

Original Article

Prevalence of Human Papilloma Virus in Sinonasal Papilloma in Southern Iranian Population

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KEY WORDS

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ABSTRACT

Statement of the Problem: Sinonasal papilloma (SNP) is a rare benign lesion characterized by high recurrence rate and malignant transformation.

Purpose: This study aimed to investigate the prevalence of human papilloma virus (HPV) infection in these lesions in South of Iran.

Materials and Method: In this cross sectional retrospective study, a total of 41 patients, 38 SNP and 3 SNP/Squamous cell carcinoma cases, from 2007 to 2014 were studied. Human papilloma virus (HPV) DNA detection was performed by nested PCR method and positive cases were analyzed for high risk HPV-16 and HPV-18.

Results: HPV was detected in 31.7%; HPV- 16 in 4.9% and HPV 18 was not detected at all. Dysplastic epithelium was detected in 53% that was not associated with HPV. Three cases were accompanied with malignant transformation that HPV genome was detected in only one case and none of them were positive for HPV16 /18 genomic DNA.

Conclusion: Current research suggests that HPV may be involved in the development of SNP. But the high risk HPV is not important in malignant transformation. More studies are needed to elucidate the possible etiologic mechanism between HPV, inverted papilloma, and squamous cell carcinoma.

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Introduction

Sinonasal papillomas (SNP) are benign, well differentiated tumor with high recurrence rate and malignant transformation is developed in 5-9% of cases. [1-2] Many studies have investigated the etiology of this disease; however, causes of tumoral transformation have not been fully elucidated. Previous studies suggested that human papilloma virus (HPV) infection plays a role in both recurrence and malignant transformation of squamous cell carcinoma (SCC). [3]

HPV infection is the most common sexually transmitted disease worldwide which is mainly transmitted through direct mucosal contact. [4-5] The viral

genome was detected in more than 50% and 90% of cervical and oropharyngeal cancers respectively. [5] The viral early genes encode two viral oncoproteins E6 and E7, which are involved in both benign and malignant proliferation of mucosal epithelium. E6 oncoprotein degraded wild-type functional p53. [5-6] E7 oncoprotein inactivates Rb protein and, promotes the G1-S phase transition with cell proliferation. [6-8] Persistent expression of viral oncogenes as well as proto-oncogenes activation is required for development of full blown malignant phenotype.

In malignant SNPs, HPV detection rates varied from 0% [3, 9-11] to 100%. [12-16] Zhong *et al.*, [17]

Table 1: Primer sequences of HPV nested polymerase chain reaction

Name	Sequence(5'-3')	Size (base pair)	Reference
MY9	GTCCMARRGGAWACTGATC	450	Manos <i>et al.</i> ,1989
MY11	GCMCAGGGWCTATAAYAATGG		
GP5+	TTTGTTACTGTGGTAGATACYAC	140	Jacobs <i>et al.</i> ,1995
GP6+	GAAAAATAAACTGTAAATCATATTC		

found that patients with HPV types 16 or 18 had a higher rate of malignant transformation than those with HPV 6/11. On the other hand, Kim *et al.* [3] reported that none of the malignant SNP cases was positive for HPV.

Several other studies [9-11, 18] also found no relationship between HPV infection and malignancy. Therefore, there is another opinion that HPV infection is an incidental colonization rather than the etiology of SNP or SNP/ SCC. [19]

This retrospective study was conducted to determine the prevalence of HPV virus in benign and malignant forms of sinonasal papillomas (SNPs) in Shiraz, South part of Iran. The positive cases were further evaluated for presence of high risk HPV16/18, to find any possible relationship with recurrence status or malignant transformation.

Materials and Method

Patient selection

The records of patients diagnosed with SNPs from 2007 to 2014 in Khalili hospital, the major referral center of Otolaryngology pathology in Shiraz, Fars province and south of Iran, were retrospectively reviewed. Total sinonasal specimens (as fixed paraffin embedded tissue blocks) which were diagnosed as inverted papilloma during this period was 41; composed of 28 male and 13 female patients. Clinical information was collected from the medical case history. However, information about potential causes of papilloma such as smoking, and exposure to organic solvents was not available. The study was approved by Ethical and Scientific Committee of Shiraz University of Medical Sciences.

DNA extraction

DNA extraction was performed from tumoral samples

using Dynabio HPV commercial extraction kit (Tehran, Iran). The β -actin DNA were amplified to assess DNA integrity with forward primer 5'-ATCATGTTTGAGACCTCCAA-3' and reverse primer 5'-CATCTCTTGCTCGAAGTCCA-3'. The polymerase chain reaction (PCR) condition was preheating 94°C for 5 min, and then followed 40 cycles 94°C for 1 min, 48°C for 45, 72°C for 30 seconds. The products were visualized by electrophoresis on 2% agarose gel.

HPV detection

HPV detection was carried out by nested PCR using the primers MY09/11 at first step and primers GP5+ /6+ at second step. DNA from a known HPV positive uterine cervix tissue was used as a positive control to assess the success of the amplification (Table 1). [20-21]

The PCR condition was preheating 94°C for 5 min followed by 50 cycles of 94°C for 1 min, 40°C for 2 min and 1min and 30 seconds at 72°C with final extension of 5 min at 72°C.

HPV16 and HPV18 genotyping

For detection of HPV 16 and HPV18 the conventional PCR was performed separately with PCR condition of preheating for 5 min at 94°C followed by 50 cycles of 94°C 1 min, 54°C for 1 min and 30 seconds at 72°C with final extension of 5 min at 72°C. The amplified fragment was analyzed on 3% agarose gel (Table 2). [10]

Statistical analysis

Data analyses were performed with statistical software (SPSS; Version 19.0, SPSS Inc., Chicago, IL, USA). The prevalence of HPV was expressed as the portion of HPV positive cases. Categorical variables were studied using chi-squared or t-test and Fisher exact test. $p \leq .05$ was considered statistically significant.

Results

Our retrospective study included 41 patients; 28 males

Table 2: Primer sequences for HPV 16 and HPV 18

	Primer sequence	Size of PCR Product
HPV16	TCA AAA GCC ACT GTC TCC TG CGT GTT CTT GAT GAT CTG CA	120 bp
HPV18	GAC ACA TTG GAA AAA CTA AC TAG TGC CCA GCT ATC TTG TG	140 bp

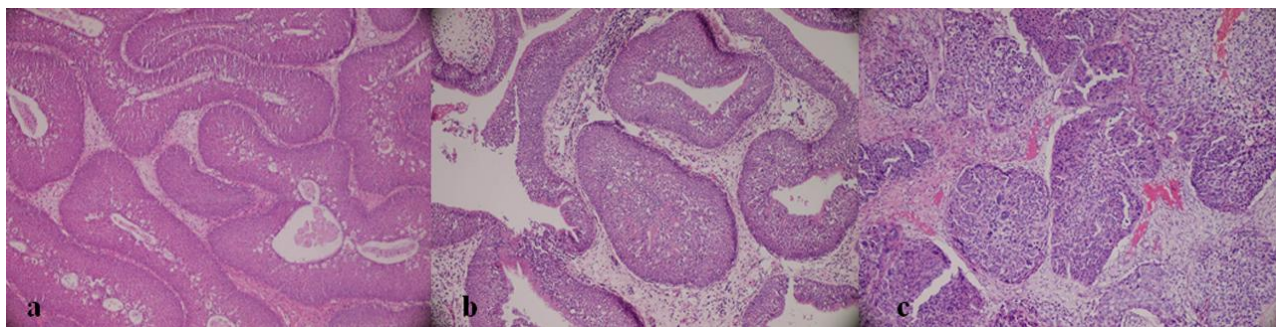


Figure 1a: Histology of inverted papilloma (H&E 200×), **b:** Inverted papilloma with mild dysplasia (H&E 200×), **c:** Squamous cell carcinoma arising from inverted papilloma (H&E 200×)

(68.3%) and 13 females (31.7%). The difference in sex distribution was statistically significant ($p=0.001$). The mean age of patients was 54 ± 10.12 years (range, 22 to 78 years). In three female patients, the papilloma was accompanied with synchronous squamous cell carcinoma (IPs /SCC).

Histologically, sinonasal papillomas were categorized as fungiform or exophytic papillomas (FPs) in 4 cases (9.8%); inverted papillomas (IPs) in 15 cases (36.6%) and mixed combination of IPs and FPs in 22 cases (53.7%).

In IPs, the invagination of squamous epithelium into edematous fibrous stroma was detected (Figure 1a).

Overall, IPs were detected in 90.3% of cases (pure or combination with FPs).

Dysplasia (Figure 1b) with different degrees was detected in 21 cases; 19 cases (48.7%) with mild dysplasia and two cases (5.1%) with moderate dysplasia.

Common HPV was amplified in 13 cases of SNPs (31.7%) that HPV 16 was detected in only two cases (4.9%). The HPV genome was detected in one case of IPs /SCCs (Figure 1c) with no amplification of HPV 16/18. There was no statistically significant correlation between different histological features of sinonasal papillomas and HPV genome ($p=0.949$) or HPV 16 ($p=0.14$) (Table 3).

Table 3: Prevalence rates of HPV DNA according to histological type of SNPs

Histological type	Common HPV Number (%)	HPV 16 Number (%)
IP + FP (n=22)	7(31.8%)	2 (9.1%)
IP (n=15)	5(33.3%)	0
FP (n=4)	1(25%)	0

FP: Fungiform papilloma; IP: Inverted papilloma

Common HPV DNA was positive in 35.7% male patients (13/28) and 23.1% (3/13) of female cases ($p=$

0.333). The mean age of patients in HPV positive cases was 52.6 ± 10.31 years and in HPV negative samples was 54.7 ± 19.31 years with no significant difference ($p=0.655$).

The HPV DNA was detected in 28.6% (6/21) and 33.3% (6/18) of SNPs that was accompanied with surface dysplasia and papillomas without atypia respectively ($p=0.465$).

HPV 16 genome was amplified in 4.8% (1/21) of dysplastic epithelium and 5.6% (1/18) of normal mucosa. There was no correlation between the presence of HPV DNA and the presence/absence of dysplasia in SNPs ($p=0.897$).

In respect of three malignant cases, HPV genome was detected in only one case and none of them was positive for HPV16 genomic DNA.

Discussion

SNP is a benign but destructive neoplasm in the nose and paranasal sinuses, associated with high postoperative recurrence rate and tendency for malignant transformation. [1, 16, 22-23] Exposure to organic solvents, and nickel compounds was considered as possible causes and smoking increased the incidence of malignant transformation. [24-25]

HPV is an epitheliotropic virus with causative role in cervical cancer. Several studies have been carried out to explore the association between HPV infection, SNPs and malignant transformation of SNPs with different conflicting results. [10, 14, 16, 26]

In current study, we found HPV genomic DNA in 31.7% cases of SNPs and HPV 16 was amplified in 4.9%. Beck *et al.* [14, 27] found HPV DNA in 63% of sinonasal papilloma and higher recurrence rate was observed in HPV positive cases.

Hasegawa *et al.* [16] believed that higher viral lo-

ad was observed in malignant lesions whereas others did not prove this correlation. [10] In our study, three cases were associated with malignant SCC and HPV genome was detected in only one case with no amplification of high risk HPV16/18 genomic DNA.

Jalilvand *et al.* [28] studied 40 samples of papilloma in Tehran, capital city of Iran. The study group composed 37 patients with benign papilloma and 3 patients in whom papilloma was accompanied with squamous cell carcinoma. The HPV genomic was detected in 18.9% of papilloma cases and 100% of papillomas were associated with SCC. In all HPV positive cases, HPV6/11 was detected in papillomas and the high risk presence of HPV16/18 in SCC samples. They concluded that HPV was important in pathogenesis of papilloma and HPV16/18 might be involved in malignant transformation. [28]

In comparison to similar study from Iran, [28] the overall prevalence of HPV was higher in our samples but the HPV6/11 was found in 4.9% of cases and high risk HPV 16/18 was not detected at all. According to difference in geographic area, other types of HPV might play a key role in the development of SNP in our population.

It has been proposed that HPV infection and especially high risk HPV16/18 plays an important role in the malignant transformation. [28] However, our results revealed that HPV was not detected in either papilloma or SCC samples; the HPV16/18 has not been considered as an etiologic factor in our population.

Lawson *et al.* [29] evaluated the published studies in sinonasal papilloma, low-risk and high-risk HPV infection and relationship between tumor recurrence and malignant transformation. The low-risk HPV was more frequently detected in exophytic papillomas when compared with inverted papillomas. They concluded that high-risk HPV was more detected in papillomas with moderate to severe dysplasia and associated with tumor recurrence.

In a meta-analysis performed by Syrianen *et al.*, [30] HPV prevalence was highest in exophytic papillomas (65.3%), followed by inverted papillomas (37.8%) and cylindrical cell papillomas (22.5%) respectively. The detection method and/ or geographic location were not important in HPV prevalence.

Xiao *et al.* [31] conducted another meta-analysis

in Chinese population and found that HPV had an important role in the occurrence and recurrence of sinonasal inverted papilloma. They also emphasized that high-risk HPV was closely associated with tumor progression. [31]

In another meta-analysis which was performed by Zhao *et al.*, [32] HPV infection, especially high risk HPV-18 was significantly associated with malignant transformation in sinonasal papillomas.

Some studies compared ISH and PCR methods for their sensitivity in viral detection and found no significant differences. [33-34] The detection rate of HPV in archived paraffin embedded specimens deteriorates from 20% at 1 year to 2% at 6 years. DNA extraction from fresh frozen specimens is the most reliable source for HPV amplification. [35]

Overall, several reasons are contributed to this inconsistency in results yielded by aforementioned studies. Our sample size of SNP/ SCC was too small to determine this association. DNA degradation occurred in paraffin-embedded tissues, which may lead to false negative results. Racial and geographical differences with presence of different environmental pollution might also have been other important predisposing factors.

Conclusion

According to the findings of our study, the prevalence of HPV in our population is in accordance with previous reports. But the high risk HPV is not important in malignant transformation. Therefore, more prospective studies are needed to understand the possible molecular mechanism between HPV, SNPs, dysplasia and squamous cell carcinoma.

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Conflict of interest

The authors disclose no potential conflicts of interest.

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