Association of human papilloma viruses with adnexal tumors

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Background: Some studies have reported a correlation between adnexal tumors and human papilloma virus (HPV) infection. We performed this study to determine the incidence of HPV infection in adnexal tumor samples to further clarify any potential link between HPV and the pathogenesis of adnexal tumors.

Methods: A total of 65 formalin-fixed, paraffin-embedded, tissue samples with a histopathologically confirmed diagnosis of adnexal tumors, obtained between 2006 and 2011, were retrieved and evaluated by polymerase chain reaction (PCR) for the presence of different types of HPV.

Results: The samples included 29 sebaceous gland tumors, 18 sweet gland tumors, and 18 follicular epithelium tumors. The HPV DNA was only detected in four out of 18 sweet gland tumors (22.2%), i.e. in 6.2% of all evaluated tumors. HPV-6 and HPV-54 were detected in one and three samples, respectively.

Conclusion: Considering the low rate of HPV in adnexal tumors, the probability of an association between HPV and adnexal tumor seems slim.

Keywords: adnexal tumors; polymerase chain reaction; human papilloma virus

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INTRODUCTION

Adnexal tumors arise from the follicular epithelium, eccrine and apocrine sweat glands, and sebaceous glands [1]. These tumors are usually benign and their clinical appearance is not often diagnostic. Adnexal tumors originate from multipotential undifferentiated cells present within the epidermis or its appendigeal structures.

Human papilloma viruses (HPVs) are small double-stranded DNA viruses that infect epithelial cells and induce a variety of proliferative lesions such as warts, laryngeal papillomas, and cervical carcinoma [2]. They also induce a wide variety of cutaneous and mucosal lesions; some of them exhibit HPV type-specific clinical and histopathologic features [3]. HPV infects the basal cells of the epithelium and multiplies in the upper more differentiated layers of the epithelium4. The DNA of cutaneous HPV types is found in both premalignant lesions and non-melanoma skin cancers. Although some studies or case reports revealed a correlation between adnexal tumors and HPV infection, their sample size was small.

In this study, we aimed to determine the presence of HPVs in adnexal tumor samples using polymerase chain reaction (PCR) to further clarify any potential link between HPV and the pathogenesis of adnexal tumors.

PARTICIPANTS AND METHODS

This cross-sectional study was conducted in our university hospital during 2013. The Ethic Committee of our university approved the protocol of the study. The patients' consent for using their samples in research projects was obtained at the time of biopsy.

A total of 65 formalin-fixed, paraffin-embedded, tissue samples with a confirmed histopathological diagnosis of adnexal tumors were retrieved from the files of the Department of Pathology of our university. The tumor samples were excised between January 2006 and December 2011. If the prepared section WAS unfavorable, a new section was obtained and the best paraffin-embedded block was evaluated for HPV DNA.

The study samples comprised adnexal tumors of sebaceous glands, follicular epithelium, and eccrine and apocrine sweat glands.

DNA was extracted from the residual paraffin-embedded tissue samples. Tissue sections (10 µm thick) were placed in 1.5-mL microtubes, deparaffinized with 1200 µL of xylene, vortexed, and centrifuged at 16000 g for five minutes. The tissue pellet was washed with 100% ethanol twice before DNA extraction. Genomic DNA was extracted by the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of the extracted DNA was evaluated with a nanodrop spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

HPV genotyping was done using the INNO-LiPA HPV Genotyping Extra assay (Innogenetics NV, Ghent, Belgium) according to the manufacturer's instructions. The INNO-LiPA HPV assay is one of the commonly used HPV genotyping tests based on the concept of reverse hybridization, planned for the detection of 28 different HPV genotypes. A 65-bp region of HPV L1 gene was amplified by SPF10 primers. Then, the resulting biotinylated amplicons were denaturated and hybridized with specific oligonucleotide probes. An additional primer pair for the amplification of the human *HLA-DPB1* gene was added to monitor the sample quality and extraction. All probes were immobilized as parallel lines on membrane strips. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase was added which bound to any previously formed biotinylated hybrid. Incubation with 5-Bromo-4-chloro-3`-indolyl phosphate-p-toluidine nitroblue tetrazolium (BCIP/NBT) chromogen yielded a purple precipitate and the results could be interpreted visually or by the LiRAS (Line Reader & Analysis Software, Innogenetics, Gent, Belgium) for LiPA HPV software v.2 (Innogenetics, Gent, Belgium). The results were presented as frequency (percentage) if positive results were obtained.

RESULTS

A total of 65 adnexal skin tumors were assessed for HPV DNA. Basic characteristics, most common involved sites, and histopathologic features of the samples presented in Table 1.

The HPV DNA was detected in four out of 18 sweet gland tumors (22.2%). Neither sebaceous gland nor follicular epithelial tumor samples were positive for HPV DNA.

In total, the HPV DNA was detected in four out of 65 adnexal specimens (6.2%). The HPV types identified in the specimens of adnexal tumor group were HPV-6 (one case) and HPV-54 (three cases). The characteristics of HPV-positive adnexal tumors are presented in Table 2.

Table 1. Clinicopathologic features of the investigated adnexal tumors.

Gender	
Female	33 (50.8%)
Male	32 (49.2%)
Age, Median (range), y	29 (3-74)
Histopathologic type of tumor	
Sweet gland tumors	18 (27.7%)
Eccrine hidradenoma	5
Eccrine poroma	4
Syringoma	4
Other tumors	5
Sebaceous gland tumors	29 (44.6%)
Nevus sebaceous of Jadassohn	26
Sebaceoma	2
Sebaceous carcinoma	1
Follicular epithelium tumors	18 (27.7%)
Pilomatricoma	12
Trichoepithelioma	4
Other tumors	2
Site of tumor	
Face	25 (38.5%)
Scalp	23 (35.4%)
Upper extremities	8 (12.3%)
Lower extremities	5 (7.7%)
Trunk	2 (3.0%)
Neck	1 (1.5%)
Genital	1 (1.5%)

Table 2. Characteristics of Adnexal Tumors Positive for Human Papilloma Virus DNA.

Type of tumor	Location of tumor	Type of HPV
Eccrine poroma	Lower extremities	HPV 6
Eccrine spiradenoma	Upper extremities	HPV 54
Syringoma	Face	HPV 54
Syringoma	Face	HPV 54

DISCUSSION

Our study revealed a low frequency of HPV in adnexal tumors (6.2%), including HPV-6 (1.55%) and HPV-54 (4.65%). Although the nature of our study does not allow adoption or rejection of a casual relation between HPV viruses and adnexal tumors, considering the prevalence of the HPV in the general population [5], the possibility of such an association seem to be slim.

There are some case reports on the association of HPV with sweat glands tumors [6-15]. One of these studies reported six poroma tumors with negative results for HPV [15], while one of the propoma tumors in our study was positive for HPV-6. This HPV type is associated with low grade malignant epithelial tumors. In another case report [8], a syringoma was positive for HPV-20 and HPV-23. One of the four syringoma tumors in our study was positive for HPV-54.

Nevus sebaceous of Jadassohn (NS), or organoid nevus, is a complex hamartoma of the skin with both epithelial and dermal skin elements that undergoes age-related changes [16]. genetic mosaicism might participate in the pathogenesis of NS; however, the specific gene or genes responsible for its clinical manifestations are unknown [17]. Asymptomatic HPV infection of the normal-appearing skin appears to be acquired very early in infancy, and vertical transmission of HPV from the mother to the fetus is possible and is the documented cause of laryngeal papillomatosis [18-20]. Many epidermal changes found in NS, specifically verrucous and papillated epidermal hyperplasia are also found in HPV-induced cutaneous lesions. In one study on NS and HPV [20], HPV DNA was detected in 82% of NS (44 cases of NS) while it was detected in none of the patients with NS (26 cases) in our study.

Furthermore, our study showed negative results for HPV DNA in other tumors of sebaceous glands, i.e. sebaceoma and sebaceous carcinoma.

There are only a few studies on adnexal tumors arising from follicular epithelium and HPV infection. Angela Rohwedder et al. [21] showed the presence of HPV DNA in trichilemmomas for the first time. The findings of other investigations do not support the hypothesis that trichilemmomas arise from HPV infection [22]. In our study, none of the 18 cases of adnexal tumors arising from follicular epithelium (trichoepithelioma, pilomatricoma, etc.) had positive results for HPV DNA.

Although there are studies on the association of HPV with skin lesions [23] or other types of cancer [24], we could not find any study on skin appendage tumors. For instance, Shayanfar *et al.* suggested an association between HHV 18 and squamous cell carcinoma of the skin in Iranian patients with a normal immune status [23].

This study had some limitations. Due to its retrospective nature, we did not have access to the adjacent, uninvolved, normal-appearing skin of patients. Another limitation was the small sample size of this study as only samples in a single dermatology center were evaluated. Hence, it could not determine the prevalence of HPV viruses in these rare skin tumors in Iran. Moreover, the cross-sectional nature of the study limited deciding on the presence or absence of any association between adnexal tumors and HPV.

In conclusion, we found a small rate of HPV infection in adnexal tumors, which makes the possibility of any association between HPV and these tumors very slim. Nonetheless, controlled studies with larger samples are required to determine such an association.

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