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Main Article

Human papilloma virus prevalence in laryngeal squamous cell carcinoma

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Abstract

Purpose: To determine the prevalence and type of human papilloma virus deoxyribonucleic acid (DNA) in cases of laryngeal squamous cell carcinoma.

Methods: We analysed the prevalence of human papilloma virus infection in archived paraffin block specimens taken from 99 cases of laryngeal squamous cell carcinoma between 1990 and 2005, using polymerase chain reaction techniques. Biopsy specimens from five proven verrucous skin lesions were used as positive controls, and peripheral blood samples from five healthy volunteers were used as negative controls.

Results: Four test samples were found to have inadequate deoxyribonucleic acid purity and were therefore excluded from the study. Human papilloma virus deoxyribonucleic acid was detected in seven of 95 cases of laryngeal squamous cell carcinoma (7.36 per cent). Human papilloma virus genotyping revealed double human papilloma virus infection in three cases and single human papilloma virus infection in the remaining four cases. The human papilloma virus genotypes detected were 6, 11 and 16 (the latter detected in only one case).

Conclusion: In our series, a very low human papilloma virus prevalence was found among laryngeal squamous cell carcinoma cases. The human papilloma virus genotypes detected were mostly 6 and/or 11, and 16 in only one case. To the best of our knowledge, this is the first report of human papilloma virus prevalence in laryngeal squamous cell carcinoma, based on polymerase chain reaction genotyping in a Turkish population.

Key words: Human Papilloma Virus; Laryngeal Neoplasms; Papillomas

Introduction

The larynx is among the most significant anatomical sites in terms of human papilloma virus (HPV) involvement.[1] The virus normally results in benign, self-limiting warts or tumours, characterised by abnormal maturation and differentiation of epithelial cells. The virus may remain latent in the basal layer of the epithelium for months or even years before histological change is detected.[2] Human papilloma virus infection is the aetiological agent of a clinically significant disease known as laryngeal papilloma or papillomatosis. Despite its benign character, malignant transformation into laryngeal squamous cell cancer (SCC) may occur in a certain proportion of cases. However, the role of HPV in the transformation process is controversial.[3]

In the present study, we aimed to determine the prevalence and the type of HPV in laryngeal SCC cases.

Materials and methods

Study protocol

The institutional ethical review board of the Gulhane Military Medical Academy approved the study protocol. The study was conducted on specimens archived between 1990 and 2005, taken from 99 cases of laryngeal SCC. None of the patients had recurrent respiratory papillomatosis or any immunosuppressant disorders. Biopsy specimens from five patients with verruca vulgaris of proven HPV content were processed as positive controls, and peripheral blood samples from five healthy volunteers were used as negative controls.

DNA extraction and amplification

Tumour blocks from 99 cases of laryngeal SCC were cut into 10 μm sections, and three sections from each case were submitted for deoxyribonucleic acid (DNA) extraction, in clean, nonsterile, capped tubes. A standard deparaffinisation procedure was performed using xylene followed by an ethanol wash. The DNA was extracted using the QIAamp DNA Mini Kit spin-column procedure (Qiagen, California, USA). The DNA concentration and purity were measured by spectrophotometry. Samples of DNA from test cases and from positive and negative controls were tested for the presence of HPV DNA by polymerase chain

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reaction using a mixed type L1 consensus primer system, which amplifies 449–452 bp fragments of the L1 open-reading frame of a broad spectrum of HPV genotypes (GenBank accession numbers V01116, X63594, M14199, K02718, X04773, J04353, M12732, NC_001952 and NC_001443; GenBank) (SP-10296 HPV L1 Gene Primer Set Kit, Maxim Biotech, California, USA). Polymerase chain reaction amplification was performed as follows: 95°C for 10 minutes; 40 cycles of denaturation at 95°C for 1 minute; annealing at 55°C for 1 minute; extension at 72°C for 1 minute; a final extension at 72°C for 5 minutes; and then a hold step at 4°C. Amplicons were analysed by a standard 2 per cent agarose gel electrophoresis procedure. Samples containing HPV DNA were submitted for HPV genotyping analysis by targetting the HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV52 and HPV58 genomes with an Multiplex polymerase chain reaction (mPCR) kit (HPV MP-70215, Maxim Biotech).

Results

The DNA purity of four test samples was inadequate; these were therefore excluded from the series. After polymerase chain reaction amplification and agarose gel electrophoresis, seven samples were found to contain HPV DNA. The overall HPV DNA positivity rate was 7.36 per cent. After genotyping by the mPCR technique, double infections were disclosed in three carcinomas: HPV6 and 11 in two cases; and HPV11 and 16 in one case. Four samples contained only one HPV genotype, confirmed to be HPV11. Histological analysis of specimens showed neither koilocytotic cellular atypia nor parakeratosis, suggestive of HPV infection. The HPV test results are listed in Table I.

Positive and negative control specimens

All five papilloma samples were found to be positive for HPV DNA, and all peripheral blood derived DNA samples were found to be negative for HPV DNA, using the same HPV screening method as for the test samples.

Discussion

There is increasing interest in the possible association between HPV infection and SCC of the larynx.[4] Multiple case series have variously reported the prevalence of HPV DNA in laryngeal cancer to be from 0 to 100 per cent.[5] There is heterogeneity in the sample collection methods and the sample sites used. As HPV infection is focal and only occurs in differentiating squamous epithelium, random biopsies have a much lower chance of detecting HPV. Also, the techniques used to isolate viral DNA differ, both in their sensitivity and in their ability to identify viral genome integration.[3]

The association of HPV with laryngeal carcinoma was first suggested following detection of the typical cytopathic effects of HPV within laryngeal carcinoma.[6] The most convincing evidence implicating HPV in laryngeal cancer is derived from studies demonstrating HPV DNA within cancer lesions, using different hybridisation techniques and polymerase chain reaction.[1,3] Even in polymerase chain reaction studies on laryngeal cancer, the HPV DNA frequency varies between 3 and 85 per cent, which is attributable to: differences in primers, genomic localisation and length of polymerase chain reaction products; differing polymerase chain reaction conditions; and false positive results originating from contamination of samples with HPV DNA.[7] In our study, paraffin block specimens from 99 laryngeal SCC cases were analysed using the polymerase chain reaction technique; five negative and five positive controls were also analysed to ensure the validity of the experimental methods.

Among head and neck cancers, HPV DNA positivity tends to show site-dependence, with the tonsils, oral cavity and larynx being the most common sites.[8,9] In the majority of cases of laryngeal papillomatosis, HPV6 or HPV11 is detected and is transcriptionally active.[10,11] Although the most important clinical manifestation of laryngeal HPV infection is laryngeal papillomatosis, HPV DNA has also been found in macroscopically apparently normal laryngeal mucosa; the incidence of polymerase chain reaction detected HPV in macroscopically normal upper respiratory tract mucosa seems to be approximately 20 per cent.[12] In carcinomas without pre-existing clinical papillomatosis, HPV DNA is detected by polymerase chain reaction in 8 to 54 per cent of cases.[13,14]

An increased incidence of papillomavirus infection in non-smoking laryngeal cancer patients raises the possibility that HPV infections are an important aetiological cause in this group of patients.[15] On the other hand, HPV DNA detection rates vary widely between different studies. Kreimer *et al.* (2005) reported a worldwide, systematic review of case series using polymerase chain reaction based methods to detect and genotype HPV in head and neck cancer.[16] The report included 5046 head and neck SCC specimens from 60 studies and showed a 25.9 per cent rate of HPV prevalence; HPV prevalence was significantly higher in oropharyngeal SCCs (35.6 per cent) than in oral SCCs (23.5 per cent) and laryngeal SCCs (24 per cent). The most common HPV type detected was HPV16, which was present in 16.6 per cent of laryngeal SCC samples. These authors also found that tumour site-specific HPV prevalence was higher among studies from North America compared with those from Europe and Asia.[16]

TABLE I

HPV DNA IDENTIFICATION

Parameter	Cases (*n*)
*HPV type**	
11 & 16	1
6 & 11	2
11	4
HPV DNA detection	
+	7
−	88
Total	95

*Double infections (i.e. more than one HPV type) were found in three cases (3.15% of HPV DNA positive cases). HPV = human papilloma virus; DNA = deoxyribonucleic acid

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- **The larynx is among the most significant anatomical sites in terms of HPV involvement. The virus normally results in benign, self-limiting warts or tumours, characterised by abnormal maturation and differentiation of epithelial cells**
- **The prevalence of HPV infection was analysed in archived paraffin block specimens taken from 99 cases of laryngeal squamous carcinoma**
- **The prevalence of HPV among laryngeal squamous carcinoma cases was found to be very low in this series**

However, this worldwide review did not include any studies from Turkey. Thus, to the best of our knowledge, the present study is the first to determine HPV prevalence, using polymerase chain reaction based methods, within laryngeal cancer in a Turkish population. Our findings showed a very low HPV prevalence in laryngeal SCCs, and thus do not support the findings of the above studies. Moreover, the prevalence of HPV16 in laryngeal cancer was less than that of HPV11.

A recent meta-analysis showed that HPV16 positivity ranged from 0 to 86 per cent in head and neck cancer cases and from 0 to 38 per cent in controls. Meta-analysis stratified by anatomical site suggested that the association between HPV16 and cancer was strongest for tonsil, intermediate for oropharynx and weakest for oral and larynx. There was also evidence of inter-study heterogeneity in the association of HPV16 with oral cancer, laryngeal cancer and oropharyngeal cancer but not with tonsil cancer.[17]

We predict that such conflicting results for HPV prevalence within laryngeal SCC will continue to be published, because HPV prevalence varies from site to site (oral, oropharyngeal and laryngeal) and from country to country. Moreover, HPV detection is more likely in those with a sexual history of more than one partner, and in those who practise oral sex.[18] Thus far, there is no biological explanation for why HPV prevalence is higher in tumours from the tonsil or oropharynx, compared with other sites in the head and neck.

There are plenty of questions about the prevalence of HPV within head and neck cancer. Further studies are needed to elucidate the role of HPV in this setting, and its connection with other known risk factors for carcinogenesis.

In conclusion, we found a human papilloma viruses prevalence within laryngeal cancer of 7.36 per cent. However, the study was conducted on paraffin block specimens, which may be a possible limitation.

References

1 Syrjanen S. Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 2005;**32**:59–66
2 Fischer M, Winterfeld FV. Evaluation and application of a broad-spectrum polymerase chain reaction assay for human papillomaviruses in the screening of squamous cell tumours of the head and neck. *Acta Otolaryngol* 2003;**123**:752–8
3 Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P *et al*. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001;**344**:1125–31
4 Gorgoulis VG, Zacharatos P, Kotsinas A, Kyroudi A, Rassidakis AN, Ikonomopoulos JA *et al*. Human papillomavirus (HPV) is possibly involved in laryngeal but not in lung carcinogenesis. *Hum Pathol* 1999;**30**:274–83
5 Hobbs CGL, Birchall MA. Human papillomavirus infection in the etiology of laryngeal carcinoma. *Curr Opin Otolaryngol Head Neck Surg* 2004;**12**:88–92
6 Syrjanen K, Syrjanen S, Pyrhonen S. Human papilloma virus (HPV) antigens in lesions of laryngeal squamous cell carcinomas. *ORL J Otorhinolaryngol Relat Spec* 1982;**44**:323–34
7 Lindeberg H, Krogdahl A. Laryngeal cancer and human papillomavirus: HPV is absent in the majority of laryngeal carcinomas. *Cancer Lett* 1999;**146**:9–13
8 Badaracco G, Venuti A, Morello R, Muller A, Marcante ML. Human papillomavirus in head and neck carcinomas: prevalence, physical status and relationship with clinical/pathological parameters. *Anticancer Res* 2000;**20**:1301–5
9 Mineta H, Ogino T, Amano HM, Ohkawa Y, Araki K, Takebayashi S *et al*. Human papilloma virus (HPV) type 16 and 18 detected in head and neck squamous cell carcinoma. *Anticancer Res* 1998;**18**:4765–8
10 Mounts P, Shah KV, Kashima H. Viral etiology of juvenile- and adult-onset squamous papilloma of the larynx. *Proc Natl Acad Sci U S A* 1982;**79**:5425–9
11 DiLorenza TP, Steinberg BM. Differential regulation of human papillomavirus type 6 and 11 early promoters in cultured cells derived from laryngeal papillomas. *J Virol* 1995;**69**:6865–72
12 Nunez DA, Astley SM, Lewis FA, Wells M. Human papilloma viruses: a study of their prevalence in the normal larynx. *J Laryngol Otol* 1994;**108**:319–20
13 Perez-Ayala M, Ruiz-Cabello F, Esteban F, Concha A, Rodondo M, Oliva MR *et al*. Presence of HPV 16 sequences in laryngeal carcinomas. *Int J Cancer* 1990;**46**:8–11
14 Garcia-Milian R, Hernandez H, Panade L, Rodriquez C, Gonzalez N, Valenzuela C *et al*. Detection and typing of human papillomavirus DNA in benign and malignant tumours of laryngeal epithelium. *Acta Otolaryngol (Stockh)* 1998;**118**:754–8
15 Fouret P, Monceaux G, Temam S, Lacourreye L, St Guily JL. Human papillomavirus in head and neck squamous cell carcinomas in nonsmokers. *Arch Otolaryngol Head Neck Surg* 1997;**123**:513–16
16 Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:467–75
17 Hobbs CGL, Sterne JAC, Bailey M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. *Clin Otolaryngol* 2006;**31**:259–66
18 Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P *et al*. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst* 2003;**95**:1772–83

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