

Investigating Human Papilloma Virus Types in Sinonasal Papilloma Using Polymerase Chain Reaction: Is It Really a Prerequisite for Nasal Papilloma Formation?

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ABSTRACT

Sinonasal papilloma is a relatively rare disease. However, it is prevalent enough for every otorhinolaryngologist to encounter it several times throughout one's medical practice. The aim of this study was to identify the presence of Human Papilloma Virus in sinonasal specimens of patients with sinonasal papilloma.

A cross sectional analytical study was performed on fresh tissue samples from 36 patients with sinonasal papilloma. Samples were studied by polymerase chain reaction for of Human Papilloma Virus detection. In conclusion, the majority of patients were of Human Papilloma Virus negative and there was no statistically significant difference in presence of squamous cell carcinoma in of Human Papilloma Virus positive and negative patients. Thus, further studies are needed to assess other potential factors that may influence the development of sinonasal papilloma.

Keywords: *sinonasal papilloma, Human Papilloma Virus, polymerase chain reaction.*

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Article received on the 9th of December 2019 and accepted for publication on the 3rd of March 2020

INTRODUCTION

Sinonasal papilloma (SNP) was first reviewed by Kramer in 1935 to make a distinction between inflammatory nasal polyposis and true nasal papilloma (1).

It is a rare tumor of the nasal cavities and paranasal sinuses, histologically derived from the Schneiderian membrane as a result of transitional metaplasia of the respiratory epithelium (Figure 1). With three distinctive characteristics that distinguish it from other sinonasal tumors: relative local aggression, high rates of recurrence, whether early or late and possible association with carcinoma (2).

Histologically, three main variants are described: exophytic, inverting and oncocyctic types. Inverting papilloma (IP) is the commonest of the three types, with a higher clinical potential to recur or erode the bone laminae (3).

The annual incidence of SNP seems to vary between 0.74 and 2.3 per 100,000 population in defined geographical regions, usually affecting adults, with a slight male predilection (4).

Although the literature on nasal papilloma has expanded rapidly, the etiology of SNP is still unknown. Certain hypotheses have been suggested, but the causality has never been established (5).

Recurrence and malignant transformation potential are the two characteristic features suggesting SNP viral origin (6). An implication of Epstein-Barr Virus (EBV) has been studied by Macdonald and co-workers, but inconclusively (7).

Human Papilloma Virus (HPV) was reported as being a possible etiological agent for papilloma (8). Recently, morphological and clinical similarities between sinonasal inverted papilloma (SNIP) and established HPV lesions (recurrent

respiratory papillomatosis, genital warts) were emphasized, and indeed, the expression of HPV structural antigens was confirmed by immunohistochemistry (IHC). However, the prevalence of HPV antigens was comparatively lower than that of respiratory papillomatosis (RRP), which was about 100% (9). The main studies and meta-analyses of recent years show HPV rates varying between 17% and 38% (10). This range of variability between reports can be attributed neither to differences in the detection method nor to those in the geographic origin of the series, but rather to dysplasia grades between series (11).

Human Papilloma Virus induces overexpression of oncoproteins E6 and E7, leading to deactivation of cell-cycle regulators such as P16, P21, P53 and retinoblastoma gene proteins (12).

Although HPV is very probably involved in IP pathogenesis, current data provide no certainty as to its precise role (13).

According to the variable reports of worldwide studies and limited researches in Iran and given the importance of this topic, we conducted a local survey to determine the prevalence of HPV species in patients diagnosed as SNIP as well as their malignant transformation potential. □

MATERIALS AND METHODS

A cross-sectional analytical study was retrospectively performed from January 2011 to December 2017 in a referral university hospital (i.e., Rasoul Akram Hospital, Tehran, Iran). The proposal of the research project was set forth in the ENT and Head and Neck Research Center, Hazrat Rasoul Hospital, The Five Senses Institute, Iran University of Medical Sciences (IUMS), Iran.

Thirty-six patients were enrolled in our study. Written informed consents were obtained and ethical approval was given by the local ethics committee. Demographic data and clinical symptoms were obtained from patients' medical records. Tissue samples were provided by the department of pathology; they were examined not only for histopathological evaluation but also for the presence of HPV DNA and its genotyping. HPV genotyping was performed by the 21 HPV GenoArray Diagnostic Kit (Granada, Spain). The GenoArray assay test is able to detect 21 types of HPV, including 13 high-risk types

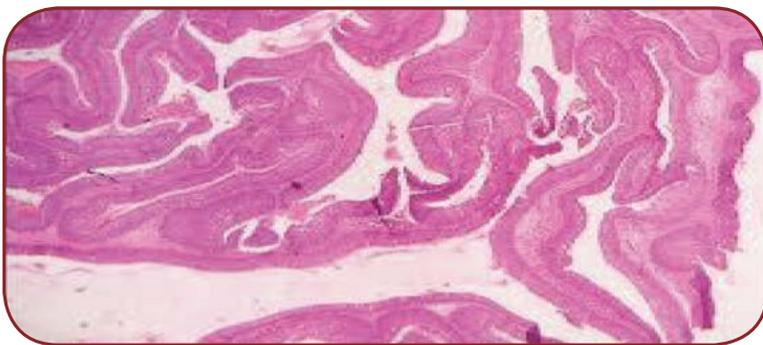


FIGURE 1. Endophytic growth of epithelial nests with smooth outer contour

(HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), six low-risk and undetermined risk types (HPV 6, 11, 42, 43, 44 and 81) and two likely high-risk types (types 53 and 66) using L1-Consensus primer based polymerase chain reaction (PCR) assay, followed by reverse dot blot hybridization that is based on the DNA Flow Technology with the semi-automated hybrispot 12 and the automated hybrispot 24.

Briefly, PCR was performed with a reaction volume of 25 µL containing DNA template, master mixture provided and DNA Taq polymerase. The amplification protocol was as follows: 9 min of denaturation at 95°, 40 amplification cycles at 95°C for 20 seconds, 30 s of annealing at 55°C and 30 s of elongation followed by 5 min at 72°C for final extension. After PCR amplification, amplicons were subjected to hybridization with the immobilized genotype-specific nucleotide probes embedded within the membrane fibers, with complementary molecules being retained by the formation of duplexes after stringent washes. Streptavidin-horseradish peroxidase conjugate was added, which binds to the biotinylated PCR products and a substrate (Nitroblue tetrazolium – 5-Bromo-4-Chloro-indolylphosphate) to generate a purple precipitate at the probe dot. Internal control dot (for monitoring of PCR) and biotin control dot (for monitoring of hybridization) should be present for each valid test. Results were then evaluated by a colorimetric change on the chip under direct visualization.

Blue-Purple spots were recognized as HPV positive. Human Papilloma Virus genotypes were then determined according to the distribution of colorimetric changes on each chip. □

OUTCOME MEASURES

The main outcome measures in our study are the prevalence of HPV infection in patients suffering from SNIP and its distribution across age, gender and histopathological types, and the distribution characteristic within different HPV subtype infections.

Data Analysis

The results obtained by the Genoarray Assay test were processed and analyzed by SPSS statistical software version 24 (IBM corp.NY).

Statistical analysis of continuous variables with normal distribution were expressed as mean±standard deviation (SD) and categorical data were defined as proportions or ratios. Baseline characteristics of study participants were compared using independent t-test or chi-square where appropriate. A p-value < 0.05 was considered statistically significant. □

RESULTS

Thirty-six (31 men and five women) patients with a mean age of 52.08±13.6 years were enrolled in our study. Three out of 36 (8.3%) sinonasal samples were detected as positive for HPV. There was no significant difference found between HPV positive rate of SNIP in male and female patients (P= 0.370, Table 1).

A HPV positive rate was found to be higher in patients aged over 39 (Table 1).

The HPV infection rate in the SNIP with dysplasia group and in the SNIP without dysplasia

Data	HPV +	HPV -	P-value
Patients with SNP	3 (8.3%)	33 (91.7%)	
Age (mean±SD)	53.9±12.8	33.33±5.5	0.011
Gender (M/F)	2/1	29/4	0.370
SCC history	1(33.3%)	4(12.1%)	0.370

TABLE 1. Demographic information of patients and their comparison with HPV



FIGURE 2. Effect of ergonomic instructions in senior students

group showed a difference that was not statistically significant ($P=0.064$, Table 1).

Four different HPV types were found in SNIP samples. Among HPV positive patients, one was a woman diagnosed as type 66, one had types 68 and 81, and the third patient had types 16 and 81 having simultaneously occurring SCC.

Among all investigated HPV types, two patients (5.56%) were positive for HPV 81 and one (2.78%) was positive for each of the HPV types 66, 68 or 16. Other HPV types were not positive for any patient, and 2.78% of cases were found to have a single HPV subtype infection, while 5.56% displayed more than one type of HPV infections simultaneously (Figure2).

There was no statistically significant difference either in the presence of SCC or in the history of smoking between the HPV positive and HPV negative groups. □

DISCUSSION

In the present study, the HPV infection status of 36 cases of SNIP was investigated retrospectively. Evidence on HPV involvement was obtained in benign sinonasal papilloma, and a few years later also in sinonasal SCC (SNSCC) (14).

The HPV DNA positivity rate of SNIP varied from 0% to 86% in the literature and it was strongly associated with HPV 6, 11, 16, 1 and 33 (15). More than 120 types of HPV have been currently identified and were classified into high-risk, low-risk and probably high-risk types based on carcinogenicity (16).

In a retrospective study, Jalilvand and colleagues examined the prevalence of HPV infection in Iranian patients suffering from SNIP and reported that 18.9% and 100% of sinonasal IP and sinonasal SCC were HPV positive, respectively; at the same time, HPV 6, 11 and HPV 16, 18 were the most commonly observed types in SNIP and SNSCC, respectively (17); but to our analysis, only 8.3% and 33.3% of SNIP and SNSCC were HPV positive retrospectively. In addition, the current study reported HPV 16, 66, 68, 81 and HPV 16 as the prominent types of HPV in SNIP and SNSCC respectively.

In a retrospective cross-sectional study, Valibeigi *et al.* investigated the presence of HPV 16, 18 DNA in sinonasal papilloma in the southern population of Iran by PCR, and HPV was detected in 31.7% of cases, while we de-

tected lesser HPV positive samples in our patients (8.3%), of whom none was positive for HPV18. Dysplastic epithelial cells were detected in 53% of subjects included in the study of Valibeigi *et al.* and three cases were associated with malignant changes, but only one of them was positive for HPV DNA; none of them was positive for HPV 16, 18 genome, which was in accordance to our study, where the only SNSCC case was negative for HPV 18 (18).

Zhang *et al.* studied the prevalence and distribution of HPV types in SNIP of Chinese population and showed that the prevalence of HPV infection in SNIP patients (64.7%) was higher than healthy control group, which was in contrast to our results. They assigned that the most common types of HPV in patients with sinonasal dysplasia were HPV 11 (40.8%) and HPV 58 (27.6%), as HPV 11 was most commonly found in Krouse stage T1 and T2 patients and HPV 58 in 45% of patients within Krouse T3 stage. Although we did not evaluate HPV typing according to Krouse sinonasal staging system, we detected three patients with different HPV types as HPV 16, 6, 68, 81, and the only SNSCC in our study was positive for HPV 16 (19).

Infection and genome integration of HPV are the prerequisites for carcinogenesis (20). Immune response of cytotoxic T-lymphocytes was shown to be significant in the prevention of squamous intraepithelial lesion development especially for HPV 16 infection (21).

The HPV infection rate in SNIP patients was found to be increased across the age group with the highest prevalence in subjects over 60 years old. This finding indicates that weakened immune response due to aging contributes to the increased trend of HPV infection incidence.

Our study has also revealed that HPV positive patients were older than HPV negative ones (22). However, some published papers insisted on a correlation between HPV and SNP (23). But the negative results of PCR in our study can confirm the theory that HPV in SNP cases is an accidental finding and it seems there is no relationship between the incriminated microorganism and SNP.

Given these paradoxical results, we need to re-evaluate the aforementioned theories in further studies. It is assumed that HPV can reside primarily in the sinuses or nasal cavity or it can be transmitted from other sources to the sinonasal area. Perhaps the latter is more probable, be-

cause in our study, in contrast to Kim's study (24), we found that most of SNP patients were HPV negative, showing that HPV could only infect a narrow spectrum of host cells.

Our study showed that HPV could be considered rather an accidental finding than an etiological factor in patients with SNP. Positive results of HPV growth in SNP may be found coincidentally. □

CONCLUSIONS

It seems that there is no correlation between HPV and SNP. Thus, further studies are needed

to find the other related causes of sinonasal papilloma. □

Acknowledgements: We hereby appreciate the sincere help of the hardworking staff of ENT and Head and Neck Research Center, The Five Senses Institute and all colleagues of the Pathology Department of Hazrat Rasoul Hospital, IUMS in Tehran, for helping us in conducting the present study.

Conflicts of interest: none declared.

Financial support: none declared.

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