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Research Article

Human Papilloma Virus and Epstein-Barr Virus in Sinonasal Inverted Papilloma

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Abstract

Background: Schneiderian papillomas are uncommon benign tumors of the sinonasal area. They tend to present local aggressiveness and recurrence, and some undergo malignant progression. This study aimed to search of humanpapillomavirus (HPV) and Epstein–Barr virus (EBV) in sinonasal inverted papilloma (IP) in order to elucidate possible role in its pathogenesis.

Methods: Forty-eight IPs were subjected to chromogenic *in-situ* hybridization (CISH) for HPV DNA (HPV-III family16), Real-time PCR for HPV, p16, anti-EBV and Ki67 immunohistochemical (IHC) studies.

Results: p16 was positive in 30 of 48 (62.5%), CISH for HPV was positive in 1 of 48 IPs (2.1%). All specimens were EBV negative. In total, 33.3% of IPs showed suprabasal Ki67 reactivity. HPV prevalence of our IP is high. EBV is not present in IP.

Conclusion: This evidence suggested that HPV infection but not EBV plays a role in pathogenesis of IP. Negative PCR results possibly depend on age of the paraffin blocks.

Keywords: Human papillomavirus; Ebstein-Barr virus; p16; Inverted papilloma; PCR

Introduction

Inverted papillomas (IPs) are benign tumors of endophytic grows of respiratory epithelium into the underlying stroma in the nasal cavity. Malignant transformation has been found in 5–15% of inverted papilloma lesions, 1.7–56% of which develop synchronous carcinomas [1]. The results of publications on the association of human papillomavirus (HPV) and Epstein–Barr virus (EBV) with IP have been variable [2,3]. Studies demonstrated that there was an association between HPV and IP [2-9], and these HPV positive IPs were associated with recurrence and dysplastic changes [2,3,7,10,11].

A systematic review and formal meta-analysis of the published literature showed that there was a significant heterogeneity between the studies using ISH and PCR [2]. This study aimed to explain to role of HPV and/or EBV associate IPs. p16 protein normally acts to block cell cycle progression at the G1 to S transition; therefore, inactivation of the p16 gene enables unregulated cell growth [12]. Ki67 is an immunohistochemical marker used to evaluate all proliferating cells that are in the active parts of the cell cycle and mitosis was used for determining the growth fraction of cell population and is potentially useful for predicting the progression to pre-neoplastic lesions and carcinoma [13].

Materials and Methods

Forty eight cases selected from the pathology archives of Dr. Lütfi Kırdar Kartal Research and Education Hospital between 2010-2013, diagnosed either as IPs or lesions suspicious for dysplasia and HPV infection microscopically (coilocytic changes) (n:6), which have no invasive carcinoma synchronous and/or metachronous. All biopsies were re-evaluated for dysplasia, inflammation, apoptosis, suprabasal mitoses. All cases were stained immunohistochemically for p16, anti-Epstein-Barr virus (EBV) and Ki67, chromogenic *in-situ* hybridization (CISH) for HPV DNA(HPV III family 16) and Real-time PCR for HPV genotyping.

Immunohistochemical studies were performed using Bond™ Polymer Refine Detection method (Leica Biosystems Newcastle Ltd, UK) with diaminobenzidine as the chromogen and hematoxyline as the nuclear counterstain. All immunohitochemical assays were performed using by the Leica BOND-MAX™ automated system. Included antibodies were anti-p16 (clone R19-D; DB Biotech, Kosice, Slovak Republic; 1:100 dilution), anti-Ki67 (clone SP6; Biocare medical, CA, USA; 1:100 dilution) and anti-EBV (clones CS1, CS2, CS3 and CS4, Leica, Microsystems, UK; 1:100 dilution). All anti bodies were diluted with Lab Vision Antibody Diluent (TA-125-AD).

EBV and p16 staining was scored as strong, weak, or negative on the basis of nuclear and/or cytoplasmic staining. Weak cytoplasmic staining or reactivity in cells less than 5% was interpreted as negative. Diffuse (more than 80% or focal 5–80%) strong staining was scored as positive. Ki67 staining was evaluated and scored according to the limited localization of basal layer (score:0) or extending to the upper layer (score:1).

All cases showed the presence of HPV DNA by CISH method and analyzed the presence of HPV DNA, HPV III family 16 for HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66. Real-time PCR for HPV performed by using the "HPV sign® Q24 complete" kit allows HPV virus detection and genotyping using "Rotor-Gene" and "PyroMark Q24" instrument system (Qiagen, Germany) for paraffin embedded tissues.

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Statistical analyses were performed using SPSS 17 for Windows. Pearson *chi*-square test was used for evaluation of the presence of dysplasia, suprabasal mitoses in squamous epithelium, p16 positivity, Ki67 positivity in the suprabasal squamous epithelium, ulceration, inflammation, presence of intraepithelial inflammatory infiltration, achantosis, parakeratosis, apoptosis and increase in capillaries. The statistical significance level was established at $p<0.05$ and confidence interval was 95%.

Results

Cases comprised 39 men and 9 women (4.3:1) ranging in age from 19 to 74 years (median 52.6 years). Four cases of IPs have low grade dysplasia (10.2%).

Thirty cases showed p16 positivity (62.5%). Two (100%) of cylindrical cell papilloma and 28 (60.9%) of IPs (Schneiderian and/or squamous) were positive for p16. Gender wise distribution of cases of IPs showed a male predominance in p16 positivity with 5 out of 25 males. p16 positivity was 64.1% (25 of 39) in males and 55.5% (5 of 9) in females. None of IPs show EBV immunohistochemically.

Ki67 and p16 expression were not significantly different among different types of papillomas (Pearson *chi*-square test; p=0.510 and p=0.333 respectively).

Immuno expression analysis of p16 and Ki67 did not reveal statistically significant differences between the expression of markers and inflammation, intraepithelial inflammatory infiltration, suprabasal mitosis, increase in capillaries. Increased expression of Ki67 was associated with the decrease of dysplasia. There was a significant correlation between Ki67 expression and apoptosis (Pearson *chi*-square test; p=0.001).We have not identified significant statistical differences in what respectively p16 stain is concerned with age and sex (Pearson *chi*-square test; $p>0.05$). p16 was positive in 3 of 4 cases with dysplasia. However, there was not a significant correlation between expression of Ki67 and p16 (Pearson *chi*-square test; $p>0.05$). The p16 reaction was identified at the some normal elements such as fibroblasts, glandular acinus, muscle fibers, ductal epithelium and endothelium. Only one lesion was focally positive for HPV by CISH and none of cases were positive by Real-time PCR for HPV.

Discussion

Forty eight cases were detected for association with HPV and/or EBV with IPs. In this study p16 was associated with IPs in 62.5% patients and HPV was in one patient, not suggesting a potential role of the HPV in IPs. According to the literature, EBV was not positive in none of the cases. Histological parameters as ulceration, inflammation, presence of intraepithelial inflammatory infiltration, acanthosis, parakeratosis, apoptosis and increase in capillaries did not significantly correlate with p16, HPV and EBV. Ki67 positivity in suprabasal of squamous epithelium was increased with degree of dysplasia according to the literature and there was correlation between Ki67 expression and presence of apoptosis.

Sham et al. studied 73 IPs with archival paraffin blocks. All specimens were EBV negative and 4.1% of IPs were HPV positive. Only focal p53 immuno reactivity of the basal and parabasal cells was found in 19% of IPs. HPV prevalence p53 expression of IPs was low. EBV was not present in IPs [2]. Kassim et al. detected HPV 16 in 40% of patients with IP, but in none of the control cases. They found a significant correlation between HPV 16 and dysplasia in IPs, but no association was found between the EBV and IP [3]. Syrjänen and Syrjänen reviewed 90 original studies and performed a meta-analysis was focused on HPV in sinonasal papillomas. HPV prevalence was 37.8% in IPs. HPVprevalence among nasal polyps and normal sinonasal mucosa was very low, 4.1% and 7.0%, respectively. There was a significant heterogeneity between the studies using ISH and PCR. In addition, there was significant heterogeneity between the studies from different geographic regions, but was not statistically significant [14].

Cheung et al. founded a uniformly high rate of p16 expression in benign Schneiderian papillomas in a patchy manner and p53 protein expression coincided with the onset of severe dysplasia and most carcinomas. They demonstrated the presence of HPV in 33% of cases, and the positivity rate varied among the different morphological types, being highest in exophytic Schneiderian papillomas. However, the HPV DNA content was so low that typing could not be performed in most cases. They concluded that HPV plays the primary role with strong p16 expression and a high rate of HPV detection [11]. Govindaraj and Wang review and conclude that the evidence exists suggesting that HPV infection plays a role in the progression of IP and confers an increased risk for recurrence and malignant transformation. PCR was the preferred detection method, and fresh or frozen specimens are the ideal source of tissue for evaluation [8]. The age of the specimen plays a role with detection. It has been demonstrated that the detection rate of HPV in IP specimens deteriorates from 20% at 1 year to 2% at 6 years in archived specimens. DNA extraction from frozen specimens serves as the most reliable source suggesting tissue banking as an ideal method to preserve tissue for HPV detection. PCR techniques, however, still possess a high sensitivity for HPV detection even in specimens with formalin fixation or paraffin embedding [6]. However, unlike PCR, these technologies cannot be applied to archival samples and are less sensitive and rapid [3].

Conclusion

There was not a significant association between the IP and HPV as well as EBV. HPV and/or EBV were not seem to be etiological factors in the pathogenesis of IPs. Also PCR techniques possess a high sensitivity for HPV detection in specimens with formal in fixation or paraffin embedding, fresh tissue detection seems to more reliable to be detect HPV genotyping.

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