



# Human papillomavirus in sinonasal inverted papillomas and squamous cell carcinoma of the larynx. In situ hybridization with human papillomaviruses DNA probes.

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#### Abbreviations:

HPV – human papillomavirus

**Key words:** human papillomavirus, inverted papilloma, carcinoma of the larynx

## Abstract

*Background and Purpose:* a substantial number of studies have reported evidence on the involvement of human papillomaviruses (HPV) in oral and sinonasal papillomas and carcinomas. Oncogenic HPV subtypes 16 and 18 seem to play an important role in carcinogenesis, whereas low risk HPV subtypes 6 and 11 are usually characteristic of benign lesions. The aim of the present study was to evaluate a role for HPV in the development of sinonasal inverted papillomas and squamous cell carcinomas of the larynx.

*Materials and Methods:* A ten sinonasal inverted papillomas and eight squamous cell carcinomas of the larynx were retrieved from archival material. All examined cases showed histological features of HPV infections (koilocytosis, binucleated squamous cells and abnormal mitoses). All cases have been analyzed using in situ hybridization. A wide spectrum probe for HPV was used initially. Further subtyping was carried out using specific probes for HPV low risk and HPV high risk.

*Results:* Of the ten studied cases of inverted papillomas, one was positive for both: wide spectrum probe and low risk probe, and one was positive for HPV high risk probe only. One of the eight cases of carcinomas was positive for both HPV DNA probes: wide spectrum probe and high risk probe. Additional positive reactions for HPV DNA using high risk probe were noted in three examined cases of squamous cell carcinomas of the larynx.

*Conclusions:* Our study confirms the role of high risk HPV subtypes in carcinomas. In contrast to other studies, one case only suggests the possible involvement of HPV 6 and HPV 11 in etiology of sinonasal inverted papillomas. The data concerning of the role of HPV in transformation inverted papillomas to squamous cell carcinomas still need further confirmation.

## INTRODUCTION

Over 500 000 patients worldwide are diagnosed with head and neck squamous cell carcinoma each year (1). Despite vast improvements in the diagnostic and therapeutic tools over the past several decades, mortality rates have not changed significantly. Several DNA viruses have been associated with the causation of cancers in humans. Of the various human DNA viruses, human papillomavirus is one of the most important tumorigenic factors. Human papillomavirus (HPV) is

a small DNA virus showing an affinity to the stratified squamous epithelium found on the mucosa and skin. Approximately 120 genetically distinct types of human papillomaviruses have been identified (2). Some types are associated only with benign squamous papillomas (3), but some human papillomaviruses have also been implicated in the genesis of several squamous cell cancers (4, 5).

It is widely accepted that HPV causes cervical cancer (6, 7). HPV has also been associated with several other types of squamous cell carcinoma and their precursors at different sites – skin, vulva, vagina, esophagus, conjunctiva, paranasal sinuses and bronchus – but the role of HPV in the pathogenesis of these lesions is less clear than it is in cervical cancer (4–6). The idea that human papillomaviruses may play a role in head and neck carcinomas has been under investigation for at least 20 years (8, 9). The similarity of the morphologic features of genital and oral HPV-associated lesions was one of the early findings that raised the possibility that HPV might be involved in oral and laryngeal squamous cell carcinoma (4, 9).

Squamous cell carcinoma of larynx has multifactor etiology and many interacting risk factors (smoking, alcohol intake, male gender, and age >54 years) are involved in their development. The latest studies suggest that high risk HPV infection and HPV co-infection (predominantly HPV 16, 18, 33, 35, 45) can be one of the causative factors in the development of head and neck squamous cell carcinoma and may play an important role in the etiology of cancer of the larynx (10). The role of papillomaviruses in the pathogenesis of head and neck squamous cell carcinomas has been uncertain, mainly because detection of HPV DNA has been highly variable. Differences in the sensitivity and specificity of HPV detection methods as well as contamination of the samples would explain the part of discrepant results reported by the researchers.

Inverted papillomas are characterized by an endophytic growth pattern and are usually a unilateral lesion with its origin in the lateral wall of the nose, ethmoid sinus or maxillary antrum. Inverted papillomas are relatively rare epithelial tumours which generate considerable interest because they are locally aggressive, have a tendency to recur and are associated with malignancy, in contrast to exophytic papillomas, where such occurrences are extremely rare. The nature and pathogenesis of inverted papilloma are debated. Viral infections are a one of potential etiologic factors. HPV subtypes 6 and 11 have been the most frequently identified HPV subtypes in oral and sinonasal papillomas (11–16).

The aim of the present study was to evaluate a role for HPV in the development of sinonasal inverted papillomas and squamous cell carcinomas of the larynx.

## MATERIALS AND METHOD

### Patients

Eight of formalin-fixed, paraffin-embedded tissue specimens of squamous cell carcinoma of the larynx and ten

of sinonasal inverted papillomas were retrieved from archival material (Chair of Pathomorphology, Medical University of Lodz). The age range for sinonasal inverted papillomas was from 29 to 69 (mean 48.0) and for squamous cell carcinomas of the larynx was from 51 to 79 (mean 60.75). Paraffin tissue sections were stained with haematoxylin and eosin and the histological diagnoses were established according to WHO classification (17). All cases showed histological features of HPV infections (koilocytosis, binucleated squamous cells, increased mitotic activity and abnormal mitoses).

### In situ hybridization

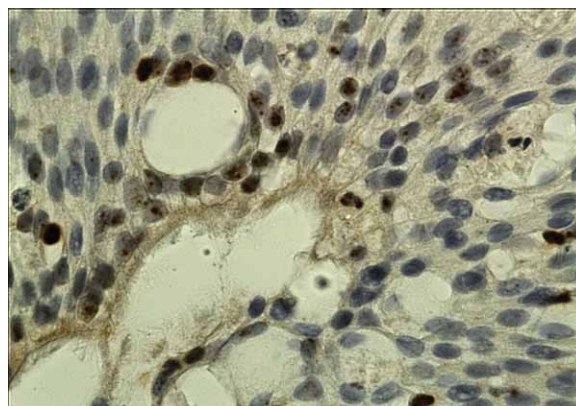
Ten sinonasal inverted papillomas and eight squamous cell carcinomas of the larynx have been analyzed using commercially available HPV DNA probes (Dako, Carpinteria, California, USA). Initially, a wide spectrum biotinylated probe for common HPV subtypes was used, according to the manufacturer's suggested protocol. The wide spectrum probe targets the genomic DNA of HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51 and 52. Further subtyping was carried out at the same way, using specific probes for HPV low risk (HPV 6 and 11) and HPV high risk (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Afterwards catalyzed signal amplification system prepared according to the instructions of the manufacturer were used (GenPoint, CSA System for in situ hybridization; Dako).

### Statistical methods

The differences between groups were tested using non-parametric Chi<sup>2</sup> test. Results were considered statistically significant if  $P < 0.05$

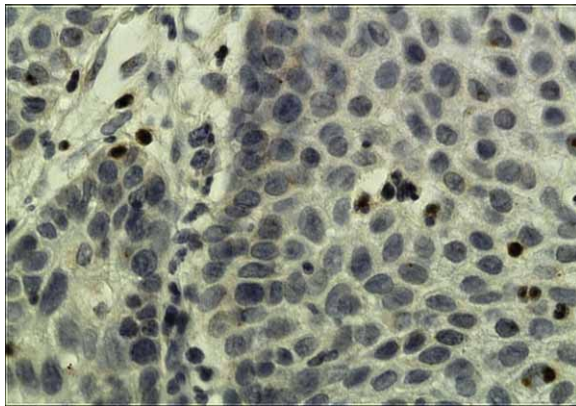
## RESULTS

Of the ten examined cases of sinonasal inverted papillomas, one was positive for HPV DNA using the wide spectrum probe and for HPV DNA subtypes 6 and 11 (Figure 1). In addition one case of ten tested inverted papillomas was positive for HPV high risk probe only (Figure 2). One of the eight cases of squamous cell carci-

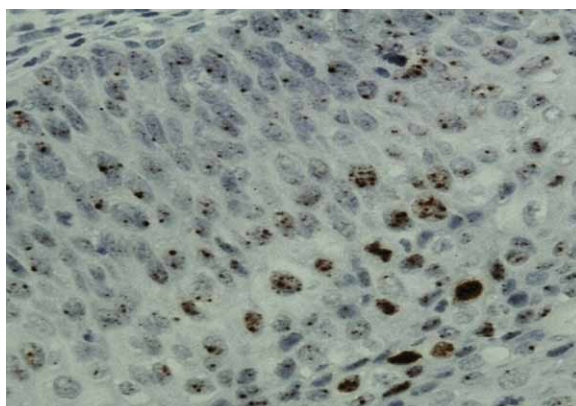


**Figure 1.** Positive DNA expression for low risk HPV probe in sinonasal inverted papillomas. In situ hybridization. Magn. 400x.

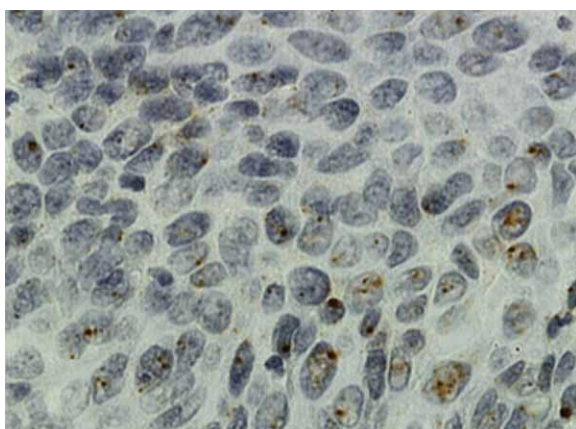
noma was positive for both HPV DNA probes: wide spectrum probe (Figure 3) and high risk probe (Figure 4). Additional positive reactions for HPV DNA using high risk probe were noted in three examined cases of squamous cell carcinomas of the larynx. No significant



**Figure 2.** Positive DNA expression for high risk HPV probe in sinonasal inverted papillomas. In situ hybridization. Magn.400x.



**Figure 3.** Positive DNA expression for wide spectrum HPV probe in squamous cell carcinoma of the larynx. In situ hybridization. Magn.400x



**Figure 4.** Positive DNA expression for high risk HPV probe in squamous cell carcinoma of the larynx. In situ hybridization. Magn.400x

differences in the frequency of HPV infection were noted in sinonasal inverted papillomas group and squamous cell carcinomas group ( $P=0.17$ ). Clinical data and the results of in situ hybridization are summarized in Tables 1 and 2.

**TABLE 1**

Summary of clinical data and results of in situ hybridization for human papillomavirus (HPV) DNA in 8 cases of squamous cell carcinomas of larynx.

Case	Age	Sex	HPV WSS probe	HPV High Risk	HPV 6/11
1	51	F	-	-	-
2	69	M	-	+	-
3	61	M	-	+	-
4	61	M	-	-	-
5	48	M	-	-	-
6	52	F	+	+	-
7	65	F	-	+	-
8	79	M	-	-	-

WSS – wide spectrum probe

**TABLE 2**

Summary of clinical data and results of in situ hybridization for human papillomavirus (HPV) DNA in 10 cases of sinonasal inverted papillomas.

Case	Age	Sex	HPV WSS probe	HPV High Risk	HPV 6/11
1	30	M	-	-	-
2	29	F	-	-	-
3	77	F	+	-	+
4	39	F	-	-	-
5	69	F	-	+	-
6	30	M	-	-	-
7	30	M	-	-	-
8	69	F	-	-	-
9	39	F	-	-	-
10	68	M	-	-	-

WSS – wide spectrum probe

**DISCUSSION**

Oncogenic human papillomaviruses, predominantly HPV 16 and HPV 18, are important factors in the pathogenesis of anogenital cancers, whereas low risk HPV (HPV 6, HPV 11) can cause benign proliferative lesions (6, 8).

A viral etiology of head and neck cancers is still under debate. Epidemiologic study reveals that the role of HPV in pathogenesis of head and neck cancers is rather uncer-

tain and controversial. The reported frequency of HPV DNA in the often studied cancers of the larynx varies between 3% – 85% (13). Our results suggest an involvement of high risk subtypes of HPV in squamous cell carcinoma of the larynx. Found in our study 50% positivity rate of high risk HPV subtypes in tissue samples from patients with squamous cell carcinoma is similar to that seen in other studies (8, 11, 13). Contrary to other studies we observed three positive results with high risk HPV probe only. It is possible that subtypes of HPV 56, 58, 59, 68 (absent in wide spectrum probe) can play an important role in pathogenesis of squamous cell carcinoma in these cases. It is also possible that demographic variables exist. In contrast to our findings, the literature data revealed that high risk HPV subtypes 16, 18, 31, 33 and 45 are rather more frequently associated with invasive carcinoma of the oral cavity, oropharynx, larynx, and nasal cavity. Numerous data suggest that HPV16 and HPV 18 are the main HPV subtypes etiologically involved in the development of squamous cell carcinomas of larynx (2, 11, 18). It is well known that the etiology of squamous cell carcinomas of the larynx is complex. It is possible that HPV infection play a synergistic role in the multifactorial etiology of laryngeal carcinoma, and HPVs are rather cofactors in the development of carcinomas.

Sinonasal squamous cell papillomas have been studied extensively for their association with HPV 6 and HPV 11 (3, 12, 13, 14). DNA of HPV was identified on average 33.3% of examined cases (13). To our knowledge the data concerning HPV DNA in inverted papillomas are rather scanty. Using PCR, Zhou *et al.* (16) showed that 30 from of 38 tested cases were infected by HPVs, in which HPV 11 positive reaction was noted in 68.2%. Using in situ hybridization, Brandwein *et al.* (11) detected HPV 6 and HPV 11 in 5 from 7 examined cases of inverted papillomas (71%), whereas Katori *et al.* (19) described HPV 6 and HPV 11 in 42% examined cases of inverted papillomas. In our study, one case only can suggest the possible involvement of HPV 6 and HPV 11 in etiology of sinonasal inverted papillomas, even though all tested cases (negative for HPV DNA also) showed morphological features of HPV infection. Lack of the expression of HPV DNA in the remaining cases could reflect a different etiology for these lesions, but it is also possible that the negative results were caused by HPV subtypes other than investigated. For instance, Guan *et al.* (20) demonstrated a possible role for HPV subtype 57 in the pathogenesis of nasal inverted papillomas. The role of HPV in the etiology and pathogenesis of these lesions is still equivocal. It must be taken into consideration that incidental expression of HPV was detected. Syrjänen *et al.* (18) described approximately 11% cases of normal oral mucosa positive for HPV.

It is well known that inverted papillomas are the lesions that have a tendency to recurrence, local aggressiveness and a tendency to transformation to squamous cell carcinoma. Histological analysis suggests that inverted papilloma tumorigenesis may occur through a series of discrete events. Kim *et al.* (21) speculated that

HPV high risk subtypes infection, can occur in benign inverted papillomas as an early event during the multi-step tumorigenesis of those lesions. Cumulative genetic defects may be required to progress from benign to dysplastic inverted papilloma and carcinoma arising from inverted papilloma. According to Lu *et al.* (22) the recurrence and malignant transformation of nasal inverted papillomas are related to HPV infection, and the higher infection rate of high risk HPV subtype is one of the reasons for malignant transformation. Wang *et al.* (15) detected by PCR HPV-related sequence in 21 out of 36 (58.3%) examined cases of inverted papillomas and in 11 from of 16 (68.8%) cases of inverted papillomas with squamous cell carcinoma. Found in our study positive reaction for high risk HPV probe may support hypothesis that HPV infection may play a role in the progression of inverted papillomas to carcinoma.

In conclusion, our study suggests the involvement of high risk HPV subtypes in squamous cell carcinoma of the larynx. In contrast to other studies, one case only showed the possible contribution of HPV 6 and 11 in etiology of sinonasal inverted papillomas. The data concerning the role of HPV in transformation inverted papillomas to squamous cell carcinomas still need further confirmation.

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