High-risk human papillomavirus infection prevalence in non-malignant tonsillar tissue: A single-center cross-sectional study

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Abstract

Background/Aim: The prevalence of human papillomavirus (HPV) in non-malignant tonsils can vary according to geographical location, age group, and risk factors. Some studies have found a relatively low prevalence of HPV, while other studies have found higher rates in non-malignant tonsils. The presence of HPV in non-malignant tonsils may be associated with precursor lesions that have the potential to develop into cancer. The aim of the current study was to detect the prevalence of HPV and p16 (one of the HPV types) in non-malignant tonsils and determine the existence of HPV in tonsil tissue using molecular and histological techniques.

Methods: One hundred-three samples from non-malignant tonsils and one sample from squamous cell carcinoma of the tonsils were analyzed for the prevalence of HPV using molecular and histological methods. Real-time polymerase chain reaction (qPCR) was performed to detect HPV in the tissue samples.

Results: HPV was not found in any tissue specimens based on histopathological and p16 immunohistochemical evaluations. HPV was not detected in all tissue samples using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

Conclusions: In our study of one hundred and four patients, HPV and p16 were not genetically detected in the tonsils that underwent surgery for reasons other than cancer. Hence, more comprehensive studies can contribute to evaluating the relationship between benign tonsil tissue and HPV infection, potentially leading to improved diagnostic and preventative measures.

Keywords: papillomavirus, HPV type 16, non-malignant tonsil, real-time PCR, high-risk human papillomavirus
Introduction

The incidence of head and neck squamous cell carcinoma (HNSCC) is a significant global health concern. HNSCC is reported to affect nearly 600,000 people each year [1]. While tobacco and alcohol use have been reported as major risk factors for HNSCC, a significant shift in the epidemiology of these cancers has occurred, especially tonsil SCC, in developed countries. In many developed nations, a substantial proportion of tonsil SCC cases have been found to now be associated with high-risk human papillomavirus (HPV) infection, particularly the HPV type 16 [2,3]. Despite significant research on HPV-associated oropharyngeal squamous cell carcinoma (OPSCC), relatively little information is available about the natural history and timeline of oropharyngeal HPV infections. Understanding the progression of HPV infection in the oropharynx is a complex and challenging area of study [4]. Detection of HPV-related oropharyngeal precancerous lesions is crucial for early detection and intervention, but this situation presents challenges that require the development of effective assessment procedures [4, 5]. The low prevalence of HPV in non-malignant (benign) tonsils means that the presence of HPV in tonsils without cancer is relatively uncommon. In patients who test positive for HPV, especially high-risk types, such as HPV-16, higher incidences of malignant (cancerous) tonsils have been found. This finding suggests that HPV-positive individuals present a greater risk of developing tonsil cancer. It is essential to find precursors or early signs of HPV-related tonsillar cancer. This step is crucial because detection of these precursors can lead to early intervention and improved outcomes in addition to the HPV vaccination, which can lead to a reduction in the risk of HPV-related cancers, including tonsillar cancer [6]. Until now, high-risk HPV is a well-known cause of cervical cancer, and efforts have been made to raise awareness about this relationship. However, current studies highlight the increasing incidence of oropharyngeal cancer [7].

The current study aimed to detect the prevalence of high-risk HPV and p16 in non-malignant tonsils and to analyze the presence of HPV in tonsillar tissue using molecular and histological techniques.

Materials and methods

Clinical specimen collection

The current study was designed as a retrospective case series. It was a single-center, retrospective cross-sectional study. Tonsil samples from 104 patients who underwent tonsillectomy between 2005 and 2020 at Amasya University Medical Faculty Hospital were included in the study. Of the 104 patients included in the study, 30 (28.8%) were women and 74 (71.2%) were men. The mean age of patients was 22.5 years. Tissue samples of the patients were stored in paraffin blocks. One hundred-three non-malignant tonsil samples and one tonsillar squamous cell carcinoma sample were examined for HPV prevalence using molecular and histological methods.

DNA isolation

A QIAamp DNA FFPE Tissue Kit (Germany) was used to extract genomic CapitalBio NanoQ drop (China). All isolated DNA samples were stored at −20 °C.

Real-time PCR

Real-time polymerase chain reaction (qPCR) was performed for the detection of HPV in tissue samples. The PCR reaction was prepared using the Gp5 + / Gp6 + primer pair and 1 × SYBR Green PCR master mix (QIAGEN) with a final concentration of 25 µl [8,9]. RT-PCR reaction was performed with positive and negative controls.

The beta-globin gene primers/probe were previously described by de Araujo et al. [8]. Isolated DNA samples were evaluated by amplification of the beta-globin gene as an internal control for DNA adequacy.

Histological evaluation of tonsillar tissues

One-half tonsil from each patient was fixed, embedded in paraffin, and examined using hematoxylin and eosin (H&E) staining to confirm benign non-neoplastic tonsillar histology.

p16 Immunohistochemistry

Four micrometer-thick serial tissue sections from formalin-fixed paraffin-embedded blocks were cut and mounted on poly-L-lysine coated glass slides. After deparaffinization and rehydration, heat-induced antigen retrieval was performed using citrate buffer (pH 6 at 100 °C for 20 min). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 10 min, and the sections were blocked for 10 min with 1.5% normal goat serum (NGS) (Invitrogen, 50062Z) diluted in phosphate-buffered saline (PBS, pH 7.4) before incubation with primary antibody. Tissue sections were incubated in a humidified chamber with primary monoclonal anti-p16 antibody (BD Pharmingen, Clone G175-405), which was diluted 1:20 with 1.5% NGS and 0.1 % Triton-X in PBS overnight at 4 °C. After the incubation period and three washes with PBS, sections were incubated with biotinylated secondary antibody and streptavidin peroxidase reagent (Abcam, USA) for 10 min. To visualize immunostaining, the sections were incubated with 3,3'-diaminobenzidine tetrahydrochloride chromogen solution (DAB). Sections were counterstained with Mayer’s hematoxylin and mounted on a mounting medium. Negative control slides were incubated with PBS instead of primary antibody. p16-positive tonsil cancer was used for positive control. All tissue sections were evaluated under a light microscope (Olympus BX51), and results based on immunohistochemistry were evaluated after the examination of tissue sections by two investigators.

Statistical analysis

HPV prevalence is defined as percentages and 95% confidence intervals (95% CI), which were calculated according to the binomial distribution. Age and sex-adjusted ratios (PR) and 95% CI prevalence were calculated based on selected patients’ characteristics using binomial regression models with a log link.

Results

HPV was not detected in all tissue samples based on qPCR. Also, HPV was not detected on histopathological slides. Histopathological and p16 immunohistochemical evaluation, archival non-malignant tonsillar tissues were analyzed for histopathological evaluation using H&E staining (Figure 1). p16 is a tumor suppressor protein that is a surrogate marker for HPV infection. Diffuse strong nuclear and cytoplasmic
immunostaining were not observed in the 105 non-malignant tonsillar tissues (Figure 1).

Fig 1. Hematoxylin- eosin staining of non-malignant tonsil (A), malignant tonsil (B) tissues. Immunohistochemical expression of p16 protein was not seen in non-malignant tonsil (C), positive control (D).

Discussion

The existence of HPV in non-malignant tonsils is often referred to as asymptomatic and is not included in the etiology of tonsillar hypertrophy or chronic tonsillitis. Hence, HPV prevalence in tonsillectomy patients is thought to reflect that of the general population. The prevalence of high-risk HPV infection in the tonsil is ≤ 1%; thus, oropharyngeal HPV-related cancer will develop in a small proportion of infected patients [6].

It has been reported that HPV infections (oral and oropharyngeal) are transmitted sexually and non-sexually [10]. Several studies have demonstrated that individuals with a history of numerous sexual partners causes a higher risk of acquiring HPV infection in the oropharynx. HPV-16 has been found to be a major causative factor for oropharyngeal SCC and it is often transmitted through sexual activity, including oral sex [1,11]. However, the relationship between sexual behavior and the existence of benign HPV in the tonsils is unclear. One study reported that most patients with HPV infection (4 out of 5 people) were young adults who had recent sexually transmitted infections. Yet, tonsil HPV infections have been reported in children in previous studies [12]. In contrast to high-risk cervical HPV infections, researchers deduced that a high proportion of tonsillar high-risk HPV infections could lead to cancer [13].

Tonsillar HPV prevalence rates were reported to be between 0% and 12% in previous studies involving only children or adults [14,15]. Researchers reported high-risk HPV based on PCR in two (1%) of 195 malignant tonsils. Furthermore, fluorescence in situ hybridization (FISH) analysis could not detect oncogenic HPV 16 and 18 in PCR-positive samples [16]. The absence or low number of HPV-positive cases in benign tonsillar diseases has also been shown in previous studies [17–19]. Another study detected HPV rates of 12.5% (high-risk HPV) and 15% (low-risk HPV) in benign tonsillar tissue from adults and children in Belgium [20]. Besides this precancerous HPV-16 type was not detected in any of the 104 patients, and the presence of HPV in tonsil tissue samples was not detected using both molecular analysis techniques (reverse transcriptase quantitative polymerase chain reaction [RT-qPCR] and nested PCR) in our study.

Another study showed that the HPV DNA rate decreased from childhood (11.5%) to middle age and old age (2.4%) [12]. Cervical HPV infection incidence increases just after the sexually active period at 20-24 years of age and then declines [21]. However, oral HPV infection incidence is almost equally distributed in all age groups [22]. The way in which rare HPV infections behave tonsil tissue is also shown by the fact that none of the 511 frozen homogenized tonsil samples taken during the mad cow disease epidemic in the UK in the 1990s to investigate prion diseases were HPV-positive [13]. Fakhry et al. [23] reported the prevalence of HPV-16 as 4.7% in 401 HIV-positive tonsil brushing samples. Consistent with previously published SPLIT findings, no association was found between HPV16 and evidence of cytological dysplasia among HIV-positive persons [23]. The largest population-based study of mouthwashes to date reported the prevalence of HPV 6.9% for any HPV type and 1.0% for HPV-16 [4].

The reticular squamous epithelium of the tonsils is more susceptible to HPV infection than other anatomical regions of the upper respiratory tract, possibly due to its porous structure and lack of structural integrity. In an HPV-positive tonsil tumor, patients typically have cervical lymph node metastases at presentation, although the primary tumor is usually small [24]. The low rate of HPV infection in benign tonsils and difficulties in detecting premalignant lesions resembling cervical intraepithelial neoplasia can be explained by the fact that HPV infection is confined to a very small area deep within the tonsils. Dysplastic tonsillar epithelium with high-risk HPV could be a leading lesion in tonsillar carcinogenesis. Researchers reported high-risk HPV in only five of 477 patients who underwent tonsillectomy for chronic tonsillitis or tonsillar hypertrophy [6]. In a study at Helsinki University, oncogenic HPV-16 was detected in 6.3% (13 out of 206) of benign tonsil samples scanned by PCR. Also, of these 13 HPV-positive cases, 11 were detected in patients who were < 26 years of age [12].

Numerous studies have shown that individuals with HPV-associated oropharyngeal squamous cell carcinomas (SCCs) tend to have a more favorable prognosis. They often experience better treatment responses and longer survival rates compared to those with non-HPV-associated oropharyngeal SCCs. While the prognostic significance of HPV in oropharyngeal cancers is well-established, its role in cancers of the sino-nasal tract (the nasal cavity and paranasal sinuses) and nasopharynx is an area of active research. It's important to enlighten whether HPV has a similar prognostic significance in cancers of the sino-nasal tract and nasopharynx. Researchers have found evidence to support the idea that individuals with virus-related diseases have better overall and disease-specific survival rates compared to other groups. However, to fully understand the implications and significance of these findings, more context and details about specific studies are needed [25,26]. In addition to this evidence, it was found that HPV-positive patients had higher survival rates, although the differences in overall survival between patients with HPV-positive and HPV-negative sino-nasal SCC were not statistically
significant [27]. However, due to the limited number of patients and the heterogeneity of tumor type and treatment modalities, no definite conclusions can be drawn about the prognostic value of HPV in sino-nasal tract carcinomas [28].

Conclusion

It is important to note that the detection of HPV in non-malignant tonsils does not necessarily indicate the presence of cancer. However, this finding underscores the importance of understanding the role of HPV in oropharyngeal health and the potential risk factors for oropharyngeal cancer. In our study consisting of one hundred and four female, genetically four patients, and both HPV and p16, one of the HPV types, were not detected in the tonsils operated for reasons other than cancer. Regular medical monitoring and HPV vaccination can be important preventive measures. As a result, it is suggested that more comprehensive studies will contribute to the relationship between benign tonsil tissue and HPV.

References