Human papillomavirus infection in nasal polyps in a Chinese population

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In order to determine the prevalence and genotype distribution of human papillomavirus (HPV) infection in patients with nasal polyps, a total of 204 patients with nasal polyps and 36 healthy controls were recruited for this study. Genomic DNA was extracted from paraffin-embedded tissue sections. HPV DNA genotyping was achieved by a flow-through hybridization and gene-chip method. HPV-positive infection was identified in 82 of 204 (40.2 %) patients, while HPV DNA was not found in healthy controls (P<0.05). Genotyping analysis showed that low-risk HPV genotype 11 was the most prevalent type of HPV in nasal polyps (45.28 %). Both single and multiple HPV genotype infections were found in these HPV-positive cases, although most (74.39 %) were infected with a single genotype. In addition, there was no correlation between HPV infection or HPV subtypes and the clinicopathological characteristics of patients, such as age, gender, number of surgery and disease course. The data from our study clearly demonstrated that HPV infection was associated with nasal polyps. Both high-risk HPV and low-risk HPV (LR-HPV) genotypes were identified in nasal polyp tissues, and LR-HPV-11 was the most prevalent type. Future research will explore the association of HPV infection with the development and progression of nasal polyps.

INTRODUCTION

Nasal polyps are a very common disease arising from the mucosa of the nasal sinuses or nasal cavity (Newton & Ah-See, 2008). These benign lesions affect approximately 1–4 % of the general population, with a slight preference towards elderly men (Hedman et al., 1999). Small nasal polyps may cause no symptoms, but larger ones frequently do. Common clinical symptoms include blockage of the nasal passages or sinuses, halitosis, anosmia, frequent sinus infections or other problems. They are most often treated with steroids or surgery, although nasal polyps removed by surgery have a 70 % chance of recurrence. The underlying mechanisms for their development have yet to be determined, but they are frequently associated with allergies, asthma, aspirin-sensitive individuals and chronic sinus infections (Hamilos et al., 1993; Mygind, 1990). Viral infection has been postulated to be one of the more important aetiological factors in the pathogenesis, progression and recurrence of nasal polyps (Stierna, 1996).

Evidence has accumulated that human papillomavirus (HPV) infection is associated with the development of several benign and malignant human tumours (Hoffmann et al., 1998; zur Hausen, 1996). HPV is also known to be highly tropic for epithelial cells, as it provides ideal internal environment for the virus replication, nucleocapsid formation and the package of virus particles. The majority of HPV genotypes cause no symptoms. There are however some sexually transmitted genotypes that cause warts or cancers in the anogenital region of men and women. HPV genotypes are divided into high-risk (HR-HPV) and low-risk (LR-HPV) types according to their carcinogenic potential.

Previous studies have shown that HPV infection may be associated with human nasal polyposis, such as inverted papilloma (Hoffmann et al., 2000; Zaravinos et al., 2009). However, the importance of HPV infection and genotype distribution in nasal polyps in China has not been clearly demonstrated. Therefore in this study, we determined the
presence of HPV infection and genotyped HPV subtypes in the nasal polyps of Chinese patients using a flow-through hybridization and gene-chip technology (HybriMax; Chaozhou Hybribio Limited Corporation). Previous studies have shown that this method has a proven high sensitivity and specificity in genotyping HPV (Liu et al., 2010), and thus has been widely applied in HPV detection and genotype identification (Bo et al., 2010; De-you et al., 2009; Tao et al., 2006). We performed this assay to identify the genotypes of HPV in 204 tissue samples from patients with nasal polyps. Previous studies have classified HPV into two main genotypes, LR and HR. In this study, we identified both specific genotypes in paraffin-embedded tissues from nasal polyps, while HPV infection was not found in healthy subjects. We found that LR-HPV-11 was the most prevalent subtype of HPV infection in these nasal polyps. These data demonstrated that HPV infection is associated with nasal polyps in this Chinese population. Further investigations will determine the role of HPV in the development and progression of nasal polyps.

RESULTS

Prevalence of HPV infection in patients with nasal polyps

We analysed the presence of HPV DNA in tissue specimens from 204 patients with nasal polyps and 36 healthy volunteers using flow-through hybridization and gene-chip technology. We then compared the prevalence of HPV infection between healthy subjects and patients with nasal polyps. HPV infection was detected in 82 of 204 (40.2 %) patients with nasal polyps, whereas HPV infection was not found in any of the 36 healthy subjects. The difference was statistically significant ($\chi^2$ test; $P<0.05$).

Prevalence of different HPV genotypes in HPV-positive patients

To further identify the role of HPV infection in the development of nasal polyps, we analysed HPV genotypes using HybriMax. We found that among the 82 cases of HPV-positive patients, a total of 106 HPV strains of 13 HPV genotypes were found. Based on previous studies defining HR-HPV and LR-HPV genotypes, we classified these genotypes and found that the overall prevalence of HR-HPV and LR-HPV types was 45.28 and 54.72 %, respectively. Five LR-HPV subtypes (11, 6, 34, 70 and 44) and eight HR-HPV subtypes (58, 52, 18, 16, 68, 53, 31 and 33) were identified (Fig. 1). In addition, LR-HPV-11 was the most prevalent type (45.28 %), followed by HR-HPV-58 (16.04 %) and HR-HPV-52 (10.38 %).

Single and multiple HPV genotype infections

We also found that single and multiple genotype infections occurred in the HPV-positive nasal polyps. Sixty-one of 82 (74.39 %) cases were infected with a single HPV genotype, while 21 of 82 (25.61 %) were infected with multiple genotypes. In addition, dual infections were the majority of multiple-genotype infections (90.48 %), followed by triple (4.76 %) and quadruple (4.76 %) infections. The multiple HPV genotype infections could be divided into three categories: (i) HR- and LR-HPV; (ii) HR- and HR-HPV; and (iii) LR- and LR-HPV. The detailed distributions of each category are presented in Fig. 2.
Correlation between HPV infection and clinicopathological characteristics of patients with nasal polyps

In order to assess the potential correlation between HPV infection and the clinicopathological characteristics of patients with nasal polyps, we collected clinicopathological data including age, gender, numbers of surgery and disease course. Our data showed that 50 of 139 male patients (35.97%) were HPV positive, and 32 of 65 female patients (49.23%) were HPV positive ($P>0.05$; Table 1). Moreover, no statistical differences were found in LR-HPV and HR-HPV subtypes between the genders. Although there were a greater number of HR-HPV cases in patients who received more than one surgery or with a prolonged disease course, the difference of surgery times between HR-HPV and LR-HPV was not statistically significant. Patients were also divided into eight groups according to their age. In the age groups 10–19 ($n=9$), 20–29 ($n=21$), 30–39 ($n=29$), 40–49 ($n=32$), 50–59 ($n=54$), 60–69 ($n=40$), 70–79 ($n=15$) and 80–89 ($n=4$) years, the percentages of patients who were HPV positive were 44.44, 33.33, 37.97, 53.13, 31.48, 42.50, 40 and 75%, respectively. There was no statistical difference among patients of the different age groups ($\chi^2=6.568; P>0.05$). It appeared that neither the age, gender, numbers of surgery nor disease course were related to HPV infection or HPV subtypes.

DISCUSSION

Prevalence of HPV infection in patients with nasal polyps

A variety of studies have concluded that the rate of HPV infection in patients with nasal polyps ranges from 0 to 92.3%, whereas the correlation between HPV infection and development and progression of nasal polyps is not completely understood. Jing et al. (2003) showed that 27.14% nasal polyp cases were positive for HPV DNA. Furthermore, Zhou et al. (2001) found that 92.3% of patients with nasal polyps were HPV-DNA positive. In contrast, Hoffmann et al. (2006) observed that HPV DNA was not detectable in specimens derived from nasal polyps. These discrepancies may be due to the limited number of samples investigated or differences in the methods used for testing HPV. Flow-through hybridization and gene-chip technology is known to be highly sensitive and specific for HPV genotyping (Liu et al., 2010), and has been widely used for HPV detection and HPV genotype identification (Bo et al., 2010; De-you et al., 2009; Tao et al., 2006). In the current study, a large-scale population of patients with nasal polyps (204 cases) was recruited, and 82 of these patients were diagnosed as HPV positive. The overall infection rate was 40.2%. These data suggest that HPV infection in nasal polyps is prevalent.

Distribution of different HPV genotypes in HPV-positive patients

In the current study, we found that LR-HPV-11 was the most prevalent genotype (45.28%), followed by HR-HPV-58 (16.04%) and HR-HPV-52 (10.38%) (Fig. 1). These data may further confirm that nasal polyps are benign lesions. Although LR-HPV-5 and LR-HPV-6 have previously been identified as the major prevalent types in Chinese patients with nasal polyps from Shanghai and Beijing areas, respectively (Jing et al., 2003; Zhou et al., 2001), the current study did not show such prevalence. The discrepancy might be due to the differences in the study populations or the method used to detect HPV infection in tissue specimens. HPV-6 and HPV-11 are prevalent in benign condylomata acuminata (Chow et al., 1987), while HPV-52 and HPV-58 are generally found in Chinese women with cervical cancer (Huang et al., 1997). These data indicate that different local environments, living conditions and diseases may be affected by these different HPV genotypes. However, we cannot rule out the possibility that HPV subtypes have specific organ-targeting characteristics.

Single and multiple HPV genotype infections

Both single and multiple HPV genotype infections existed in the HPV-positive patients with nasal polyps in this study. Dual infections were the majority of the multiple HPV genotype infections (90.48%). Quadruple infections with both HR- and LR-HPVs were also found, suggesting that HPV genotypes have no competitive inhibition in the host, in contrast to those found in previous studies of nasal polyps (Jing et al., 2003; Zhou et al., 2001) and cervical cancers (Liu et al., 2010; Sun et al., 2010). Additionally, co-infection by HPV-11 and HPV-58, a typical pattern in HR- and LR-HPV dual infections, was quite prevalent in these patients, implying that there were no inhibitory effects between HR- and LR-HPV genotypes.

Correlation between HPV infection and clinicopathological characteristics of patients with nasal polyps

Our results demonstrated that clinicopathological characteristics of patients with nasal polyps, such as the

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Table 1. Correlation between HPV infection and clinicopathological characteristics of patients with nasal polyps

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<tr>
<th>Characteristic</th>
<th>HPV positive</th>
<th>HPV negative</th>
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<tbody>
<tr>
<td></td>
<td>LR-HPV (n)</td>
<td>HR-HPV (n)</td>
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<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
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<td>26</td>
<td>24</td>
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<tr>
<td>Female</td>
<td>13</td>
<td>19</td>
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<td>No. surgeries</td>
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<tr>
<td>1</td>
<td>32</td>
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<tr>
<td>$\geq 2$</td>
<td>7</td>
<td>13</td>
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<td>13</td>
<td>12</td>
</tr>
<tr>
<td>1–10</td>
<td>19</td>
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<td>$&gt;10$</td>
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patients’ gender, age, number of surgeries and disease course were not associated with HPV infection or HPV subtypes (Table 1). Male and female patients presented equal susceptibility to HPV infection, which is consistent with previous reports (Jing et al., 2003). It has been suggested that HPV prevalence curves exhibit a U-shaped age-specific pattern, with higher prevalence in younger and older women than in middle-aged women (Castle et al., 2005). In contrast, there was no significant difference in HPV prevalence in females of varied ages from the Tibet Autonomous Region of China (Jin et al., 2009), which agrees with the results obtained in the present study. The potential correlation between HPV infection and age prevalence requires further investigation.

The HPV E6 gene is one of the most relevant viral gene products that contribute to the immortalization and transformation of HPV-infected cells (Münger et al., 2004). The activation of E6 appears to be critical for in vivo induction of epithelial hyperplasia (Nguyen et al., 2003), and thus may lead to the recurrence of nasal polyps. In the current study, there was no significant association between HPV infection and the number of surgeries, with a trend toward lower HPV-positive rates in patients with a greater number of operations. This phenomenon might be due to the self-limited ability of HPV virus in host cells, but we cannot exclude the possibility that our results could be related to some missed follow-up cases after surgery. Although a previous report showed that both HR- and LR-HPV subtypes were related to the progression of benign nasal inverted papilloma with atypical hyperplasia (Sun et al., 2010), no significant differences were found between HPV infection and nasal polyp recurrence.

In addition, there was no statistical difference between HPV infection and disease course. Nevertheless, enhanced HPV infection was observed in patients with a disease course of 1–10 years. When the course of disease was longer than 10 years, the HPV infection rate was reduced, suggesting that virus clearance by the immune system may play a role in eliminating HPV. However, the number of HR-HPV cases was increased with a prolonged disease course, which might be related to the capability of HR-HPVs in suppressing host immune responses (Suprynocz et al., 2008) and blocking the apoptotic signalling pathway (James et al., 2006).

METHODS

Study population. A total of 204 subjects who met the diagnostic criteria of nasal polyps were recruited from Shanghai Pudong New Area Gongli Hospital between the years 2005 and 2010. Among these patients, 139 were male and 65 were female. The median age of these patients was 49.12 years (range: 10–87 years, SD=16.18). The clinical history of nasal polyps ranged from 1 month to 50 years, with a mean of 8.58 years. All of the 204 patients had undergone surgery for the removal of nasal polyps at least once, and in 60 cases such surgery had been performed twice. In addition, 36 healthy individuals (22 male and 14 female) with a median age of 45.75 years (range: 18–67 years, SD=10.46) were also included. Nasal polyps from patients or middle turbinate mucosa from healthy subjects were removed and used for experiments. This study was approved by the Ethics Committee of Shanghai Pudong New Area Gongli Hospital. All subjects agreed to participate in this study by providing written informed consent.

HPV-DNA extraction. Formalin-fixed and paraffin-embedded tissue blocks were obtained from the Department of Pathology, Shanghai Pudong New Area Gongli Hospital, Shanghai, China. Paraffin sections were then prepared from these blocks. After grinding in liquid nitrogen, these paraffin sections were dewaxed in dimethylbenzene for 10 min and rehydrated in a series of ethanol solutions [100, 95 and 75% (v/v) ethanol for 10 min each]. Tissue DNA was extracted by using a QIAamp DNA Mini kit (Qiagen) according to the manufacturer’s instructions.

PCR, flow-through hybridization and gene chip. Approximately, 1 µg DNA from the paraffin section was incubated with 24 µl PCR mixture (23.25 µl PCR pre-mix and 0.75 µl Taq DNA polymerase) obtained from Hybribio Biotechnology. Specific HPV primers were provided by Hybribio, but the company did not disclose the primer sequences. The PCR mixture was then amplified in a Veriti 96-well PCR Thermal Cycler (Applied Biosystems) for an initial denaturation at 95 ºC for 9 min followed by a total of 40 cycles at 95 ºC for 20 s, 55 ºC for 30 s and at 72 ºC for 30 s. At the final cycle of the PCR amplification, the PCR mixture was set at 72 ºC for 5 min and then stored at 4 ºC.

HPV genotyping was achieved by a flow-through hybridization and gene-chip method. The low-density gene chip was pre-fixed with 37 type-specific oligonucleotides and the genotype was analysed using HybriMax (Chaozhou Hybribio Limited Corporation). The results were then evaluated by a colorimetric change on the chip under direct visualization. Blue–purple spots were recognized as HPV positive. The gene chip identified 22 genotypes of LR-HPV and 15 genotypes of HR-HPV.

Statistical analysis. Correlations of HPV infection between normal and nasal polyp tissues or between the clinicopathological parameter and HPV infection, and the associations of the different subtypes of HPV infection in nasal polyps were analysed by using z² tests with SPSS 11.5 software. A P-value <0.05 was considered statistically significant.

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REFERENCES


