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THE ASSOCIATION BETWEEN HUMAN PAPILLOMA VIRUS AND LARYNGEAL MASSES

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Abstract

The Human Papilloma Virus (HPV) can play a role in the development of head and neck tumors such as oropharyngeal tumors. Nevertheless, a real impact between Human Papilloma Virus and other head and neck sites such as the larynx is not well studied.

This study aimed to assess the association of Human Papilloma Virus and laryngeal tumors. It is a prospective, case control study in which a random sample of 34 patients who have laryngeal masses was included in the period between November 2017 to December 2018. The study was carried out In the Department of Otolaryngology, Basrah Teaching Hospital.

Patients were evaluated by a questioner according to gender, age, marital status, full medical history, and examination. A biopsy then was taken for histopathology study to confirm presence of tumors or other lesions. Malignant tumors were evaluated according to staging and grading systems. All lesions were examined for the presence of HPV by Immunohistochemistry (IHC) study.

The results of this study revealed that there was HPV infection in 30% of the patients. Moreover, HPV was found in 30% of laryngeal squamous cell carcinoma and in 30% of benign lesions. Statistical tests showed no significant association between HPV and Laryngeal squamous cell carcinoma.

In conclusion, although there was thirty percent of patients having HPV infections, but there was no significant association between HPV and laryngeal malignant tumours. A larger epidemiologic and more multicentric study is mandatory to evaluate the true prevalence of HPV infection in the mucosa of larynx and laryngeal malignancies.

Keywords: Human, papilloma Virus, laryngeal tumors , immunohistochemistry, laryngeal mass

Introduction

Human papillomavirus (HPV) infection is a sexually transmitted infection that is responsible for a growing subdivision of head and neck squamous cell carcinomas (HNSCCs). Failure to clear the virus leads to unregulated cell proliferation, more mutations, and cancer formation. While the different HPV proteins contribute to viral replication, the E6 and E7 viral oncoproteins are crucial in malignant transformation. Together they can lead to epithelial cell immortalization. E6 binds to p53 and promotes its degradation via ubiquitin-dependent protease pathways[1]. The wild-type p53 is usually activated by DNA damage or cellular stress and acts as a tumour suppressor. P53 also modulates cell cycle and regulates apoptosis[2]. E6 activates the hTERT promoter inducing telomerase activity, which also leads to cell immortalization[3].

The E7 viral protein binds and inactivates pRb (retinoblastoma protein) which is an important tumour suppressor. pRb blocks cell cycle progression by binding to E2F transcription factor and therefore blocking progression from G1 to S-phase[4]. Therefore disruption of pRb-E2F complex leads to unregulated cell cycle entry into S-phase leading to increased cell proliferation. Usually,

unregulated S-phase would cause apoptosis via the p53 pathway. However, suppression of p53 activity by E6 interrupts this pathway and prevents cell death. Consequently, affected cells lose their ability to repair DNA damage then accumulate mutations and chromosomal instability. This can eventually lead to cancer formation.

Transmission of HPV; While sexual behaviours and HPV infection have been strongly linked with tumours of the oropharynx, there is little epidemiological evidence linking these with tumours of the larynx[5,6].

The HPV infection can access basal and parabasal cells of multilayered epithelium in three different sites[7] which are sites of mucosal injury, metaplastic epithelium and the squamocolumnar junction. When the HPV virus infects an active epithelial cell, it may cause a latent infection with viral replication connected with the cell cycle[8].

The proliferative process initiated by the episomal form of HPV starts at the parabasal and basal layers of squamous epithelium of the oropharynx, oral cavity, or at the reserve cell layer in the respiratory epithelium of the larynx. In the larynx, the proliferation of the reserve cell (basal layer) which induced by HPV develops an alteration with a multilayered squamous cell epithelium formation and eventually papilloma can develop[7].

Clinically, papillomas tends to arise from the junction of respiratory and squamous epithelium and areas of iatrogenic induced squamous metaplasia[9].

The genital HPVs are divided into high-risk and low-risk types based on their association with invasive cervical cancer. By definition, HPV is low risk if it has never been isolated from cervical carcinoma and high risk if it ever has been. Persistent infection with any one of about 15 high-risk (carcinogenic) types accounts for virtually all cervical cancers[10]. Examples of low-risk HPV: 6, 11, 42, 43, 44, 53, 54, 57, 66. High-risk HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68[11]

Patients and methods

This prospective case control study included 34 patients presented with laryngeal masses attended to the otolaryngology Department, Basrah Teaching Hospital in the period between November 2017 to December 2018.

Inclusion criteria: Patients with hoarseness who do not respond to medical treatment, and patients with suspected malignant lesions through history and clinical examination.

Exclusion criteria; Patients refusing examination and endoscopy, patients who refuse to consent to the study, patients with laryngeal invasion from other primary sites such as thyroid and hypopharynx.

Patient evaluation

A complete history is taken from the patients. A complete general and full otorhinolaryngology examination was done. Flexible nasoendoscopy and 90-degree rigid laryngoscopy were done using (ENTERMED diagnostic unit). All examinations were photographed. Assessment of the lesion size, location, overlying mucosa and status of surrounding tissue was done; also examination of supraglottic with epiglottis, the status of vocal folds, subglottic examination and pyriform fossae was done.

Suspected lesions such as exophytic, fungating and ulcerative lesions sent for larynx-computed tomography to assess the extent of the lesion, cartilage erosion, nodal status, and adjacent tissue involvement.

Direct laryngoscopy was done; a therapeutic excision, such as in the cases with laryngeal polyps, and biopsy taking in suspected malignant lesions.

Sections were stained using hematoxyline and eosin stain and examined under a light microscope for histopathological diagnosis. Lesions

diagnosed as tumours, were evaluated and classified by WHO classification of laryngeal tumours[12].

Immunohistochemistry study was done using a special kit Dako® Monoclonal Mouse Anti-Human Papillomavirus (HPV) Clone K1H8. Code M3528. Immunogen Alkaline disrupted HPV type 1[13].

Anti-HPV antibody used in our study was found to be immunoreactive with paraffin sections of formalin-fixed HPV-infected tissues with HPV type 6, 11, 16, 18, 31, 33, 42, 51, 52, 56 and 58. Positive immunostaining was primarily confined to the nuclei of infected cells[14,15].

Under general anaesthesia, direct laryngoscopy was done. After visualizing the lesions, the pathology was evaluated about site, size, type, invasion and involvement of the surrounding tissue. Excision of a mass was done. Biopsy was saved in formalin container, labelled and sent for histopathology study and Immunohistochemestry. Sections were stained using hematoxyline and eosin following the procedure of Avwioro[16].

Immunohistochemistry procedure; Slide preparation: removal of paraffin, rehydration, and unmasking of antigen. After that, inactivation of endogenous peroxidase was done.

Primary antibody reaction: The slides were allowed to drain and shacked off excess fluid with a brisk motion, then primary antibody solution was applied to the appropriate slides covering the tissue sections. Secondary antibody reaction (Biotin/Streptavidin Detection) using 100 µl of biotinylated applied to each slide.

Substrate preparation: 20µl from DAB chromogen was added to each 1ml of substrate buffer. Counterstaining by Mayer's hematoxyline was applied to cover the section.

Results

The study comprised 34 patients. Thirty one males and three females. Data analysis was done using IBM SPSS Statistics Data Editor V.23.

Human papilloma virus immunohistochemistry was studied and showed that ten patients (29.4%) were positive, five patients were diagnosed as having benign lesions and the other five patients were diagnosed as malignant lesions. Twenty four patients (70.6 %) were negative as shown in figures 1, 2 and 3 and Table I.

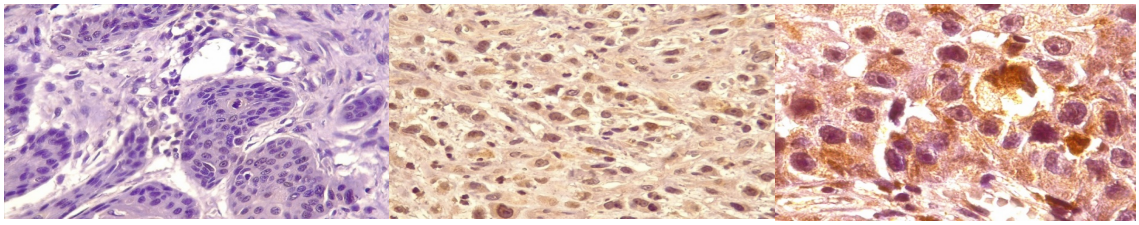

Figure 1: Clinical slide of squamous cell carcinoma with negative HPV, we can note that the cells do not take IHC stain.

Figure 2: Clinical slide of poorly differentiated squamous cell carcinoma. With HPV positive IHC . The infected cells shows stained nucleus.

Figure 4: Clinical slide of moderately differentiated squamous cell carcinoma. With HPV Positive IHC staining.

Table I: HPV immunohistochemistry staining

	Number	Percentage
Positive	10	29.4
Negative	24	70.6
Total	34	100.0

Because the study was based on HPV which is considered as a sexual transmitted disease, it is critical to mention that revealed that 95% of the patients were married and two patients were widows. According to lifetime sexual partners, 17 patient has one partner, 10 patients had two partners and seven patients more than two sexual partners. There is no significant association between marital status and number of sexual partners and HPV. Twenty-nine patients (85%) were smokers, whereas five patients were nonsmokers (15%). There was no significant relation between smoking history and HPV infection.

The masses were divided to benign and malignant lesions according to histopathology study that showed benign lesions in seventeen patients (50%), the other seventeen patients (50%) showed malignant growth. There was equal distribution of HVP infected cases (50% each) and there was no statistical significant association between HPV and tumour behaviour.

According to histopathology diagnosis, squamous cell carcinoma was found in seventeen patients (50%), a laryngeal polyp in fifteen patients (44%) and laryngeal papilloma in two patients (6%). Histopathology study and HPV association is studied and found that five patients out of 17 patients diagnosed as squamous cell carcinoma have HPV infection. Four patients out of 15 patients diagnosed of laryngeal polyps are HPV positive and one patient out of two patients diagnosed as papillomas has HPV infection. There was no significant relation between HPV and histopathology diagnosis as shown in table II.

Table II: Histopathological study and HPV.

		Number	Percent	HPV Positive	HPV Percent	P-Value
Behaviour						1.000
	benign	17	50%	5	50%	
	malignant	17	50%	5	50%	
Histopathology diagnosis						0.861
	Polyp	15	44.1%	4	40%	
	Papilloma	2	5.9%	1	10%	
	Sq. cell Ca	17	50%	5	50%	

According to primary tissue status, seventeen patients out of thirty four were diagnosed as squamous cell carcinoma, five patients out of seventeen who have squamous cell carcinoma presented with T1 (30%), eight patients presented with T2 (47.1%) and two patients (12%) presented with T3 and T4.

HPV in patient diagnosed as squamous cell carcinoma was found in one of four patients diagnosed as T1, Three patients out of five patients were diagnosed as T2 and one of two patients diagnosed as T4 has HPV positive infection. Statistical tests show no significant relation between HPV and tissue status of squamous cell carcinoma.

The node status was also evaluated, fifteen patients out of seventeen patients who were diagnosed as squamous cell

carcinoma (88%) presented with no nodal metastasis, and one patient (6%) presented with N1 and N2. Three out of fifteen patients with no nodal metastasis have HPV positive (20%). The remaining two HPV positive patients were N1 and N2. Statistical tests was done and show no significant relation between HPV and nodal status.

Grading of malignant samples (n=17) was documented as moderately differentiated carcinoma in 11 patients (64.7%), poorly differentiated and well differentiated in 3 patients for each (17.6%).

Grading of squamous cell carcinoma and HPV was studies and showed that one patients with HPV positive infection was well differentiated and two patients were moderately and poorly differentiated, respectively. Statistical test was done and show no significant relation between HPV and grade of squamous cell carcinoma.

Association between HPV and staging of malignant lesions was done and showed that 20% of HPV positive cases was on stage one, where as 40% of HPV presented with stage two. Statistical test was done and show no significant relation between HPV and stage of squamous cell carcinoma as demonstrated in Table III.

Table III: Squamous cell carcinoma and HPV

		Number	Percent	HPV Positive	HPV Percent	P Value
Tissue Status						1.00
	T1	5	29.4%	1	20%	
	T2	8	47.1%	3	60%	
	T3	2	11.8%	0	0%	
	T4	2	11.8%	1	20%	
Nodal Status						0.083
	N0	15	88.2%	3	60%	
	N1	1	5.%	1	20%	
	N2	1	5.9%	1	20%	
Grading						0.442
	Well differentiated	3	17.6%	1	20%	
	Moderately differentiated	11	64.7%	2	40%	
	Poorly differentiated	3	17.6%	2	40%	
	Undifferentiated	0	0%		0%	
Stage of SCC						0.383
	1	5	29.4%	1	20%	
	2	7	41.2%	2	40%	
	3	2	11.8%	0	0%	

Discussion

This study screened three low-risk types which may cause benign lesions such as papillomatosis[17]. Laryngeal Papillomatosis is the most prevalent benign tumour of the larynx in children, and mother-to-child transmission is probably involved in juvenile-onset papillomatosis[18]. Low risk genotypes screened in the study: HPV type 6, 11, and 42.

Eight high-risk genotypes including HPV 16 which is the most frequently detected type in laryngeal carcinomas[19]. High-risk

genotypes screened in the study: HPV type 16, 18, 31, 33, 51, 52, 56 and 58.

About the relation between HPV and smoking, 90% of positive HPV were smokers, although in another study there were similar proportions of patients with HPV positive and negative tumours reported a history of cigarette smoking (82% vs. 95%, respectively)[20], this may due the unequal smoking distribution in the sample (76.5 % of patients were smokers).

According to the site and side of the lesion, there was no significant association with HPV positivity, and the predominant glottic lesions (70%) in HPV positive samples may be due to more glottic samples (79.5%) compared with supraglottic lesion (14.7%). Moreover, this may refer to early presentation of glottic lesions compared with other surgical sites.

We found that about 30% of the cases (10 out 34 patients) were HPV positive. This can be divided according to histopathological study to three groups; First group contains patients diagnosed as laryngeal polyps which conserved a normal mucosa contains no neoplastic growth, there were four patients (26.7%) out of fourteen patients diagnosed as laryngeal polyps have HPV infected mucosa. This agree with other study that show that HPV DNA is also detected in normal mucosa (incidence:20%) and laryngeal SCCs[18]. This result was approximately the same result as in Nunez et al[21] which showed that post-mortem specimens of macroscopically normal laryngeal mucosa were HPV positive in 25% of cases. Another study show a wide range of values of HPV in normal oral cavity mucosa, from 0%[22-24] up to 70%[25]. This variation in results may be due to the effect of different methods of sample collection (PCR, Western blotting or IHC), due to the anatomic sites of sample collection (base of tongue, hypopharynx or elsewhere), and the sensitivity of the technique been used.

Other study showed vocal cord nodules and polyps have harboured HPV type 6 or HPV type 11 DNA in 23% of patients[26]. This goes with our result that shows 26.7% of patients with laryngeal polyps were HPV positive.

The second group is patients who were diagnosed as laryngeal papilloma which is caused primarily by HPVs 6 and 11, with a small fraction (less than 5%) caused by HPV16 or other types[27].

The third group is patients diagnosed as malignant lesions which is squamous cell carcinoma. In 29.4% of patients diagnosed as squamous cell carcinoma there is HPV infected laryngeal mucosa. In spite of this high proportion but there is no significant association between the two.

There are multiple studies about the association between the HPV and laryngeal SCC. The role of HPV infection in laryngeal cancers has been supported by many other studies but does not have strong association as that identified with cancer of the oropharynx and oral cavity[28,29].

Another Systematic Review and Meta-Analysis by Li X et al shows that prevalence of HPV infection in laryngeal cancer was found to be cancer in 28.0%[30]. Moreover, a systematic review of articles published up to 2004 confirmed that the prevalence of HPV in SCC of the larynx could be as high as 46.9%[31].

On the other hand, In 2005, a total of 5046 head and neck cancers were pooled in a meta-analysis[32]; of these, 1435 were laryngeal cancers. HPV DNA was identified in 24% of tested laryngeal tumours.

A recent systematic literature review identified more than 40 publications in which laryngeal SCC was examined for the presence of HPV DNA, and found the prevalence of HPV DNA in 1,712 cases of laryngeal SCC to be 23.6%[33] which was higher than the weighted prevalence of HPV DNA in oral cavity SCC, which was 20.2%[33].

According to the pathology grading and staging, our study reveals that there are no significant relations. This goes with other larger study which showed the same results[34].

Conclusions: This study shows that about 30% of all laryngeal masses have Human Papilloma Virus infections. About 26% of non-neoplastic lesions presented as laryngeal polyps have HPV positive immunohistochemistry staining. We found that about one third of patients diagnosed as laryngeal malignancy as squamous cell carcinoma have HPV infected cells. In spite of these high percentages, the statistical tests shows that no significant relations present between HPV and laryngeal malignancies. The main withdrawals of this study are the small sample size, a short period that lacks prolonged follow-up of HPV positive patients to evaluate the prognosis of the disease, and limitation of facilities to screen more samples from other provenances in the country.

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