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Research Article

High-Prevalence of Various High-Risk Sub-types of Human Papilloma Virus in Patients with Acquired Cholesteatoma in Greece

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Abstract

Although there are a lot of theories, the pathogenesis of cholesteatoma remains unclear. Few studies exist worldwide regarding the presence of Human Papilloma Virus (HPV) in cholesteatoma with controversial results. The presence of DNA of various high and low risk HPV sub-types was detected by real-time PCR to lesions obtained from sixty-two patients with acquired cholesteatoma. In addition, the presence of bacterial DNA was also detected using 16S rRNA PCR. Control group included tympanic membrane skin samples from patients operated by tympanoplasty. Cholesteatoma samples of thirty patients (48.3%) were found to be positive for one at least HPV sub-type. Twenty-five samples were positive for HPV16 (40.3%), nine were positive for HPV6 (14.5%), one for HPV18 (1.6%), one for HPV31 (1.6%), one for HPV51 (1.6%) and one for HPV59 (1.6%). None of the patients of control group was found to be positive for any sub-type HPV (0%). On the other hand, all specimens (cholesteatoma and tympanic membrane skin mucosa) were negative for the presence of bacterial DNA. Our results demonstrate a high prevalence of various high and low-risk HPV sub-types in cholesteatoma tissue samples in Central Greece.

Keywords: HPV; sub-types acquired holesteatoma; Greece

Introduction

Cholesteatoma is an abnormal, noncancerous skin growth tumor, that can develop in the middle section of the ear, behind the eardrum [1]. It may be a birth defect (congenital cholesteatoma) or acquired cholesteatoma, which is most commonly associated with repeated middle ear chronic inflammation caused by bacteria or viruses [2,3].

Papillomaviruses are small, non-enveloped, epitheliotropic, double stranded DNA viruses that infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation [4,5]. It is well known that Human Papilloma Virus (HPV) infection has been mainly associated with cervical cancer but also with anal and oropharyngeal cancer [6,7]. In 1995, Stremlau., *et al.* reported for the first time the presence of HPV DNA [8]; however, up to date, it remains unclear whether the HPV is a possible etiology for the development of cholesteatoma.

Aim of the Study

Aim of the present study was to determine the prevalence and the distribution of various HPV sub-types in patients with acquired cholesteatoma in Central Greece.

Materials and Methods

This study was conducted prospectively on tissues collected from patients who were operated with acquired cholesteatoma from January 2008 to January 2017 in the Department of Otorhinolaryngology of the University Hospital of Larissa (UHL), Central Greece. Patients with congenital cholesteatoma were excluded. We note that UHL is a tertiary care unit that serves a population of 1.000.000 inhabitants.

For the purpose of the present study, fresh tissues from patients who were operated with acquired cholesteatoma were sent to the Department of Microbiology of UHL. Extraction of total DNA was assessed using commercial kits (Invitrogen), according to the manufacturer's instructions. The efficiency of DNA extraction and the possible presence of inhibitors in the sample were confirmed by the detection of β2-globin gene [9]. The extracted DNA samples were tested by quantitative real-time PCR (Applied Biosystems 7500 Fast Real-Time PCR system) according to the manufacturer's instructions, using two commercially available assays, the HPV High Risk Screen Real Time PCR (Sacace Biotechnologies), which detects the sub-types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and the HPV 6/11 Real-Time PCR kit (Sacace Biotechnologies), which detects the sub-types 6 and 11. In addition, the pres-

ence of bacterial DNA was also tested in all specimens using 16S broad range PCR followed by sequencing [10]. In addition, sixty clinical samples included tympanic membrane skin, which were obtained from patients who were operated by tympanoplasty the same period, were used as control group; DNA extraction followed by molecular detection of HPV and bacteria was performed as previously described. We note that, as the origin of cholesteatoma is the tympanic membrane skin, we chose patients who were operated by tympanoplasty as control group.

All calculations were computed by R-base program for Linux. The results were presented as mean SD or percentages. SPSS 20 statistical software (IBM, Chicago, IL, USA) was used for statistical analysis. P values lower than 0.05 were considered as statistically significant.

The study was approved by the University Hospital of Larissa and School of Medicine, University of Thessaly review boards (approval protocol No 19/2-2-2017). A written informed consent within demographic information of all participants (age, gender, history etc) was obtained.

Results

Our study group consisted of sixty-two patients (38 men and 24 women) aged from 19-77 years old (mean age 40.1 20.4), while, the control group included sixty patients (18 men and 24 women), aged from 30-55 years old (mean age 42 4). DNA extraction, indicated by β2-globin gene detection, was successfully done in all samples. Cholesteatoma samples of 30 patients (48.3%) were found to be positive for one at least HPV subtype. Twenty-five samples were positive for HPV16 (40.3%), nine were positive for HPV6 (14.5%), one for HPV18 (1.6%), one for HPV31 (1.6%), one for HPV51 (1.6%) and one for HPV59 (1.6%). It is interesting that, among the HPV-positive samples, six were positive for both HPV16 and HPV6, while, one was positive for HPV6, HPV16 and HPV18. None of the patients of control group was found to be positive for any HPV sub-type (0%). Statistical significant difference on the prevalence of HPV was observed between the two study groups ($p < 0.005$).

Finally, all specimens (cholesteatoma and tympanic membrane skin) were negative for the presence of bacterial DNA, indicating that bacteria don't have essential role in the pathogenesis of cholesteatoma.

Discussion

In our study, a high prevalence of various high-risk HPV subtypes was detected in tissues collected from patients with acquired cholesteatoma, while, none specimen of the control group was found to be positive. As mentioned above, the HPV prevalence in cholesteatomas varies significantly between studies. Rydzewski., *et al*. have demonstrated that, among a group of fifty-three patients, of whom thirty-nine suffered from granulation tissue chronic otitis media, seven from cholesteatomatous otitis media, six from middle ear malignant neoplasm, and one from middle and/or external ear benign neoplasm, the presence of HPV DNA reached to 41.5% [11]; specifically, among cholesteatomatous chronic otitis media HPV DNA types 6 or 11 was identified in 70%. On the other hand, Bai., *et al.* reported 29,5%, prevalence of HPV DNA in forty-four cases of middle ear cholesteatoma, while Bergmann., *et al.* reported 36% of cholesteatomas to contain HPV DNA using PCR [12,13].

In addition, Ferekidis., *et al.* demonstrated that among fourteen specimens (seven aggressive and seven simple cholesteatomas), three aggressive were HPV-positive, while, all simple cholesteatomas were HPV negative [14]. On the contrary, Chao., *et al*. reported that only one out of thirty-six cholesteatomas (2,7%) contained 6-HPV DNA, while, Franz., *et al.* showed that only one out of twenty-nine (3,4%) biopsies of cholesteatomas was positive for HPV DNA [15,16].

Conclusion

Our results demonstrate a high prevalence of various HPV subtypes (48.3%) in patients with acquired cholesteatoma in Central Greece, suggesting that the virus might play a role in the pathogenesis of cholesteatomas. Other studies are required to clarify if the viral presence influences the progression of cholesteatoma and if the virus is responsible for recurrences.

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