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Research paper

Prevalence and genotypes of human papillomavirus in saliva and tumor samples of head and neck cancer patients in Hungary

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ABSTRACT

In addition to traditional risk factors such as smoking, alcohol consumption and betel nut use, human papillomavirus (HPV) infection also plays a role in the development of head and neck squamous cell carcinomas (HNSCCs). Although among European countries the highest incidence and mortality rates of head and neck cancer types were recorded in Hungary, data regarding HPV prevalence in HNSCCs is scarce. We collected biopsy and saliva samples from patients diagnosed with HNSCC or oral potentially malignant disorders (OPMDs) and tested them for the presence of HPV using the PCR consensus primer set MY09/11 and the GP5+/6+ primer pair. HPV genotypes were assessed by sequencing of the amplified PCR fragments. Oral mucosa and saliva samples from tumor- and OPMD-free individuals were also analysed. HPV was detected in 11 out of 60 HNSCC samples (18%). All of the HPV positive tumors carried HPV type 16. 5 out of the 57 saliva samples collected from HNSCC patients was HPV positive (8.8%); among them, in addition to HPV16, HPV13 was also detected. Tumors located to the oropharynx had the highest HPV positivity rate with 50% (7 out of 14), which was significantly higher than the HPV prevalence in oral mucosa samples collected from controls (0 out of 20; $p > 0.001$) or in OPMD biopsies (0 out of 21, $p > 0.001$). 2 out of 57 control saliva samples (3.5%, subtype HPV13 and 11) and 3 out of 39 saliva samples from OPMD patients (7.7%, subtype HPV18, 81 and 10) were HPV positive. Our data suggested that HPV16 infection may contribute, in concert with cigarette smoking, to the development of a subset of head and neck cancers in Hungary. HPV16 infection per se does not account, however, for the high HNSCC incidence rate recorded in this country.

1. Introduction

Head and neck cancer is a broad term that includes cancers arising in the mucosal lining of the oral cavity, pharynx, larynx, paranasal sinuses and nasal cavity; lip and salivary gland carcinomas also belong to this category (Bose et al., 2013). Most of these malignancies are head and neck squamous cell carcinomas (HNSCCs). Head and neck cancer is the sixth most common cancer type with approximately 630,000 new cases annually; there are significant differences, however, between geographical regions in terms of HNSCC incidence (Vigneswaran and Williams, 2014). In Europe, the highest age-standardized incidence and mortality rate for oral cavity and pharyngeal cancer was reported from Hungary (Ferlay et al., 2013; Bonifazi et al., 2011; Garavello et al., 2010). In 2012, Hungary also had the highest age-standardized incidence rate (ASR) of laryngeal cancer for men in Europe, and it was among the top five countries worldwide regarding the ASR of hypopharyngeal cancer (Ferlay et al., 2013; Shield et al., 2017). Thus, compared to other European countries, there is a relatively high number of new head and neck cancer cases in Hungary. The exact causes for this phenomenon remain to be explored. A series of factors may influence the development of head and neck carcinomas, including traditional risk factors such as tobacco use and alcohol consumption which act synergistically (Petersen, 2009). In addition, alteration of the oral microbiome associated with poor oral hygiene and periodontal

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disease, dietary factors, and viral or fungal infections may also increase the risk of HNSCC development (Meurman, 2010; Hettmann et al., 2016). Furthermore, recent data suggested a role for genetic susceptibility associated with certain polymorphic alleles or complex alterations of the genome in the genesis and clinical outcome of HNSCCs (Chandrasekharappa et al., 2017; Cancer Genome Atlas Network, 2015).

Even though in the last decades the incidence of head and neck cancer decreased in the Western World, mostly due to the decrease in tobacco use, an unexpected increase in oropharyngeal cancer cases was also observed, especially among non-smokers and non-drinkers. Accumulating evidence has proven a causal link between the infection of the oral mucosa with human papillomavirus type16 (HPV16), a carcinogenic "high-risk" oncovirus, and a subset of head and neck tumors, especially those of the oropharynx (reviewed by zur Hausen, 2009; Pytynia et al., 2014; Ramqvist et al., 2015). The transmission of mucosal HPV types, including high-risk (HR) types involved in the development of anogenital carcinomas and low-risk (LR) types that are not associated with an increased risk of cervical and other cancers, is primarily sexual, and oral-genital sex may lead to HPV infection of the oropharynx (reviewed by Feller et al., 2010). In addition, mouth to mouth contact, autoinoculation, and vertical birth-transmission may also contribute to HPV infection of the oral cavity and the oropharynx (Feller et al., 2010). HPV-associated oropharyngeal malignancies were predominantly associated with HPV16 infection, although an association with several other HR HPV types including HPV18, HPV33 and HPV52 was observed as well (Michaud et al., 2014).

In Central-European countries, epidemiological data related to HPV prevalence in HNSCC patients is scarce. Thus, the purpose of this study was to evaluate the presence of oral HPV infection in a South-East Hungarian population and its association with HNSCC and oral potentially malignant disorders (OPMDs) including oral lichen planus and leukoplakia that may progress to oral cancer (Dionne et al., 2015). We determined HPV prevalence and subtypes in biopsy samples from patients with HNSCC and OPMD. Salivary samples from the same patients and saliva and normal mucosa samples from a control group were also analysed.

2. Materials and methods

2.1. Study design and collection of samples

The study protocol was approved by the Institutional Review Board of the University of Szeged, Szeged, Hungary. Prior to the investigation, an informed consent was signed by each patient that participated in the study. The study groups consisted of HNSCC patients (group 1), OPMD patients (group 2) and controls (group 3).

Unstimulated whole saliva samples and punch biopsies from HNSCCs or OPMDs were taken from patients attending the Department of Oto-Rhyno-Laryngology and Head-Neck Surgery, Faculty of Medicine, and Department of Oral Surgery, Faculty of Dentistry, University of Szeged, Hungary, respectively. Saliva samples were frozen and stored at −70 °C until investigation. The biopsy samples used for molecular studies were also frozen and stored at −70 °C until DNA isolation; alternatively they were formalin fixed for histological examination. The histopathologic diagnosis of biopsies was established at the Department of Pathology, Faculty of Medicine, University of Szeged. Control saliva or saliva and oral mucosa samples were taken from individuals free of periodontitis, gingival inflammation or oral mucosal disease who attended the Faculty of Dentistry, University of Szeged, Hungary. Patients whose head and neck tumor was of unknown origin or had a histology other than squamous cell carcinoma were excluded from the study.

Table 1
Prevalence and types of HPV in HNSCC patients, patients with OPMD and controls.

	HPV prevalence (%)	HPV type detected
HNSCC patients		
Saliva	5/57 (8.8%)	HPV16 (3 cases), HPV13 (2 cases)
Tumor biopsy	11/60 (18%)	HPV16
Oropharyngeal cancer		
Saliva	4/12 (33%)	HPV16 (3 cases), HPV13 (1 case)
Tumor biopsy	7/14 (50%)[a]	HPV 16
Hypopharyngeal cancer		
Saliva	1/24 (4%)	HPV13
Tumor biopsy	2/25 (8%)[b]	HPV16
Laryngeal cancer		
Saliva	0/21 (0%)	
Tumor biopsy	2/21 (9.5%)	HPV16
Patients with oral potentially malignant disorders (OPMDs)		
Saliva	3/39 (7.7%)[c]	HPV18, HPV10, HPV81
Biopsy	0/21 (0%)	
Controls		
Saliva	2/57 (3.5%)	HPV13 and HPV11
Tissue	0/20 (0%)	

HPV: human papillomavirus.
HNSCC: head and neck squamous cell carcinoma.
OPMD: oral potentially malignant disorder.
[a] A HPV PCR-positive tumor biopsy sample and a HPV PCR-negative tumor biopsy sample had no corresponding saliva pair.
[b] A HPV PCR-negative tumor biopsy sample had no corresponding saliva pair.
[c] The 3 HPV PCR-positive saliva samples had no corresponding OPMD tissue sample pair.

2.2. Patient characteristics

We analysed 57 saliva samples and 60 tumor biopsy samples collected from HNSCC patients (study group 1; mean age 60.26 years, 51 males and 9 females). The localization of the tumors is shown in Table 1. There were 14 patients with oropharyngeal carcinoma, 25 patients with hypopharyngeal carcinoma and 21 patients with laryngeal carcinoma. The mean age of patients with oropharyngeal carcinoma was 58 years; there was no significant difference between the mean age of HPV-associated oropharynx cancer (OPC) patients (57.8 years; 7 patients, see Table 1) and the mean age of HPV-negative OPC patients (58.1 years, 7 patients). Patients with a head and neck tumor of unknown origin or with histology other than squamous cell carcinoma were excluded from the study. HNSCCs were assigned to stages based on the TNM classification criteria (Stevenson, 2016). 5% (3/60) of carcinomas belonged to stage I, 11.7% (7/60) belonged to stage II, whereas 14 out of 60 (23.3%) were assigned to stage III. The remaining HNSCCs (36 out of 60, 60%) were categorized as stage IV carcinomas. Thus, most of the HNSCCs analysed represented more advanced stages (stage III and IV). Regarding histological grade, 7 out of 60 carcinomas (11.66%) represented well differentiated grade 1 tumors. The majority (36/60, 60%) was moderately differentiated, grade 2 carcinoma. 16 out of 60 (26.66%) HNSCCs were poorly differentiated (grade 3) and there was an undifferentiated (grade 4) carcinoma, too (1/60, 1.66%).

39 saliva samples and 21 lesion biopsy samples were collected from OPMD patients (study group 2; mean age 61 years, male to female ratio 1:3). The diagnosis was oral lichen planus (OLP) in 36 cases; in addition, oral leukoplakia was recorded in 2 cases and fibroma in 1 case. All of the tissue samples derived from OLP patients.

57 saliva samples and 20 oral mucosa samples collected from control patients were analysed as well (study group 3; mean age: 35.2 years, male to female ratio 1:1).