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The Prevalence of upper respiratory tract lesions in pathology department, Beni-Suef University Hospital
(Histopathological and immunohistochemical Evaluation)

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Abstract

The nasal cavity, paranasal sinuses, pharynx, and larynx form a complex upper respiratory tract (URT) system and are susceptible to infections, tumor-like conditions and true neoplastic conditions. The aim of this study is to detect the prevalence of URT lesions, to detect the prevalence of different lesions in different ages and sexes and to detect the etiology and possible high risk factors whenever possible. A total of 202 specimens of URT lesions were received at the pathology department, Beni-Suef University Hospital during the period from June 2017 to June 2021. All were evaluated histopatholgically. Of which, 59 were also evaluated immunohistochemically using anti-HPV Clone K1H8 antibody. Nasal polyp was the most prevalent lesion followed by vocal polyp. There was a significant relation of squamous metaplasia with nasal polyps and also there was a significant relation of dysplasia with vocal polyps indicating careful follow up after excision. There was no significant relation between HPV and different lesions with squamous morphology suggesting it as uncertain risk factor.

Keywords

Upper respiratory tract, Lesions, HPV, Immunohistochemistry.

1. Introduction:

The nasal cavity, paranasal sinuses, pharynx and larynx, all of which are connected, form a complex upper respiratory tract (URT) system. This region is made up of a variety of elements such as epithelial, glandular, lymphoid, cartilage and bone. It is also susceptible to infections, tumor-like conditions and true neoplastic conditions. Histopathological evaluation is mandatory to differentiate non-neoplastic lesions from neoplastic masses for effective care and proper management [1].

A variety of viruses, bacteria and fungi can cause URT lesions [2]. Infection with human papilloma virus (HPV) may cause both benign and malignant URT tumors in humans [3]. HPV infection has reached a considerable proportion worldwide, particularly among women, in whom it is the primary cause of cancer, making HPV a current public health priority. As a whole, the epidemiological distribution of HPV infection obviously varies around the world [4].

2. Materials and Methods:

A total of 202 specimens of patients with URT tract lesions were received at the Pathology department, Beni-Suef University Hospital during the period from June, 2017 to June, 2021

Archival formalin-fixed paraffin-embedded (FFPE) tissue blocks of all cases were collected and included in this study. Available well established pathological and clinical data as age and sex were included.

This study had some limitations due to low number of included cases as a result of closure of pathology laboratory at Beni-Suef University Hospital in the COVID era in 2020, because this hospital was designated to isolate corona patients. Also, there was insufficient clinical data including personal habits as smoking and alcohol consumption, medical history as diabetes mellitus and occupational risk factors.

2.1 Histopathological Evaluation:

Paraffin blocks of the lesions were sectioned at 4 µm thickness by rotatory microtome. Sections were stained with routine Hematoxylin and Eosin (H&E) stain for pathological re-evaluation. PAS special stain was used for detection of any suspected fungal infection.

2.2 Immunohistochemical Evaluation:

Immunohistochemical study was done to detect HPV infection. Since HPV prefers squamous epithelium [5], we chose 78 lesions that had squamous morphology for immunohistochemical study. Sections from only 59 lesions (17 nasal polyp s with squamous metaplasia, 7 inverted sinonasal papillomas, 18 vocal polyps, 2 sinonasal squamous cell carcinoma, 5 pharyngeal squamous cell carcinoma and 10 laryngeal squamous cell carcinoma) were mounted on positively charged slides and were stained by

ready to use, mouse anti-HPV monoclonal antibody Clone K1H8 that detects HPV subtypes 6, 11, 16, 18, 31, 33, 42, 51, 52, 56 and 58 (Thermo scientific, Cat. MS-1826-R7, Fremont, CA , USA). The remaining 19 cases were excluded due to bad processing or tiny tissue. Immunohistochemical staining was performed by standard autostaining protocols using Dako autostainer link 48. Parallel positive sections of positive HPV skin wart cases were used as external positive control for each set of slides.

2.3 Interpretation of HPV positivity:

HPV positivity was detected as brownish staining mainly in the nuclei of infected cells. Occasionally, the cytoplasm of infected cells was also observed to be immunoreactive [6]. Scoring of HPV immunohistochemical expression was not applied in our study because only 1 out of 59 studied patients with lesions showing squamous morphology was positive for HPV.

2.4 Slide examination and imaging:

Slides were examined by Olympus BX53 light microscope and images were captured using Leica digital pathology slide scanners (Aperio LV1 IVD).

Statistical analysis:

The collected data was coded then entered and was analyzed using the statistical package for the social sciences version 25 for windows (IBM Corp. Released 2017. Armonk, NY, USA).

Descriptive statistics for the demographic and pathological characteristics of cases were first analyzed. Age was expressed as mean and standard deviation (mean ± SD) then was categorized into 3 categories. All categorical data was expressed as number and percent. Associations between categorical variables were run by Chi-square test. P-value was considered significant at less than or equal 0.05. Graphs were used to illustrate simple information.

3. Results

Demographic data of the participants in our study are demonstrated in table (1). The age of the participants ranged from 2.5 to 83 years with a mean age of 40.8±17.6 years. More than half of them were in the age category from 21 to 50 years. One hundred thirteen cases (55.9%) were females and 89 cases (44.1%) were males with female to male ratio of 1.3:1.

As shown in table (2), the most commonly affected site in our study was nasal and sinonasal site 142(70.3%) followed by larynx 54 (26.7%) then pharynx 6(3%). The most prevalent lesion was nasal polyp 79(39.1%) followed by vocal polyps 32(15.8%). Only twenty eight out of 202 lesions were neoplastic (13.9%).

The most prevalent type of nasal polyps was allergic type 60 (75.9 %). Only 6 out of 20 cases of fungal rhinosinusitis were invasive forms. The incidence of recurrence was low

(5.4%) among patients with nasal polyps and vocal polyps.

Our study showed that there were 9 cases (8%) of the vocal polyps had dysplastic changes and 18 cases (16%) of the nasal polyps with squamous metaplasia as illustrated in table (3).

In this study, there was a significant association between older age categories and neoplastic lesions (p<0.001) (table 4). Figure (1) showed that there was a significant association between younger age categories and nasal polyps (p=0.042).

As illustrated in figure (2), the neoplastic lesions were significantly associated with male sex (p=0.009). This study demonstrated that vocal polyps and laryngeal squamous cell carcinoma were statistically related to male sex (p<0.001). Also, there was a significant association between fungal rhinosinusitis and female sex (p<0.001) (figure 3).

There was a significant association of squamous metaplasia with nasal polyps and also, there was a significant association of dysplasia with vocal polyps (p<0.001) (figure 4).

Our study illustrated that there was only 1 out of 59 lesions with squamous morphology showed positive HPV immunohistochemical expression (1.7%). The relation between HPV and different lesions with squamous morphology was non-significant (p=0.182) (figure 5).

Table (1) Demographic data of the participants (N =202):

Variables		**Values (%)**
Age (years)	Mean ± SD	40.8±17.6
	Range	2.5-83 years
	≤20 years	27 (13.3%)
	>20-50	107 (53.0%)
	>50	68 (33.7%)
Gender	Male	89 (44.1%)
	Female	113 (55.9%)

Table (2) Sites and histopathological features of the studied lesions:

Variables		Number (N=202)	Percent (%)
Site	Nasal and sinonasal lesions Pharyngeal Laryngeal	142 6 54	70.3 3.0 26.7
Nature	Non-neoplastic Neoplastic	174 28	86.1 13.9
Lesion histopathology	Acute rhinosinusitis Allergic rhinitis Chronic non-specific laryngitis Chronic rhinosinusitis Fungal rhinosinusitis Rhinoscleroma Nasal polyp Vocal polyp Inverted sinonasal papilloma Laryngeal squamous cell carcinoma Pharyngeal squamous cell carcinoma Sinonasal squamous cell carcinoma	4 5 10 11 20 13 79 32 8 12 6 2	2.0 2.5 5.0 5.4 9.9 6.4 39.1 15.8 4 5.9 3 1.0

Table (3) Histopathological epithelial changes among patients with nasal and vocal polyps:

Items		Number (N=111)	Percent
Histopathological epithelial changes	Absent With dysplasia (in Vocal Polyp) With squamous metaplasia (in Nasal Polyp)	84 9 18	75.7 8.1 16.2

Table (4) Relation between age and the nature of the lesion:

Nature	Age (years)			Total	p-value
	≤ 20	>20-50	> 50		
Non-neoplastic	27	97	50	174	<0.001
	100.0%	90.7%	73.5%	86.1%	
Neoplastic	0	10	18	28	
	0.0%	9.3%	26.5%	13.9%	
Total	27	107	68	202	
	100.0%	100.0%	100.0%	100.0%	

Figure (1) Relation between age and the lesion histopathology

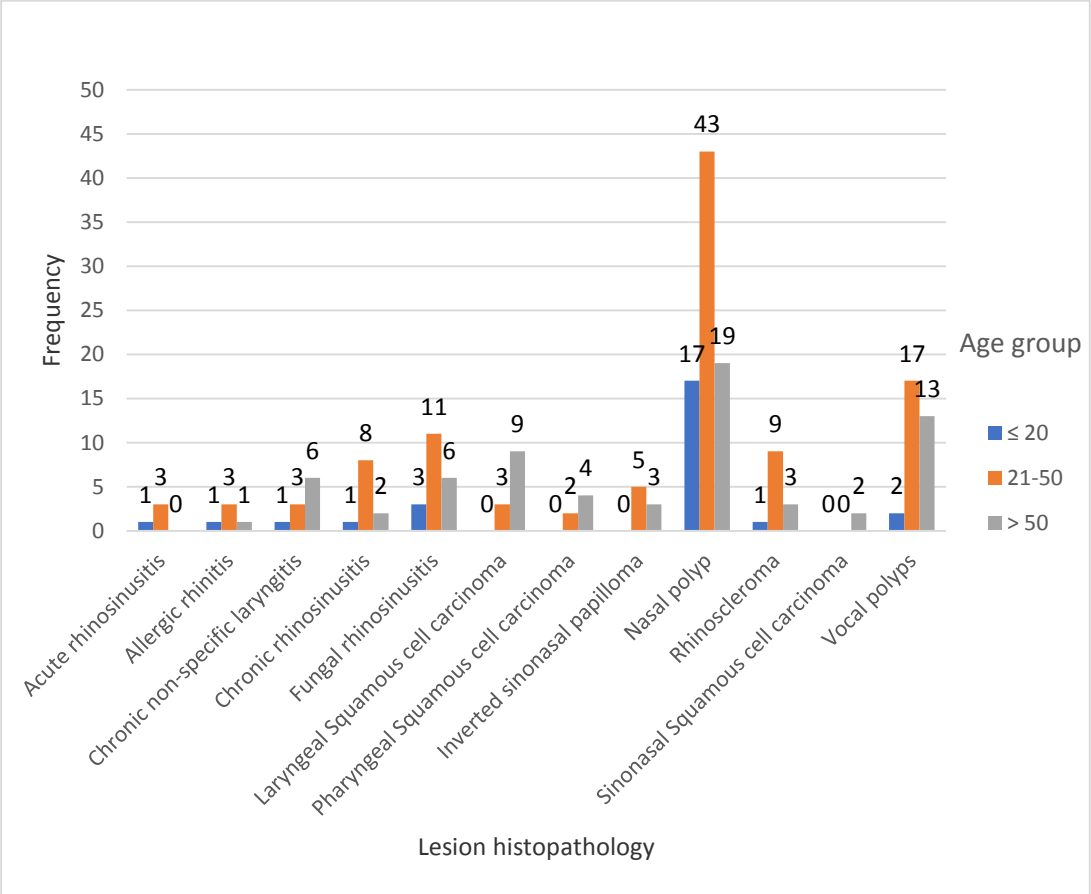


Figure (2) Relation between sex and the nature of the lesion

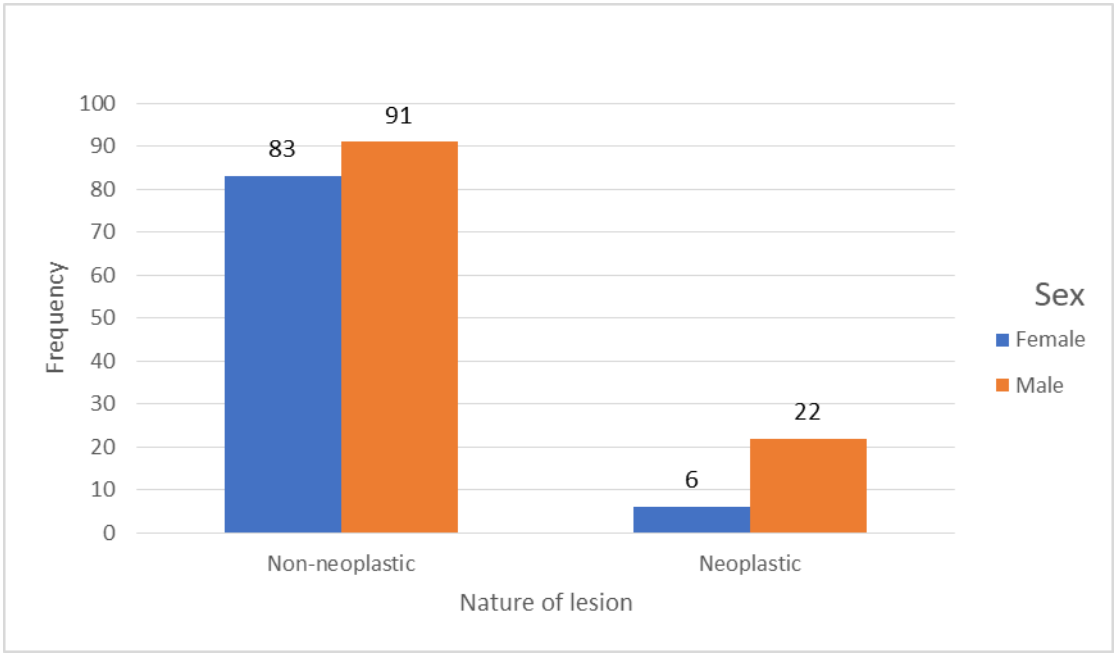


Figure (3) Relation between sex and the lesion histopathology

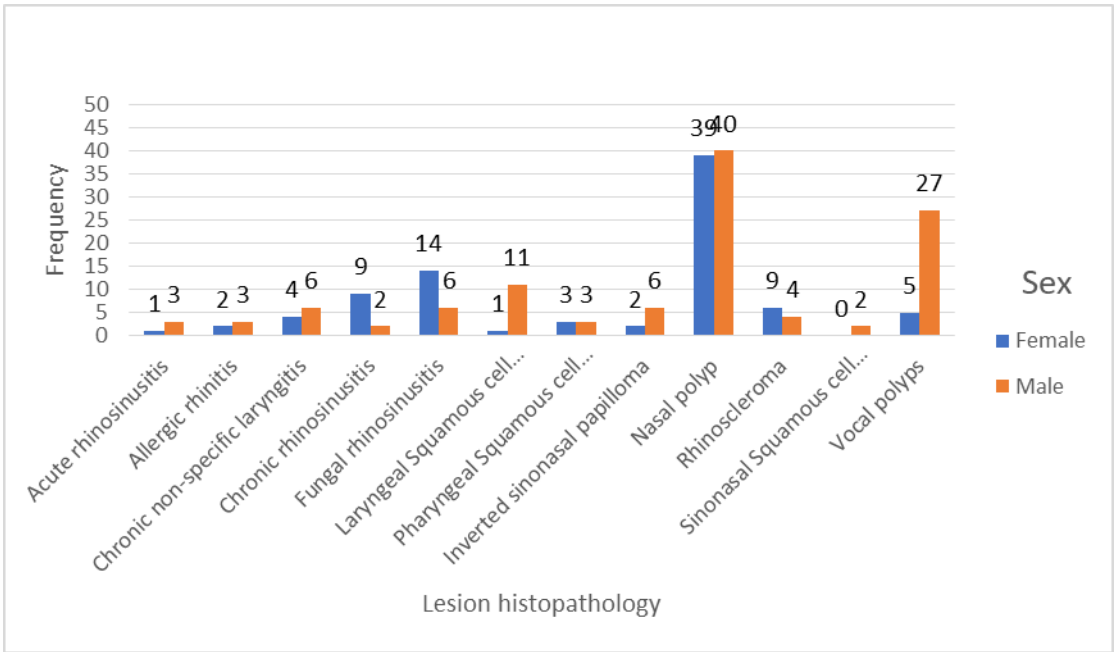


Figure (4) Relation between the type of polyp (nasal or vocal) and histopathological epithelial changes

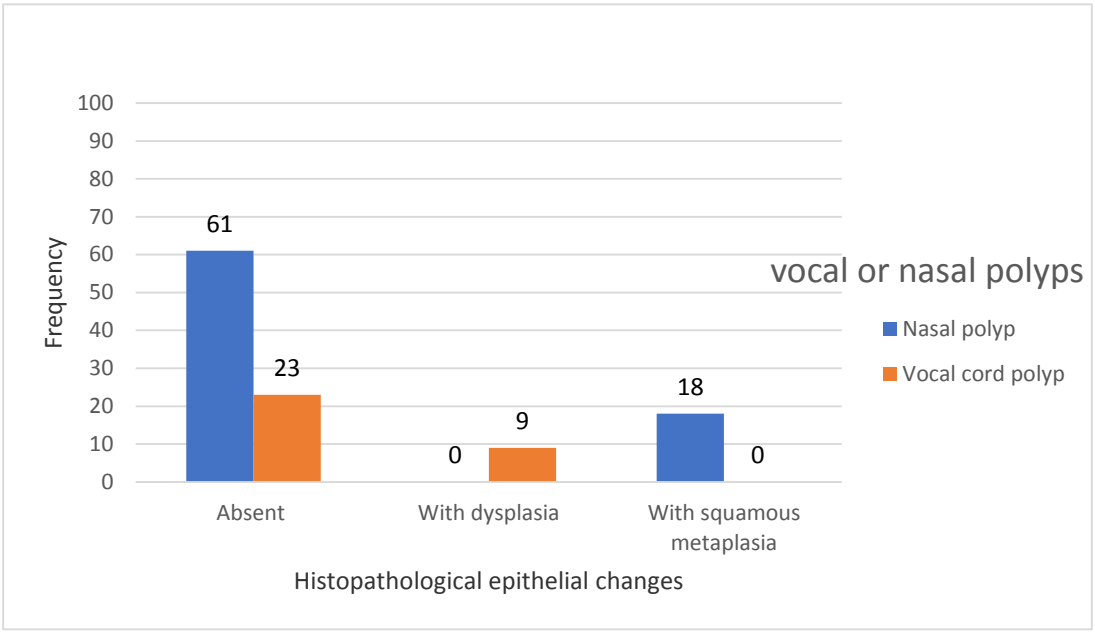
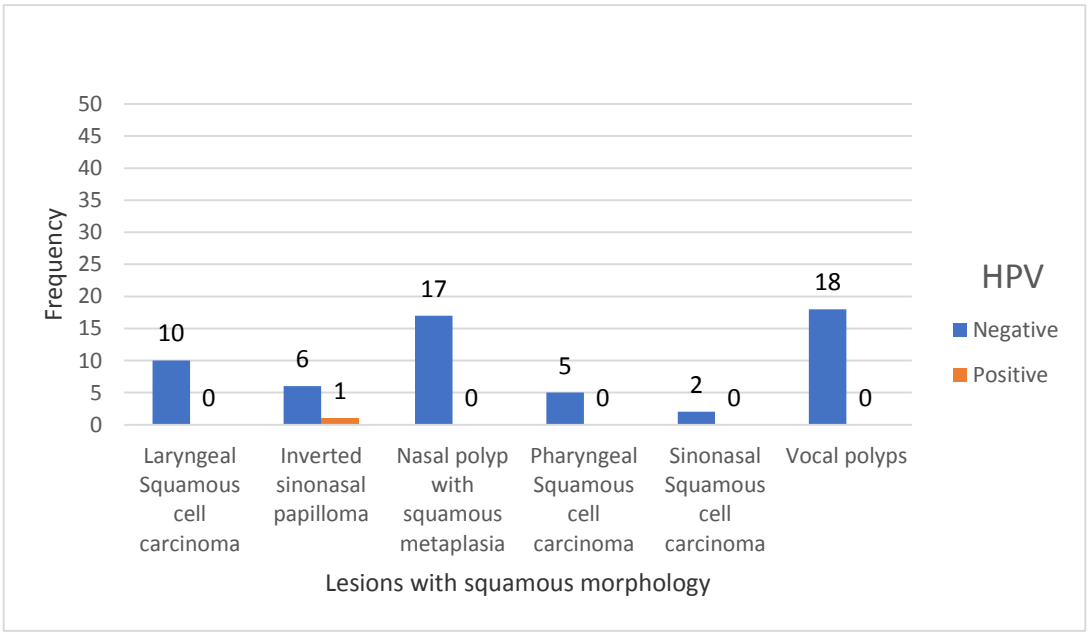


Figure (5) Relation between HPV immunohistochemical expression and lesions with squamous morphology



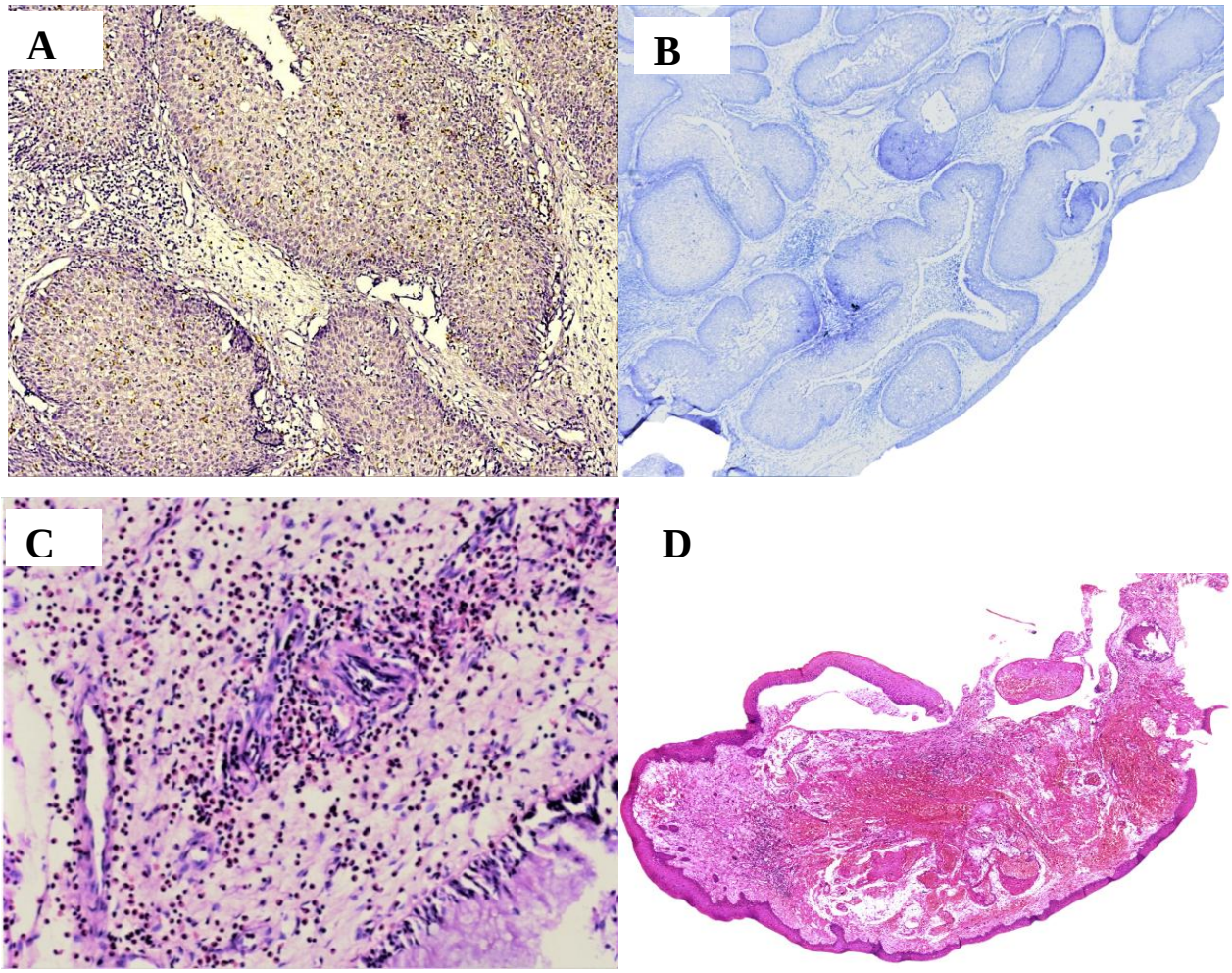


Figure A: Inverted sinonasal papilloma with endophytic growth of squamous epithelial nests shows positive HPV immunohistochemical expression (IHC, OM x100). **Figure B:** Inverted sinonasal papilloma with endophytic growth of squamous epithelial nests shows negative HPV immunohistochemical expression (IHC, OM x25). **Figure C:** Allergic nasal polyp is lined by respiratory epithelium. The subepithelium shows thick-walled blood vessels, marked edema and dense chronic inflammatory cell infiltrate with excess eosinophils (H&E stain, OM x200). **Figure D:** Vocal polyp is covered with stratified squamous epithelium (H&E stain, OM x25).

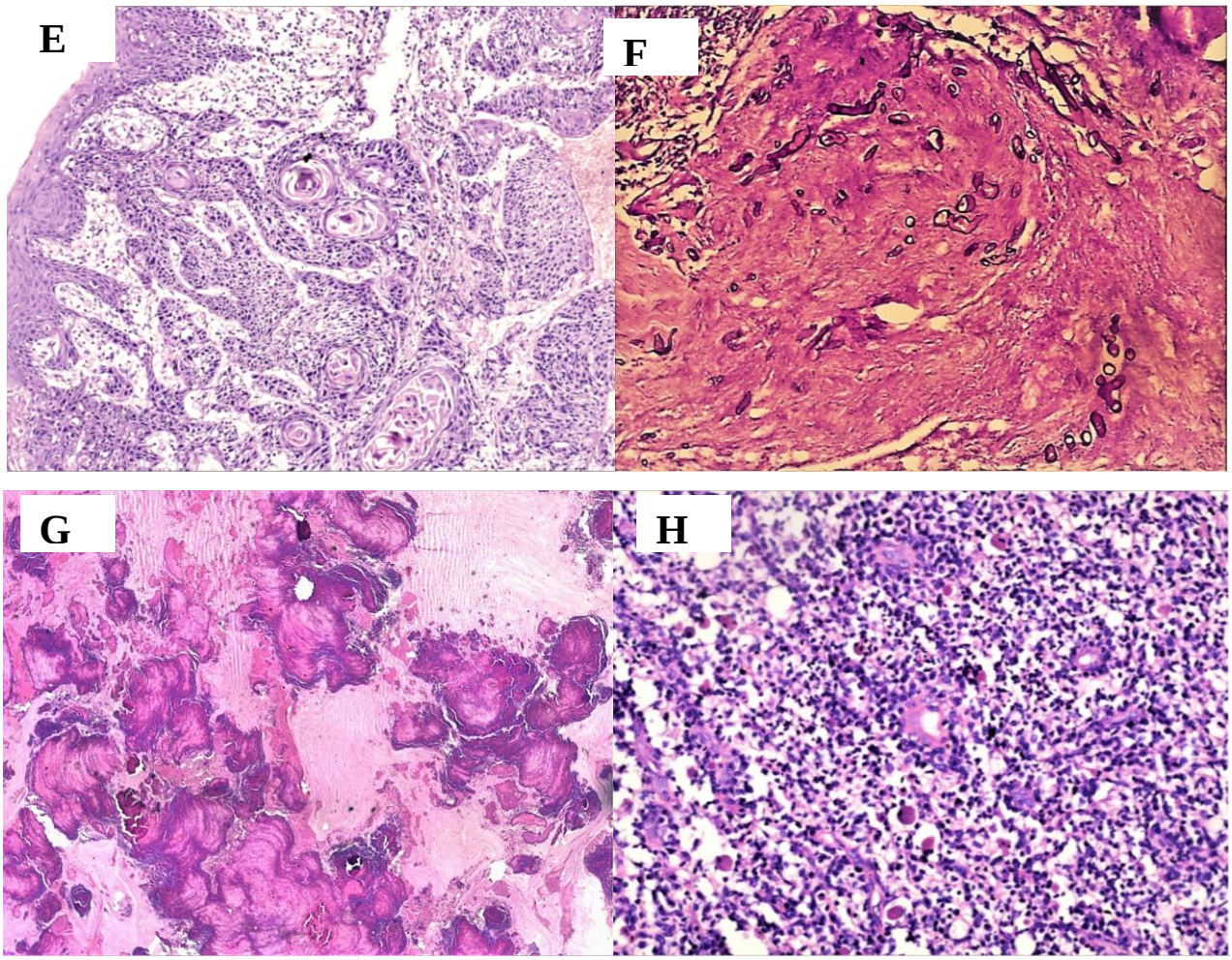


Figure E: Laryngeal invasive moderately differentiated keratinizing squamous cell carcinoma (H&E stain, OM x100). **Figure F:** Invasive fungal rhinosinusitis shows broad branching hyphae and spores highlighted by PAS special stain (OM x200). **Figure G:** Allergic fungal rhinosinusitis reveals eosinophilic mucin that contained epithelial debris, degranulated eosinophils and other inflammatory cells arranged in lamellar pattern (H&E stain, OM x25). **Figure H:** Rhinoscleroma reveals dense mixed inflammatory infiltrate including number of mikulicz cells (foamy macrophage), lymphocytes, plasma cells and Russell bodies (H&E stain, OM x200).

4. Discussion:

In this study, 202 patients with URT lesions were included. Our results revealed that 53% of patients were in the age group from 21-50 years with a female predilection (55.9%). The majority of lesions (70.3%) were located in sinonasal region, followed by larynx (26.7%). Among the studied lesions, only 13.9% were neoplastic. **Anbukkarasi et al.,** [1] studied 186 cases and reported findings close to ours but male sex was predominant. To our knowledge, no other researches have discussed these previous points to compare with our results.

Our study showed that the most prevalent type of nasal polyps was allergic type 60 cases (75.9 %). Parallel to our results, **Pushpalatha et al.,** [7] demonstrated that 55 out of 85 nasal polyps were allergic type (68.75%). Other studies showed variable results, **Jaison et al.,** [8] **and Khade et al.,** [9] found that non-allergic type was 28 out of 50 (56%) and 33 out of 41 (80.4%), respectively.

For fungal rhinosinusitis cases, 14 (70%) with non-invasive lesion and only 6 (30%) cases of fungal rhinosinusitis were invasive (all of them were COVID positive). Similarly, **Vandarkuzhali et al.,** [10]**, Waghray et al.,** [11]**,** and **Lupoi et al.,** [12]**,** found 16 out of 22 (72.7%), 45 out of 50 (90%) and 72 out of 74 (97.3%) of fungal rhinosinusitis with non-invasive lesion, respectively. **Sebastian et al.,** [13]**,** illustrated that invasive fungal rhinosinusitis affects immunocompromized patients, and COVID has a higher chance of developing invasive fungal infections, most likely due to its lower immunological competence.

Our study revealed a significant association between older age categories and neoplastic lesions ($p<0.001$). That was consistent with **Anbukkarasi et al.,** [1] who showed most of squamous cell carcinoma cases were more than 60 years old (80.6% of 31 patients).

As regarding the relation between younger age categories and nasal polyps, it was significant ($p=0.042$). Also, **Khade et al.,** [9]**,** reported 38.3% of 41 patients of nasal polyps belonged to 20-50 age group. However, **We et al.,** [14] found that increasing trend of nasal polyps prevalence with increasing age was statistically significant ($p\text{-value}< 0.001$).

The relation between male sex and neoplastic lesions, it was significant ($p=0.009$).On the same side, **Anbukkarasi et al.,** [1] showed that out of 31 cases, the maximum occurrence of tumor was noted in males, 26 cases (83.9%). **Rettig et al.,** [15] illustrated that males have a higher incidence of neoplastic lesions than females likely due to higher rates of substance abuse, particularly tobacco use, among men.

Also, there was a significant association between male sex and vocal polyps and laryngeal squamous cell carcinoma ($p<0.001$). Similar results were shown by **Sharma et al.,** [16] and **Ma et al.,** [17] who demonstrated that 96.66% and 90% of 30 and 110 patients with laryngeal squamous cell carcinoma were males, respectively. Also, it was related to results obtained by **Malik et al.,** [18] who reported that among 28 cases of vocal polyps, males were 67.86% and females were 32.14%. On the other hand, **Martins et al.,** [19] showed that 57% of vocal polyps were female. These differences could be explained by lifestyle variations.

In this study, the association between fungal rhinosinusitis and female sex was significant ($p<0.001$). Matching with our results, **Lupoi et al.,** [12] demonstrated in a study of 74 patients, the incidence among females was

more than double than that in males. While, **Vandarkuzhali et al.,** [10] and **Waghray et al.,** [11] found that fungal rhinosinusitis was higher among males, 16 out of 22 cases (72.7%) and 35 out of 50 cases (70%), respectively.

By studying histopathological epithelial changes of nasal polyps and vocal polyps, there were 9 cases of the vocal polyps had dysplastic changes (7 of them were low grade and 2 of which were high grade) and 18 of the nasal polyps showed squamous metaplasia. There was a significant association of squamous metaplasia with nasal polyps and also, vocal polyps were significantly associated with dysplasia ($p<0.001$). That was consistent with **Effat et al.,** [20] who found dysplasia in 35% of 15 smokers with vocal polyps. Cigarette smoking is major risk factor for laryngeal dysplasia development. **Jiang et al.,** [21] illustrated an increase of squamous metaplasia in nasal polyps over time may be due to the potential link between tissue inflammation and epithelial damage. Also, **Perić et al.,** [22] detected the histopathological findings of epithelial atypia in 44 out of 212 (20.7%) of nasal polyps. While, **Nunes et al.,** [23] reported no dysplasia in 75 patients with vocal polyps and **Yang et al.,** [24] reported that dysplasia in vocal polyp was only 5% of 1115 patients.

Our study showed that most of cases with nasal polyps and vocal polyps were not recurrent 105 (94.6%) and only 6 cases were recurrent (5.4%). Within the same track, **Delwar et al.,** [25] demonstrated 3.7% recurrence (2/54) in vocal polyps. However, **Lee et al.,** [26] showed 22.22% of 18 vocal polyps were recurrent. Other studies by **Calus et al.,** [27], **Sarhan et al.,** [28] and **Zhao et al.,** [29] illustrated higher incidence of recurrent nasal polyps among 38, 100 and 85 patients (78.9%, 29% and 45.9%), respectively. Lower rate of recurrence of polyps may be due to proper treatment.

In our study, the relation between HPV and different lesions with squamous morphology (n=59) was not significant. One case of inverted sinonasal papilloma showed positive HPV expression. Parallel to our results, **Sham et al.,** [30] and **Wang et al.,** [31] illustrated 4.1% and 6.2% of 73 and 46 patients with inverted sinonasal papilloma were HPV positive, respectively. **Hoffmann et al.,** [32] showed that all 20 specimens derived from nasal polyps (100%) were HPV-negative. **Giacomini et al.,** [3] reported that 100% of 8 patients with LPs were HPV negative. **Takahashi et al.,** [33] found only 6 of 64 sinonasal squamous cell carcinoma (9.4 %) cases to be positive for high risk HPV. **Xu et al.,** [34] demonstrated in a study of 674 patients with LC in China, HPV infection prevalence was only 4.9% in laryngeal squamous cell carcinoma patients. **Jansen et al.,** [35] found that the combined effects of tobacco use and alcohol consumption were major risk factors, responsible for 70–80% of

pharyngeal carcinoma of the total study patients (n=2382) in the United States.

Other studies showed variable results; **Roh et al.,** [36] demonstrated 39.9% of 54 patients with inverted sinonasal papilloma were HPV positive. **Knör et al.,** [37] and **Jaiswal et al.,** [38] reported 15.1% and 45% out of 166 and 60 nasal polyps were HPV positive, respectively. **Iravani et al.,** [39] demonstrated HPV positive in 23.07% of 20 patients with vocal polyps. **Lewis et al.,** [40] and **Kılıç et al.,** [41] showed HPV was positive in 22.8% and 31.7% of 171 patients and 770 patients with sinonasal squamous cell carcinoma, respectively. **Nakagawa et al.,** [42] illustrated 22 out of 70 pharyngeal squamous cell carcinomas were positive (31.42%). **Abdulsamad et al.,** [43] demonstrated HPV positive in 29.42% of 20 patients with laryngeal squamous cell carcinoma. We could explain these differences in the prevalence of HPV by life style (as oral sexual behaviors) and environmental variations.

5. Conclusion and Recommendations:

Our findings revealed that The URT lesions were more common in females in the age category from 21 to 50 years. Nasal polyp was the most prevalent lesion followed by vocal polyps. Nasal polyps are more common in younger age. Older age group was most commonly affected by neoplastic lesions. Male sex was more commonly affected by neoplastic lesions, vocal polyps and laryngeal squamous cell carcinoma. While female sex was more commonly affected by fungal rhinosinusitis. Higher proportion of nasal polyps and vocal polyps showed squamous metaplasia and dysplasia, respectively indicating careful follow up after excision. There was no significant relation between HPV and different lesions with squamous morphology suggesting it as uncertain risk factor. Further larger studies are recommended to investigate other risk factors as personal habits like smoking and alcohol consumption, medical history like diabetes mellitus, occupational, genetic, environmental or infectious rather than HPV including COVID and others.

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