A Cross-Sectional Study on Sinonasal Inverted Papilloma: Does Human Papilloma Virus Play a Role in Its Etiology?

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Abstract

Aims: To correlate the HPV genotypes with recurrence of disease and malignant transformation. Methods: A prevalence cross-sectional study. The tumour tissue was isolated from the paraffin-embedded tissue (PET). The DNA was extracted from the tissue using the QiAamp DNA Mini Kit (Qiagen, Germany). Gel electrophoresis was performed to determine the presence of genomic DNA. HPV detection and genotyping were carried out using SACACE HPV High Risk and Low Risk Typing Real-TM kit (SACACE, Italy). Two different types of kits were used, that is HPV 6,11 Real-TM and HPV 16,18 Real-TM kits. Results: A total of 44 patients, 36 were male and 8 were female with a ratio of 5:1. 61.4% was Malay, 22.7% was Chinese, 11.4% Indian 4.5% other races. 15 out of 44 patients had HPV positive (34%). The recurrence rate of positive HPV infection compared to negative HPV was not statistically significant ($p>0.05$). There was a significant correlation of HPV 16 and 18 infection with malignant transformation ($p<0.05$). A high detection rate of a high-risk HPV type (67%) was observed in patients with inverted papilloma with malignant transformation. Conclusion: The prevalence of HPV in inverted papilloma is 34%. Our result supports that HPV infection is an aetiological factor in sinonasal inverted papilloma. A high-risk HPV plays a role in the oncogenesis of sinonasal inverted papilloma. Further studies should be conducted to further elaborate this relationship.

INTRODUCTION

Inverted papilloma (IP) is a benign epithelial tumour composes of well-differentiated columnar or ciliated respiratory epithelium having variable squamous differentiation. The incidence of IP approximately 0.2 to 0.6 per 100,000 population per year.[1] They most commonly arise from the lateral nasal wall (90%) with frequent extension into the maxillary, anterior, and posterior ethmoid sinuses.[2] They are typically unilateral (95%). There are three main clinical characteristics that attribute the tumours which have a tendency to recur (16-60%), their destructive capacity to surrounding structures and their propensity to be associated with malignancy (5-13%).[3] IP occurs predominantly in male patients, with a male to female ratio of 3:1. The age range of presentations extends from the second to the seventh decade of life. The mean age at the time of clinical presentation is nearly 50 years.[4]

HPV is a small double stranded DNA virus which is approximately 8Kbp in size. They are associated with specific mucosal and epithelial lesions ranging from benign proliferative lesions to invasive carcinomas. Approximately 120 genetically distinct types of human papillomaviruses have been identified, and the genomes of more than 80 have been completely sequenced.[5] Benign lesions are usually associated with low risk types of HPV which are the Type 6 and 11.[6] HPV types 16 and 18 are classified as the high-risk types and are commonly associated with malignant lesions. All the identified types of HPV have appeared to be only epitheliotropic and infect the mucosal or cutaneous epithelium of the anogenital tract or upper respiratory

tract.[7]. PCR-based detection of HPV E6 oncogene expression in frozen tissue samples is generally regarded as the gold standard for establishing the presence of HPV.[8]

The recurrence and malignant transformation of nasal inverted papillomas were related to HPV infection.[9] The presence of this virus may play a role in the biological behaviour of these epithelial proliferations. In a meta-analysis of 1041 sinonasal papilloma analysed for HPV, 347 (33.3%) cases were positive.[10] However, the association between HPV infection and IP is still not universally acceptable due to the high disparate rates of HPV detection of between 0–79%.[2] Due to this great variance, it was our aim to determine the prevalence of HPV in sinonasal inverted papilloma, to correlate the HPV genotypes with recurrence of disease and to correlate the HPV genotypes with malignant transformation.

MATERIALS AND METHODS

This was a prevalence cross-sectional study. All patients who were diagnosed to have sinonasal inverted papilloma.

Biopsy samples

A total number of 44 paraffin-embedded tissue (PET) samples. Specific PET blocks containing IP tissues were identified and chosen for sectioning and PCR analysis.

DNA extraction

The DNA from the tissues was carried out using the QiAamp DNA Mini Kit (Qiagen, Germany).

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Gel Electrophoresis

Gel electrophoresis was performed on all the samples to determine the presence of genomic DNA.

DNA Quantification

The quality of the DNA was determined using Nano-Drop ND-1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The extracted DNA was stored in -20 °C until further analysis.

HPV Detection

HPV genotyping was carried out using SACACE HPV High Risk and Low Risk Typing Real-TM kit (SACACE, Italy). Two different types of kits were used, that is HPV 6,11 Real-TM and HPV 16,18 Real-TM kits.

HPV genotyping

HPV genotyping was done using SACACE HPV High Risk and Low Risk Typing Real-TM kit (SACACE, Italy). This method enables the detection on twelve High Risk HPV (HR-HPV) genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and two Low Risk HPV (LR-HPV) (6, 11) targeting E7 region.

The strips then were inserted into the Strata-gene MX3000P for the amplification step. The amplification programmed was shown in Table 2.

Results obtained from RT-PCR were then keyed-in to the software (Microsoft® Excel HPV Typing Real-Time MX Results Matrix.xls) provided by the SACACE kit.

RESULTS

Demographic data

A total of 44 patients were included in the study. There were 36 male patients and 8 female patients with a ratio of 5:1 (Figure 1). The mean age of males with IPs was 60 (range,18 to 72 years) and the mean age for females was 56 (range, 41 to 68). There were 27 (61.4%) Malay patients, 10 (22.7%) Chinese patients, 5 (11.4%) Indian patients and the remaining 2 (4.5%) patients were of other races (Figure 2).

Inverted papilloma with recurrence and malignant transformation

Seven of 44 patients (16%) had tumour recurrence throughout the study period. This group of patients were classified as IP with recurrence. Three patients (6.8%) were found to have synchronous SCC and were classified as IP with SCC). The remaining 34 patients (77.2%) with benign lesions and without recurrence were grouped as IP (Table 1).

HPV status

HPV genotyping was carried out using Real-Time PCR (RT-PCR) technique with HPV Low Risk (6,11) and High Risk (16,18) Typing Real-TM kit. From the histological samples of 44 patients, 15 patients were found to have at least one subtype of HPV. HPV was detected in 15 (34%) of cases (Table 1).

Four patients were found to have two subtypes present from the histological specimens. One of the patients had Type 6 and Type 11 HPV, another patient had Type 6 and Type 16 present while two more patients had type 16 and Type 18 HPV detected (Table 2). None of the patients had more than two subtypes present.

The most prevalent type of HPV detected in the samples was Type 11 which was detected in eight (18%) patients. There were five (11%) patients that were positive for HPV Type 6. Four patients had HPV Type 16 (9%) while only two (5%) patients had HPV type 18 detected.

Eleven out of 34 (32%) patients with IP had at least one positive HPV detected. Two out of seven (29%) patients with tumour recurrence had at least one type of HPV positivity. Two out of three (67%) patients with IP with SCC had at least one type of HPV detected.

Association of HPV with tumour recurrence

There were seven patients who had tumour recurrence and were classified as IP with recurrence (Table 1). From this study, we found that only two out of the seven (29%) patients with tumour recurrence were positive for HPV. One of the patients had HPV 16 while another patient had HPV 6 and 11. The recurrence rate of inverted papilloma positive for HPV infection was 15% compared to IPs with negative HPV infection (18%). The result was not statistically significant ($p>0.05$). (Table 3)

Correlation of HPV with inverted papilloma with malignant transformation.

From this study, three out of 44 patients had IP with synchronous SCC. They were classified into the group IP with SCC (Table 1). During this study period, no patients had metachronous SCC. Two out of these three patients had positive HPV results. Both HPV 16 and 18 were detected in the two patients.

Our results showed an association between HPV 16 and 18 infection with malignant transformation ($p<0.05$). The HPV 16 infection and malignant transformation was statistically significant with a p value of 0.013 while HPV 18 infection and malignant transformation showed a p value of 0.003 (Table 1).

DISCUSSION

Inverted papilloma is a rare benign sinonasal tumour known for its local aggressiveness, high recurrence rate and the propensity for malignant transformation. It's pathogenesis still remains to be elucidated though these characteristics however potentially implicate an infectious aetiology. Numerous studies have been conducted, and the role of HPV infection still remains debatable. The recurrence and malignant transformation of nasal inverted papilloma is related to HPV infection, and the higher infection rate of high risk HPV subtype is one of the reasons for malignant transformation.[9]

A meta-analysis of 20 studies found that 131 IPs were positive for HPV from a total of 341 IPs, (38%).[11] Another meta-analysis study, a total of 1,041 IPs and IPsSCC were analysed and HPV DNA was detected in 347 (33.3%) of cases.[12] In our study, we found that the overall detection rate of HPV in IPs was 34.1% (15/44). This correlates with the detection rate as seen in other studies.

Sham et al found that the detection rate of HPV in benign IP was 31.7% (13/41) which is higher than the average of 26%.[13]The most prevalent type of HPV detected in our samples was Type 11 which accounted

for 18% (18 of 44) followed by 11% (5 of 44) who had HPV Type 6. Study by Zhou et al showed that the commonest type of HPV detected was Type 11.[14] In the current study, we found that 12 patients had at least one type of the low risk group of HPV (Type 6 and 11) and four patients had at least one type from the high risk group (Type 16 and 18). This results correlates with other studies that also showed that HPV subtypes 6 and 11 were the most frequently identified HPV subtypes in sinonasal papilloma.[10]

All the patients who had the low risk type of HPV belonged to the benign IP group. Out of the four patients who were positive for the high risk group of HPV, two had IP with SCC and two others belonged to the benign group. Studies have shown that the high risk types are usually more commonly associated with concomitant malignancy. This further raises the question of whether the benign group which are positive for HPV type 16 or 18 may potentially progress to premalignancy and malignancy. Histological analysis has suggested that IP tumorigenesis may occur through a series of discrete events. HPV high risk subtypes infection, can occur in benign inverted papilloma as an early event during the multistep tumorigenesis of these lesions. Cumulative genetic defects may be required to progress from benign to dysplastic inverted papilloma and to carcinoma.[15]

Inverted papilloma has the propensity to convert to squamous cell carcinoma in 5–9% of cases. Malignant alteration is found to be caused by the specific viral genes E6 and E7 of high risk HPV types 16 and 18 and their respective proteins, causing cell immortalisation with increased efficiency when they are expressed together.[7] In cervical cancers, it is well established that the malignancy originate in the transformation zone, where the columnar cells of the endocervix form a junction with the stratified squamous epithelium of the vagina, giving rise to squamous metaplasia. Cells at this junction will undergo rapid turnover and are vulnerable to the effects of carcinogens.[16] This theory may also be true for sinonasal epithelium, and may explain how HPV is responsible for the potential malignant transformation of IPs to malignancy.[17]

Barnes et al reported that of all carcinomas that were associated with IPs, 61% were synchronous and 39% were metachronous. There were 6.8% of patients (3 of 44) who had IP with malignant transformation. All the three patients had synchronous SCC and there were no patients with metachronous tumour.[11] There was evidence of HPV integration into inverted papilloma causing malignant transformation to squamous cell carcinoma which involved two out of three tumours in their study (66.7%). HPV type 16 was the predominant genotype found in those studies, followed by HPV types 18 and 11.[17] HPV was detected in 54% (12/22) of benign IP and 70% (7/10) of IP with SCC. By contrast, the rate of HPV detection in sinonasal SCC that are unrelated to IP is lower (22.3%, 95% CI 15.9–28.6).[18] HPV was detected in 21 out of 36 (58.3%) of inverted papilloma, and in 11 of 16 (68.8%) cases of inverted papilloma with squamous cell carcinoma.[19] In our study, we also found a high detection rate of high risk HPV infection (66.7%) in patients with IP with SCC as compared to 31.7% (13/41) in benign IP. This further supports that HPV infection plays an important role in malignant transformation of inverted papilloma.

Inverted papilloma is known for its high recurrence rate of about 16-60%. Most surgeons agree that recurrence is due to either incomplete removal of tumour or disease developing from predisposed mucosa, both of which occur more often with limited resections. However, studies have shown a high incidence of recurrence rate despite complete surgical removal. Many studies have shown correlation of HPV infection with recurrence. Study by Syrjanen et al found that all the four recurrent cases were HPV positive (three had HPV 11, one had HPV 16).[20] There was recurrence in 13 out of 15 HPV positive IP patients and in none of the 10 HPV negative patients.[18] In this study, we found that the recurrence rate was 15.9% (7/44). The detection rate of HPV infection in IP with recurrence was 28.6% (2/7). The detection rate seen in our study is lower than that reported in other studies.

There have been many theories as to why there is such a discrepancy in detection rates of HPV infection. The lower number of specimens testing positive for HPV may be attributable to several factors. DNA extraction from frozen specimens would be the most reliable source. Tissue banking would therefore serve as an ideal method to preserve tissue for optimal HPV detection. A few technical factors have been found to account for the observed striking variations in HPV detection rates.[21] PCR technique of HPV detection has been found to be sensitive to false positive results from a variety of sources. This may be due to errors in

result interpretation or, from true HPV negative specimens which may have been contaminated with HPV DNA. The current study focused on Malaysian population of multi-ethnicities (Malays, Chinese and Indians) while most other studies have detected higher detection rate of HPV IP in North American and European populations.

Lack of the expression of HPV DNA in the remaining samples may also be due to HPV subtypes other than that investigated. For instance, Xiang et al demonstrated a possible role for HPV subtype 57 in the pathogenesis of nasal inverted papilloma.[22] In this study, we only examined specimens for only four HPV types (6, 11, 16, 18). The number of patients that were enrolled in this study was small due to the rarity of this disease. Patients who have been found positive for HPV infection should be closely followed up in view of potential recurrence and malignant transformation. Regular screening of upper aerodigestive tract for SCC related malignancy should also be performed in this group of patients.

CONCLUSION

The prevalence of HPV detected in inverted papilloma in our patients is 34%. Our results support that HPV infection is an aetiological factor in sinonasal inverted papilloma. A high detection rate of a high-risk HPV type (67%) was observed in patients with inverted papilloma with malignant transformation. A high-risk HPV plays a role in the oncogenesis of sinonasal inverted papilloma. Further studies should be conducted to further elaborate this relationship.

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