Turkish Journal of Medical Sciences

Volume 46 | Number 2 Article 11

1-1-2016

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TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 310-314

doi:10.3906/sag-1406-103

The role of human papilloma virus and herpes viruses in the etiology of nasal polyposis

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Received: 25.06.2014 • **Accepted/Published Online:** 25.05.2015 • **Final Version:** 17.02.2016

Backround/aim: The aim of this study was to investigate the etiological role of human papilloma virus (HPV), herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus-6 (HHV-6) and -7 (HHV-7) in the occurrence of nasal polyposis.

Materials and methods: Nasal polyp samples from 30 patients with nasal polyposis and normal nasal mucosa from 10 patients without nasal polyps were obtained. DNA was extracted from tissues. Real-time polymerase chain reaction was performed for all runs.

Results: No HSV-1, HSV-2, or VZV was detected in the samples. Among the patient samples, EBV and HHV-7 DNA were detected in 18 (60%), HHV-6 was detected in 20 (66.7%), and HPV was detected in 4 (13.3%) samples. Among the controls, CMV DNA was positive in one (10%). EBV was positive in 5 (50%), HHV-6 and HHV-7 were positive in 7 (70%), and HPV was positive in 2 (20%) samples. No significant difference was found among the groups with any test in terms of positivity.

Conclusion: The association of *Herpesviridae* and HPV with the pathogenesis of nasal polyps was investigated in this study and no relationship was found. Thus, these viruses do not play a significant role in the formation of nasal polyps.

Key words: Nasal polyposis, pathogenesis, *Herpesviridae*, human papilloma virus

1. Introduction

Nasal polyposis, which is a common chronic inflammatory disease of the nasal and paranasal sinus mucosa, is associated with nonneoplastic mucosal lesions. The causes of nasal polyp formation remain unknown. Genetic predisposition has been suggested, although scant data exist to support such theories (1). Activated epithelial cells may be the major source of mediators that induce the influx of inflammatory cells (mostly eosinophils), as well as the proliferation and activation of fibroblasts, leading to nasal polyp formation. Infectious agents (including viruses, bacteria, and fungi) may be potential primary factors that activate nasal epithelial cells (1,2). This inflammation causes mucosal edema and leads to the thickening of the basal membrane, atypical gland formation, goblet cell hyperplasia, inflammatory cell infiltration, and subepithelial edema formation. Viral infections are considered to play a role in the pathogenesis of polyps by stimulating inflammation (1). The probable initial triggers that upregulate inflammation of the lateral wall of the nose to develop nasal polyposis are reported to be allergic reaction, viral infection, bacterial infection, fungal infection, and environmental pollution (3,4). Researchers believe that those factors disrupt the epithelial lining and initiate an inflammatory response (4). If the inflammation does not subside, stromal edema may consolidate and result in polyp formation (4). Multiple theories have been proposed for the formation of nasal polyps (5). Human papilloma viruses (HPVs) are responsible for many benign and malignant lesions of the skin (6). Herpes viruses are a diverse family of large DNA viruses, all of which can cause lifelong latent infections (7). Herpes virus infections have been reported to play a role in lesions of the upper respiratory tract (8–10). However, their role in the formation of human nasal polyposis is not fully understood (8).

The present study aimed to investigate the presence of HPV, herpes simplex virus 1 and 2 (HSV-1/2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus-6 and -7 (HHV-6/7) in tissue samples obtained from 30 nasal polyposis patients using polymerase chain reaction (PCR),

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and to determine the etiological roles of these viruses by comparing the results of tissue samples obtained from 10 healthy individuals.

2. Materials and methods

The current study was approved by the Ethical Committee of Clinical Research of Abant İzzet Baysal University. Signed informed consent was obtained from all patients.

2.1. Patients

Thirty patients with chronic rhinosinusitis who had undergone functional endoscopic sinus surgery for nasal polyposis and 10 patients who had undergone septoplasty for nasal septum deviation without nasal polyps were included in the present study. The diagnosis of polyps (CRSwNP) was based on patient history, clinical examination, nasal endoscopy, and computed tomography according to the current EP3OS guidelines (9). Nasal septum deviation was diagnosed by endoscopic examination, and computed tomography was evaluated by an experienced otolaryngologist. Nasal polyp samples were taken during the functional endoscopic sinus surgery using forceps. After surgery, the definitive diagnosis of the polyps was confirmed by histopathological examination. For the control group, nasal mucosa samples obtained during surgery for nasal septum deviation from 10 subjects without nasal polyps were used. Patients with inverted Schneiderian papilloma or cystic fibrosis were excluded from the study. Samples taken during surgery were sent to a microbiology laboratory for microbiological examination and stored at –70 °C under sterile conditions until the study tests were performed.

2.2. DNA extraction from tissues (nasal polyp and controls) and PCR

DNA was extracted from tissues using a commercially available system provided by QIAGEN (QIAamp DNA Mini Kit; Hilden, Germany). The tissues were digested with 180 µL of buffer ATL (provided with the kit) and 20 µL of proteinase K (20 mg/mL) overnight at 56 °C. After digestion, 200 µL of buffer AL and 100% ethanol were added. The solution was transferred to a QIAamp spin column and centrifuged for 1 min. Next, the column was washed with 500 µL of buffer AW1, spun for 1 min, washed with buffer AW2, and spun for 3 min. The DNA that adhered to the column was eluted with 200 µL of buffer AE at room temperature by centrifugation for 1 min. For PCR, aliquots of 200 µL were used.

Real-time PCR was performed in all runs. To this end, the HSV QLP 1.1 Kit (Fluorion, İontek, İstanbul, Turkey) targeting the HSV DNA polymerase gene region (91 bp) was used. A total of 10 µL of DNA was added to 14 µL of amplification mix. Distilled water was added to a final volume of 25 µL. The sensitivity of the kit was 1.0×10^3 copies/mL for HSV type 1 and 8.0×10^3 copies/mL for HSV type 2. The CMV QNP 3.0 Kit (Fluorion) targeting the CMV DNA polymerase gene region (139 bp) was used. A total of 0.3 µL of the internal control and 5 µL of DNA were added to 14.7 µL of amplification mix. Distilled water was added to a final volume of 25 µL. The sensitivity of the kit was 4.5×10^1 copies/mL. The Epstein–Barr Virus QNP 1.0 Kit (Fluorion) targeting EBV long internal repeat 1 region (IR1) primers (233 bp) was used. A total of 1 µL of the internal control and 12.5 µL of DNA were added to 16.5 µL of amplification mix. The sensitivity of the kit was 3.8×10^2 copies/mL. The HPV QNS 1.1 Kit (Fluorion) targeting HPV L1 region primers (150 bp) was used. A total of 5 µL of DNA was added to 12.5 µL of amplification mix. Distilled water was added to a final volume of 25 µL. The sensitivity of the kit was 5×10^2 pg/mL. The RealQuality RS-VZV Kit (Code RQ-S35; AB Analitica, Padua, Italy) targeting the VZV ORF 29 gene region (encoding the major DNA binding protein) was used. A total of 1 µL of internal control and 5 µL of DNA were added to 13.5 µL of amplification mix. Distilled water was added to a final volume of 25 µL. The RealQuality RS-HHV-6 Kit (Code RQ-S15; AB Analitica) targeting HHV-7 U57 was used. A total of 1 µL of internal control and 5 µL of DNA were added to 13.5 µL of amplification mix. Distilled water was added to a final volume of 20 µL. The RealQuality RS-HHV-7 kit (Code RQ-S19; AB Analitica), targeting the HHV-6 U67 open reading frame gene region was used. A total of 1 µL of internal control and 5 µL of DNA were added to 13.5 µL of amplification mix. Distilled water was added to a final volume of 25 µL. Amplification was performed using an Icycler thermal cycler (v 3.0a; Bio-Rad Laboratory, Hercules, CA, USA). Sequence analysis for typing was performed in HPV-positive samples (ABI PRISM BigDye Terminator Cycle Sequencing Kit and ABI Prism 310 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA).

2.3. Statistical analysis

Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as numbers and percentages. Differences between groups in terms of viruses were analyzed using the chi-square test and Fisher's exact test. The results were evaluated using 95% confidence intervals, and $P < 0.05$ was deemed to indicate statistical significance.

3. Results

No HSV-1, HSV-2, VZV, or CMV DNA was detected in the patient samples. HSV-1, HSV-2, and VZV DNA were not detected in the control samples. EBV DNA was detected in 18 (60%) patient samples, HHV-7 DNA in 18 (60%), HHV-6 DNA in 20 (66.7%), and HPV DNA in 4 (13.3%). No HSV-1, HSV-2, or VZV DNA was detected in the control samples. CMV DNA was positive in one (10%) control

sample. EBV DNA was positive in 5 (50%) individuals of the control group, whereas 7 (70%) individuals of this group were positive for HHV-6 and HHV-7. Two (20%) control subjects were found to be positive for HPV DNA (Table).

Regarding the HPV typing results, type 16, 31, and 66 HPV, all of which were in the high-risk group, were each detected in one patient. One patient was positive for type 81 from the low-risk group. One control subject was positive for type 16 from the high-risk group, and one was positive for type 29 from the low-risk group.

No significant difference was found between the groups in terms of the positivity rates of CMV, EBV, HHV-6, and HHV-7 DNA (P = 0.25, P = 0.8535, P = 0.8455, P = 0.8455, and P = 0.6091, respectively).

4. Discussion

Nasal polyposis is a common disease with a high medical failure rate and high recurrence rate. The prevalence of nasal polyps is reported to be within a range of 1%–32%; however, in a study conducted using nasal endoscopy, which is accepted as the gold standard for the diagnosis of nasal polyposis, the frequency was reported to be 2.7% in 1387 individuals (11). Additionally, polyps smaller than 5 mm are reportedly unlikely to be clinically relevant (4).

Chronic infections of the nose and paranasal sinuses are commonly observed in patients with nasal polyps (2). The most frequent pathogens are reported to be *Staphylococcus aureus*, beta-hemolytic streptococci, and *Haemophilus influenzae* (2). *S. aureus*, together with enterotoxin, is considered to behave as a superantigen in the formation of polyps (2,12). Additionally, even though numerous studies on the etiology of nasal polyposis focusing on HSV, EBV, HPV, and adenovirus have been performed to date, the viral cause of the disease remains controversial (2,13). Thus, the investigation of any probable etiological microorganism will be useful for the treatment and prevention of nasal polyposis. In our study, we performed PCR to detect HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, and HPV DNA.

HSV-1 leads to oral cold sores, whereas HSV-2 causes genital herpes (8). In the present study, no sample was positive for HSV-1 or HSV-2. Zaravinos et al. (8) reported only 2 positive samples for HSV-1 and HSV-2. Several studies reported no evidence for the presence of HSV-1 and HSV-2, suggesting no association between infection by these viruses and the pathogenesis of nasal polyposis (2). Additionally, Wang et al. (14) suggested that HSV-1 infection might lead to significant damage of the nasal epithelium, consequently facilitating invasion of *S. aureus* into the nasal mucosa; moreover, they suggested that nasal polyp tissue was more sensitive to invasion of HSV-1 and epithelial damage by HSV-1 than turbinate mucosa.

VZV, the cause of chicken pox and herpes zoster, was not found in any of our samples. Similarly, Zaravinos et al. (8) reported no positivity in nasal samples. According to several records, VZV does not seem to be involved in the formation of nasal polyps (8).

CMV is a member of the beta herpes virus family, and most adults may be seropositive owing to exposure to the virus at some period during their lives. CMV can lead to serious infection, particularly in immunocompromised

Table. Distribution of the viruses in nasal polyp samples.

	Results			
	Patient group (n = 30)		Control group (n = 10)	
	Positive	Negative	Positive	Negative
HSV-1*	0	30	0	10
HSV-2*	0	30	0	10
VZV*	0	30	0	10
CMV*	0	30	1	9
EBV*	18	12	5	5
HHV-6*	20	10	7	3
HHV-7*	18	12	7	3
HPV*	4	26	2	8

*HSV-1: herpes simplex virus 1; HSV-2: herpes simplex virus 2; HPV: human papilloma virus; VZV: varicella zoster virus; EBV: Epstein–Barr virus; CMV: cytomegalovirus; HHV-6: human herpes virus 6; HHV-7: human herpes virus 7.

patients, when the latent virus reactivates (15). Despite the accumulation of in vitro findings, the role of CMV in the formation of carcinoma has not been established (15). In the present study, we detected CMV in one of the controls and in none of the patients. Zaravinos et al. (8) also found no significant results for CMV with regard to the pathogenesis of polyps. By contrast, Kulkarni et al. (10) reported a case in which CMV led to nasal polyposis in a patient after renal transplantation. Very few reports have attributed nasal polyps to CMV (8).

Tao et al. (16) investigated the prevalence of EBV infection in 13 cases of nasal polyps using southern blot hybridization and PCR. EBV DNA was detected in 2 of 13 (15%) cases by Southern blot hybridization and in 9 of 13 (69%) by PCR, indicating that a higher rate may be obtained by the PCR method. Zaravinos et al. (8) found this rate to be 35% (8/23) using PCR. In our study, we also used PCR method, and we found EBV in 60% of nasal polyp patients; 50% of the control subjects had EBV in our study, indicating that EBV may not be a causative agent for nasal polyposis.

Tao et al. (16) detected EBV in 88% of normal nasopharyngeal mucosa tissue in their population, whereas nasal polyps were much rarer. Consequently, they stated that it was unlikely that EBV contributes to nasal polyp formation. They concluded that the very low number of EBV-positive cells in each positive case did not support the hypothesis of a role for EBV in the pathogenesis of nasal polyposis. Host immunity and several other factors are considered to be important in the process (16). No direct evidence has yet been reported regarding the presence of EBV in the epithelium of the upper respiratory tract in asymptomatic individuals (16). EBV has been found in normal nasopharyngeal mucosa and in nasal lesions such as nasal lymphoma and nasal polyps (16,17).

HHV-6 and HHV-7 are also members of the beta herpes virus family, and they were found in most of our patients and control subjects with no significant differences. By contrast, Zaravinos et al. (8) did not detect those viruses in any of their nasal mucosa samples. According to the few published reports, no evidence exists concerning the significant association of HHV-6 and HHV-7 with nasal polyp formation.

Sinonasal papilloma is classified into 3 types: inverted, fungiform, and cylindrical. Buchwald and Norris (18) reported a prevalence of 78% (39/50) HPV positivity in fungiform papilloma in their metaanalysis, but they reported no HPV positivity in cylindrical types. The latter finding supports the etiological role of HPV in sinonasal papilloma. Hoffman et al. (19) found HPV in one of 33 patients. In 2006, Hoffmann (20) could not detect HPV in nasal polyposis patients a second time (0/20). Sun et al. (21) reported that the HPV-positive rate of inverted papilloma was 64.36% (65/101), and they concluded that the occurrence of inverted papilloma was related to HPV infection. Similarly, Ogura et al. (22) found HPV prevalence to be 80% in the recurrent inverted type and 14.3% in nonrecurrent papilloma. The latter report demonstrates that the relationship between HPV and recurrence in inverted papilloma is not nasal polyposis. In the present study, we found HPV positivity to be 13% in nasal polyposis patients and 20% in the controls.

Studies have demonstrated that the presence of HPV in patients with nasal polyps ranges from 0% to 92.3%, whereas the correlation between HPV infection and the development and progression of nasal polyps is not completely proven (23). Jing et al. (24) showed that 27% of nasal polyp cases were positive for HPV DNA. Zhou et al. (25) found that 92.3% of patients with nasal polyps were HPV DNA-positive, even though they could not detect HPV in their control group. In the study of Pei et al. (23), 40.2% of 204 patients with nasal polyps were diagnosed as HPV-positive. These data suggest that HPV infection in nasal polyps is prevalent (23). By contrast, Hoffmann et al. (19) could not find HPV DNA positivity in any specimen derived from nasal polyps. The discrepancy may be due to the low number of samples included in the study or differences in the methods used for detecting HPV (23). The underlying causes of the discrepancy may also be explained by the low amount of HPV in the polyp sample, heterogeneity of the sinonasal lesions analyzed, or involvement of different or not yet identified genotypes of HPV in this pathology (26).

The present study is a rare report investigating the association of various viral groups with the pathogenesis of nasal polyposis. For each virus, we performed PCR as a sensitive method to detect a low amount of viruses in the samples. Our study did not demonstrate any significant role for the viruses evaluated in the formation of polyps. The present study was performed to investigate the association of a large number of viral groups with the pathogenesis of nasal polyps, and we conclude that these viruses may not play a role in the formation of nasal polyps. However, new studies with larger series may be helpful for determining the role of EBV in the formation of nasal polyposis.

Acknowledgment

This study was supported by the Scientific Research Project Fund of Abant İzzet Baysal University (Project No. 2011.08.01.421).

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