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Original Article

The prevalence of human papillomavirus infection in Iranian patients with sinonasal inverted papilloma

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Abstract

Background: Inverted papilloma (IP) is an uncommon disease which arises in the mucosal membrane of the nasal cavity and paranasal sinus. It has been proposed that human papillomavirus (HPV) is the causal agent in the pathogenesis of IP and plays a key role in the progression from benign IP to malignancy. As there are no prior studies that focus on an Iranian population, this study intended to characterize the prevalence of HPV types in benign and malignant forms of IP.
Methods: In this retrospective study, we included a total of 40 IP patients [37 benign IP and 3 IP/squamous cell carcinoma (SCC)] who were referred to Amiralam Hospital in Tehran from 2004−2006.
Results: HPV was detected in 18.9% and 100% of IP and IP/SCC cases, respectively. In all HPV positive cases of IP and IP/SCC cases, HPV6/11 and HPV16/18 were detected, respectively. Therefore, HPV types were different between the IP and IP/SCC patients, and this difference was statistically significant ($p = 0.002$).
Conclusion: This study suggests that HPV6 and 11 may be involved in the development of IP, but HPV16 and 18 likely play an important role in the progression from benign to malignant form of IP.

Keywords: benign; human papillomavirus (HPV); HPV types; inverted papilloma; malignancy

1. Introduction

Inverted papilloma (IP) is a benign epithelial growth in the basement membrane of the nasal cavity and paranasal sinus that inverts into the underlying stroma with varying degrees of metaplasia.[1−3] This type of cancer is characterized by local aggressiveness, a tendency for recurrence even after radical treatment and an association with malignancy. Based on the histological grading system, IP ranges from inflammatory lesions with metaplasia to malignant squamous cell carcinoma (SCC).[1,4]

IP is a rare disease in the general population, with an incidence rate of 0.75−1.5/100,000 per year.[5] This tumor is more prevalent in male (male-to-female ratio = 3.4:1) and the white Caucasian population.[3] IP cases are usually diagnosed in the 5th−7th decades with the mean age of 53 years. However, IP has been sporadically reported in children, adolescents, and elderly individuals.[5]

IP typically arises from the lateral nasal wall in the area of the middle turbinate or ethmoid recesses and frequently extends secondarily into the sinuses, mainly the maxillary and ethmoid and, to a lesser extent, the sphenoid and frontal.[6] The

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most common clinical symptom of IP is unilateral nasal obstruction that has been observed in 80−98% of cases. Other common symptoms are clear rhinorrhea, nasal drip, epistaxis, headaches (especially frontal), epiphora, anosmia, proptosis, and diplopia.[7−9] In patients with malignant IP, epistaxis is the most frequent symptom.[3,7]

The etiology of these lesions is not well understood, but several risk factors have been proposed including exposure to organic solvents, welding fumes, nickel compounds, cigarette smoking, and infectious agents.[10]

As mentioned above, IP has three distinctive characteristics including a high tendency to recur after radical therapy, confined aggressiveness, and association with malignant transformation.[1,2,11] These features strongly suggest the involvement of an infectious agent, as it closely resembles the clinical pattern of recurrent respiratory papillomatosis.[12] Human papillomavirus (HPV) has been considered as a potential etiological agent associated with malignant changes of IP.[13] This assumption has been supported by proof that not only HPV genome and antigens have been commonly detected in IP specimens,[14−17] but also HPV infection increases the chance of recurrence and malignant transformation of IP.[17−20] Consequently, it has been proposed that HPV infection is the causal agent in the pathogenesis of IP.

Regarding the prevalence of HPV in IP patients, there are no studies that have been conducted in Iran. Thus, this study aimed to characterize the prevalence of HPV types in benign and malignant forms of IP in an Iranian population, in order to find a relationship between HPV types with IP presentation, recurrence status, and malignant progression.

2. Methods

2.1. Study population

A series of 40 formalin-fixed paraffin-embedded samples of sinonasal IP [37 benign IP and 3 malignant IP (SCC)] was obtained from patients referred to the Amiralam Hospital in Tehran, Iran from 2004 to 2006. All samples were collected following approval by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

The demographic features of patients were retrieved from their medical records. In order to investigate the recurrence of IP, patients were followed up for a duration of 17.68 ± 10.1 months (range, 6−24 months) and the recurrent IP was diagnosed by endoscopy test.

2.2. HPV DNA detection

Tissue preparation and DNA isolation were performed according to a previously reported procedure.[21] The integrity of extracted DNA was assessed by amplification of a 268 bp fragment of beta-globin that has been described elsewhere.[22] Following a DNA integrity assay, all samples were suitable for polymerase chain reaction (PCR) analysis.

Nested PCR using the consensus MY09/MY11 primer pair (outer primers) and the GP5+/GP6+ primer pair (inner primers), targeting about 150 bp fragment of HPV L1 gene, was applied to detect HPV DNA in the specimens. The first round of PCR reactions was carried out in a 25 μL reaction mixture including 100−200 ng of DNA template, 2mM $MgCl_2$, 10 pmol of each primer (MY09/MY11), 50μM of each dNTP and 2 U of *Taq* DNA polymerase (5 Prime GmbH, Hamburg, Germany). PCR amplification cycles included: an initial 3-minute denaturation at 94°C, followed by 40 cycles of 55°C for 1 minute, 72°C for 1 minute, 94°C for 1 minute, and a final annealing at 55°C for 1 minute with 5 minutes elongation at 72°C. The second round of PCR reactions was carried out in a 50 μL reaction mixture including 100−200 ng of DNA template, 3mM $MgCl_2$, 25 pmol of each primer (GP5+/GP6+), 50μM of each dNTP and 2 U of *Taq* DNA polymerase (5 Prime GmbH). PCR amplification cycles included: an initial 3-minute denaturation at 94°C, followed by 40 cycles of 40°C for 2 minutes, 72°C for 1.5 minutes, 94°C for 1 minute, and a final annealing at 40°C for 2 minutes with 5 minutes elongation at 72°C. The PCR products were run on a 1.5% agarose gel and stained with ethidium bromide.

2.3. Genotyping of HPV

The PCR amplification products were sequenced using a BigDye Terminator version 3.1 Cycle Sequencing Kit and a 3130 Genetic Analyzer Automated Sequencer as specified by Applied Biosystems manuals (Foster City, CA, USA). Nucleotide sequences were edited with Bioedit software (Tom Hall, Ibis Biosciences, Carlsbad, CA) and converted to FASTA format. Then, sequences were compared to other HPV types using the Blast server (http://www.ncbi.nlm.nih.gov/blast/).

2.4. Statistical analysis

The presence of any association between HPV positivity with age, sex, histology, and recurrence of IP were analyzed employing the Fisher's exact test (Epi Info 7, Statistical Analysis System Software, Centers for Disease Control and Prevention, Atlanta, GA). The differences were considered statistically significant when $p < 0.05$.

3. Results

A total of 40 IP patients (37 benign IP and 3 IP/SCC) were included in this study. The demographic features of cases including age, sex, histology, recurrent status, common symptoms, and the frequency of HPV DNA are shown in Table 1. Our study found that IP was 2.33 times more prevalent among men than women, and the mean age of patients was 64.56 ± 17.1 years (range, 28−49 years). All patients had a unilateral involvement of the nasal cavity, and the most common anatomical locations of IP involvement were the maxillary sinus, ethmoid sinus, and middle nasal turbinate. The most common clinical symptoms of IP were nasal obstruction, paroxysmal nocturnal dyspnea (PND), mouth breathing, and epistaxis.

The integrity of extracted DNA was confirmed by PCR from the beta-globin gene for all samples. Overall, HPV was