled with blood (Figure 4).

Out of 95 laryngeal biopsies, 41 (43.2%) malignant tumors noticed, which is the predominant one. Out of 41 malignant tumors, 39 were SCC, basaloid squamous type and adenocarcinoma were one each. Highest number of malignant tumors were seen between 41 and 60 years (Table 2).

There is male preponderance in malignant tumors when compared to females. Males were 36 (87.8%) and females were 5 (12.1%), and the ratio is 7.2:1. Among 36 males, 34 (94.4%) were smokers. Smoking and male variables in relation to malignant tumors were statistically significant and the $P < 0.001$.

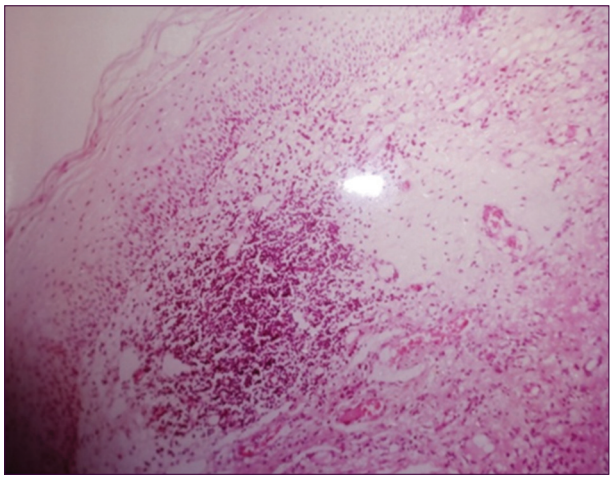

Figure 1: Chronic non specific laryngitis

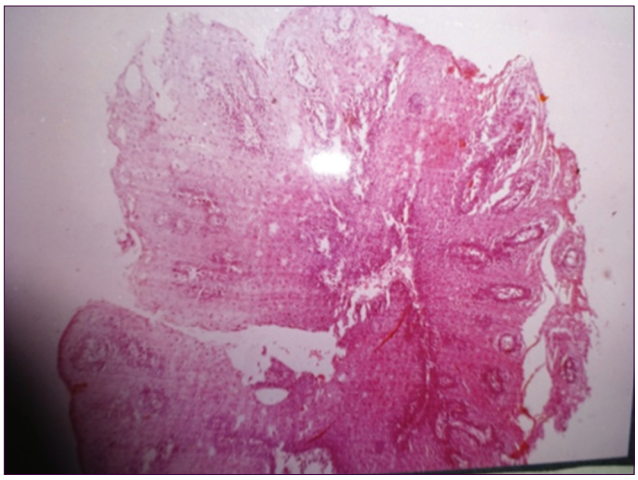

Figure 3: Squamous papilloma of larynx

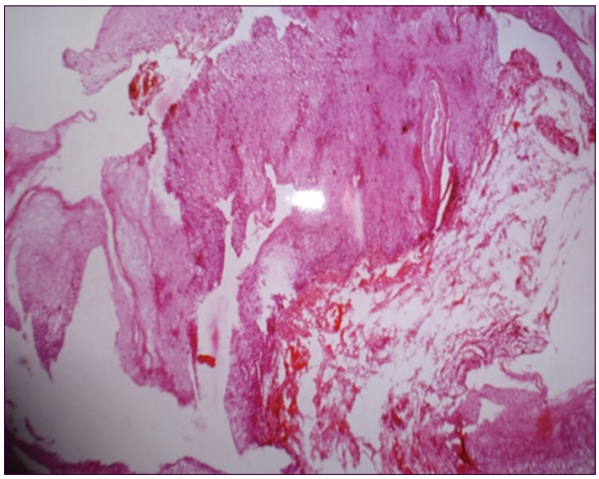

Figure 2: Chronic hypertrophic laryngitis

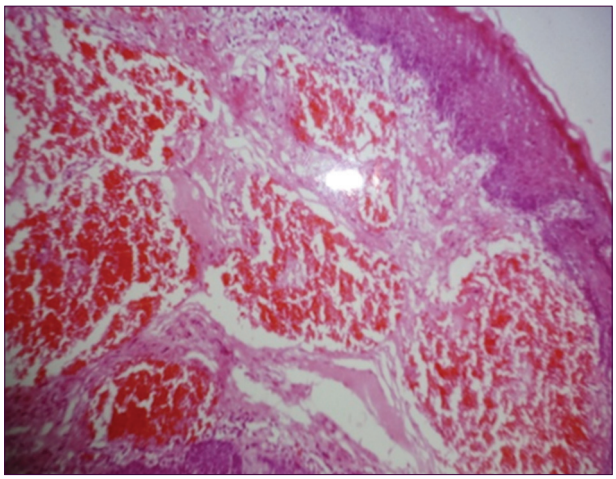

Figure 4: Haemangioma of larynx

Most frequent site for occurrence of malignant tumors is glottis followed by Supraglottis (Table 3).

Histological picture of SCC showed tumors consisting of irregular masses of squamous cells (Figures 5 and 6). The invading tumor masses were composed of varying proportions of normal squamous cells and atypical squamous cells. The majority of the tumors were Grade II (Table 4).

DISCUSSION

Acute laryngitis is infective or non-infective etiology should diagnose clinically and using other diagnostic aids, but not biopsy where it is contraindicated. Any chronic non-specific inflammatory reaction of laryngeal mucosa may be called chronic laryngitis.

Hoarseness presenting for more than 2 months demands specialist attention,[8] whereas biopsy forms the cornerstone of an appropriate workup. In the present study, 23 laryngeal biopsies were reported as non-specific laryngitis, and all patients were presented with hoarseness of voice.

In the present study, peak incidence of laryngeal lesions was shown in between 41 and 60 years, which coincides with Brock's observation.[9] Most of the members (91.3%) in this study were smokers, and many studies were also noticed strong association of chronic non-specific laryngitis with tobacco smoking.[10,11]

In this study, dysplasia of Grade II was seen in only one biopsy. The relation between chronic laryngitis and the malignant transformation of such larynx is well known since 1923.[12-14] Proper follow-up provides greater information about the fate of such larynx.

In the present study, one case of TB larynx was noted in posterior part of vocal cord of a 60 years male patient. Tuli *et al.* noticed TB larynx at posterior part of true vocal cord, the arytenoid cartilage and inter arytenoid space.[15]

Scleroma of the larynx is usually associated with nasal scleroma,[16] but several publications have discussed the involvement of larynx, trachea, and bronchi.[17] The larynx being involved in 15-80% and the tracheobronchial tree in 2-20% of patients with rhinoslceroma.[18] Only two cases of

Table 2: Age-wise distribution of malignant tumors of larynx

Age in years	Number of malignant tumors (%)
0-20	Nil
21-40	9 (21.9)
41-60	25 (60.9)
61 and above	7 (17.07)
Total	41 (100)

Table 3: Subsite distribution of malignant tumors of larynx

Site	Number of cases (%)
Supraglottis	13 (31.7)
Glottis	27 (65.8)
Subglottis	1 (2.4)
Transglottic	-
Total	41 (100)

Table 4: Grading of squamous cell carcinoma

Grades	Number of cases (%)
I	10 (25.6)
II	26 (83.8)
III	3 (7.6)
IV	-
Total	39 (100)

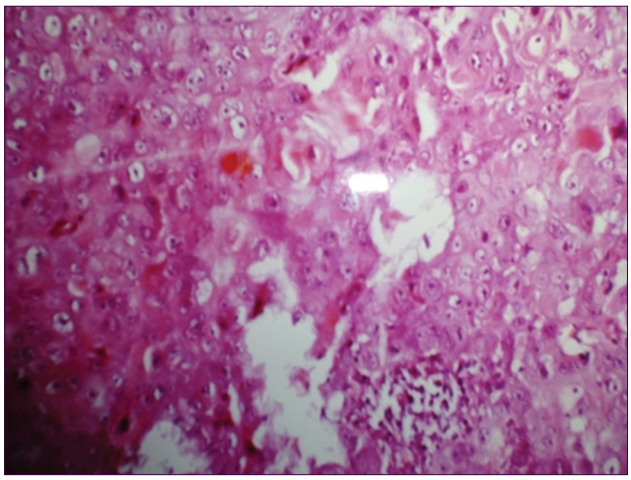

Figure 5: Squamous cell carcinoma of larynx

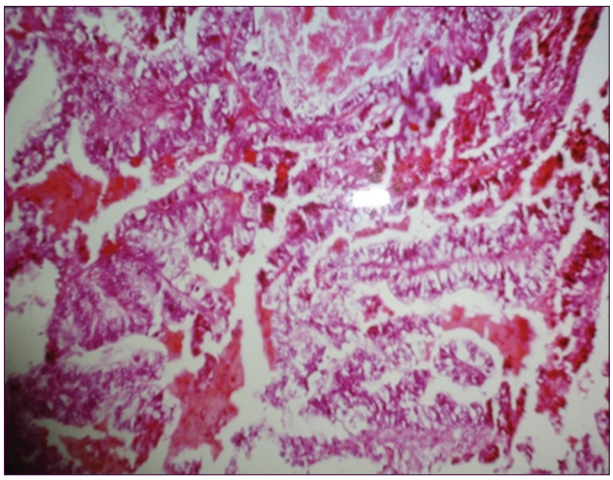

Figure 6: Adenocarcinoma of larynx

isolated laryngeal involvement without nasal disease have been communicated.[16,19] In this study, scleroma of larynx was observed in biopsy of nasal cavity and also glottis.

As per this study, all 8 patients with vocal nodules were aggressive voice users. Regarding etiology laryngoceles, different workers have different opinions which include increased transglottic pressure, congenital defect in laryngeal ventricle, and carcinoma of larynx and can occur at any age.[20] As per this study, an association of cancer larynx with laryngocele was not appreciated.

In the present study, out of 95 laryngeal lesions, 8 benign tumors were observed, 7.4% were squamous papillomas, and 1.1% were hemangiomas, and the ratio of benign and malignant tumors was 5:1. In Sharma *et al*.[21] study, the ratio of benign to malignant tumors was 2:3. Out of the benign tumors, 70% were vocal polyps, 20% were squamous cell papilloma, and 10% were chronic inflammation. Most common benign tumor found by Arnold *et al*.[22] was papilloma, Cocks *et al*.[23] found vocal cord polyps, and Nerurkar *et al*.[24] reported recurrent respiratory papilloma.

Laryngeal cancers formed 7.01% of all cancers detected. Out of 95 laryngeal biopsies, 41 (43.2%) malignant tumors noticed, which is the predominant one. Out of 41 malignant tumors, 39 were SCC, basaloid squamous type and adenocarcinoma were one each. Many other studies also reported SCC as a predominant type (Table 5).

A highest number of malignant tumors were seen between 41 and 60 years in this study. Cancer of larynx forms about 2.2% of all cancers in men and 0.4% in women.[22] Other studies also documented that malignant tumors most commonly notice in the age group of 40-60 years, incidence of malignancy increases with increasing age[31] and in women malignant tumors were noticed one decade younger than males.[23]

In this study, there is male preponderance in malignant tumors when compared to females. Males were 36 (87.8%) and females were 5 (12.1%), and the ratio is 7.2:1. This line is supported by Bakshi *et al*.[30] and Goiato and Fernandes.[32]

Table 5: Incidence of SCC in various studies

Study	Percentage of SCC
Kaufman and Burke[25]	90
Kumar *et al*.[26]	95
Domanowski[27]	96
Jaiswal and Hoang[28]	99
Wang *et al*.[29]	99
Bakshi *et al*.[30]	69 non-keratinizing SCC 27 Keratinizing SCC

SCC: Squamous cell carcinoma

Sharma *et al*.[21] documented that males were predominantly affected with benign (7:3) and malignant tumors (29:1) than females.

Among 36 males, 34 (94.4%) were smokers. Smoking and male variables in relation to malignant tumors were statistically significant in this study. Among smokers, death rate is 20 times higher than non-smokers.[33] Sharma *et al*.[21] reported that smoking is plays an important role in benign (25%) and malignant tumors (73.2%). The symptoms of laryngeal tumors vary from mild hoarseness of voice to life-threatening distress.

The most frequent site for occurrence of malignant tumors of larynx is glottis (65.8%) followed by supraglottis (31.7%) in the present study. The majority of the tumors were Grade II (83.8%). This is in similar to Bastian observations.[34] In contrast to our study, sharma *et al*.,[21] Thompson *et al*.,[35] and Bakshi *et al*.[30] reported more number of supraglottic tumors followed by glottic, subglottic, and transglottic tumors.

In the present study, among malignant tumors 26 (63.4%) were moderate differentiated, 11 (26.8%) were well differentiated, and 4 (9.7%) were poorly differentiated. Most glottic carcinomas were well to moderately differentiated, whereas a high percentage of those located in the other regions of larynx were moderate to poorly differentiated.[36]

CONCLUSION

From this, we conclude that among laryngeal lesions, malignant tumors were most common followed by chronic non-specific laryngitis. Among malignant tumors, SCC observed most commonly. Malignant tumors were associated with smoking and hoarseness of voice and were more in males than females. The most frequent site for occurrence of malignant tumors of the larynx is glottis and supraglottis. In recent days, pre-operative biopsy procedures give good results because of fiber optic endoscopic assisted biopsies, help to confirm malignancy and also help to differentiate the tumors. Accurate diagnosis with biopsy on clinical suspicious lesions will help to treat patients early and promptly. Health education in communities in relation to tobacco usage will help to reduce the incidence of malignant tumors.

ACKNOWLEDGMENTS

We are expressing our gratitude toward staff of the Department of ENT, for helping to do this staff. We also express thanks to staff of the Department of Pathology, for their cooperation while doing this study.

REFERENCES

1. Sasaki CT, Isaacson G. Functional anatomy of the larynx. Otolaryngol Clin North Am 1988;21:595-612.
2. Sasaki CT. Anatomy and development and physiology of the larynx. In: Goyal R, Shaker R., editors. Part. 1. Oral Cavity, Pharynx and Esophagus. GI motility Online: Nature Publishing Group; 2006.
3. Clarance T Sasaki, Roy D Carlson. Malignant neoplasms of the larynx. Cummings, Otolaryngology, Head and Neck Surgery. p. 1925.
4. Ogura JH, Mallen R, Spector GJ. Tumor of larynx and pharynx. In: Ballenger JJ, editor. Diseases of Nose, Throat, Ear. 12th ed. London: Butterworths; 1997.
5. Robin PE, Olofsson J. Tumours of the larynx. In: Hibbert J, editor. Laryngology and Head and Neck Surgery. Vol. 6. Great Britain: Heinemann International; 1997. p. 1-9.
6. GBD Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;385:117-71.
7. SEER Stat Fact Sheets: Larynx Cancer. NCI. Available from: http://www.seer.cancer.gov/statfacts/html/laryn.html. [Last retrieved on 2014 Jun 18].
8. Vrabec DP, Davidson FW. Inflammatory diseases of larynx. In: English GM, Otolaryngology. Vol. 3. Philadelphia, PA: Lippincott-Raven Publishers; 1990.
9. Brock PV. Scott-Brown's Otolaryngology. 5th ed. London: Butterworth; 1987.
10. Auerbach O, Hammond EC, Garfinkel L. Histologic changes in the larynx in relation to smoking habits. Cancer 1970;25:92-104.
11. Lederer FJ, Soboroff BJ. Medical problems related to diseases of the larynx. Otolaryngol Clin North Am 1970;3:599-608.
12. Crissman JD. Laryngeal keratosis and subsequent carcinoma. Head Neck Surg 1979;1:386-91.
13. Gabriel CE, Jones DG. Hyperkeratosis of the larynx. J Laryngol Otol 1962;76:947-57.
14. Norris CM, Peale AR. Keratosis of the larynx. J Laryngol Otol 1963;77:635-47.
15. Tuli BS, Gupta V, Singh H, Chary G, Chand AK. Laryngeal manifestations in pulmonary Kochs. Indian J Otolaryngol Head Neck Surg 1993;2.
16. Ballenger JJ. Diseases of the Nose, Throat, Ear, Head and Neck. 14th ed. Philadelphia, PA: Lea & Febiger.
17. Shum TK, Whitakar CW, Meyer PR. Clinical updates in rhinoscleroma. Otolaryngol Head Neck Surg 1982;93:663-5.
18. Holinger PH, Gelman HK, Wolfe CK Jr. Rhinoscleroma of the lower respiratory tract. Laryngoscope 1977;87:1-9.
19. Agarwal MK, Samant HC, Gupta OP, Gupta S. Solitary scleroma of the larynx. Ear Nose Throat J 1981;60:315-7.
20. Holinger LD. Pharyngocele, laryngoceles and saccualr cysts. In: English GM, Otolaryngology. Vol. 3. Philadelphia, PA: Lippincott-Raven Publishers; 1990.
21. Sharma DK, Sohal BS, Bal MS, Aggarwal S. Clinico-pathological study of 50 cases of tumours of larynx. Indian J Otolaryngol Head Neck Surg 2013;65 Suppl 1:29-35.
22. Arnold WJ, Laissue JA, Friedmann I, Naumann HH. Larynx. In: Diseases of the Head and Neck. Vol. 8. Stuttgart: Georg Thieme Verlag; 1987. p. 8-13.
23. Cocks H, Quraishi M, Morgan D, Bradley P. Leiomyosarcoma of the larynx. Otolaryngol Head Neck Surg 1999;121:643-6.
24. Nerurkar N, Kalel K, Pathania V, Bradoo R. Recurrent respiratory papilloma in pregnancy. Bombay Hosp J 2006;48:187-90.
25. Kaufman JA, Burke AJ. The etiology and pathogenesis of laryngeal carcinoma. In: The otolaryngologic clinics of North America. Curr Concepts Laryngeal Cancer I 1997;30:1-13.
26. Kumar V, Abas AK, Fausto N. Robins and Cotran's Pathologic Basis of Disease. 7th ed. India: Saunders; 2004. p. 786-7.
27. Domanowski G. Malignant tumours of the larynx. In: Coleman JA, Talavera F, Calhoun KH, Slack, Meyers AD, editors. Head and Neck Oncology. Section. 1-10. eMedicine World Medical Library; 2006.
28. Jaiswal VR, Hoang MP. Primary combined squamous and small cell carcinoma of the larynx: a case report and review of the literature. Arch Pathol Lab Med 2004;128:1279-82.
29. Wang MC, Liu CY, Li WY, Chang SY, Chu PY. Salivary gland carcinoma of the larynx. J Chin Med Assoc 2006;69:322-5.
30. Jaimanti, Panda NK, Sharma S, Gupta AK, Mann SB. Survival patterns in treated cases of carcinoma larynx in north India - A 10 years followup study. Indian J Otolaryngol Head Neck Surg 2004;56:99-104.
31. Shirley D. Cartilaginous lesions of the larynx. Grand rounds archives BCM. In: Bobby R, editor. Alford Department of Otolaryngology: Head and Neck Surgery. Houston: Baylor College of Medicine; 1997.
32. Goiato MC, Fernandes AU. Risk factors of laryngeal cancer in patients attended in the oral oncology centre of Aracatuba. Braz J Oral Sci 2005;4:741-4.
33. Ridge JA, Glisson BS, Lango MN, Fiegenberg S. Head and neck tumorsin. In: Pazdur R, Wagman LD, Camphausen KA, Hoskins WJ, editors. Cancer Management: A Multidisciplinary Approach. 11th ed. ???: Franklin Wong; 2008.
34. Bastian RW, Collins SL, Kaniff T, *et al*. Indirect videolarynscopy versus direct endoscopy for larynx and pharynx cancer staging towards elimination of preliminary direct laryngoscopy. Ann Otol Rhinol Laryngol. 1989;8:693.
35. Thompson LD, Wenig BM, Heffner DK, Gnepp DR. Exophytic and papillary squamous cell carcinomas of the larynx: A clinicopathologic series of 104 cases. Otolaryngol Head Neck Surg 1999;120:718-24.
36. Chung CK, Stryker JA, Abt AB, Cunningham DE, Strauss M, Connor GH. Histologic grading in the clinical evaluation of laryngeal carcinoma. Arch Otolaryngol 1980;106:623-4.

How to cite this article: Varalakshmi KP, Naik VS, Swapna RS, Sravani P, Padmaja MN. Laryngeal Biopsies with Special Reference to Malignant Tumors: A Histopathological Study. Int J Sci Stud 2016;4(3):197-202.

Source of Support: Nil, **Conflict of Interest:** None declared.