ASSESSMENT OF HUMAN PAPILLOMA VIRUS IMMUNOREACTIVITY IN LARYNGEAL SQUAMOUS CELL CARCINOMAS

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ABSTRACT

Objective: Human Papilloma Virus (HPV) infections have been suggested to be associated with the squamous epithelial lesions. Certain HPV types have been detected in squamous cell carcinoma (SCC). The goal of this study is to assess pan HPV expression in laryngeal squamous cell carcinomas and adjacent mucosal epithelium and to correlate its expression with tumor grade, nodal status, and tumor stage.

Methods: The present study includes 39 cases of laryngeal SCC treated by radical laryngectomy. The tumor tissues were originally formaline-fixed and paraffin-embedded. The sections representing both tumor and the adjacent mucosa were analyzed for pan HPV expression by immunohistochemical method.

Results: Pan-HPV staining was positive in 14 cases (35%) either at the tumor cells or at the adjacent mucosa. Among these, positive staining only at the tumor cells was observed in 1 case (7%), and 2 cases (15%) were positive both at the tumor cells and at the adjacent mucosa. The remaining 11 cases (78%) showed positivity only at the adjacent mucosa, Regarding the tumor stage, the adjacent mucosa showed positivity in 8 cases (53 %) among 15 T3 and T4 tumors while only 2 cases (13%) were positive at tumor cells. A proportional distribution was seen among the groups regarding tumor grade and nodal status.

Conclusion: The present study demonstrates that although not providing direct evidence, adjacent mucosa should also be investigated for achieving higher detection rate in laryngeal SCC. The detection of HPV at tumor cells by this method is of limited value. The advanced stage was not correlated statistically with HPV expression, although a higher rate of HPV positivity in T3-T4 tumors was found. This study also did not indicate any correlation with tumor grade and nodal status. However for a definite conclusion, wider series should be studied.

Key Words: Laryngeal cancer, human papilloma virus, immunohistochemistry

INTRODUCTION

In the past decades, both DNA and RNA viruses have been implicated in many human cancers. Strong epidemiologic association were determined in many studies between several viruses and tumors.

Human Papilloma Virus (HPV) represents a family of more than 50 related DNA viruses and have been known to be oncogenic since the study of Shope in the 1930s (1). Certain HPV (especially type 16 and 18) have been suggested among possible etiologic factors in either genital cancers or head and neck tumors (2).

An overall rate of 50% for HPV have been demonstrated in oral, nasal, laryngeal and oesophageal carcinoma (3). HPV is also detected in normal mucosa, benign lesions as well as carcinomas of the larynx (4-6).

There are not many reports demonstrating all genotypes of HPV in a certain group of tumors, because in most of the studies the observers have studied certain genotypes using situ hybridization and but rarely immunohistochemistry.

The goal of the present study is to assess HPV immunoreactivity in laryngeal squamous cell carcinomas and adjacent mucosal epithelium and to investigate its correlation with tumor grade, tumor stage, and nodal status.

MATERIALS AND METHODS

The present study includes 39 cases of laryngeal squamous cell carcinomas treated by radical laryngectomy. The Hematoxylen-Eosin stained sections obtained from the archieves of Dokuz Eylül University Hospital, Department of Pathology were reviewed, and the most representative section of the tumor and adjacent mucosa including normal squamous epithelium were selected for the immunohistochemical method. Tumor staging was based upon UICC (Union of Internationale Coutre le Cancer Classification) and the grading was done from I to III according to the degree of differentiation (7).

The tumor tissues were originally formaline-fixed and paraffin-embedded. The selected blocks were recut on poly-lysine-coated slides and deparaffinized sections were incubated with 0.3% hydrogen peroxide for 5 minutes in order to inactivate endogenous peroxidase activity. After incubating with prediluted pan HPV kit (DAKO Inc.) for 15 minutes at room temperature in a moist chamber, linking antibody and streptavidin (Zymed Inc) were applied for 10 minutes. The slides were washed three times in PBS after each step. The reaction was visualized with amino etil carbazole (AEC) (Zymed Inc.) and the sections were then counterstained with hematoxylin.

RESULTS

Two patterns of immunostaining for HPV were observed: nuclear and cytoplasmic. Nuclear staining was observed in 2 cases (5%), whereas it was cytoplasmic in 12 cases (30%) ,and the immunostaining was focal in overall cases.

The immunostaining profile was summarized in Table I. HPV staining was positive in 14 cases (35%) either at the tumor cells or at the adjacent mucosa. Among these, 2 cases (15%) were positive both at the tumor cells and at the adjacent mucosa (Fig 1). The positive staining only at the adjacent mucosa was observed in 11 cases (78%), and the remaining 1 case (7%) showed positivity only at the tumor cells (Fig 2).

Tables II, III and IV summarize the relationship of immunohistochemical positivity with tumor stage, nodal status and grade. Regarding the stage of the tumor, there were higher immunopositivity for HPV in advanced stages. But the difference was statistically insignificant ($p=0.07$, Chi-square test). The overall positivity in 24 T1 and T2 cases was 25% in either tumor or adjacent mucosa, whereas it was 66% in the remaining 15 T3 and T4 tumors. Among the latter group, the adjacent mucosa showed positivity in 8 cases (53%) while only 2 cases (13%) were positive in tumor cells. HPV immunopositivity did not show any significant correlation ($p>>0.05$) with nodal involvement and tumor grade. A proportional distribution was seen among the groups regarding these two parameters.

DISCUSSION

Certain subtypes of HPV have been detected in laryngeal squamous cell carcinomas and the adjacent normal mucosa (8-13). This suggests its possible role in carcinogenesis. One of the unique characteristics of HPV is its requirement of squamous differentiation for viral assembly and cytopathic manifestations. HPV mRNA is therefore found most abundantly in mature squamous epithelium showing strong cytoplasmic and nuclear signals (14). In several studies HPV has been found to be integrated into the tumor cell genome and transcribed, leading to expression of two oncogenes, E6 and E7 proteins. It seems that the mechanism of action of E6 and E7 oncogenes involves binding to and inactivation of the retinoblastoma (Rb) and p53 gene products. After integration and proliferation, the amount of HPV related products decreases and detection of mRNA by in situ hybridization and immunohistochemical method becomes difficult (1). The analysis of the adjacent mucosa for HPV detection may be more significant than detecting in tumor areas. We therefore especially focused on the adjacent non-neoplastic mucosa including normal squamous epithelium for achieving a higher detection rate. The impressive finding was the significantly higher positivity at this localization, in concordence with the viral assembly hypothesis which indicates the integration in carcinogenesis along with decreased viral protein synthesis.

It is evident that, in the head and neck region, HPV plays a role in pathogenesis of both primary benign neoplastic proliferation and neoplastic lesions (5, 6, 14). HPV immunoreactivity shows latent infection at the normal mucosa and needs additional factors, such as irradiation or trauma in order to predispose malignancy (second hit hypothesis) (14). HPV detection for a chosen subtype or types may be of value, but this excludes all other types rising many questions about the importance of the others. Therefore in the present study, pan HPV antibody identifiying all subtypes was used for the detection of HPV expression by immunochemisty.

Clayman et al (4) reported that the detection of HPV was significantly related to decreased survival, independent of disease stage. However, in the present study, a higher rate of HPV positivity in T3-T4 tumors was encountered parallel to the data at the literature (15).

It has been suggested at the literature that HPV is expressed most frequently in poorly differentiated squamous cell carcinomas (5). We did not find any significant correlation between HPV expression and either tumor grade or nodal status. However for a definite conclusion, wider series should be studied.

To conclude, the present study demonstrates the incidence of HPV in tumoral areas and adjacent normal mucosa of laryngeal squamous carcinomas, especially in T3 and T4 tumors, disregarding any statistical correlation with tumor stage, tumor grade and nodal involvement. For achieving better detection rate in carcinomas by immunohistochemistry, although not providing direct evidence, the adjacent normal mucosa should be especially investigated.

Table I. HPV immunoreactivity and immunostaining profile in positive cases (No: 14)

	HPV Immunoreactivity
At only tumor	1/14 (7%)
At only adjacent mucosa	11/14 (78%)
Both at tumor and adjacent mucosa	2/14 (15%)

Table II. Tumor stage and HPV immunoreactivity in overall cases (No: 39)

	HPV Immunoreactivity		
Tumor Stage	Tumor	Adjacent Epithelium	Total Number
T1	0 (0%)	3 (21%)	14
T2	1 (10%)	2 (20%)	10
T3	0 (0%)	2 (66%)	3
T4	2 (16%)	6 (50%)	12

Table III. Nodal stage and HPV immunoreactivity in overall cases (No: 39)

	HPV Immunoreactivity		
Nodal Stage	Tumor	Adjacent Epithelium	Total Number
N_0	2 (9%)	7 (33%)	21
N_1, N_2	1 (5%)	6 (33%)	18

Table IV. Tumor grade and HPV immunoreactivity in overall cases (No: 39)

	HPV Immunoreactivity		
Tumor Grade	Tumor	Adjacent Epithelium	Total Number
I	1 (5%)	6 (30%)	20
II	1 (11%)	4 (44%)	9
III	1 (10%)	3 (30%)	10

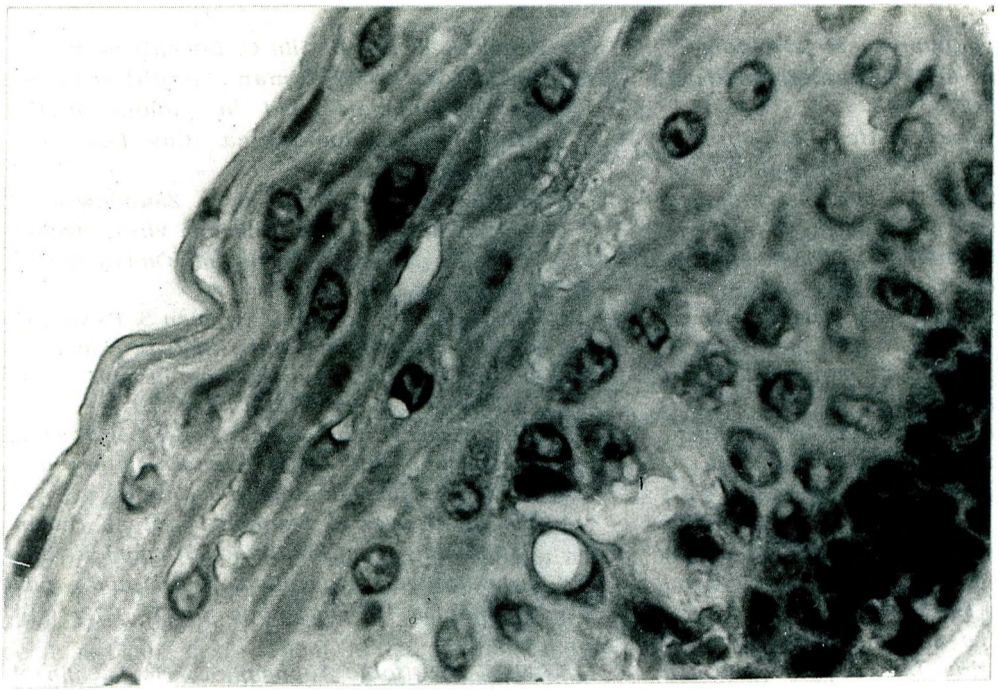

Fig. 1: HPV immunoreactivity in normal squamous epithelium adjacent to the tumor (cytoplasmic staining). X 400 magnification.

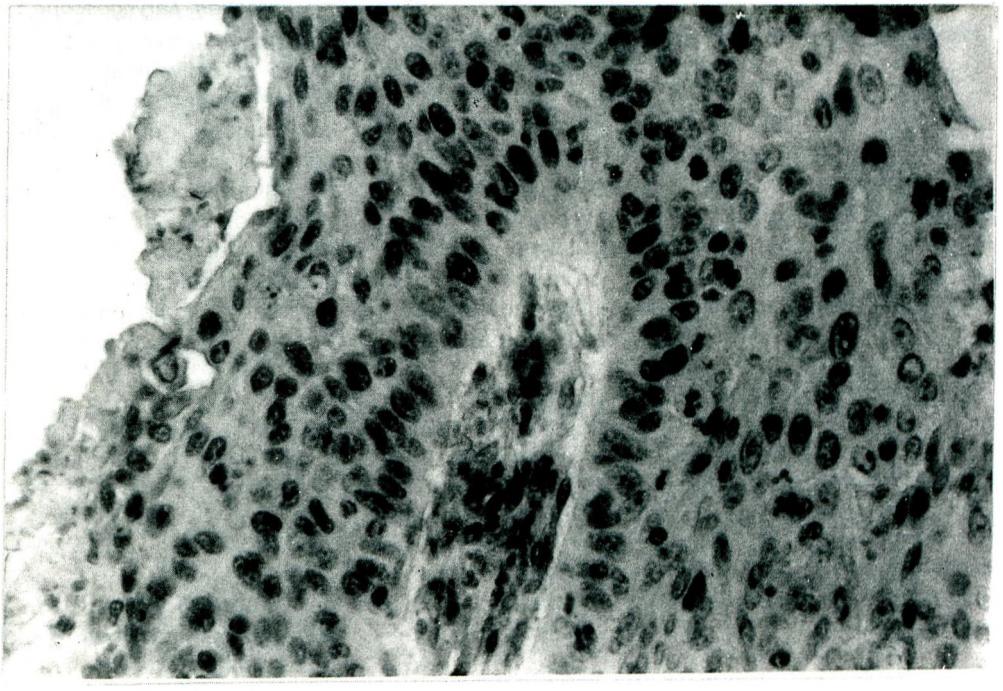

Fig. 2: Nuclear immunostaining of HPV at tumor. X 200 magnification.

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