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Pathology

Correlation of Histopathological Types of Laryngeal and Nasopharyngeal Mass with PCR Detected Epstein - Barr virus (LMP-1) – A Hospital Based Prospective Study

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Abstract **Original Research Article**

Introduction- Larynx is one of the most common sites of head and neck carcinoma. Nasopharyngeal carcinoma is relatively uncommon. Juvenile nasopharyngeal angiofibroma is a very rare disease but gradually increase in the incidence in the current decade as compared to the 1980s suggesting that some evolving host-agent- environmental interaction or probably a novel etiologic agent operating in this part of the world. ***Materials and method*****:** Total 53 patients from ENT department, having laryngeal or nasopharyngeal masses are included in this prospective study. During operation, part of the tissue is taken in phosphate buffer solution for detection of EBV-LMP-1 by PCR and part of tissue is taken in formalin for histopathology. ***Result:*** Out of 35 cases of laryngeal masses, 32 patients (91.4%) are male, 14 patients (40%) are in 6th decade, 22 patients (62.86%) are smoker, 20 patients (57.14%) presented with hoarsness of voice, 8 patients (22.86%) have mass in both vocal cord, 21 patients (60%) have moderately differentiated squamous cell carcinoma, 6 (17.14%) have vocal cord nodule, 2 (5.71%) have fibro-epithelial polyp with dysplasia, 2 (5.71%) have dysplasia, 2 (5,71%) have squamous cell papilloma with koilocytic changes. Remaining 2 patients have cavernous haemangioma and inverted papilloma. Out of 18 cases of nasopharyngeal masses, 14 patients (77.78%) are male, 5 patients (27.78%) are in 2nd decade and 4 patients (22.22%) are in 6th decade, 9 patients (50%) are smoker, 10 patients (55.56%) presented with swelling in cervical region, fever and weight loss, 9 patients (50%) have undifferentiated non-keratinizing nasopharyngeal carcinoma, 6 patients (33.33%) have nasopharyngeal angiofibroma. Remaining 3 patients have keratinizing squamous cell carcinoma and non-Hodgkin lymphoma. But EBV- LMP-1 is not found in any laryngeal or nasopharyngeal cases in this study. ***Conclusion*****:** Most of the laryngeal masses are diagnosed as moderately differentiated squamous cell carcinoma and most of the nasopharyngeal masses are non-keratinizing nasopharyngeal carcinoma followed by nasopharyngeal angiofibroma. EBV infection may not always be a leading cause of laryngeal or nasopharyngeal mass.

Keywords: PCR, EBV- LMP-1, laryngeal mass, nasopharyngeal mass.

INTRODUCTION

In India, head and neck carcinomas accounted for 30% of all carcinomas. One of the most common sites of head and neck carcinoma is larynx. Laryngeal carcinoma is the ninth and seventh most common cause of carcinomas in males in Asia and India respectively [1]. While nasopharyngeal carcinoma is relatively uncommon worldwide, it is endemic in certain populations including southern China, Southeast, the Middle East, and North Africa. Juvenile nasopharyngeal angiofibroma is a very rare disease. Its incidence has been cited to be 0.05% of all head and neck neoplasms as per many study reports [2]. Juvenile nasopharyngeal angiofibroma has witnessed a four-fold increase in the incidence in the current decade as compared to the 1980s.This suggests some evolving host-agent-environmental interaction or probably a novel etiologic agent operating in this part of the world.

The role of many factors, especially tobacco use and alcohol consumption has been clearly shown in the development of laryngeal carcinoma. Also it is known that certain viruses have oncogenic potentials, and the relationship between laryngeal carcinoma and viruses has been a popular subject of research for many years [3-5]. The Epstein-Barr virus (EBV) has long been related to nasopharyngeal carcinoma [6].

AIMS AND OBJECTIVES

1. To find out the different histological types of laryngeal mass
2. To find out the different histological types of nasopharyngeal mass
3. To study the association of EBV- LMP-1 with different histological types of laryngeal and nasopharyngeal mass.

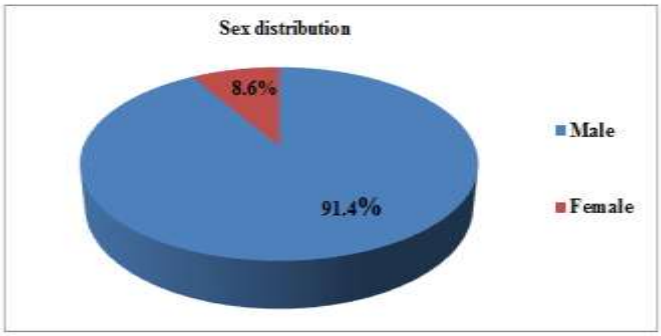
MATERIALS AND METHODS

It is observational prospective hospital based study from January'18 to June'19 in the department of pathology, biochemistry and Otorhinolaryngology. Those patients, having laryngeal or nasopharyngeal mass, admitted to the Otorhinolaryngology department for the biopsy, included in this study. In operation theatre, part of the mass is taken in phosphate buffer solution for conventional PCR and another part is kept in formalin for histopathology. Tissue in phosphate buffer solution is stored in – 20^0C.

The primer to be used for EBV- LMP-1:

Forward primer – 5' CCG AAG AGG TTG AAA ACA AA 3'
Reverse primer – 5'GTG GGG GTC GTC ATC ATC TC 3'

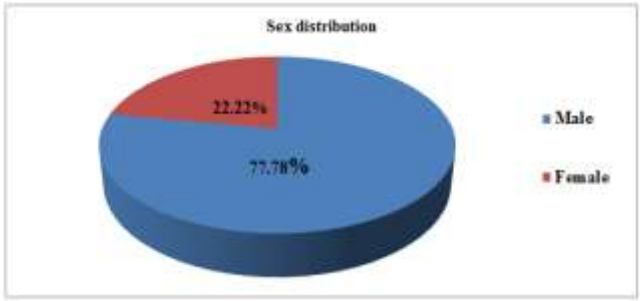
RESULT ANALYSIS



Graph-1(a): Sex distribution of the patients with laryngeal mass

Out of 35 patients with laryngeal mass, majority of the study population (91.4%) are males.

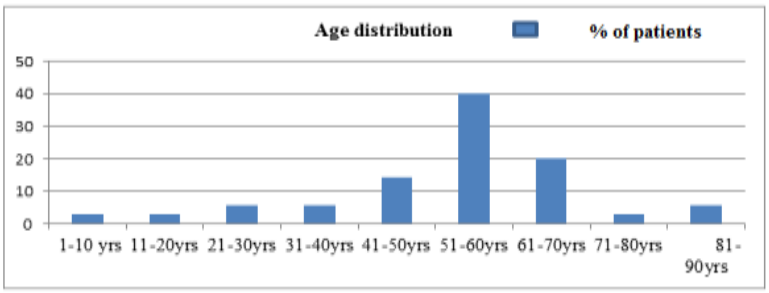
Inference: Male preponderance is noticed.



Graph-1(b): Sex distribution of the patients with nasopharyngeal mass

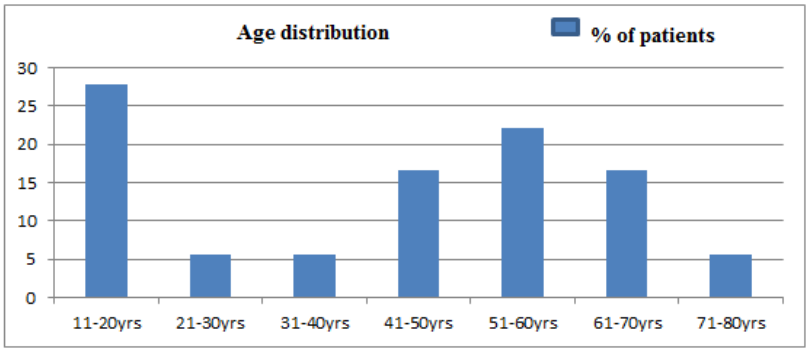
Out of 18 patients with nasopharyngeal mass, majority (77.78%) are male and male to female ratio 3.5.

Inference: Male preponderance is noticed.



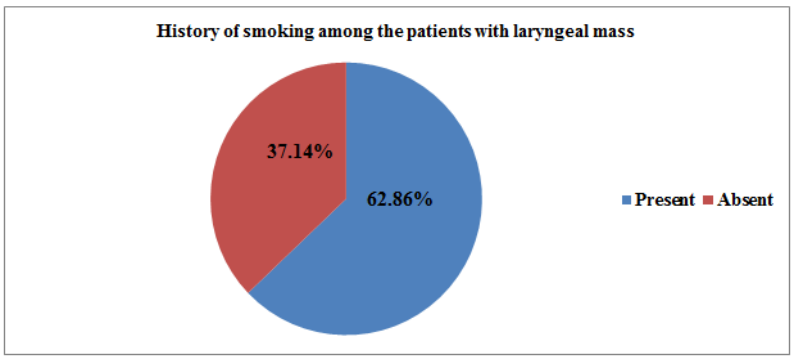
Graph-2(a): Age distribution of the patients with laryngeal mass

Inference- Majority of patients with laryngeal mass is in 6th decade.



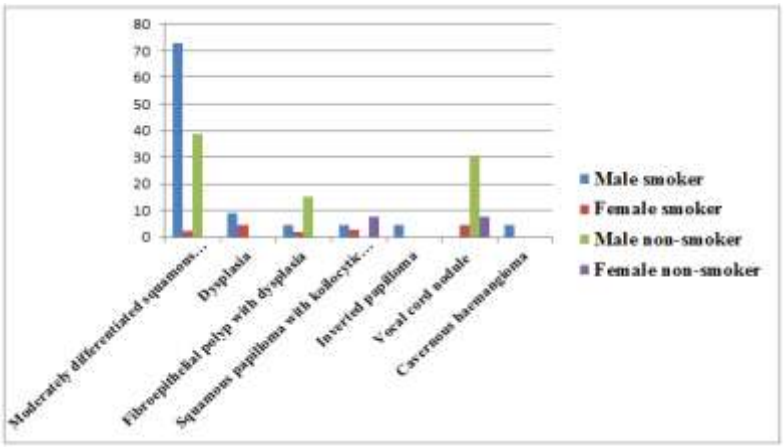
Graph-2(b): Age distribution of patients with nasopharyngeal mass

Inference- Majority of patients with the nasopharyngeal mass is in 2nd decade, followed by 6th decade.



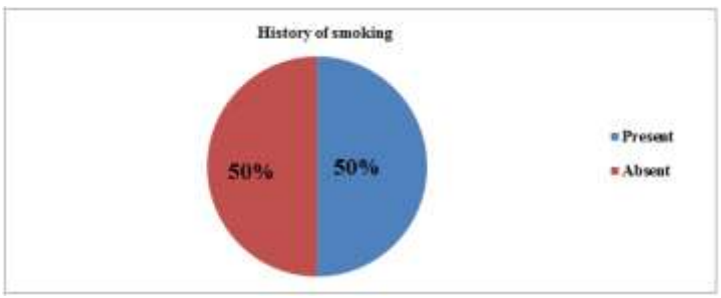
Graph-3(a): Distribution of history of smoking among patients with laryngeal mass

Inference- Majority of the patients with laryngeal mass has positive history of smoking (62.86%).



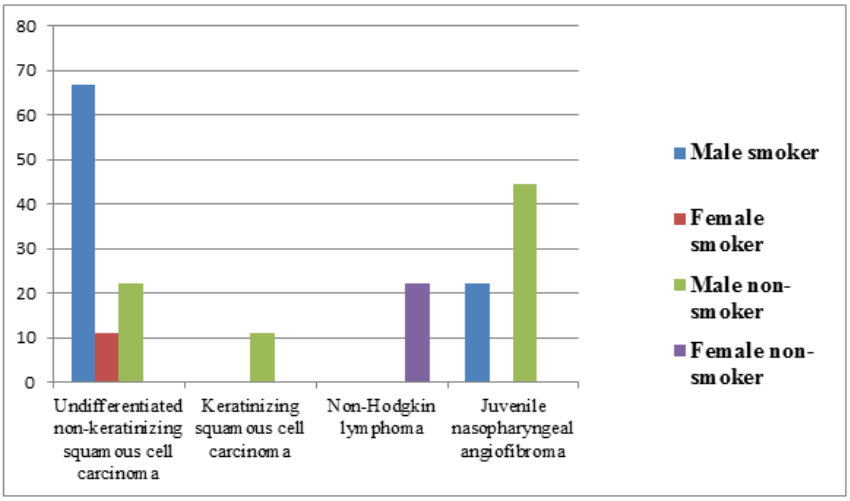
Graph-3(b): Distribution of sex with history of smoking among patients with laryngeal mass

Inference- 72.7% male smoker patients have moderately differentiated squamous cell carcinoma.



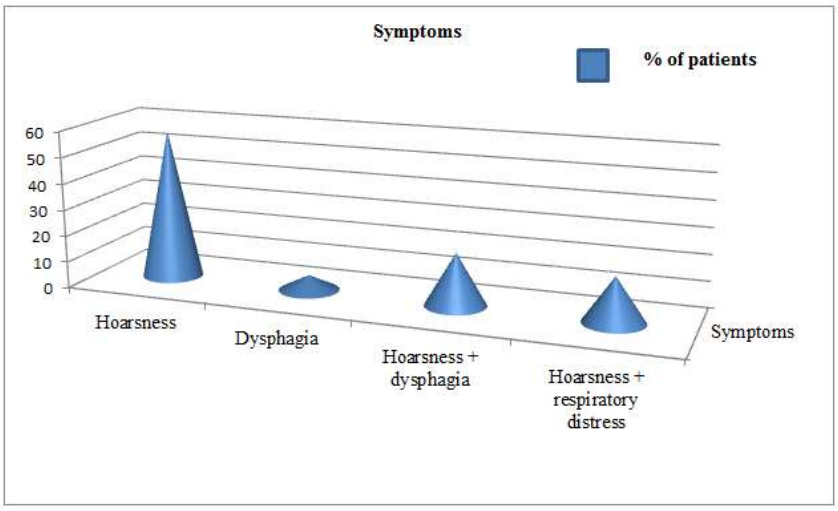
Graph-3(c): Distribution of sex with history of smoking among patients with nasopharyngeal mass

Inference- In the present study, 50% patients with nasopharyngeal mass have positive history of smoking, whereas, another 50% patients have no history of smoking



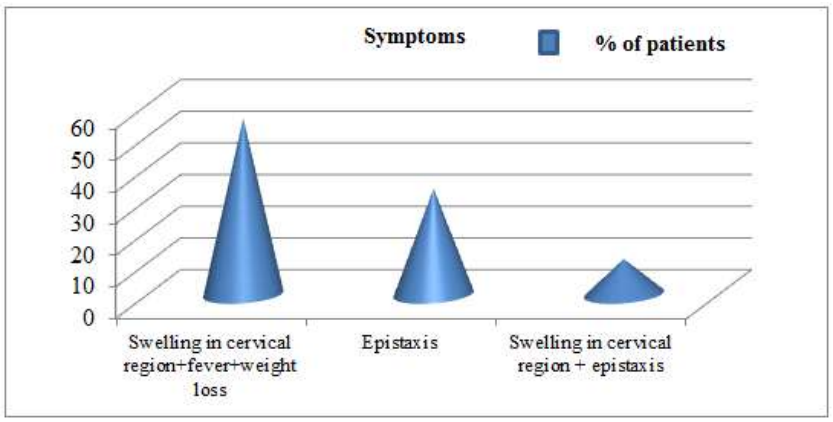
Graph-3(d): Distribution of sex with history of smoking among patients with nasopharyngeal mass

Inference- In this study, 66.7% male smoker patients have undifferentiated non-keratinizing squamous cell carcinoma and 44.44% male non-smoker patients have juvenile nasopharyngeal carcinoma.



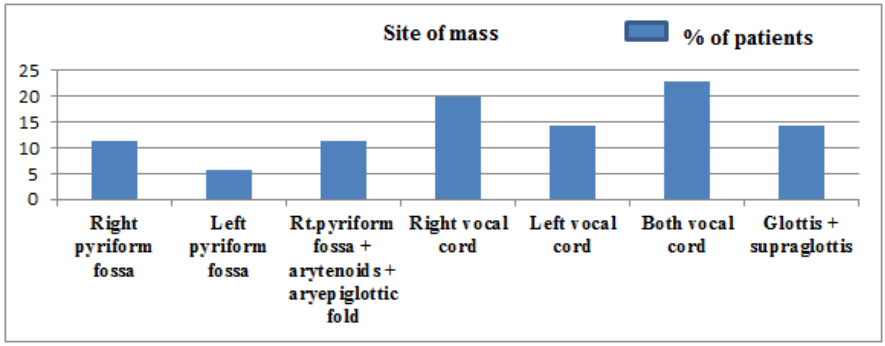
Graph-4(a): Distribution of symptoms among patients with laryngeal mass

Inference- Majority of patients with laryngeal mass was with the symptoms of hoarsness of voice.



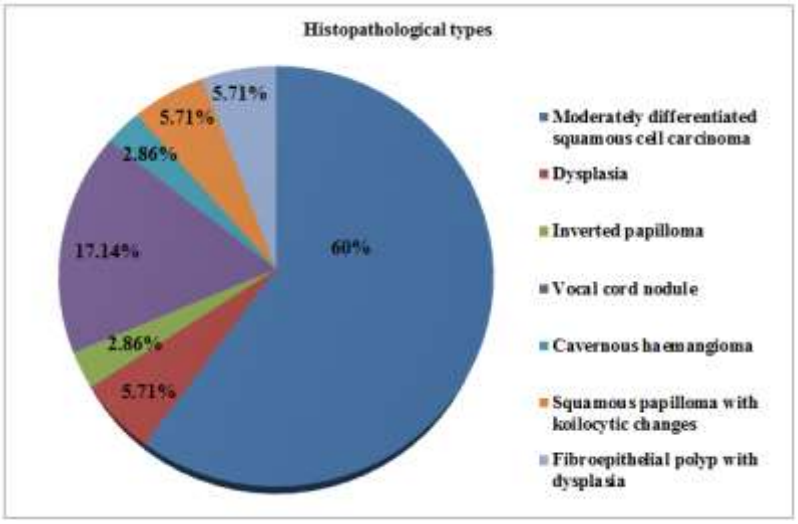
Graph-4(b): Distribution of symptoms among patients with nasopharyngeal mass

Inference- Majority of patients with nasopharyngeal mass was with the symptoms of swelling in cervical region, fever and weight loss.



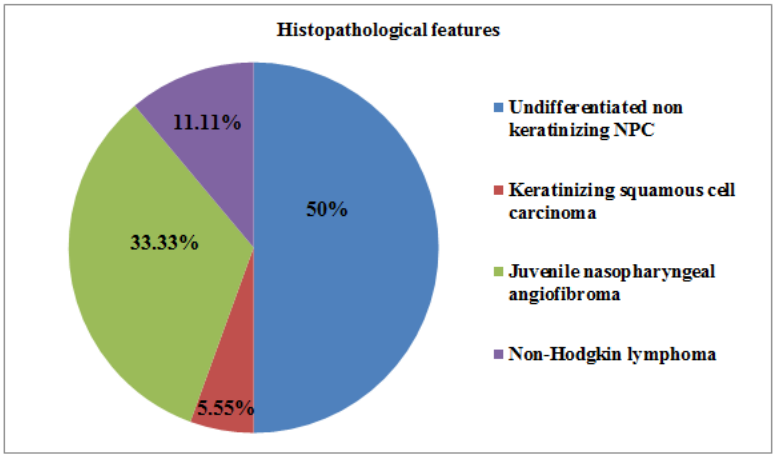
Graph-5: Distribution of site of mass by fibre optic laryngoscopic findings among patients with laryngeal mass

Inference- Majority of the laryngeal mass are located at vocal cord (22.86%), follow by right vocal cord (20%), left vocal cord (14.29%) and glottis and supraglottis (14.29%).



Graph-6(a): Distribution of histopathological types among patients with laryngeal mass

Inference- Majority of patients with laryngeal mass have moderately differentiated squamous cell carcinoma (60%) followed by vocal cord nodule (17.14%).



Graph-6(b): Distribution of histopathological types among patients with nasopharyngeal mass

Inference- Majority of patients with nasopharyngeal mass has undifferentiated non-keratinizing NPC (50%), followed by juvenile nasopharyngeal angiofibroma (33.33%).

DISCUSSION

35 patients with laryngeal mass and 18 patients with nasopharyngeal mass are included in the study during the period of one and half year and history, clinical examination, histopathological examination and PCR are performed to ascertain the association of EBV-LMP- 1 with different histopathological types.

In the present study of 35 patients with laryngeal mass, there are 32 (91.4%) males and 3 (8.6%) females and in patients with nasopharyngeal mass, there are 14 (77.78%) males and 4 (22.22%) female among 18 patients. Majority of patients with laryngeal mass (40%) are in the 51 to 60years and nasopharyngeal mass are 11-20 years (27.78%). A study by Nitin Arora et al (2017) showed that laryngeal cancers were more common in males (93%), than in female (7%) and it is most prevalent in age group of 51 to 60 years (45%) [7]. A study conducted by Hannu S et al with 302 (95%) males and 16 (5%) females showed that the mean age of men at the time of diagnosis was 62.8 years and that of women 60.8 years [8] Doloi, P.K. *et al*. showed incidence of vocal cord nodule more in male with a male-to-female ratio 4:1 [9]. Xie SH *et al*. showed that the overall male to female ratio of the annual age-standardized incidence rates of nasopharyngeal carcinoma ranged 2.2-3.1. The male to female ratio of NPC incidence increased with age until peaking at ages 55-59 years and a decline thereafter [10]. A study by Coutinho-camillo CM *et al*. in juvenile nasopharyngeal angiofibroma showed that adolescents and young adults between 14 and 25 years are affected, and there is a distinct male predominance [11].

In this present study, 62.86% patients with laryngeal mass have history of smoking. Among them, 72.7% male smoker patients have moderately differentiated squamous cell carcinoma, 9.09% male smokers have dysplasia, 4.55% male smokers have squamous papilloma with koilocytic changes, 4.55% male smoker patients have inverted papilloma 4.55% female smokers have vocal cord nodule, 4.55% male smoker patients have cavernous haemangioma. But 38.5% non-smoker male also have moderately differentiated squamous cell carcinoma, 15.4% non-smoker male have fibro epithelial polyp with dysplasia, 7.69% female nonsmoker patients have squamous papilloma with koilocytic changes, 30.8% non-smoker male and 7.69% female nonsmoker have vocal cord nodule. National Cancer Registry Program enlists laryngeal cancer as one of the tobacco-related cancer [12] Rao *et al.* found that biddi and cigarette smoking to be associated with cancer of the larynx.[13] Pyeko Menach *et al*. showed that 33 (66%) of the experimental group patients had a positive history of current cigarette smoking compared to controls (6%) and among patients who smoked and did not drink alcohol, 4(20%) had glottic cancer (p=0.001) with an OR of 19.75, which was statistically significant [14]. A study by Silvano Gallus *et al*., based on the largest published dataset on laryngeal cancer in women, confirms that tobacco smoking is the most important risk for women as for men and tobacco appears to have a greater role in women than in men[15]. Drasko Cikojevic *et al*. showed that the proportion of smokers was lowest in benign lesion group (72.13%) and highest in malignant lesion group (97.14%) and there was a statistically significant difference in the prevalence of smoking habit between patients with laryngeal tumours and those with benign or precancerous laryngeal lesion ($p< 0.001$)[16].

In the present study, half of the study population with nasopharyngeal mass have a positive history of smoking and another half(50%) have no history of smoking and 66.7% male smoker and 11.11% female smoker and 22.22% male non smoker patients have undifferentiated non keratinizing squamous cell carcinoma. 11.11% male non smoker has keratinizing squamous cell carcinoma and 22.22% female non smoker patient has non-Hodgkin lymphoma and 22.2% male smoker and 44.44% male non smoker patients have juvenile nasopharyngeal angiofibroma. A study by M Long *et al*. showed that significantly increased risk was only found among male smokers (OR 1.36), not among female smokers (OR 1.58) and significantly increased risk also existed in the differentiated (OR 2.34) and the undifferentiated type of NPC (OR 1.15)[17].

Common presenting symptoms in the present study in case of laryngeal mass are hoarsness in 57.14%, followed by hoarsness and dysphagia in 20% patients, hoarsness and respiratory distress in 17.14% patients, only dysphagia in 5.71% patients and in cases of nasopharyngeal angiofibroma, majority of the patients (55.56%) presented with swelling in cervical region, fever with weight loss, whereas, 33.33% patients have epitstaxis and 11.11% have swelling in cervical region and epistaxis. In 2016, Reddy DS et al showed that out of 50 cases of benign lesions of larynx, 34 (68%) were males and female were 16(32%) and all patients were presented with hoarsness of voice [18]. The symptoms of laryngeal cancer depend on the size and location of the tumour. Symptoms are-hoarsness or other voice changes, a lump in the neck, a sore throat or feeling that something stuck in the throat, persistent cough, stridor, bad breath, earache (referred), difficulty swallowing [19]. S. Marc Stokes *et al*. showed that nasopharyngeal angiofibroma presents as a nasal mass or obstruction o with repeated episodes of epistaxis [20]. Swelling of the lymph nodes in the neck is the initial presentation in many patients of nasopharyngeal carcinoma [21].

In this present study, most common site of laryngeal mass detected by fibre optic laryngoscopy is bilateral vocal cord (22.86%), followed by right vocal cord (20%), glottis and supraglottis (14.29%) and left vocal cord (14.29%), right pyriform fossa (11.43%),

right pyriform fossa with arytenoids and aryepiglottic fold (11.43%). Most laryngeal cancers originate in the glottis (true vocal cords, anterior and posterior commissures). Supraglottic cancers (epiglottis, arytenoids and aryepiglottic folds, and false cords) are less common, and subglottic tumours are least frequent [22]. Ayotunde James Fasunla *et al*. showed that transglottis (91.8%) was the most common anatomic tumor location [23]. Dinesh Kumar Sharma *et al.* showed that the majority of the benign tumours in the larynx arose from the glottic region (70%) followed by that of supraglottic region (25%) [24].

In this study, nasopharyngeal masses are detected by endoscopic biopsy and CECT Scan of head and neck and all the masses are located at fossa of Rossenmullar.

In this study, among the patients with laryngeal masses, 60% of them have moderately differentiated squamous cell carcinoma, 5.71% patients have dysplasia, 2.86% patients have inverted papilloma, 17.14% patients have vocal cord nodule, 2.86% patients with cavernous haemangioma, 5.71% patients have squamous papilloma with koilocytic changes and another 5.71% patients have fibro epithelial polyp with dysplasia. Similarly, Dinesh Kumar Sharma et al showed that squamous cell carcinoma was the most common malignant tumour detected in the larynx and in cases of malignant tumours 100% were squamous cell carcinoma [24]. Manish Sharma *et al*. have showed in their study that 32% patients have vocal cord nodule and 2% have haemangioma [25]. Aniket R. Buche *et al*. showed that 45% cases have vocal cord nodule and 5% patients have laryngeal papillomatosis among the patients with benign lesions of larynx [26].

In this study, among the patients with nasopharyngeal mass 50% patients have undifferentiated non keratinizing squamous cell carcinoma, 5.55% have keratinizing squamous cell carcinoma, 11.11% patients have non-Hodgkin lymphoma and rest 33.33% patients have juvenile nasopharyngeal angiofibroma. Rudresha Antapura Haleshappa et al showed that 84% cases had the WHO Type 3 histology while only 16% cases had the WHO Type 2 histology and none of the cases had well-differentiated keratinizing squamous cell carcinoma. Common symptoms at presentation were neck swelling in 80% cases and nasal obstruction and/or epistaxis in 28% cases [27].

In this study, EBV- LMP- 1 is not identified by conventional PCR method in any laryngeal or nasopharyngeal mass. Gok U *et al*. found that EBV DNA present in 11 patients (50%) with laryngeal carcinoma and in 7 patients (41.2%) with vocal cord nodule [28].

Togay Muderris *et al*. investigate the EBV with real time polymerase chain reaction in tumor tissue of 25 patients with laryngeal carcinoma and 17 patients with benign laryngeal lesions and showed that there was no significant difference between the control group and patient group in terms of EBV PCR positivity ($p>0.05$)[29].

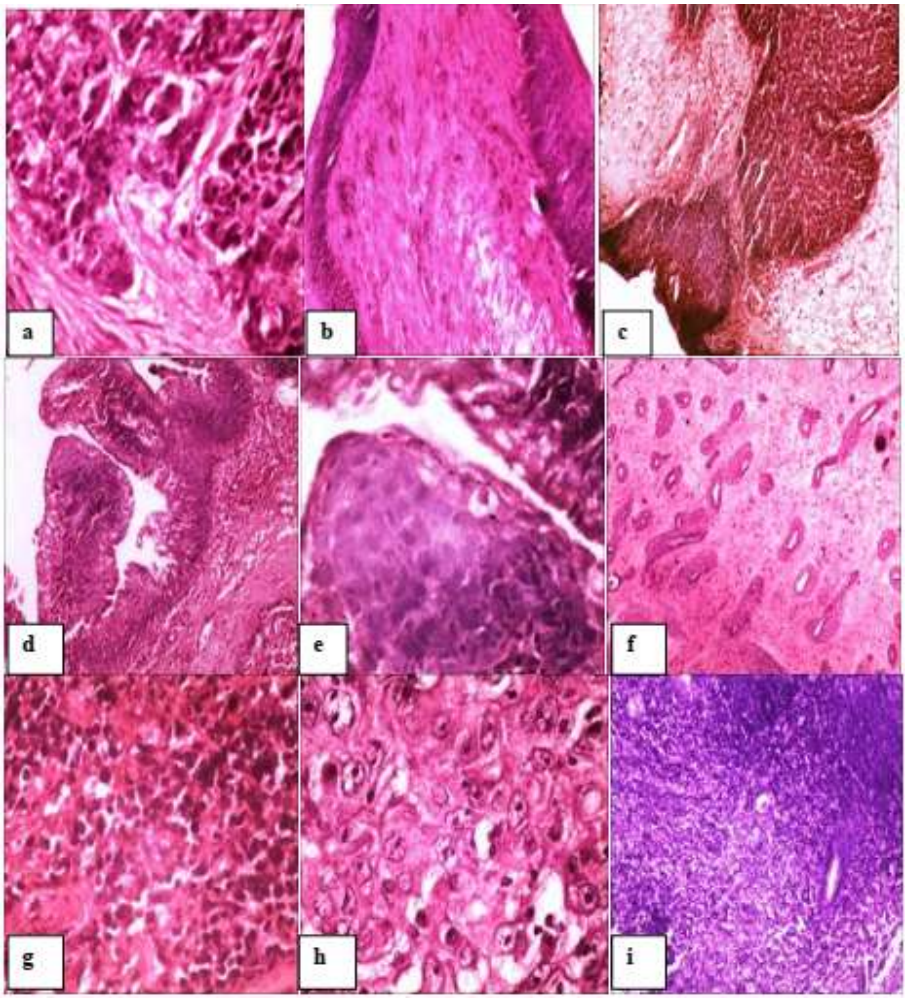
In their study, Mathew H. Stenmark *et al*. they take paraffin-embedded tumor specimens from 62 patients with primary NPC to analyze EBV using in situ hybridization for EBV encoded RNA and high-risk HPV with $p16^{INK4a}$ immunohistochemistry and multiplex PCR and micro array for determination of HPV types. They found that 43% cases were EBV positive/ HPV negative, 30% were EBV negative and HPV positive and 17% were both EBV and HPV negative. Non-keratinizing Type II and III NPC predominated among EBV positive tumors (100%) and HPV tumors (66.7%), while keratinizing Type I NPC was most prevalent among EBVand HPV negative [30].

Carlos R *et al*. have submitted 15 angiofibroma specimens to PCR for EBV, in situ hybridization was also employed for EBV. The PCR technique produced a false positive reaction in 5 cases, with all cases non-reactive with EBV-ISH [31].

Ali Edreis *et al*. was using same primer, that is used in this study for EBV LMP-1 and they showed that among 82 NPC tissue specimens, EBV DNA was identified in 51 samples (62.2%)[32].

CONCLUSION

Though this study is too small to conclude, conventional PCR may not enough to diagnose EBV LMP-1 from tissue and EBV LMP-1 may not be a leading cause of laryngeal or nasopharyngeal mass.



Photomicrograph : a) Moderately differentiated squamous cell carcinoma, H & E stain (40X), b) Vocal cord nodule, H & E stain (10X), c) Inverted papilloma, H& E stain (10X), d) Squamous papilloma with koilocytic changes, H & E stain (10X), e) Squamous papilloma with koilocytic changes, H & E stain (40X), f) Juvenile nasopharyngeal angiofibroma, H & E stain (4X), g) Undifferentiated non-keratinizing nasopharyngeal squamous cell carcinoma, H & E stain (40X), h) Undifferentiated non- keratinizing nasopharyngeal squamous cell carcinoma, H & E stain (40X), i) Non-Hodgkin lymphoma of nasopharynx, H & E stain (10X)

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