Prevalence of Human Papilloma Virus and Human Herpes Virus Types 1–7 in Human Nasal Polyposis

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This study aimed to investigate the prevalence of human papilloma virus (HPV), herpes simplex virus-1/-2 (HSV-1/-2), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus-6/-7 (HHV-6/-7) in 23 human nasal polyps by applying PCR. Two types of control tissues were used: adjacent inferior/middle turbinates from the patients and inferior/middle turbinates from 13 patients undergoing nasal corrective surgery. EBV was the virus most frequently detected (35%), followed by HPV (13%), HSV-1 (9%), and CMV (4%). The CMV-positive polyp was simultaneously positive for HSV-1. HPV was also detected in the adjacent turbinates (4%) and the adjacent middle turbinate (4%) of one of the HPV-positive patients. EBV, HSV, and CMV were not detected in the adjacent turbinates of the EBV-, HSV- or CMV-positive patients. All mucosae were negative for the VZV, HHV-6, and HHV-7. This is the first study to deal with the involvement of a comparable group of viruses in human nasal polyposis. The findings support the theory that the presence of viral EBV markedly influences the pathogenesis of these benign nasal tumors. The low incidence of HPV detected confirms the hypothesis that HPV is correlated with infectious mucosal lesions to a lesser extent than it is with proliferative lesions, such as inverted papilloma. The low incidence of HSV-1 and CMV confirms that these two herpes viruses may play a minor role in the development of nasal polyposis. Double infection with HSV-1 and CMV may also play a minor, though causative, role in nasal polyp development.

INTRODUCTION

Nasal polyps, a common chronic disease of the nasal or paranasal sinus mucosa, are non-neoplastic mucosal lesions. These inflammation-induced mucosal swellings histopathologically consist of basement membrane thickening, atypical gland formation, goblet cell hyperplasia, inflammatory cell infiltration, and subepithelial edema. The epithelium of the mucosal swelling is, however, normal [Stierna and Carlsoo, 1990; Hosemann et al., 1994; Hellquist, 1996]. These lesions often originate from the middle meatus and the ethmoid sinus region of the nasal cavity [Larsen and Tos, 1991]. Nasal polyposis is almost always present in conjunction with chronic rhinosinusitis [Fokkens et al., 2005]. Since the molecular alterations required for nasal polyp development have only recently been investigated, the molecular mechanisms behind nasal polyps are poorly understood. Along with various factors, including K-RAS codon 12 mutations/elevated expression levels [Zaravinos et al., 2007], increased expression of VEGFA and TGFβ1 [Watelet et al., 2004; Little et al., 2008; Zaravinos et al., 2008b] and BRAF and RKIP under-expression [Zaravinos et al., 2008a], virus infection is considered to play a causative role in the pathogenesis of nasal polyposis, excepting malignant sinonasal neoplasms [Kozak et al., 1991; Becker et al., 1994; Hoffmann et al., 2000, 2006; Zhou et al., 2001; Lu et al., 2007].

Human papilloma virus (HPV) infections are classified into those that (i) produce specific clinically visible lesions, (ii) remain subclinical, and (iii) are latent

KEY WORDS: nasal polyps; adjacent inferior/middle turbinates; HPV; EBV; HSV-1/-2; CMV; VZV; HHV-6/-7; PCR

Abbreviations used: HPV, human papilloma virus; HSV-1/-2, herpes simplex virus-1/-2; VZV, varicella-zoster virus; EBV, Epstein–Barr virus; CMV, cytomegalovirus; HHV-6/-7, human herpes virus-6/-7; PCR, polymerase chain reaction; ISH, in situ hybridization.

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Accepted 1 April 2009
DOI 10.1002/jmv.21534
Published online in Wiley InterScience (www.interscience.wiley.com)
Zoster virus (VZV), a viral infection has in the past been reported to play a role in lesions of the upper respiratory tract [Tao et al., 1996; Gao et al., 2000; Kulkarni et al., 2003]. However, their role in the development of human nasal polyposis is poorly understood.

To contribute further to the knowledge on viral infection of the mucosa of the upper airways, the presence of a group of viral DNA [HPV and seven herpes viruses: herpes simplex virus-1/-2 (HSV-1/-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), and human herpes virus-6/-7 (HHV-6/-7)] was determined in nasal polyps of the nasal cavity and the paranasal sinuses, in mucosa from the adjacent inferior and middle turbinates, as well as in clinically intact mucosa from control inferior and middle turbinates obtained from disease-free patients. Molecular analysis revealed the frequent occurrence of EBV in nasal polyp samples, implying that EBV viral infection plays an important role in the pathogenesis of the disease.

**METHODS**

**Patients**

Biopsies of nasal polyps and mucosa from the inferior and middle adjacent turbinates were obtained from 23 patients with chronic rhinosinusitis/nasal polyposis (17 men and 6 women; average age, 51 years; range, 21–73 years). Polyps arose from the middle turbinate, middle meatus, or ethmoidal sinuses and were multiple and bilateral. Following a histopathological examination of the specimens, the nasal polyps were classified into grades I–III according to their size [Malm, 1997] (Table I). Patients suffered from sneezing, rhinorrhea, headaches, nasal obstruction, and hyposmia or anosmia, and had undergone nasal polypectomy at least once in the past. Patients with nasal polyps had not taken any drugs (topical or systemic corticosteroids) for at least 1 month prior to surgery.

Thirteen patients with nasal septum deformity who underwent nasal corrective surgery and had no history of nasal or allergic symptoms of any kind served as the control group. During surgery, a biopsy of the mucosa of the inferior and middle control turbinates of each patient was performed. Patients in this group had not taken corticosteroids for at least 1 month prior to surgery.

Total and specific IgE levels were determined by RAST in the sera of the patients. The patients were subjected to hypersensitivity skin tests for a number of allergens, and those with allergy or asthma were excluded from the study. Clinical parameters such as age, gender, smoking habits, and alcohol consumption were available for the patients and control subjects.

The Ethics Committee of the University of Crete approved the present study. All participants (patients and control subjects) were informed of the scope of the study and gave their written informed consent.

**Genomic DNA Extraction, Oligonucleotide Primers, and PCR Amplification**

Nasal mucosa tissues were digested with 0.1 mg/ml proteinase K (Promega, Madison, WI) and 400 μl of digestion buffer containing 150 mM NaCl, 400 mM Tris–HCl, 60 mM EDTA, and 15% SDS, pH 8.0, in a 1.5-ml eppendorf tube. Samples were then incubated at 60°C for 2 days. Fresh proteinase K was added three times daily. The samples were extracted once with phenol/chloroform and once with chloroform. DNA was precipitated by the addition of 20 μl of 5 M NaCl and 1 ml of ethanol, recovered by centrifugation for 15 min at 4°C, washed once with cold 70% ethanol, and resuspended in 50 μl of double distilled water. DNA concentration was calculated using the NanoDrop™ 1000 Spectrophotometer. Specimens were examined for the presence of amplifiable DNA using a set of primers for the β2-microglobulin gene.

Amplification of HPV and herpesviridae family viral DNA in the specimens was originally performed by polymerase chain reaction (PCR) using specific primer pairs for each E6 gene region of the HPV-6, -11, -16, -18, and -33 subtypes, or herpes family DNA primers specific

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**TABLE I. Clinicopathological Characteristics of the Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Nasal polyps and adjacent turbinates</th>
<th>Control mucosae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.3 years; range, 21–73</td>
<td>33.4 years; range, 12–68</td>
</tr>
<tr>
<td>Smoke (yes/no)</td>
<td>8/15</td>
<td>5/8</td>
</tr>
<tr>
<td>Alcohol (yes/no)</td>
<td>7/16</td>
<td>4/9</td>
</tr>
<tr>
<td>Aspirin tolerance/intolerance</td>
<td>6/17</td>
<td>13/0</td>
</tr>
<tr>
<td>Stage of nasal polyp (grades I–III)</td>
<td>I:3</td>
<td>II:9</td>
</tr>
<tr>
<td></td>
<td>II:11</td>
<td></td>
</tr>
</tbody>
</table>
for each virus. The samples were initially examined for the
presence of non-type-specific HPV DNA using the
general HPV primers, GP5+/+6+ [de Roda Husman et al.,
1995]. Appropriate negative and positive controls were
included in each PCR reaction in order to exclude
contamination events and to establish the specificity
of primer-directed amplification. Recombinant plasmids
containing HPV type-specific sequences served as
positive controls for the corresponding PCR reactions.

The University Hospital of Heraklion, Crete, and used as
patients was kindly provided by the Virology Clinic of
known CMV-, HSV-, VZV-, HHV-6-, and HHV-7-positive
template for EBV amplification. DNA from previously
Supernatant from the B19 marmoset cell line (ATCC,
Wesel, Germany) was used as a relevant control
among HPV strains were used as the positive control.

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contamination events and to establish the specificity
of primer-directed amplification. Recombinant plasmids
containing HPV type-specific sequences served as
positive controls for the corresponding PCR reactions.
for statistical analysis. In the patient group, infection was correlated with clinical characteristics including age, gender, tobacco and alcohol consumption, aspirin tolerance/intolerance, and nasal polyp grade. P-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS v16.0 software (SPSS, Inc., Chicago, IL).

RESULTS

This study was conducted to determine the prevalence of HPV and seven herpes virus types (HSV-1, HSV-2, EBV, VZV, CMV, HHV-6, and HHV-7) in human nasal polyposis and the adjacent turbinates using PCR.

Three nasal polyp samples (13%) and one sample of each adjacent turbinate [inferior, 1/23 (4%); middle, 1/23 (4%)] of an HPV-positive nasal polyp patient were found to be HPV positive (P = 0.044; Pearson's bivariate correlation). Further HPV typing of the samples did not reveal any of the oncogenic types of the virus (HPV-6, -11, -16, -18, and -33) (Fig. 1).

Of note, eight nasal polyp samples (8/23, 35%) were EBV positive. Correlation analysis revealed this to be highly significant (P < 0.001; Pearson's bivariate correlation), supporting the hypothesis that the presence of EBV markedly influences the pathogenesis of nasal polyposis.

Two nasal polyp samples (8%) were found to be HSV-1 positive (P = 0.040; Pearson's bivariate correlation) and one sample (4%) was found to be CMV positive. None of the adjacent nasal turbinate mucosae were positive for the presence of HSV-1 or CMV (Fig. 2). The CMV-positive nasal polyp sample was positive simultaneously for HSV, but otherwise the simultaneous presence of two or more DNA viruses was not detected. No nasal polyp samples from the adjacent or control turbinate mucosae were found to be positive for the remaining viruses (VZV and HHV-6/-7). The control mucosae (from the inferior and middle turbinates) were negative for the presence of HPV, EBV, HSV-1/-2, CMV, VZV, or HHV-6/-7.

Table III shows the exact number of infected samples as a percentage, as well as the statistically significant correlations detected among the tissue groups studied. Further statistical analysis failed to reveal any association with the influence of HPV or herpes virus on nasal polyp grade or other clinicopathological characteristics.

DISCUSSION

Despite extensive research on human nasal polyposis, with case percentages reaching 4.3% in population studies in the USA and Europe, the cause of the disease remains unknown. Many exogenous and endogenous factors have been proposed as being responsible for their growth [Watelet et al., 2004; Zaravinos et al., 2007, 2008b; Little et al., 2008], including bacterial or viral infection. In an animal experiment, the induction of nasal polyps in the maxillary sinus of rabbits was demonstrated by infecting the rabbits with various bacteria and preventing the ventilation of the sinuses [Stierna, 1996].

Using PCR, the present study confirmed the absence of a significant HPV infection percentage in nasal polyps. Previously reported observations regarding the lack of correlation between HPV infection and nasal polyp formation [Hoffmann et al., 2006] were also confirmed by PCR and Southern blot hybridization. On the other hand, Becker et al. [1994] reported 20 HPV-negative cases of nasal polyps using the MY09 and MY11 primers, as opposed to the GP5+/6+ primers used in the present study. Findings regarding the incidence percentage of HPV with a variable degree of dysplasia [Zhou et al., 2001] also appear to contradict the results of the present study. A higher degree of proliferation and apoptosis has been demonstrated in inverted papilloma compared to nasal polyps [Guichard et al., 1998]. Cellular proliferation was also significantly higher than apoptosis in the inverted papilloma, which is characteristic of their neoplastic structure. The principal hypothesis regarding the ability of HPV to induce cellular proliferation in various lesions of the mucosa is based on the fact that the virus uses the replication system of the host cell, as the virus itself does not code for replication enzymes. However, the host cell, primarily the stroma of basal cells in the squamous cell epithelium, arrests replication so that the virus may pass through the epithelium. To prevent this termination of cellular proliferation, and thus to maintain and enhance the proliferation rate of the host cell, the viral oncogenic proteins E6 and E7, respectively, interact with the cell proliferation regulators p53 and pRB [Vousden, 1993]. This hypothesis regarding viral infection is supported by the absence of HPV DNA infection in non-neoplastic lesions, such as nasal polyps. The data of the present study demonstrating the presence of viral...

Fig. 1. A: Amplification with the general primers GP5+/6+ yielded three NP samples positive for the HPV virus. Lanes 1 and 2: HPV-positive NP samples; lane 3: HPV-positive control; lane 4: HPV-negative control. Lane L, 100 bp DNA ladder. B: Further typing of the three HPV-positive NP samples with type-specific primers did not give any positive outcome. Lanes 1, 2, 5, 6, 9, and 10: Negative samples for the oncogenic types HPV-16, -18, and -33, respectively; lanes 4, 8, and 12: negative controls for each of the subtypes pre-mentioned; lane L, 100 bp DNA ladder. NP, nasal polyp.
infection in nasal polyps confirm the hypothesis that the HPV virus is correlated to a lesser degree with infectious lesions of the mucosa than with proliferative lesions.

EBV is a member of the gamma herpes virus family and causes infectious mononucleosis and two types of cancer, including Burkitt’s lymphoma [Panagiotakis et al., 2007]. In the present study, eight nasal polyp samples (35%) were found to be EBV positive, with a statistically significant correlation between the presence of EBV and nasal polyp formation (\( P < 0.001 \)). The presence of EBV infection in nasal polyps was demonstrated at an even higher percentage using various techniques in patients from Hong Kong, China [15% with Southern blot, 69% with PCR, and 85% with in situ hybridization (ISH)] [Tao et al., 1996]. In contrast, in a study with Canadian nasal polyp patients, EBV was not detected by ISH for EBV DNA [Kozak et al., 1991]. These differences in EBV detection rates are likely due to geographical criteria, as well as to differing detection and sampling methods. In the present research, PCR was applied, since it is a more sensitive molecular approach, rather than FISH, in order to study virus expression in nasal mucosa. The results of the present study, in combination with previously reported studies [Tao et al., 1996], indicate that a low level of persistent lytic infection due to EBV may occur in the nasal mucosa. As it is possible for other lymphocytes to be infected by a virus released from a lytic EBV infection at that site, EBV-positive cells are able to persist through this mechanism, in turn contributing to the persistence of EBV. However, whether this leads to the formation of nasal polyps is unknown. As EBV has been detected in 88% of normal nasopharyngeal mucosa [Tao et al., 1996], while nasal polyps are much rarer, it is considered unlikely that EBV contributes to the etiology of nasal polyp formation. The EBV virus has been detected in normal nasopharyngeal mucosa and in the nasal lesions of nasal lymphoma, nasopharyngeal carcinoma, and nasal polyps. The number of cells containing the EBV virus in nasal polyps or normal nasopharyngeal mucosa [Tao et al., 1995b,c] is significantly lower than the corresponding number in nasal lymphomas [Tao et al., 1995a] and nasopharyngeal carcinoma [Wu et al., 1991], despite proliferative EBV infection in nasal polyps or normal nasopharyngeal

### TABLE III. Rates of HPV and *Herpes Viruses* DNA Detection, in Nasal Polyps, Adjacent and Control Turbinate Mucosae (Middle and Inferior)

<table>
<thead>
<tr>
<th>Virus type</th>
<th>Nasal polyp (n = 23)</th>
<th>Adjacent turbinates (AIT and AMT) (n = 46)</th>
<th>Control turbinates (CIT and CMT) (n = 26)</th>
<th>Significance*</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected (n)</td>
<td>Infected (n) %</td>
<td>Infected (n)</td>
<td>%</td>
<td>Infected (n)</td>
</tr>
<tr>
<td>HPV-GP5+/6+</td>
<td>3</td>
<td>13</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><em>Herpesviridae family</em></td>
<td></td>
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<tr>
<td>HSV-1</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSV-2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>CMV</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EBV</td>
<td>8</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VZV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>HHV-6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>HHV-7</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tbody>
</table>

PC, Pearson correlation.

*Pearson’s bivariate correlation.

mucosa. This difference in viral load may be due to immunological surveillance by the healthy host of non-neoplastic nasal and nasopharyngeal mucosa, by which the cells carrying the EBV load are constantly destroyed. The small number of cells bearing EBV in non-neoplastic nasal and nasopharyngeal mucosa may also reflect the pressure of the immunological surveillance of the host. However, the frequent detection of low EBV infection levels in the nasal mucosa, at least in the context of an inflammatory lesion, supports the theory that the nose is one of the sites of EBV persistence. Thus, EBV may be responsible for tumors at this site. This finding could probably provide the basis for future clinical applications in the detection of EBV.

HSV-1 causes oral and occasionally genital cold sores, whereas HSV-2 is the most common cause of genital herpes. In the present study, two positive nasal polyp samples (8%) were detected by PCR. Previously, HSV was not detected in nasal polyps from Canadian patients using the ISH technique [Kozak et al., 1991]. Of note is that the number of published reports referring to the involvement of HHV infection in the formation of nasal polyps is limited. Therefore, the relatively low incidence (8%) detected in the present study should be regarded as arbitrary.

VZV causes chicken pox and herpes zoster. No VZV-positive nasal polyp samples were found in the present study, suggesting that this virus is not involved in the pathogenesis of the mucosa. To the best of our knowledge, there are no previous reports correlating the presence of this virus with the formation of nasal cavity lesions.

CMV belongs to the beta herpes virus family and causes serious symptoms, particularly in immunocompromised patients [Drew, 1992, 2007]. Apart from two reports, one on a CMV infection case with rare clinical manifestation in the form of nasal polyp [Kulkarni et al., 2003], and another on a CMV case with chronic sinusitis and nasal polyposis in a patient [Yoskovitch and Cantrell, 1998], no other reports attribute nasal polyposis to the presence of CMV. In the present study, one CMV-positive nasal polyp sample (4%), a double-infection case also positive for HSV-1, was recognized.

HHV-6, another member of the beta herpes virus family, causes a childhood disease called roseola infantum as well as multiple sclerosis. It is associated with exanthema subitum [Yamanishi et al., 1988; Pellett et al., 1992] and EBV- and CMV-negative cases of mononucleosis in young adults [Pellett et al., 1992; Krueger et al., 1994]. HHV-7, which was identified in 1990, is also a member of the beta herpes virus family and is closely related to HHV-6 and CMV [Griffiths et al., 2000]. Neither HHV-6 nor HHV-7 were detected in the present study, nor has their presence in nasal mucosa been reported in the past, suggesting that these herpes viruses are not associated with benign nasal lesions.

To the best of our knowledge, this is the first study to investigate the involvement of a comparable group of viruses in human nasal polyposis. The present findings support the causative role played by EBV in the formation of nasal polyps, as well as its presence in the adjacent mucosa. The level of statistical significance reached supports the theory that the presence of the EBV virus markedly influences the pathogenesis of nasal polyposis, while the low incidence of HPV detection confirms the hypothesis that HPV is correlated to a lesser extent with infectious mucosal lesions than with proliferative lesions, such as inverted papilloma. The low prevalence of HSV-1 and CMV in this study, in conjunction with the results of a limited number of previous reports, confirms the minor role played by the two herpes viruses in the development of these benign tumors. Double infection of nasal polyposis patients with HSV-1 and CMV could additionally play a minor, though causative, role in the development of the disease. Lastly, the results reveal that VZV and HHV-6/7 are not involved in the pathogenesis of nasal polyps.

REFERENCES


