

Human Papilloma Virus Prevalence in Hyperplastic Tonsils and Adenoids in Children and Young Adults

Çocuklar ve Genç Erişkinlerde Hiperplastik Tonsil ve Adenoidlerde İnsan Papilloma Virüsü Sıklığı

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ABSTRACT Objective: Our aim was to determine HPV prevalence and genotypes in nontumoral tonsillar and/or adenoid tissue in young adults to open a discussion about the rationale of HPV detection in tonsil and adenoid tissues because of the cancer development risk. **Material and Methods:** Archived resection materials of 258 patients treated between 2003 and 2007 were retrieved for HPV detection and typing. Sections of formalin-fixed, paraffin-embedded tissue samples of each resected material were used as the DNA source. Six patients were excluded because of the inadequate quality of the extracted DNA. The age range of the remaining 252 patients was between 5 and 21 years (11.94 ± 4.20). After PCR screening, four different sets of primers covering 18 HPV types were used for HPV genotyping by multiplex polymerase chain reaction (MPCR). **Results:** Sixteen of the 252 surgical samples, namely nine of 114 tonsillectomies (7.9%), four of 87 adenoidectomies (4.6%), and 3 of 51 adenotonsillectomy tissues (5.9%) were found to contain the HPV genome (6.3%). Genotypes revealed that HPV-16 was the dominant genotype, found in 11 cases, followed by HPV-6/11 in four cases, and HPV-31 in one case. **Conclusion:** The prevalence of HPV in children and young adults that suffered from upper airway obstruction due to nontumoral tonsil and/or adenoid overgrowth was found to be 6.3% in our study. This study reveals the need for further molecular assays for HPV under the light of 6.3 % of detected HPV prevalence and the high risk HPV type 16 predominance. Since the oncogenic risk, we strongly suggest evaluation of HPV in tonsillectomy and adenoidectomy material similar to oropharyngeal squamous epithelium.

Key Words: Human papillomavirus 16; tonsillectomy; adenoids

ÖZET Amaç: Amacımız, genç erişkinlerde tümöral olmayan tonsil ve adenoid dokusunda kanser gelişme riskinden dolayı human papilloma virüsü (HPV) taranmasının mantığına açıklık getirmek için tümöral olmayan tonsil ve adenoid dokusunda HPV sıklığı ve genotipini saptamaktır. **Gereç ve Yöntemler:** 2003-2007 yılları arasında tedavi olmuş 258 hastanın arşiv materyallerinde HPV taraması ve tiplendirilmesi yapıldı. DNA kaynağı olarak, her bir rezeksiyon materyalinin formalinde fikse, parafin bloklü doku örnek kesitleri kullanıldı. Ekstrakte edilen DNA kalitesinin yeterli olmayışı nedeniyle altı hasta çalışma dışı bırakıldı. Kalan 252 hasta 5-21 (11.94 ± 4.20) yaşları arasındaydı. Tarama polimeraz zincir reaksiyon (PCR)larından sonra, 18 HPV tipini kapsayan dört farklı primer seti kullanılarak multiplex polymerase chain reaction (MPCR) ile HPV genotipleme yapıldı. **Bulgular:** Yüz ön dört tonsillektominin dokuzu (%7.9), 87 adenoidektominin dördü (%4.6) ve 51 adenotonsillektominin üçü (%5.9) olmak üzere toplam 252 cerrahi örneğin 16'sında (%6.3) HPV genomu varlığı tespit edildi. En sık olarak 11 olguda HPV 16 genotipi izlenirken, daha az olarak dört olguda HPV 6/11 ve 1 olguda ise HPV 31 genotipleri saptandı. **Sonuç:** Bu çalışma %6.3'lük HPV sıklığı ve yüksek riskli HPV tip 16 baskınlığı tespit edilmesi ışığında HPV için ilave moleküler yöntemlere gereksinimi ortaya koymaktadır. Kanser riskinden dolayı, tonsillektomi ve adenoidektomi materyallerinde orofaringeal yassı epitele benzer şekilde HPV değerlendirilmesini ısrarla öneriyoruz

Anahtar Kelimeler: İnsan papillomavirüsü tip16; tonsillektomi; adenoidler

The lymphoid tissue of the nasopharynx and oropharynx is composed of the adenoids, tubal tonsils, lateral bands, palatine tonsils, and lingual tonsils. Tonsillitis and adenoiditis are very common in young adults and children, and many pathogens can cause these infections. Increasing evidence has suggested that human papillomaviruses (HPV) can infect the tonsillar epithelium.¹ However, there have only been a few reported cases of HPV infection of the tonsils and adenoids in young adults and children with the exception of laryngeal papillomatosis, which is a well-known HPV infection of the upper airway.²

HPV is estimated to be the most common sexually transmitted infection.³ Most people who become infected with HPV will not have any symptoms, and the infection will either clear on its own or the virus will result in benign, self-limiting warts or tumors, characterized by abnormal maturation and differentiation of epithelial cells. Despite its benign character, HPV has been associated with head and neck squamous cell carcinomas (HNSCC), especially of the oropharynx, with highest distribution in the tonsils.⁴ However, the role of HPV in the cancer transformation process is controversial, and there is increasing interest in the possible association between HPV infection and SCC of the oropharynx.

In the present study, we attempted to determine HPV prevalence and types in nontumoral tonsil and/or adenoid tissue in young adults. Our aim was to open a discussion about the rationale of HPV detection in tonsillary and adenoid tissues because of the cancer development risk. In addition, we aimed to have a clue for further evaluations to realize the possibility of longitudinal transmission during birth, since HPV remains latent in the epithelium for years.⁵

MATERIAL AND METHODS

STUDY PROTOCOL

This study was conducted on specimens archived between 2003 and 2007, taken from 258 individuals who suffered from upper airway obstruction and who were treated with bilateral tonsillectomy

and/or adenectomy. None of the patients had respiratory papillomatosis or any immunosuppressant disorders. Six patients were excluded due to inadequate quality of the DNA in their archival material. The age range of the remaining 252 patients was between and 5 to 21 (11.94 ± 4.2). The materials of each case in the study were treated together, regardless of operation type, such that samples taken from bilateral tonsillectomy, adenoidectomy, or both were submitted for DNA extraction in the same tube.

DNA EXTRACTION AND MULTIPLEX POLYMERASE CHAIN REACTION (MPCR)

Four 10 μm -thick sections of formalin-fixed, paraffin-blocked tissue samples of each resected material were used as the DNA source. Approximately 20 to 80 μm thick tissue samples, representing all available tissue blocks, were submitted for DNA extraction in the same tube. DNA extraction was performed using a standard procedure (QIAamp DNA mini kit, QIAGEN, Hilden, Germany). The extracted DNA concentration, determined using spectrophotometry at 260 nm, was approximately 100 ng/ μl , and was used for the PCR reaction. The integrity of the DNA extracted from each sample was determined using both DNA electrophoresis and PCR amplification of the human beta-globin housekeeping gene.

One of the most sensitive PCR assays, the nested MY/GP PCR, which enables the amplification of broad spectrum HPV genotypes by using primers targeted to the viral E6/E7 oncogene regions, was used for the first round of HPV genomic screening.⁶⁻⁸ Following HPV screening, the HPV-positive samples were submitted for genotyping. The previously described GP-E6/E7 genotyping strategy, based on MPCR product size, was used for HPV genotyping.⁷ Briefly, four different sets of primers covering 18 HPV genotypes, including HPV types 16, 18, 31, 59, 45 (first MPCR set); 33, 6/11, 58, 52, 56 (second MPCR set); 35, 42, 43, 44 (third MPCR set); and 68, 39, 51, 66 (fourth MPCR set) were used. Detailed information about screening using the MY/GP nested PCR assay and genotyping using the nested MPCR (nMPCR) assays are

TABLE 1: HPV screening and typing strategies.

MY/GP assay (nPCR) for HPV screening
1 st round PCR: MY9/MY11
2 nd round (nest) PCR: GP5/GP6
HPV genotyping assay (nMPCR)
MPCR Group 1: HPV Types: 16, 18, 31, 45, 59
MPCR Group 2: HPV Types: 33, 6/11, 52, 56, 58
MPCR Group 3: HPV Types: 35, 42, 43, 44
MPCR Group 4: HPV Types: 39, 51, 66, 68

both shown in Table 1.⁷ Each of the screening and genotyping PCR amplicons were loaded onto 2% agarose gel and electrophoresed for 45 minutes under 100 V and constant current in TBE buffer. Amplicons were visualized under UV light after ethidium bromide staining. The presence of previously known base pair length DNA bands was taken as the evidence of HPV genome presence (Table 1).

RESULTS

Sixteen of the 252 surgical samples, specifically nine of 114 tonsillectomy, four of 87 adenoidectomy, and three of 51 adenotonsillectomy tissues, were found to contain the HPV genome by MY/GP nPCR screening assay (6.34%). Genotyping results of these positive samples using nMPCR HPV assay revealed that HPV-16 was the dominant genotype found in eight tonsillectomy samples, two adenotonsillectomy samples, and one adenoidectomy sample. HPV-6/11 was detected in three adenectomy samples and one tonsillectomy sample. HPV-31 genome was found in only one adenotonsillectomy sample. None of the samples in the study were found to contain multiple HPV genotypes (Table 2).

DISCUSSION

HPV may play a role in the pathogenesis of tonsillar cancer because the mucosal squamous epithelium is easily exposed to viral infection. The possibility of longitudinal transmission during at birth is also another issue in the etiology of oropharyngeal SCC development since HPV remains latent in the epithelium for years. Multiple case series have reported the prevalence of HPV DNA in head and neck cancers as 0-100%.⁴ Among head and neck cancers, HPV DNA positivity tends to show site dependence, with the tonsils, oral cavity, and larynx being the most common sites.^{9,10} Kreimer et al¹⁰ reviewed 5 046 head and neck SCC cancers from 60 studies and realized that the HPV coexistence was an important issue with a 25.9% prevalence. Other studies also stress HPV relation in the SCCs, especially HPV type 16.^{2,11-13}

Since the squamous cancer-HPV coexistence is well documented as well as oncogenic propensity of certain types of HPV virus, HPV screening and typing of oropharyngeal squamous epithelium in hyperplastic tonsil and/or adenoid tissues seems meaningful to determine whether a preventive treatment is needed or not.

The location of the tonsillar tissue in the oropharynx is ideal for sampling mucosa potentially exposed to HPV, and these specimens provide an abundant source of mucosal tissue for further molecular testing. Sampling the hyperplastic tonsil and/or adenoid tissue for HPV detection seems to be a reliable assay for further risk assessment. However, there are a few studies that evaluated the presence of HPV DNA in tumor-free tonsil and/or adenoid tissue. Syrjanen searched medical literature until the end of 2002 and

TABLE 2: HPV genotype distribution in resected materials.

Test material	MY/GP screening	HPV genotyping		
	HPV positive/ total	Type 16	Type 6/11	Type 31
Tonsillectomy materials	9/114 (7.89 %)	8	1	-
Adenoidectomy materials	4/87 (5.59 %)	1	3	-
Adenotonsillectomy materials	3/51 (5.88 %)	2	-	1
Total	16/252 (6.34 %)	11/16	4/16	1/16

reported that only 200 normal tonsillar samples and /or biopsy samples from tonsillar tissue were analyzed for the presence of HPV DNA.² Overall, 8.5% (17 of 200) of the samples contained HPV DNA, either type 16 (12 samples) or 6/11 (five samples).² We analyzed English medical literature and Syrjanen's report for articles that analyzed HPV DNA in nontumoral tonsillar and/or adenoid tissue using PCR based techniques.¹⁴⁻²² We found nine reports in which a total of 555 non-tumoral tonsillar samples and 50 adenoid samples were analyzed for the presence of HPV DNA. In total, 5.9% (33 of 555) of the samples contained HPV DNA, and the most prevalent type was HPV 16 (31 samples). Concordantly, our results showed that 6.34% (16 of 252) of the samples contained the HPV virus genome and HPV-16 was the dominant genotype.

There is a wide range of variation, between 0-15%, in the rate of HPV DNA detected in tumor free tonsil and adenoid tissue in different studies.²⁰⁻²² HPV DNA detection method may be the cause of this variation. In the present study we used the gold standart PCR method for HPV detection and genotyping to avoid false results.

Chen et al¹⁹ showed the prevalence of HPV DNA as 6.3% in patients undergoing tonsillectomy for chronic tonsillitis or tonsillar hypertrophy. In their study, they included patients from all age groups ranging from 2 to 72 years of age.¹⁹ Sisk et al²⁰

tested 50 normal tonsil samples and 15 papilloma specimens using PCR, and found that two of normal tonsil samples, were positive for HPV DNA and all papillomas were positive for DNA of HPV types 6/11. In contrast to these results, Ribeiro et al²¹ examined the prevalence of HPV using PCR in the tonsils of 100 children undergoing tonsillectomy, and found none of the tonsil samples were positive for HPV.²¹ In the present study the age range was slightly older when compared to Ribero's study group, however it was quite matching with the Chen's study group. This result indicates that HPV rates in oropharyngeal squamous muco-sa may vary between populations.

The passenger or driver dilemma for HPV-related squamous epithelial lesions has long been investigated with great outcomes realizing the real oncogenic potential of certain HPV types, being most frequently the HPV-16 and HPV-18. Since the oncogenic risk, we strongly suggest evaluation of HPV in tonsillectomy and adenoidectomy materials as a sample of oropharyngeal squamous epithelium. In addition to previous studies on non-tumoral oropharyngeal epithelium, the present study reveals the need for further molecular assessment for HPV since HPV prevalence was detected as 6.34%, with a high risk HPV type 16 predominance. This study also enlightened a further need for evaluating the longitudinal HPV transfectin as a risk factor for cancer.

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