SHORT COMMUNICATION

The Frequency of Human Papilloma Virus in Laryngeal Squamous Cell Carcinoma

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ABSTRACT

The association of HPV in laryngeal squamous cell carcinoma (LSCC) is investigated in several studies but controversial results are established. This study aimed to investigate the HPV DNA positivity in LSCC patients diagnosed and treated in two otorhinolaryngology referral centres in northern region of Turkey. The study was planned as a retrospective investigation of LSCC patients. Fifty-two formalin-fixed, paraffin-embedded (FFPE) tissue blocks of laryngeal cancers-diagnosed and treated between 2010 and 2016, were included. Polymerase chain reaction (PCR) method was used for detection of HPV genotypes. PCR amplification was successful in 40 of 52 patients. Among the 40 LSCC samples, HPV DNA was detected in one patient (2.5%). The evaluated HPV positivity in LSCC as low; but larger studies are needed to confirm this.

Key Words: *Laryngeal squamous cell carcinoma, polymerase chain reaction, HPV genotypes.*

Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck squamous cell carcinoma (HNSCC) subtypes.[1] In Turkey, LSCC is found the eighth most common cancer in men; incidence rate was 6,2 per 100,000 men in 2014.[1]

Human Papilloma Virus (HPV) has recently emerged as a new important etiological factor in the development of HNSCC.[2] It is linked especially with oropharynx and oral cavity cancers.[2] The incidence of HPV-related HNSCC is rising in the world and this rise is linked to changing sexual behaviours, such as a high number of sexual partners, multiple oral sex partners, anogenital warts, etc.[2] Today, it is known that HPV-related HNSCC is a separate disease with different molecular features, clinical presentation, treatment and prognosis.[2] On the other hand, the link between HPV and LSCC is less clear; HPV positivity in LSCC has been examined in several studies and positivity reported between 0-79.[2-5] These results vary due to sample size, identification methods, geographic and cultural differences, and inclusion criteria. Only few studies evaluated the HPV prevalence in LSCC in Turkey. This retrospective study aimed to evaluate HPV infection rate in our LSCC patients.

This retrospective investigation included LSCC patients who had been diagnosed and treated in University Hospital and Training and Research Hospital – otorhino-laryngology referral centres – between January 2010 and December 2016 in Samsun. Approval was granted by the 19 Mayis University Ethics Committee. The clinical characteristics of the patients were obtained from the computerised database. LSCC tissue samples were used that were fixed with 10% Neutral Buffered Formalin (NBF) and embedded with paraffin. All the blocks were analysed in the Pathology Department of Mayis University Hospital. After the initial evaluation of 52 patient records, 40 blocks of the patients were found to be adequate for HPV testing.

Table I: Patient and tumor characteristics.

Variable	All cases (n=40)	
	N	%
Age (years)		
<60	11	27.5
>60	29	72.5
Gender		
Male	39	97.5
Female	1	2.5
Smoking		
Yes	40	100
No	0	0
Stage		
I/II	11	27.5
III/IV	29	72.5
Treatment		
Surgery only	14	35
Radiotherapy only		
Surgery + radiotherapy/chemoradiotherapy	14	12.5
Radiotherapy ± chemotherapy	6	15
Chemotherapy	1	2.5
Relapsed		
Yes	13	32.5
No	27	67.5
Registered death		
Yes	9	22.5
No	31	77.5
HPV positive		
Yes	1	2.5
No	39	97.5

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Table II: Characteristics of the HPV positive patient.

HPV subtype	Age	Gender	Tumor location	Stage	Smoking	Treatment	DFS (months)
16	70	Male	Glottis	III	Yes	Surgery+RT	48

HPV DNA tests were performed with real time PCR (Cobas® 4800 v2.0, HPV Test). Type 16, type 18 and a pool panel of other 12 high risk HPV (types 31,33,35,39, 45,51,52,56,58,59,66 and 68) detected and report with automatised system. Genotype results were reported as positive for HPV 18, Positive for HPV 16, or Positive for pooled 'other' 12 HR-HPV subtypes and negative. β globin was an internal control for the validity of cellular DNA for PCR. Negative and positive kit controls were included for each run.

In this study, 52 LSCC blocks were retrieved; but due to suboptimal fixation in 12 cases, 40 were eligible for analysis. DNA extraction, purification and PCR testing were successful in these 40 blocks (76% of 52 blocks).

The patients tested for HPV DNA comprised 39 males and 1 female, with a mean age of 61years (range, 42-74 years). All the patients were smokers. Alcohol consumption and sexual behaviour were not reported in the medical files. The most common primary tumor location was glottic area (65%), 35% of the patients were treated with surgery only, and during the follow-up period, 13 of the 41 patients suffered recurrence, second primary lung cancer was detected in 2 patients and 5 patients died during the follow-up period (Table I).

Of the 40 LSCC patients, only one patient was HPV positive (2.5%) (Table II). The primary tumor was located in the glottic area. HPV 16 subtype was detected in this male patient aged 70 years, with a smoking history and stage III laryngeal cancer. After surgery, the patient received adjuvant radiotherapy and was still alive at 48 months without relapse.

Perhaphs in the last 20 years, one of the most important discovery in head and neck oncology is the association between HPV and oropharyngeal cancers and lesions.[2,3] They have better outcomes compared to HPV negative patients, staging differs and clinical trials are investigating the "treatment deintensification" for HPV-mediated oropharyngeal cancers.[2] Unfortunately, its relationship to LSCC is less certain.

In this retrospective study, HPV positivity was investigated in 40 LSCC patients in the central-northern region of Turkey. HPV DNA was detected in one patient (2.5%). These results may suggest that HPV-related LSCC has not yet arised as a significant health problem in our region. The 2.5% incidence is one of the lowest reported positivity in the literature but in agreement with Rodrigo *et al*. whom found 1.6% prevalence in laryngeal cancer in northern Spain.[4]

A large case control study from two high incidence regions (Central Europe and Latin America) reported a very low HPV prevalence (3.8%) in laryngeal and hypopharyngeal cancers that supports us.[5] These different HPV positivity results are possibly attributed to the differences in the population characteristics: age, smoking habbits, sexual behaviour, the sample size, the sample type and, mainly, the HPV detection methods used. All the patients in this study were smokers and most of them are older than 60 years. The variation in oral sexual behaviour may affect the incidence, but sexual data was not available in this study.

According to a Turkish national screening programme, cervical HPV prevalence was found to be 3.8% in 2 million women older than 30 years.[6] This rate was the same in all 81 provinces in Turkey.[6] This is lower than rates reported in Europe; that HPV-positivity is 10% in women of the same age.[6] These screening programme findings support the low HPV positivity in laryngeal cancer patients in this study.

In this study, HPV positive patient's tumor location was glottic area. It has been speculated that the glottic area of the larynx is more suitable for HPV infection. It may be due to the squamocolumnar junction in the laryngeal ventricle that is similar to the transition zone in the cervix.[5]

This study has several limitations. First, the sample size was small and this may be the reason for the low HPV positivity rate. Secondly, the quality of fixation was suboptimal and we were unable to detect DNA in 12 of 52 patients. Finally, it was planned as a retrospective study, so risk factors such as oral sex and the lifetime number of sexual partners are not known.

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