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Assessing The Role of Human Papillomavirus (Types 6 And 11) In Tumorigenesis and Its Relationship to P53 Tumor Suppressor Gene Protein Expression in A Series of Benign and Malignant Laryngeal Tumors.

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ABSTRACT:

Background: Laryngeal benign and malignant neoplasia have a complex etiology. The two most significant ones are drinking and smoking. Laryngeal cancer has been linked to mucosal high-risk oncogenic Human Papillomavirus (hrHPV) types. Additionally, it has been shown that low-risk HPV (lrHPV) varieties are frequently found in head and neck malignancies. The etiology and carcinogenesis of various HPV types have been the subject of numerous investigations.

Objectives: To evaluate a potential role of lrHPV6/11 in the pathogenesis and/or tumorigenesis of the tissues of laryngeal benign and malignant tumors by examining the co-expression of low-risk HPV 6/11 gene products and tumor suppressor gene protein (p53) expressions.

Methods: Using chromogenic in situ hybridization for screening and immunohistochemistry for evaluating p53 overexpression, a retrospective study was carried out on 100 and 57 tissue block specimens with various laryngeal tumors to identify and genotype for screening and lrHPV6/11 by molecular technique.

Results: The polyps group had a higher percentage of positive signal HPV6/11/p53 intensities (4:44.4%) than the malignant (5:33.3%), nodules (2:28.6%), and seemingly healthy (1:9.1%) groups. In the malignant and polyp groups, the high positive signal percentage of HPV6/11/P53 score was moderate (++) at 2:100% and 1:33%, respectively. There were notable differences in the positive signal percentage of HPV6/11/P53 low score between the nodules group (1:100%) and the polyps group (1:33.3%).

Conclusion: The co-expression of lrHPV6/11DNA and p53 cannot trigger tumorigenesis, but it facilitate the aggregation of somatic mutagenesis subordinate to elevated DNA-damage and inhibition of cellular pathway for DNA damage response mechanism (cDDR) , therefore , co-infection of lrHPV type 6 as well as HPV11 with elevated level of p53 in benign and malignant laryngeal tumors could reflect a possible role in pathogenesis and tumorigenesis of potentially precancerous lesions

INTRODUCTION:

Laryngeal tumors are abnormal growths on vocal cords or surrounding tissues, classified broadly into benign (non-cancerous) and malignant (cancerous). Both of them typically present with voice change and persistent hoarseness However, malignant tumors are life-threatening, carry a high risk of spreading, and require urgent medical evaluation (1,2). Laryngeal cancer holds

the 2ed in the category of the emergence of malignant neoplasms in the neck and head, comprising roughly one –5% in all types systemic carcinomas observed in Otorhinolaryngology (3).

The causative role of laryngeal malignant and benign neoplasia is multifactorial. Laryngeal tumors can result from the combined action of smoking, drinking, gastro- esophageal reflux, occupational exposure to toxic fumes, air pollution and infection with various type of viral infection (4,5,6). Although the involvement of viruses in laryngeal tumors has been thoroughly investigated, contradictory results have surfaced, depending on the population group and the methods used. About 20 years ago, HPV infection was discovered in mucosal laryngeal tissues. It is unknown how much of the virus is present in normal population epithelium (7). It has been determined that HPV viruses may be a risk factor for the development of a subset of head and neck squamous cell carcinoma (SCCHN), particularly those of the oropharynx (8). Human papillomaviruses are tiny, non-enveloped viruses having a circular, double-stranded DNA genome that is about 8000 bp long. Over 250 entire papilloma genomes have been identified. HPV16, 18, 33, and 53 are the most common of the 15 "high-oncogenic risk" genotypes that are carcinogenic, while the remaining genotypes are low risk (lr) HPV genotypes that cause non-cancerous diseases.Notably, the most common genotypes of the human papillomavirus (HPV) are 6 and 11 (9.10,11).Usually, benign laryngeal papillomas rather than malignant tumors are caused by lr HPV, particularly HPV6 and HPV11. However, in 1% to 4% of instances, a rare neoplastic change into squamous cell carcinoma (SCC) might happen (12). It has been demonstrated that HPVs operate as a promoting factor in the multistep process of laryngeal squamous cell carcinoma (LSCC) formation; the activity of two oncogenic viral proteins, E6 and E7, is the main cause of virus-associated carcinogenesis. By binding to the ubiquitin-ligase enzyme (E6-AP), these proteins have the capacity to promote the breakdown of the p53 protein. This inhibits p53-dependent signaling pathways, which become unregulated after cellular homeostasis is lost, ultimately contributing to the development of laryngeal squamous cell carcinoma (13). Elevated levels of p53 have been observed in cases with HNSCC. It has been indicated that p53 gene abnormalities may be indicative of a relatively poor clinical outcome, or esophageal SCC. Furthermore, expression of the mutated form of p53 gene has been previously found precancerous lesions within upper respiratory and digestive tracts, suggesting that p53 gene abnormalities represent a very early event during pathophysiology of head and neck SCC (14,15).

Laryngeal Papilloma (LP)also known as recurrent respiratory papillomatosis or glottic papilloma is a rare medical condition. It leads to the development of various tumors or papilloma over time; without treatment, this can pose a life-threatening risk, as these uncontrolled growths can obstruct the airway (16). Laryngeal papilloma might be results from infection with human papillomavirus (HPV) has been documented especially type 6 and type 11 which enhance hyperplasia growth and formation different shapes of benign tumors in the larynx .The exact molecular basis that responsible of tumorigenesis steps in LP has yet to be uncovered .Vocal cord polyps are the more common .It is frequently grown in men who have a history of excessive voice use and chain smoking; Polyps and nodules are pedunculated lesions which may be unilateral or bilateral that are morphologically similar to the epithelial lining of the larynx . These lesions frequently appear on the inferior vocal cords and could exhibit distinct microvascular features (17,18). So, the presence study represents an important contribution to understanding the potential role of low risk HPV6,11 in pathogenesis and tumorigenesis of laryngeal malignant and benign tumors in Iraq.

STUDY MATERIAL AND METHODS:

Histological specimens:

157 laryngeal histology specimens (formalin fixed and paraffin embedded, or FFPE) from patients having laryngectomy or endoscopic biopsy were used in this study, which was conducted as a retrospective analysis. The samples included 45 cases of laryngeal cancer (carcinoma), 72 cases of benign laryngeal tumors, including 35 polyps and 37 nodules, and 40 specimens taken during autopsies that, upon postmortem histopathological examination, appeared to be healthy tissue, effectively acting as a seemingly healthy control group.

Patients with laryngeal tumors ranged from two-82 years. Archived records obtained from histopathology laboratories of various hospitals and medical centers comprising: Al-Hariri hospital, Medical city, Al-Kindy teaching hospital, teaching AL-Kadhemiya hospitals, Al-Yarmok hospital, along with records archived in privately owned laboratories. Biopsy specimens of histologically normal- appearing laryngeal tissues that are suspected free from significant pathological changes were collected from the archival records Forensic Medicine of the Institute in Baghdad province between January 2024 and January

2026.Diagnoses were based on the patients recorded histopathological reports, following a confirmatory re-examination of each tissue specimen by a specialist histopathologist.

STUDY METHODOLOGY:

Human Papilloma virus DNA (HPV-DNA) was detected molecularly and genotypically for blocks of laryngeal tissues using an advanced chromogenic in situ hybridization (CISH) method (ZytoVision GmbH, Germany). Digoxigenin-labeled long DNA probes (T-1144-400; ZytoVision GmbH, Bremerhaven Kit, Germany) were used to target particular DNA sequences in tissue samples in order to detect the following genotypes of HPV: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82). Concurrently, low-risk HPV6/11 genotypes were identified using particular DNA probes tagged with digoxigenin [(T-1055-400), ZyoVision GmbH., Bremerhaven, Germany]. In the Laboratory Research Facilities at the Communicable Diseases Unit of the College of Medicine in Baghdad, the (CISH) analysis was carried out in accordance with the manufacturer's instructions. Digoxigenin-labeled housekeeping gene-specific probes were substituted to induce reactive staining. With the exception of the diluted probe, which was left out of the negative control solution, all reagents were added.In the context of the screening HPV test, the positive signals [(T-1151-40: ZytoFast Plus Implementation kit AP-Permanent red)] were seen as bright red staining with red permanent solution and counterstaining with hematoxylin, while the signals of CISH HPV6/11 [(ZytoFast Plus Implementation kitHRP-green:T-1073-40)] were seen as bright green staining with counterstaining with nuclear signals in the complementary sequence sites. A light microscope was used to quantitatively evaluate in situ hybridization signals for different molecular markers at 10× and 40× magnifications. Positive cells were counted at 1000× magnification. The percentage of positive signals and staining intensity scores, which indicate the number of positive cells and the intensity of the reaction, respectively, were used to assess the in-situ hybridization signals. The intensity scores ranged from no staining to high staining. Ten randomly chosen microscopic fields with 100 cells each were used to count the positive cells. Cases were then categorized into one of the following three percentage-based scoring categories: [Score (1) =1-25%, Score (2)=26-50%, Score (3)>50%] (19). This was done by calculating the mean proportion of affirmative cells throughout the ten fields. To assess p53 protein overexpression in different laryngeal malignancies, immunohistochemical (IHC) labeling was used. To assess p53 protein overexpression in different laryngeal cancers, immunohistochemical labeling was used. The HRP/DAB-based Exposure detection system for mouse and rabbit antibodies (Abcam, product no. ab80436) in combination with a monoclonal antibody against p53 (clone Pab240) [product no. ab26] were used for staining operations in accordance with the manufacturer's protocol. Nuclear and cytoplasmic p53 staining intensity was assessed and classified as either strong or missing. The percentage of cells showing positive reactivity was used to calculate the staining scores. Samples with weak to moderate nuclear staining received a score of +1 (10–75%), samples with strong nuclear staining received a score of +2 (>75%), and samples with no nuclear staining received a score of 0. The statistical significance of the discrepancies between the variables under study was evaluated using chi-square tests, independent-samples t-tests, and analysis of variance (ANOVA).

RESULTS:

1-distribution of age and sex among study groups:

In terms of the correlation between age and the study groups, the findings showed that all groups' ages ranged from 2 to 82 years. The control group had the lowest mean age (27.25 ± 12.15 years; range: 2–66 years), while the group of patients with laryngeal malignant tumors had the greatest mean age (56.91 ± 17.12 years; range: 8–82 years). A statistical study showed that the groups' mean ages differed significantly ($p < 0.05$) (Figure 1). Gender-wise, males (110, 43.43 ± 19.91 years) had a greater mean age than females (47, 39.68 ± 19.25 years) (Figure 2); however, this difference was not statistically significant ($p > 0.05$).

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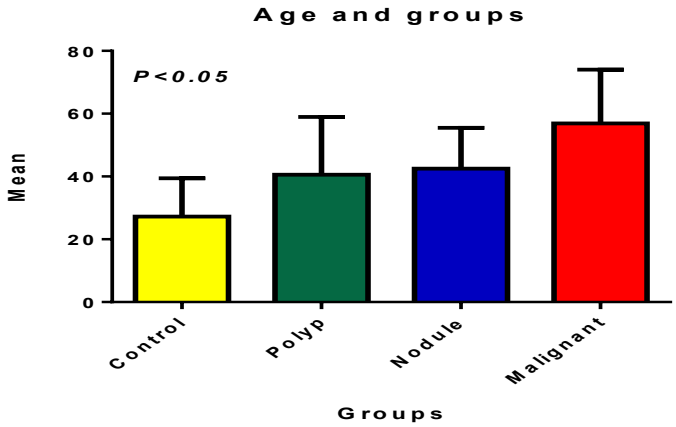

Figure 1 . Distribution of laryngeal tumor tissues in relation to mean of age among study groups .

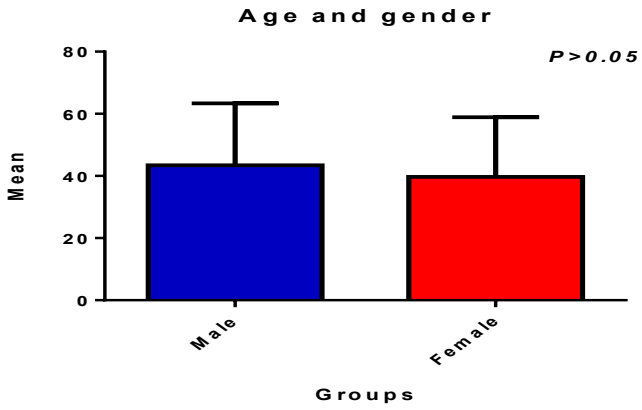

Figure2. Distribution of laryngeal tumor tissues in relation to sex among study groups.

From figure (3) noted that the site of specimen collection were elevated percentage of laryngeal tumors (%) within malignant patients was left vocal cord (18: 40%) while other groups were right vocal cord Nodules (17:45.9%) and Polyps (16: 45.7%) larger than the others specimen [Nodules (6, 16.2%), Polyps (10, 28.6%) and malignant patients (15, 33.3%)]. NS: Not statistically significant (P > 0.05).

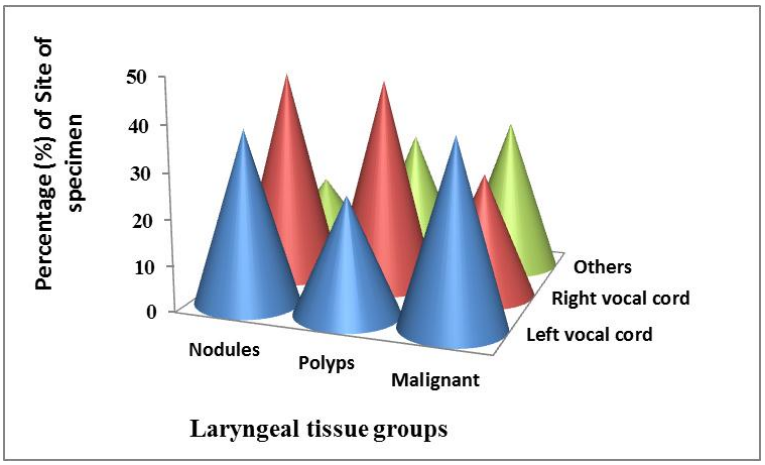

Figure3. Distribution of laryngeal tumor tissues according to anatomical site of specimen

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2- Low risk HPV6/11DNA-CISH /p53-IHC:

a. Lr HPV6/11DNA-CISH /P53 protein-IHC intensities

The prevalence of lrHPV6/11DNA positive infected cells with strong staining signal intensities of were noted in 5 cases (33.3%) while no staining signal were detected in 22(73.3%) cases with laryngeal malignant tumors. In the laryngeal polyp group, strong staining intensity for (lrHPV 6/11) DNA/p53protien -IHC expression was recorded in 4 cases (44.4%), whereas no staining signal was found in 22 cases (84.6%). In the laryngeal nodule group, strong positive staining intensity was noted in two cases (28.6%), while no staining signal was observed in 25 cases (83.3%). In apparently healthy individuals (control group), strong positive staining intensity was observed in one case (9.1%), while no staining signal was detected in 29 cases (100%) regarding lrHPV 6/11 DNA/p53-IHC. Statistical analysis revealed no significant differences among the studied groups P (> 0.05) as in table 1.

Table 1. Comparative Analysis of HPV6/11 DNA-ISH Signal Intensity and p53-IHC Expression Intensity across the Studied Groups.

Diagnostic categories				P53 stain intensity		χ^2 test (P-value)
				NO stain	Strong stain	
Non- tumor controls	HPV6 / 11 stain intensity	NO stain	No	29	10	NS* P > 0.05 (P = 0.103)
			%	100 %	90.9 %	
		Strong stain	No	0	1	
			%	0.0 %	9.1 %	
	Total		No	29	11	
			%	100 %	100 %	
Benign Nodules	HPV6 / 11 stain intensity	NO stain	No	25	5	NS* P > 0.05 (P = 0.496)
			%	83.3 %	71.4 %	
		Strong stain	No	5	2	
			%	16.7 %	28.6 %	
	Total		No	30	7	
			%	100 %	100 %	
Benign Polyps	HPV6 / 11 stain intensity	NO stain	No	22	5	NS* P > 0.05 (P = 0.074)
			%	84.6 %	55.6 %	
		Strong stain	No	4	4	
			%	15.4 %	44.4 %	
	Total		No	26	9	
			%	100 %	100 %	
Laryngeal Malignancy	HPV6 / 11 stain intensity	NO stain	No	22	10	NS* P > 0.05 (P = 0.642)
			%	73.3 %	66.7 %	
		Strong stain	No	8	5	

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			%	26.7 %	33.3 %	
	Total		No	30	15	
			%	100 %	100 %	

S* = Significant, NS*=non-significant

b. LrHPV6/11DNA/p53 protein -IHC scores:

Among laryngeal malignancy cases, the expression signal of positive staining score of HPV6/11DNA -infected cells and Positive p53 immunoreactivity was observed 2 cases (100.0%) and 1 case (7.1%), respectively, showing moderate score (++) and low score (+) staining, respectively. Conversely, absent staining was exhibit in 21 cases (72.4%). Within benign polyp's tissue cases demonstrated positive lrHPV6/11 DNA–p53 IHC staining, with moderate score (++) staining noted in 1 case (33.3%) and low score (+) staining signal in 2 cases (33.3%), while absent staining was recorded in 22 cases (84.6%). laryngeal benign nodules, positive moderate score (++) and low score (+) staining for lrHPV6/11 DNA–p53 IHC signal were identified in 1 case (16.7%) and 1 case (100.0%), respectively, whereas negative staining was recorded in 25 cases (83.3%).

Finally, regarding non-tumor control tissues which appeared normal upon gross examination low-score staining (+) for low-risk human papillomavirus (lrHPV6/11) DNA and p53 protein was observed in one case (25%), whereas absent staining results were recorded in 29 cases (100%). Statistical analysis revealed significant differences among the studied groups ($P < 0.05$), with the exception of the laryngeal malignancy group, where the difference was not statistically significant, as shown in table 2.

Table 2. Comparative Analysis of HPV6/11 DNA-ISH Signal score and p53-IHC Expression score across the Studied Groups.

Diagnostic categories				P53 stain scores			χ^2 test (P-value)
				(-) Absent	+	+ +	
Non- tumor controls	HPV6 / 11 scores	(-) Absent	No	29	7	3	S* (P<0.05) P = 0.011
			%	100 %	100 %	75 %	
		+	No	0	0	1	
			%	0.0%	0.0%	25.0%	
	Total		No	29	7	4	
			%	100 %	100 %	100 %	
Benign Nodules	HPV6 / 11 scores	(-) Absent	No	25	5	0	S* P < 0.05 (P = 0.016)
			%	83.3%	83.3%	0.0%	
		+	No	2	0	1	
			%	6.7%	0.0%	100 %	
		++	No	3	1	0	
			%	10.0%	16.7%	0.0%	
	Total		No	30	6	1	
			%	100 %	100 %	100 %	

Benign Polyps	HPV6 / 11 scores	(-) Absent	No	22	3	2	S.* P < 0.05 (P = 0.027)
			%	84.6%	50.0%	66.7%	
		+	No	0	2	0	
			%	0.0%	33.3%	0.0%	
		++	No	4	1	1	
			%	15.4%	16.7%	33.3%	
	Total		No	26	6	3	
			%	100 %	100 %	100 %	
Laryngeal Malignancy	HPV6 / 11 scores	(-) Absent	No	21	11	0	NS* P>0.05 (P = 0.126)
			%	72.4%	78.6%	0.0%	
		+	No	1	1	0	
			%	3.4%	7.1%	0.0%	
		++	No	7	2	2	
			%	24.1%	14.3%	100%	
	Total		No	29	14	2	
			%	100 %	100 %	100 %	

S* = Significant, NS*=non-significant, (-) =Negative signal, (+) =Positive signal

DISCUSSION:

People of all ages are susceptible to laryngeal tumors (21). Approximately 4.5% of all malignant tumors are laryngeal carcinoma, one of the most prevalent malignant neoplasms of the upper respiratory tract (22). Hoarseness is one of the most prevalent symptoms in otolaryngology, and it can be caused by a variety of underlying laryngeal disorders, ranging from benign, treatable conditions like vocal cord nodules, polyps, and chronic laryngitis to potentially fatal cancers, especially squamous cell carcinoma of the larynx (23).

One of the biggest issues facing global health is viral infections. It is responsible for about 15% of cancer cases globally. In this regard, the human papillomavirus (HPV) is especially noteworthy because it is known to cause cervical cancer; around 70% of cases are caused by high-risk genotypes (hrHPV), mainly HPV16 and HPV18. (24). Thus, low-risk HPV has been found in many cases of benign and malignant head and neck cancers (25).

According to the literature that is currently available, this study is the first in Iraq to look at the relationship between lrHPV6/11 gene products and p53 protein expressions using CISH and IHC, respectively, for archived (formalin-fixed, paraffin-embedded, or FFPE) pathological tissue samples from both benign (nodules and polyps' growths) and malignant tumors of the larynx.

1- Age Distribution among Patients with Benign and Malignant Laryngeal Tumors :

The patients in this study ranged in age from 2 to 82 years, as shown in Figure 1. When compared to patients with benign laryngeal lesions, such as polyps and nodules, whose mean age was primarily dispersed between the 40–49 age group, the mean age of patients with malignant laryngeal tumors was (56.91 ± 17.12) years, indicating a highly significant difference. The results of this study are consistent with those of De Silva et al. (2019), who found that patients with laryngeal cancer ranged in age from 8 to 84 years (26).The findings were consistent with the Shalatah et al., 2022 study, which found that the average age of laryngeal cancer was 58.93±11.18 and that the age range was 31–80 (27). In contrast, Xiao et al.'s 2019 study, which looked at the disease burden and risk factor trends among middle-aged and older adults (28), found that the incidence of laryngeal cancer

increased steadily with age, especially in the 50–90 age group and among both sexes between 1990 and 2021 (28).Oga et al. (2016) found that Nigerian patients with head and neck cancers, especially laryngeal carcinoma, had a mean age of 43.3 years, which contradicts the aforementioned research assertion.(29)

A follow-up research by Çobanoglu et al. (2026) on patients who had surgery for benign laryngeal tumors and non-cancerous vocal cord lesions revealed that the mean age was often between 35 and 48 years.(30).

The present investigation is consistent with the 4.5-year descriptive study carried out by Sachdeva et al. (2022), which comprised 36 patients whose mean age was 42 years and who underwent laryngoscopy and histological exams that revealed benign laryngeal lesions (31). In contrast to the current investigation, another clinical study by Pawan et al. (2009) found that individuals between the ages of 21 and 30 had the highest prevalence of benign laryngeal tumors (32). Additionally, the majority of patients with laryngeal benign lesions, precisely 28 patients (35%), fell between the 21–30 age category, according to Hiremath's (2025) study, which closely matched our data.In 53 cases, vocal abuse was found to be a prevalent predisposing factor. The most common symptoms were hoarseness or voice alteration (affecting 82.5% of the total, or 66 cases), Vocal cord polyps were the most frequently observed benign lesion, recorded in 17 cases (21.25%) (33).

A possible link between age and the development of laryngeal cancer has been suggested by earlier research showing differences in the age distribution of individuals with various laryngeal tumor types. This could be explained by long-term, ongoing exposure to risk factors like alcohol consumption and smoking, as well as environmental carcinogenic agents such radiation, chemicals, and viruses, all of which are major causes of laryngeal cancer (34). Changes in cellular DNA, which can be regarded as another important factor in the development of such malignant tumors, may be influenced by the marked impairment in the immune response seen in these age groups due to the cancer-associated decline in host defense mechanisms (Immunosenescence) (35).

While benign tumors are most commonly caused by abuse of the voice or exposure to chemicals and viruses, gastroesophageal reflux disorder (GERD) can produce laryngeal hyperplasia and neoplasia in addition to heartburn and an increased risk of esophageal cancer (36). In terms of genetic disorders, those who have inherited DNA repair errors and certain genetic syndromes are at a very high risk of developing head and neck malignancies, particularly laryngeal and throat cancers. Diseases like dyskeratosis congenita and Fanconi anemia demonstrate how these mutations hinder the body's capacity to repair DNA damage, putting patients at risk for early-onset cancers (37).

2- Sex Distribution among Patients with Benign and Malignant Laryngeal Tumors:

As depicted in figure .2, Male patients with benign and malignant laryngeal tumors constituted a larger proportion of the study population compared to female patients, accounting for 110 cases (43.43%) and 47 cases (39.68%) respectively without any significance differences ($p > 0.05$). These sex-related findings align with the results of numerous studies as in (38,39,40,41). Although the precise reasons underlying the differences in the incidence of benign and malignant laryngeal tumors between males and females remain unclear, the higher incidence observed among males may be associated with greater exposure to established risk factors, particularly cigarette smoking, water pipe smoking as well as high levels of alcohol intake. These risk factors are more prevalent among men and may also be associated with repeated exposure to inhaled irritants in occupational and domestic environments, as well as with nutritional deficiencies (42). Certain occupations predominantly held by men distinct from those typically pursued by women may pose a risk factor for laryngeal tumors (whether malignant or benign). This risk arises from prolonged, high-level exposure to substances such as chemical agents, wood dust and paint vapors associated with th petroleum, plastic, metal and textile manufacturing sector agents like asbestos that may also increase the risk of developing tumors of the larynx and hypopharynx.(43)

3- Distribution of laryngeal tumor tissues according to anatomical site of specimen:

Figure .3 shows that the most common site for laryngeal tumors among patients with malignant tumors was the left vocal cord (18 cases; 40%). In contrast, the other groups comprising those with nodules (17 cases; 45.9%) and polyps (16 cases; 45.7%) showed higher rates of involvement on the right vocal cord compared to other sites [nodules (6 cases; 16.2%), polyps (10 cases; 28.6%), and malignant tumor patients (15 cases;33.3%)].Statically , there wasn't significant differences .

There is no established medical rule or consistent trend indicating that laryngeal cancer occurs more frequently on the left vocal cord; tumors arise on either the left or right true vocal cord based on relation to individual risk factors including ; tobacco and alcohol intake rather than a biological predisposition toward a specific side (17). In regard to the current results , While Joos

etal.,(2009) demonstrated that the laryngeal carcinomas lesion was found to be firmly attached to the right true vocal cord(44). The current results of this investigation are close agreement with those reported by Kumar e*tal* , (2015) who demonstrated that patients with laryngeal cancer cases were predominantly located on the left side of larynx(45) In reference to laryngeal benign tumors, the most elevated percentage of laryngeal right vocal cord site of nodules and polyps were higher 17(45.9%) and 16(45.7%) respectively than the percentage of their left vocal cord site and others of nodules and polyps.The current results align with the findings of Idu-Filho et al. (2013), who found that the proportion of benign laryngeal lesions (polyps and nodules) was higher on the right vocal fold (61.9%) compared to the left vocal fold (34.92%) and other laryngeal sites (3.17%)(46). Additionally, a study by Divi et al. (2011) found that the highest proportion of benign laryngeal lesions occurred on the right vocal fold(47).In contrast, Singhal et al. (2009) found that benign laryngeal lesions were more frequently located on the left vocal fold (44%) than in other laryngeal regions (40% and 16%)(48).

The incidence rate of laryngeal lesions varies according to the anatomical sites particularly those situated on true vocal cord. The reported predominant anatomical sites of laryngeal cancer has varied across different studies. Tumor progression in the vocal cords may be influenced by their distinctive anatomy and relatively limited lymphatic outflow compared with other laryngeal regions.These anatomical characteristics may restrict the metastatic potential of early-stage lesions. The intrinsic elastic layer of the larynx may also affect the direction of tumor spread, with lesions originating from the free edge of the vocal cords potentially extending into the underlying vocalis muscle and paraglottic space. Progressive invasion of the deeper tissues can also result in varying degrees of vocal cord mobility impairment, ranging from mild mucosal stiffness to complete vocal cord fixation. Also specimen collection and other histological and physiological factors such as paralysis that occurs in vocal cord from nerves origin or the infection with many bacteriological and virological agents that encouraging dysplasia and hyperplasia of these sites in addition to the nutritional and mechanical behavior of swollen (49,50) .

4- Detection of lrHPV6/11DNA-ISH / P53-IHC in laryngeal neoplasms :

This longitudinal study employs a repeated-measures design for detecting the DNA of low-risk HPV types 6 and 11, in addition to evaluate tumor suppressor gene p53 expression across different study groups , in order to assess whether the co-presence of the virus disrupts the host cell cycle and assess potential role of investigated HPV genotypes in pathogenesis and tumorigenesis of laryngeal lesions during the early stages of their development. The present study results showed high percentage of HPV6/11/p53 intensities among polyps group(4:44.4%) more than malignant (5:33.3%),nodules (2:28.6%)and apparently healthy groups(1:9.1%) .while high positive percentage of HPV6/11/P53 moderate(++)score in malignant and polyps groups was (2:100%) and (1:33%)respectively . The positive percentage of HPV6/11/P53 low score was in nodules group(1:100%) and polyps group (1:33.3%)respectively with significant differences . The results showed variations in staining intensity and grading (+1 versus +2) for low-risk human papillomavirus (lrHPV6/11) and p53 protein, with a high proportion (exceeding 70%) of negative results (absence of signal) regarding intensity and score observed across the study groups. In regard to HPV6/11 DNA , the negative HPV6/11DNA-CISH percentage indicates that virus DNA of HPV6/11 not integrated with host cell genome and has not disrupted it. This results consist with many studies (51,52,53). One proposed explanation is that the DNA of low-risk human papillomaviruses (lrHPV) persists as independent, circular DNA molecules (extrachromosomal) within laryngeal hyperplastic tissues. Unlike high-risk types such as HPV16, which frequently integrate into the host cell genome during malignant transformation a fact established by numerous studies (54). In general, the cellular transformation mechanisms triggered by human papillomaviruses rely on the E6 as well as E7 gene products . These proteins performed a wide range of functions and engage in numerous interactions . The specific oncogenic HPVgenotypes can interact with host cell proteins . The oncoproteins E6 together with E7 have been demonstrated to impair molecular regulatory pathways governing cellular proliferation through interference with the activity of two major tumor suppressor proteins, p53 and retinoblastoma protein (pRb). Efficiently lr HPV types promote epithelial cell proliferation and generate substantial numbers of progeny virions. The E6 along with E7 proteins of low-risk HPV play pivotal roles in the viral life cycle; however, they exhibit considerably limited activity in driving cellular transformation and their association with genomic instability is less pronounced (55). The E7 protein of low-risk human papillomavirus (HPV) exhibits limited affinity for retinoblastoma protein (pRb) and does not stimulate its degradation. Also , E6 protein of lrHPV types display limited affinity for p53 and have a markedly reduced ability to mediate its degradation. Moreover, E6 proteins from low-risk HPV types are not generally associated with the activation of telomerase activity. On other hand ,the elevated percentage of intensity / score of HPV6/11 DNA in some cases of malignant cases (5:33.3%/2:100%(++),1:7.1%(+))and in polyps cases (4:44%/1:33.3(++),2:33%) ,while in nodules cases the positive signal for intensity /scoring was (2:28.6%/1:100%).This co-expression of (lrHPV6/11)DNA and the p53 exhibits

limited capacity to promote neoplastic progression, however, this facilitates acquisition of somatic mutations resulting from an elevated burden of DNA damage and the inhibition of the cellular DNA damage response (DDR) at the end increasing p53 level in infected host cell with these low risk genotypes(56). Numerous cases involving low-risk genotypes specifically types 6 as well as 11 have also been documented in context of head and neck malignant and benign tumors including laryngeal lesions(57). Other explanation , Under certain conditions, lr(HPV) may be implicated in cellular transformation.It may be plausible that under specific biological circumstances , cellular transformation may occur as a result of low risk HPV types . This issue is of particular importance because low risk genotypes share specific traits with high risk ones particularly within E7 gene region which in turn influences cellular behavior . E7 proteins derived from low-risk HPV types, including HPV6 and HPV11, have been reported to synergize with the Ras oncogene in the transformation of primary baby rat kidney (BRK) cells, although they exhibit substantially reduced transforming efficiency and lower pRb-binding affinity compared with E7 proteins of high-risk HPV types, particularly HPV16 (58.59).Researchers proposed that concurrent viral coinfections may exert a synergistic pathogenic effect, thereby augmenting cellular hyperproliferation and pre-neoplastic mutations or accelerating malignant transformation(60).Coinfection with lrHPV can enhance viral persistence and exacerbate benign epithelial lesions (e.g., condylomata acuminata) through shared mechanisms of immune evasion. The presence of HPV6, HPV11, or both has been reported in 50–100% of recurrent respiratory papillomatosis (RRP) cases . Whereas , The development of squamous cell carcinoma (SCC) represents a rare but clinically significant complication of recurrent respiratory papillomatosis (RRP). Previous studies have reported malignant transformation rates of approximately 3–6% among patients with adult-onset RRP, while this complication is exceedingly uncommon in juvenile-onset disease, with reported rates of less than 1%. Despite the predominant association of RRP with low-risk HPV types 6 and 11, progression to malignancy may occur under the influence of additional predisposing factors (61,62).However, lrHPV6/11 types completely lack the oncogenic potential to drive malignant transformation, a process that typically requires latent high-risk HPV types infection (53).

Regarding p53 test results, observing an absent or low p53 protein expression (a negative result) in the study groups is to be expected; In normal conditions , DNA of lrHPV6/11 does not integrate into the host cell chromosome Furthermore, the p53 protein has a short half-life (approximately 30 to 50 minutes), rendering it undetectable via immunohistochemistry . It can be inferred cases with negative p53-IHC among laryngeal tumors subjects in present study cohort accounting for over 72.4% of the total may result (aside from technical failures) from a biallelic deletion of the TP53 gene, a marked reduction in p53 protein levels (whether mutated or wild-type), p53 harboring non-sense mutation or the presence of a N-terminally truncated p53 protein that evades detection by the monoclonal antibody used in the p53 assay(63). Other potential explanations exist: the p53 protein might be present sporadically in a small fraction of cells; it could be mutated in a much larger number of cells but accumulate to detectable levels in only a small subset; or the detected p53 protein might be wild-type but undergo abnormal stabilization or exist at elevated levels(64).

While increased signal intensity and score of p53 protein levels, this finding warrants further interpretation and analysis. The current study reveals the presence of both the mutant and wild-type (normal) forms of human p53 protein, as the monoclonal antibody used for detection interacts with both variants. The p53 increases half-life to 6–8 hours when a mutation occurs .The results reveal elevated levels of mutant p53 protein expression in certain cases of benign and malignant tumors study groups coinciding with reduced transcription levels of the wild-type p53 gene within transforming cells a phenomenon associated with the process of malignant tumorigenesis. In contrast, the findings indicate that benign tumor cells and normal tissues exhibit either no DNA damage or only low levels there of levels insufficient to trigger an increase in p53 gene transcription(65,66). To more explanation , more than 1,000 mutations in p53 gene have been identified in connection with human tumors therefore, it must demonstrate mutations that affect p53. Two distinct forms of mutations involving the p53 gene have been identified : null mutations as well as non-null mutations. Notably, the mutant p53 protein (resulting from non-null mutations can be detected using immunohistochemistry (IHC)) exhibits prolonged stability with preferential nuclear accumulation tumor cells. In contrast , missense mutations(null) may result in complete loss of p53 immunoreactivity (cannot be detected by IHC) lead to the production of a truncated (shortened) and unstable p53 protein(67). Theoretically, it should be possible to distinguish between tumors showing negative p53 immunohistochemistry results (null mutations), those showing low levels (wild-type), and those showing overexpression (non-null mutations). Consequently, some researchers consider low p53 levels a favorable prognostic indicator, while others view them as unfavorable; this discrepancy depends on the relative proportions of null mutations versus wild-type cases within the low-p53 group(68). Numerous studies have been assessed the clinical relevance of p53 overexpression in patients with laryngeal malignancies . Also, Variations in p53 protein expression levels across studies

may be attributed to fundamental methodological and biological variables, including sample size, tumor heterogeneity, and the sensitivity of detection assays(66,69) .Although instances of HPV DNA integration (specifically types 6 and 11) have been documented in cases of juvenile laryngeal papillomatosis such as in the study by (Rahmoun etal.,2024) this aligns with the findings of the current study(70). We observed positive test results for low-risk HPV DNA (types 6 and 11) alongside elevated p53 protein expression. This accumulation of protein within the cell indicates the onset of genomic instability, abnormal cell cycle alterations, the initiation of pathological processes, and tumor development driven by the accumulation of mutations especially missense mutations that could occur in these positive cases among study groups(56) . This phenomenon that may be associated with impaired p53 –mediated tumor suppressive function, the acquisition of oncogenic activity , or the capacity for inappropriate p53 activation; all these factors lead to severe dysregulation of cell proliferation and growth. Mutant p53 is known to form tetramers complexes with wild-type p53, thereby exerting a dominant-negative effects that compromises normal p53 –mediated cellular functions likely through conformational disruption of the p53 DNA-binding region (or in other cellular proteins), thereby reducing the ability to activate or repress target genes (67,71,72). Another potential explanation lies in the fact that the p53 protein engages in an MDM2-dependent negative feedback mechanism, ensuring precise regulation of their respective levels within the cell; however, the loss of transcriptional activity can lead to reduced Mdm2 levels, resulting in the stabilization and increased abundance of the mutant p53 protein regardless of whether the mutant has lost its original function or acquired a new one(73,74).

CONCLUSION

The co-expression of lrHPV6/11DNA and p53 possessed limited tumorigenic potential but may indirectly contribute to the acquisition of somatic mutations by promoting DNA damage and impairing the cellular DNA damage response mechanisms. Therefore co- infection of lrHPV6 as well as lrHPV11 with elevated level of p53 in benign and malignant laryngeal tumors could reflect a possible role in pathogenesis and tumorigenesis of certain precancerous lesions. However, further investigations with larger cohorts are warranted to accurately assess the prevalence and genotype distribution of high-risk oncogenic HPV within these tissues enhancing the robustness and clinical significance of the results.

REFERENCES:

[1] Yadagiri Goud ,P. and Sruthi G. Spectrum of Benign and Malignant Laryngeal Lesions in Patients Presenting with Hoarseness of Voice: A Cross-Sectional Study. Volume 15 Issue 9 (September, 2025) | Pages 235 – 239.DOI : 10.61336/ejcm/25-09-38

[2] Luke LM, C S A, George S. A cross-sectional study on the aetiology and predisposing factors of hoarseness of voice. Indian J Otolaryngol Head Neck Surg. 2024 Feb;76(1):309–13. doi: 10.1007/s12070-023-04152-y. PMID: 38440669; PMCID: PMC10908878.

[3] Yin CY, Zhang SS, Zhong JT, Zhou SH. Pepsin and laryngeal and hypopharyngeal carcinomas. Clin Exp Otorhinolaryngol. 2021;14(2):159-168. doi:10.21053/ceo.2020.00465.

[4] Zhou T, Wang X, Zhu Q, Zhou E, Zhang J, Song F, et al. Global trends and risk factors of laryngeal cancer: a systematic analysis for the global burden of disease study (1990-2021). BMC Cancer. (2025) 25:296. doi: 10.1186/s12885-025-13700-4.

[5] Frank AL, Joshi TK. The global spread of asbestos. Ann Glob Health. (2014) 80:257–62. doi: 10.1016/j.aogh.2014.09.016 11.

[6] Lin C, Cheng W, Liu X, Li H, Song Y. The global, regional, national burden of laryngeal cancer and its attributable risk factors (1990-2019) and predictions to 2035. Eur J Cancer Care (Engl). (2022) 31:e13689. doi: 10.1111/ecc.13689.

[7] Laskaris,S.; Sengas ,I. Maragoudakis ,P.; Tsimplaki ,E.; Argyri ,E.; Manolopoulos ,L.; Panotopoulou ,E. Prevalence of human papillomavirus infection in Greek patients with squamous cell carcinoma of the larynx. (2014)ANTICANCER RESEARCH 34: 5749-5754 .

[8] Gomaa ,MA. ; El Gindy ,K.E.; Abdel Nabi U.; Mohammed ,H. ; Abdel Twab ,N.; Mahmoud ,R.and Mohiy ,K. Human Papillomavirus Subtype 16 and the Pathologic Characteristics of Laryngeal Cancer OTO Open. 2017 May 8;1(2):2473974X17707925. doi: 10.1177/2473974X17707925.

[9] . Doorbar J, Quint W, Banks L et al. The biology and life-cycle of humanpapillomaviruses. Vaccine 2012; 30 (suppl 5): F55–F70.

[10] Forman D, de Martel C, Lacey CJ et al. Global burden of human papillomavirus and related diseases. Vaccine 2012; 30 (suppl 5): F12–F23.
[11] Boda D, Docea AO, Calina D, Ilie MA, Caruntu C, Zurac S, et al. Human papilloma virus: Apprehending the link with carcinogenesis and unveiling new research avenues. Int J Oncol. 2018 Feb 28;52(3):637-55. doi: 10.3892/ijo.2018.4256.
[12] Silva, L. L. da, Teles, A. M., Santos, J. M. O., & Costa, R. M. G. da. (2023). Malignancy associated with low-risk HPV6 and HPV11: A systematic review and implications for cancer prevention. Cancers, 15(16), 4068. https://doi.org/10.3390/cancers15164068.
[13] Nowińska ,K.; Ciesielska , U.; Podhorska-Okołów, M. .and Dzięgiel,P. The role of human papillomavirus in oncogenic transformation and its contribution to the etiology of precancerous lesions and cancer of the larynx: A review. Advances in Clinical and Experimental Medicine. 2017; 26(3), 539-543. DOI: 10.17219/acem/67461
[14] de Oliveira LQR, Farshadi F, Mlynarek A, Mascarella MA, Hier M, Coletta RD, et al. p53 Positivity Predicts Poor Survival in Oropharyngeal Squamous Cell Carcinoma Dependent on HPV Status. Cancers. 2026; 18(10):1660. doi: 10.3390/cancers18101660.
[15] Zhong, L., Li, H., Chang, W., Ao, Y., Wen, Z., & Chen, Y. TP53 Mutations in Esophageal Squamous Cell Carcinoma. Frontiers in Bioscience-Landmark, 2023; 28(9), 219. doi: 10.31083/j.fbl2809219.
[16] Toutounchi ,S.J.; Eydi ,M.; Golzari ,S.E.; Ghaffari ,M.R. and Parvizian, N. Vocal Cord Paralysis and its Etiologies: A Prospective Study. J Cardiovasc Thorac Res. 2014 Mar 4;6(1):47–50. doi: 10.5681/jcvtr.2014.009.
[17] Carpén ,T.; Hadj-Allal,Z.; Nikkilä ,R.; Hansen ,J.; Heikkinen ,S.; Lynge ,E.; Martinsen,J.; Selander,J. and etal . Occupational Variation in the Incidence of Laryngeal.Cancer in the Nordic Countries. Oncol Ther (2026) 14:707–720https://doi.org/10.1007/s40487-026-00436-9.
[18] Derkay ,C.S . Recurrent Respiratory Papillomatosis. The Laryngoscope; Volume 111, Issue 1 pp. 57-69. https://doi.org/10.1097/00005537-200101000-00011.
[19] Mohammed Ali, S.H. ; Taha, T.A; Ahmed,B.SH. Al-qurtas,M. and Al-Hamdani, F. MOLECULAR DETECTION AND GENOTYPING OF HUMAN PAPILLOMA VIRUS (16/ 18, AND 6/11) IN PATIENTS WITH LARYNGEAL LESIONS USING DNA- IN SITU HYBRIDIZATION. International Research Journal of Natural and Applied Sciences .2016;Vol. 3, Issue 11:71-93.
[20] Taha, T.A. Molecular and Virological Study of Nuclear Co-Localization of High Oncogenic Risk Human Papillomavirus 16/18(E6/E7) Genes and Overexpressed P53 Protein in Tissue From Malignant and benign laryngeal tumors. 2021; VOL.25:Issue 5:2380-2400.
[21] Kayembe Kabutakapua, E., Mpiana Junior, S., Kalala Kabala, H., Kabongo Beya, F., Ntumba Malemka, A., Tshingamb Kamin, Y., Sekele, P., & Nyembue Tshipukane, D. .Frequency and histopathological type of laryngeal tumours at the University Hospital of Kinshasa, Democratic Republic of the Congo. Orapuh Journal,(2025) 6(2), e1215. https://doi.org/10.4314/orapj.v6i2.15.
[22] He, Z., Hu, Y., Yang, X., Han, B., Li, S., Huang, S., & Chen, X. . Global trends and cross-country inequalities in laryngeal cancer: A systematic analysis of the 2021 Global Burden of Disease study represented by China. Tobacco Induced Diseases,(2025); 23(July), 205796. DOI:10.18332/tid/205796 .
[23] Goud, P. Y., & Sruthi, G. . Spectrum of benign and malignant laryngeal lesions in patients presenting with hoarseness of voice: A cross-sectional study. European Journal of Cardiovascular Medicine,(2025); 15(9), 235-239. DOI : 10.61336/ejcm/25-09-38.
[24] Damian, D. .The role of viruses in cellular transformation and cancer. Cancer Reports, (2025). 8(2), e70150. doi: 10.1002/cnr2.70150 .
[25] Monavari, S. H. R., & Keyvani, H. . Human papillomavirus investigation in head and neck squamous cell carcinoma: Initial report from the low risk HPV types associations. Asian Pacific Journal of Cancer Prevention,(2025); 18(9), 2573-2579. 10.22034/APJCP.2017.18.9.2573. doi: 10.22034/APJCP.2017.18.9.2573.
[26] De Silva , G.H. ; Miranda, M.G.; Rodrigues , D.S.; De Souza ,G.R. and Ribeiro ,G.V. . Epidemiological factors in patients with larynx cancer treat by surgery , radiotheraby or therapeutic associations . Archives of otolaryngology and rhinology . (2019) 5(2) : 043-049. doi : 10.17352/2455-1759.000095
[27] Shalatah,M.; Mohamed, S.; Abdelgelil, A.; and Mohamed, D.. Role of P16, P53 and Cyclin D1 in Detection of HPV Induced Laryngeal Squamous Cell Carcinoma: An Immunohistochemical Study.2022(may) AIMJ.p:138-151. DOI:10.21608/aimj.2022.102738.1628 .

[28] Xiao,H.; Wang ,H.; Zhang H.; Wu ,SH. and Yu ,B. Global, regional, and national burden of laryngeal cancer in middle-aged and older adults from 1990 to 2021: an analysis of age and sex differences and attributable risk factors. Front. Public Health, 30 May 2025 . Volume 13 :1-19 https://doi.org/10.3389/fpubh.2025.1601029 .

[29] Oga, E.; Schumak, L., Alabi, B.; Obaseki, D.; Umana, A.; Bassey, I-A. Gbugh, G., Oluwole, O.; Akeredolu, T.; Adebamowa, S. and etal. ."Paucity of HPV- related head and neck cancer (HNC) in Nigeria". PLOS one(2016). 11(4): e015 2828.

[30] Çobanoğlu,H.B.; Keskin,K.; Köprücü,E.R. and Semerci,H.T. Benign laryngeal lesions: review of 329 cases in a single center. Northwestern Med J. 2026;6(1):52-57. DOI: 10.54307/NWMJ.2025.200.

[31] Sachdeva,H.. Nirupama,M.; Padmanabha,N ; Sreeram,S. Lobo,F.D. Pai ,R.& Sreedharan, S.Histopathological Study of Benign Tumours of the Larynx: A Descriptive Study in Coastal Karnataka. Indian J Otolaryngol Head Neck Surg.2022 Oct;74(Suppl 2):2019-2023. doi: 10.1007/s12070-020-01979-7. Epub 2020 Jul 21.

[32] Pawan,S..; Bhandari, A.; Chouhan, M.; Sharma, M. and Sharma, S. "Bengin tumors of the larynx: a clinical study of 50 cases:. Indian J. Otolaryngol Head & Neck Surg.;(2009) vol. 61. (1): 26- 30. DOI:10.1007/s12070-009-0013

[33] Hiremath, B. . Clinicopathological spectrum of benign lesions of the larynx and the role of microlaryngeal surgery in patients with hoarseness. Bengal Journal of Otolaryngology and Head Neck Surgery (2024). Vol. 32 No. 3. DOI: https://doi.org/10.47210/bjohns.2024.v32i3.55.

[34] Carpén ,T. ; Hadj-Allal ,Z.; Nikkilä ,R. Hansen,J. Heikkinen,S.; Lynge ,E. ; Martinsen ,J.; Selander ,J.; Mehlum , I.; Wojewodzic ,M.;and etal. Occupational Variation in the Incidence of Laryngeal Cancer in the Nordic Countries. Oncology and Therapy 2026(April);14(2):707-720 DOI:10.1007/s40487-026-00436-9

[35] Qianwen Liu 1,3,#, Jingfeng Li 1,3,#, Xiuqiao Sun 1,#, Jiayu Lin 1,3, Zhengwei Yu 1, Yue Xiao 1,3, Dan Li 1,3, Baofa Sun 2,✉, Haili Bao 1,✉, Yihao Liu

a. Immunosenescence and cancer: molecular hallmarks, tumor microenvironment remodeling, and age-specific immunotherapy challenges. J Hematol Oncol. 2025 Aug 22;18:81. doi: 10.1186/s13045-025-01735-w.

[36] Kuo,C-L. . Acid Reflux Increases Susceptibility to Laryngopharyngeal and Oropharyngeal Cancers. Archives of Otorhinolaryngology-Head & Neck Surgery. 2020;4(1):2.DOI: 10.24983/scitemed.aohns.2020.00123

[37] Choi,J. and Jung,M. . Head and Neck Cancer in Fanconi Anemia: Clinical Challenges and Molecular Insights into a DNA Repair Disorder. Cancers (Basel). 2025 Sep 18;17(18):3046. doi: 10.3390/cancers17183046.

[38] Sharma ,D. Sohal S.; Bal ,M. and Aggarwal,S. Clinico-Pathological Study of 50 Cases of Tumours of Larynx. Indian J Otolaryngol Head Neck Surg. 2011 Dec 13;65(Suppl 1):29–35. doi: 10.1007/s12070-011-0420-6.

[39] Verro ,B. ; Fiumara ,S.; Saraniti ,G. and Saraniti ,C. Laryngeal Cancer in Women: Unveiling Gender-Specific Risk Factors, Treatment Challenges, and Survival Disparities. Curr. Oncol. 2025, 32(1), 19; https://doi.org/10.3390/curroncol32010019.

[40] Cobanoglu,H.B; Koprucu,E.R. Non-squamous Cancers of the Larynx. CurrentOncology Reports (2024) 26:625–632. https://doi.org/10.1007/s11912-024-01535-4.

[41] Shofoluwe,N.A.; Lawal,J.; Quadri,O.R.; Manir Anka Hamza,M.A.; Abdullah,M.A. Yahaya,S.T. and Sanni,R. Saleem Zubair1 Mustapha Lawal Jimoh7 Demographic and Clinical Characteristics of Benign Laryngeal Lesions: Insights from a Decade of Experience in a Tertiary Hospital. Niger Med J 2024; 65(6):961-972. https://doi.org/10.60787/nmj.v65i6.582 .

a. https://doi.org/10.1007/s40487-026-00436-9.

[42] Charbotel ,B.; Fervers , B.; and Droz,J. . Occupational exposures in rare cancers: A critical review of the literature. Critical Reviews in Oncology/Hematology;Volume 90, Issue 2, May 2014, Pages 99-134. https://doi.org/10.1016/j.critrevonc.2013.12.004.

[43] Kostev ,K. Jacob ,L; Kalder ,M.; Sesterhenn ,A.; Seidel ,D. Association of laryngeal cancer with vocal cord leukoplakia and associated risk factors in 1,184 patients diagnosed in otorhinolaryngology practices in Germany. Mol Clin Oncol. 2018 Mar 21;8(5):689–693. doi: 10.3892/mco.2018.1592.

[44] Joos, B. Joos, N.; Bumpous, J.; Burns, C.; French, C. and Farghely. (2009). "Laryngeal squamous cell carcinoma in a 13 year- old Child associated with Human papillomaviruses 16 and 18: A case reported and review of the literature". Head and Neck Pathol; 3: 37-41. DOI 10.1007/s12105-008-0093-y.

[45] Kumar, J.; Bhutia, N. Bhutia, T.; Malik, P.; Singh, M. and Devi, H. (2015).‖ Malignant Tumors of the Larynx: A Clinicopathological Study of 30 Cases‖ Middle East. Journal of Cancer; 6 (2): 71-78.

[46] Ido Filho, J. Carvalho. B; Mizoguchi, F.; Catani, G.; Filho, E.; Malafia. O. and Stahlke Jr. (2013). "Characteristics of polypoid lesions in patients undergoing microsurgery of larynx". Int. Arch. Otorhinolaryngeal; 17 (3): 279- 284.

[47] Divi,V. ; Sataloff,R.T. and Oxenberg, J. Large Vocal Fold Polyp . ENT-Ear, Nose & Throat Journal, Volume 90, Issue 1 :E30. https://doi.org/10.1177/014556131109000119.

[48] Singhal, P.; Bhandari, A.; Chouhan, M.; Sharma, M. and Sharma, S. (2009). "Bengin tumors of the larynx: a clinical study of 50 cases:. Indian J. Otolaryngol Head & Neck Surg.; vol. 61. (1): 26- 30.

[49] Mousavi ,S.; Ilaghi ,M.; Aslani ,A.; Najafi ,M.; Yekta ,Z. and Nejadghaderi,S. Laryngeal cancer incidence trends in the United States over 2000–2020: a population-based analysis. Arch Public Health. 2024 Jul 10;82:106. doi: 10.1186/s13690-024-01333-1.

[50] Mastronikolis,N.S. ; Papadas,T.A ; Goumas,P.D. ; Triantaphyllidou,I-E. ; Theocharis,D.A. ; Papageorgakopoulou,N. Vynios,D.H. Head and Neck: Laryngeal tumors: an overview . Atlas Genet Cytogenet Oncol Haematol. 2008-12-01

[51] Babawale , P.I. Antonieta Guerrero-Plata , A.G. Respiratory Viral Coinfections: Insights into Epidemiology, Immune Response, Pathology, and Clinical Outcomes. Pathogens. 2024 Apr 12;13(4):316. doi: 10.3390/pathogens13040316 .

[52] Donne ,A.J.; Hampson ,L; Homer J and . Hampson I.N. The role of HPV type in Recurrent Respiratory Papillomatosis. International Journal of Pediatric Otorhinolaryngology Volume 74, Issue 1, January 2010, Pages 7-14. DOI:10.1016/j.ijporl.2009.09.004. Low risk HPV-E6 traps p53 in the cytoplasm and induces p53-dependent apoptosis

[53] da Silva ,L.L.; Teles ,A.M. ;. Santos ,J.M.; de Andrade ,M.S.; Rui Medeiros ,R.; Faustino-Rocha ,A.I; Oliveira ,P.A.; etal. Malignancy Associated with Low-Risk HPV6 and HPV11: A Systematic Review and Implications for Cancer Prevention. Cancer, August 2023;15(16):4068. DOI:10.3390/cancers15164068

[54] Baba ,S.K.; Alblooshi ,S.S. ; Yaqoob ,R.; Behl ,S.; Al Saleem ,M.; Rakha,E.; Malik ,F.; Singh ,M.; Akhtar ,M. ; etal. Human papilloma virus (HPV) mediated cancers: an insightful update. J Transl Med. 2025 Apr 29;23:483. doi: 10.1186/s12967-025-06470-x.

[55] Zhang ,B.; Chen ,W. and Roman ,A. The E7 proteins of low- and high-risk human papillomaviruses share the ability to target the pRB family member p130 for degradation. Proc Natl Acad Sci U S A. 2005 Dec 28;103(2):437–442. doi: 10.1073/pnas.0510012103

[56] Uehara ,K.; Tanabe , Y.; Hirota ,S.; Higa ,S.; Zensei Toyoda ,Z.; Kurima ,K.; Kina ,S.; Nakasone ,T.; Akira Arasaki ,A.; Kinjo,T. Co-expression of low-risk HPV E6/E7 and EBV LMP-1 leads to precancerous lesions by DNA damage. BMC Cancer. 2021 Jun 10;21(1):688. doi: 10.1186/s12885-021-08397-0.

[57] Gillisona,M.; Alemany ,L.; Snijders ,P.; Chaturvedi ,A.; Steinbergf,B. ; Schwartz ,S.; Castellsagu,X. Human Papillomavirus and Diseases of the Upper Airway: Head and Neck Cancer and Respiratory Papillomatosis. Vaccine. Volume 30, Supplement 5, 20 November 2012, Pages F34-F54. https://doi.org/10.1016/j.vaccine.2012.05.070.

[58] Yim,E-K. and Park, J-S.The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis. Cancer Research and Treatment : Official Journal of Korean Cancer Association 2005;37(6):319-324.DOI: https://doi.org/10.4143/crt.2005.37.6.319.

[59] Thomas ,J.; Oh ,S.; Terhune ,S.; Laimins,L.A. Cellular Changes Induced by Low-Risk Human Papillomavirus Type 11 in Keratinocytes That Stably Maintain Viral Episomes. J Virol. 2001 Aug;75(16):7564–7571. doi: 10.1128/JVI.75.16.7564-7571.2001.

[60] Ebrahimi ,F.; Rasizadeh ,R.; Sharaflou ,S.; Aghbash ,P.; Ali Shamekh ,A.; Jafari-Sales,A. ; and Baghi,H. . Coinfection of EBV with other pathogens: a narrative review. Front. Virol., 08 November 2024Volume 4 - 2024 | https://doi.org/10.3389/fviro.2024.1482329.

[61] Fernandes ,Q.; Gupta ,I.; Murshed,K.; Abo Samra ,H.; Al-Thawadi ,H.; Vranic ,S.; Petkar ,M.; Babu ,G.; and Al Moustafa,A. Coinfection of HPVs Is Associated with Advanced Stage in Colorectal Cancer Patients from Qatar https://doi.org/10.3390/pathogens12030424

[62] Huebbers,C.; Preuss,S.; Kolligs,J.; Vent,J.; Stenner,M.; Wieland,U.; Silling , S.; Drebber ,U.;. Speel,E. and Klussmann,J. Integration of HPV6 and Downregulation of AKR1C3 Expression Mark Malignant Transformation in a Patient with Juvenile-Onset Laryngeal Papillomatosis. HPV6 and AKR1C3 in Juvenile-Onset RRP. PLOS ONE. February 2013 ; Volume 8 (Issue 2) e57207. doi: 10.1371/journal.pone.0057207.

[63] Giacomelli ,A.; Yang,X. Lintner,R.; McFarland,J.; Duby,M.; Kim,J.; Howard,T.; Takeda,D.; Ly,S.; Kim,E.; Brian, G.; Hurhula,B.; Sharpe,T.;etal. Mutational processes shape the landscape of TP53 mutations in human cancer. Nat Genet. 2018 Sep 17;50(10):1381–1387. doi: 10.1038/s41588-018-0204-y .

[64] Miron Prokocimer, Varda Rotter. Structure and Function of p53 in Normal Cells and Their Aberrations in Cancer Cells: Projection on the Hematologic Cell Lineages. Blood. Volume 84, Issue 8, 15 October 1994, Pages 2391-2411. https://doi.org/10.1182/blood.V84.8.2391.2391 .

[65] Assimakopoulos ,D.; Kolettas,E.; Zagorianakou,N.; Evangelou, A.; Skevas,A.; N and Agnantis,j. Prognostic significance of p53 in the cancer of the larynx. Anticancer Res. 2000 Sep-Oct;20(5B):3555-64. Pathogens 2023, 12, 424.

[66] Jia,H.; Wu ,S.;. Ma,G.; Yang ,P.; Li ,X.; Zeng,M.; n Ji ,X. and Xing ,X. p53 Immunohistochemistry staining patterns and prognosis significance in 212 cases of non-endometrioid endometrial cancer. Pathology - Research and Practice. Vol;263 (7447):155595. DOI:10.1016/j.prp.2024.155595.

[67] Kennedy ,M. and Lowe,S. Mutant p53: it's not all one and the same . Cell Death Differ. 2022 Mar 31;29(5):983–987. doi: 10.1038/s41418-022-00989-y.

[68] Babamohamadi ,M.; Babaei ,E.; Salih , B.; Babamohammadi , M.; Azeez ,H. and Othman, G.Recent findings on the role of wild-type and mutant p53 in cancer development and therapy. Front Mol Biosci. 2022 Sep 26;9:903075. doi: 10.3389/fmolb.2022.903075.

[69] Mir Mohammad Jalali1 *, Abtin Heidarzadeh2 , Mansour Jamali Zavarei3 , Hadi Sarmast4 P53 Overexpression Impacts on the Prognosis of Laryngeal

[70] Squamous Cell Carcinomas. Asian Pacific Journal of Cancer Prevention,2011. Vol 12(7): 1731-4.

[71] Rahmoun,M. ; Aussel,A.;Bouzidi,S.; Pedergnana,V.; Malassigné,V.; Julien Puech,J.; , Veyer,D.; Péré,H.; Lepine,C.;Fabian Blanc,F. and etal,. Genomic diversity of HPV6 and HPV11 in recurrent respiratory papillomatosis: Association with malignant transformation in the lungs and clinical outcomes. Tumour Virus Research .Volume 18, December 2024, 200294. https://doi.org/10.1016/j.tvr.2024.200294.

[72] Chan ,W.; Siu,W.; Lau,A.; Poon,R. How many mutant p53 molecules are needed to inactivate a tetramer?. Mol Cell Biol.2004 Apr;24(8):3536-51. doi: 10.1128/MCB.24.8.3536-3551.2004.

[73] Pietsch ,C. and Murphy ,M. Low risk HPV-E6 traps p53 in the cytoplasm and induces p53-dependent apoptosis. Cancer Biol Ther. 2008 Dec;7(12):1916–1918. doi: 10.4161/cbt.7.12.7169.

[74] Terzian,T.; Suh,Y.; Iwakuma,T.; Post,S.; Neumann,M.; Lang,G.;Carolyn S. Pelt,V. and Lozano,G. The inherent instability of mutant p53 is alleviated by Mdm2 or p16 INK4a loss. Genes Dev. 2008, 22:1337-1344. doi:10.1101/gad.1662908.

[75] Scheel ,A.; Bellile ,E.; McHugh ,J.; Walline ,H.; Prince ,M.; Urba ,S.; Wolf ,G.; Eisbruch ,A.; Worden ,F.; Carey ,T.; Bradford ,C. Classification of TP53 mutations and HPV predict survival in advanced larynx cancer. Laryngoscope.2016 Sep;126(9):E292-9. doi: 10.1002/lary.25915.