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Human Papilloma Virus Epidemiology and Subtyping in Head and Neck Squamous Cell Carcinoma by Anatomical Site in Central Greece

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Abstract

Objectives: To assess Human Papilloma Virus (HPV) prevalence and subtypes distribution in a population of Head and Neck Squamous cell carcinoma (HNSCC) patients in Central Greece.

Methods: Paraffin-embedded biopsy samples from 90 patients with confirmed HNSCC, were retrospectively analysed for the presence of various HPV sub-types via real time PCR. In addition, a total of 206 controls, who visited the ENT Department the same period, were also tested for the presence of HPV sub-types in their pharyngeal cavity, by the same molecular assay.

Results: According to the anatomical site, HNSCC originated from four different sites: oral cavity, oropharynx, larynx, and hypopharynx. The presence of HPV was detected in 42.2% of samples (38/90), while the most prevalent subtype was HPV-16 (89.5%; 34/38), followed by HPV-18 (%), HPV-31 and HPV-33(%). Amongst the HPV(+) HNSCC samples, most originated from the oral cavity (45%, 17/38) and the oropharynx (32%, 12/38). On the other hand, among the non-HNSCC controls, 9.7% (20/206) were HPV positive, while the most prevalent sub-types were HPV-31, HPV-56, HPV-45 and HPV-41 (Table 1); moreover, six samples were positive for more one subtypes. Statistical analysis revealed that HPV (+) patients were 7 times more likely to have HNSCC [OR 6.8, 95%CI 3.65–12.67, p<0.0005], and HPV-16 (+) patients were 124 times more likely to have HNSCC [OR 124.5, 95%CI 16.67–929.3, p<0.0005].

Conclusion: High prevalence of HPV was detected in HNSCC patients in Central Greece, where HPV-16 predominate. Our results demonstrate the necessity of the 9-valent HPV vaccination.

Keywords: Hnscc; Human Papilloma Virus; Human Papilloma Virus subtypes; Human Papilloma Virus Epidemiology

Introduction

Human papilloma virus (HPV)-associated cancer accounts for 5% of all cancers worldwide [1,2], and for 29.5% of new cancer cases attributable to infections [3]. HPVs are DNA virus that infect epithelial cells [4]. More than 207 HPV -subtypes have been identified so far, and their association with tumour progression has been under research for more than 50 years [4,5]. 40 HPV subtypes infect the anogenital tract, and within these, there are oncogenic HPV subtypes which are classified as either high-risk, causing intraepithelial neoplasia and cervical cancers (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82), or low-risk, causing genital warts (HPVs 6, 11) [4]. HPV 16 and HPV 18 together account for 70% of cervical cancers [6].

The first evidence for an association between HPV and head and neck squamous cell carcinoma (HNSCC) was published in the 1980s [7,8], while, many more publications followed [9-12]. Global epidemiological data indicate that oral HPV infection is one of the main causes for the dramatic increase in oropharyngeal cancer that has controversially been characterized as epidemic [13,14].

The oropharynx is the main site of HPV-associated HNSCC, and, to a lesser extent the oral cavity and larynx [15]. 90% of oral cavity, pharyngeal, and laryngeal cancers are squamous cell carcinomas [16]. HPV -16 is the subtype most commonly associated with HNSCC, present in more than 80% of cases [13,17].

Alcohol and tobacco abuse are the commonest risk factors for these malignancies [16]. However, patients with HNSCC who are also positive for HPV-associated disease are less likely to abuse alcohol and tobacco and are usually younger than HPV-negative subjects [18,19]. Furthermore, HPV (+) patients show a better

response to treatment and better survival outcome [16,20], and HPV status detection has been suggested as independent predictor of overall survival [21].

Globally, the geographical distribution of HNSCC is diametrically different: the incidence of cervical cancer is higher in the less developed world, while the incidence of head and neck cancer is highest in North America and Europe [15]. Epidemiology data on HPV infection in Greece mostly focus on cervical cancer [22-24], and a recent systematic review revealed a data gap regarding HPV prevalence on oropharyngeal sites [24]. In Greece, no data exist concerning the prevalence of HPV in HNSCC. Aim of the present study was to assess HPV-subtypes prevalence in a population of HNSCC patients in Central Greece between 2010-2014.

Methods

Setting

HNSCC samples: We used retrospectively paraffin-embedded tumour samples from patients who had undergone surgery for HNSCC between 2010–2014. Only Squamous Cell Carcinoma (SCC) samples were included in our analysis that was accompanied by a complete histological analysis and a complete patient history.

Non-HNSCC samples: Pharyngeal swab samples were collected from individuals (93 men and 113 women) 12–80 years old, all habitants of Central Greece, with clinically normal mouth mucosa who had visited our ENT Department between 2010–2014, and were tested for HPV-subtypes by the same molecular method mentioned above. All participants provided written informed consent, and completed a questionnaire with demographic data (age, sex, occupation, residence, behavior, HPV-vaccination etc). The protocol was approved by the Ethics Committee of the University Hospital of Larissa, Greece (N 3450).

HPV-Subtypes Molecular Detection

All paraffin and pharyngeal samples were sent to the Microbiology Laboratory of the University Hospital of Larissa-Greece for further analysis. 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. 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 [25-27].

Statistical Analysis

The chi-square test, Fisher's exact test, or independent t-test was used as appropriate for the comparison of demographics between HPV (+) and HPV (-) samples. The risk of HPV infection was estimated using logistic regression. The statistical package SPSS version 14.00 (SPSS Inc, Chicago, IL) was used for all statistical analyses. All tests were two-sided. The level of statistical significance was set at $P<0.005$.

Results

HPV Prevalence and Subtype Identification

A total of 90 HNSCC, originated from four different sites (oral cavity, oropharynx, larynx and hypopharynx) and 206 non-HNSCC samples were included. According to the data obtained, 42.2% (38/90) of HSCNN samples were HPV (+), most of which (89.5%; 34/38) were positive for the HPV-16 subtype (Table 1); three samples positive for HPV-16 were also positive either HPV-6 or HPV-11. Among the 38 HPV-positive HNSCC, 17 (45%) originated from the oral cavity, 12 (32%) from oropharynx, 5 (13%) from larynx and 4(10%) from hypopharynx (Table 2 describes the distribution of HPV-subtypes in each anatomical site). Sex, smoking status, and alcohol use did not differ statistically significantly between HPV (+) and HPV (-) HNSCC patients (Table 3).

Table 1: HPV subtype detection and comparison between HNSCC (N=90) and Non-HNSCC (N=206) samples.

	HNCCCgroup (N=90)	Non-HNSCC (N=206)	P
HPV positive	38	20	-
HPV 16	34	1	<0.0005
HPV 18	1	3	1.000
HPV 31	1	7	0.442
HPV 33	1	2	1.000
HPV 39	0	3	0.556
HPV 45	0	4	0.318
HPV 51	0	4	0.318
HPV 56	0	5	0.328
HPV 58	0	1	1.000
HPV 59	0	3	1.000
HPV 6 or 11	4	0	0.014

Table 2 : HPV subtype distribution by HNSCC anatomical site.

	Larynx	Oropharynx	Hypopharynx	Oral cavity
HPV 16	5	11	4	14
HPV 18	0	0	0	1
HPV 31	0	1	0	0
HPV 33	0	0	0	1
HPV 6, 11	0	2	0	2

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Table 3: Comparison of demographic characteristics of HPV (+) and HPV (-) amongst the HNSCC patients (N=90).

	HPV (+)	HPV (-)	P
n, (%)	38 (42.2)	52 (57.8)	
Age, mean, (SD)	61 (14)	61 (12)	0.851
Male	30	42	1.000
Female	8	10	
Smoking	21	30	0.833
Alcohol	15	16	0.501

On the other hand, 9.7% of non-HNSCC control samples (20/206) were HPV (+), where the most prevalent subtypes were HPV-31, HPV-56, HPV-45 and HPV-41 (Table 1); among them, six samples were positive for more than one subtypes. It is interesting that the HPV-16 was only detected only in one sample of this group.

According to the questionnaire obtained, none of the HNSCC and non-HNSCC patients was vaccinated by the tetra-valent HPV vaccine. HPV (+) sub-types were found significantly more frequently in the HNSCC samples than in the non-HNSCC samples (42.2% vs. 10%, p<0.0005). The same was found for HPV 16 (+) (37.8% vs. 0.5%, p<0.0005) and for HPV 6 and 11 (+) (4.4% vs. 0%, p=0.014) (Table 1).

Logistic Regression Results

Statistical analysis revealed that HPV (+) samples were 7 times more likely to have HNSCC [OR 6.8, 95%CI 3.65–12.67, p<0.0005], and samples positive for HPV 16 were 124 times more likely to have HNSCC [OR 124.5, 95%CI 16.67–929.3, p<0.0005]. Dependence on the site of the HNSCC, oropharynx samples were 10.2 times more likely to be HPV (+) and 187.9 times more likely to be HPV -16 (+) (Table 4).

Table 4: Logistic regression results for the likelihood of HPV or HPV 16 infection,

	HPV (+)		HPV 16 (+)	
	Odds Ratio (95%CI)	P value	Odds Ratio (95%CI)	P value
Oropharynx	10.2 (3.97–25.95)	<0.0005	187.9 (22.37–1578.56)	<0.0005
Oral cavity	7.5 (3.42–16.56)	<0.0005	119.6 (15.05–949.91)	<0.0005
Hypopharynx	6.2 (1.61–23.83)	0.008	136.7 (13.21–1414.29)	<0.0005
Larynx	3.32 (1.08–10.18)	0.036	73.2 (8.00–670.24)	<0.0005

HNSCC vs. Non-HNSCC.

Discussion

According to our results, high prevalence of HPV (42.2%) was detected in patients with HNSCC in Central Greece, a rural Greek area with 1.000.000 inhabitants. We note that approximately twenty/ thirty patients per year admitted to the ENT Department of University Hospital of Larissa-Greece are diagnosed with HNSCC. The HPV prevalence in our HNSCC samples was quite similar to the 40.0% HPV prevalence reported in a meta-analysis of 39 studies conducted in 19 European countries [28]. The prevalence differed according to the anatomical sites, e.g. was 66.4% in tonsillar cancers, 25.7% in tongue cancers, and 15.3% in pharyngeal cancers [28]. A systematic review of 105 studies conducted in 23 countries in Europe and North America investigated HPV prevalence in oropharyngeal squamous cell carcinoma [29]. The study reported an increasing trend in HPV prevalence in the European countries from 28% before 1995 to 49.5% after 2005 [29]. However, data from other Greek studies demonstrate lower HPV prevalence in HNSCC: 22.2% (14/63) reported by Blioumi et al and 11.1% (5/45) reported by Kouvousi et al [30,31]. This variation could be explained by the method used which varies between in situ hybridization to PCR, immunohistochemistry or Southern blot [25,31]. Geographical variations have also been reported to affect HPV HNSCC prevalence rates. For example, in the North-East Italy Baboci et al reported 8.5% prevalence for HPV DNA in 247 HNSCC cases [32]. In India, Bahl et al reported 22.8% HPV prevalence in oropharyngeal patients [33]. In Nigeria, Oga et al did not detect HPV in Head Neck Carcinoma (HNC) patients [34]. In Senegale, Ndiaye et al reported very low prevalence (3.4%) [17] while, similar prevalence was reported by Ribeiro et al in samples collected from Argentina, Brazil, Cuba, Russia, Slovakia, Czech republic, Romania, and Poland [35]. On the contrary, in France, St Guily et al reported 46.5% HPV prevalence, in oropharyngeal carcinomas [36]. In South Wales (UK), Evans et al detected 55% HPV DNA [37].

On the other hand, among non-HNSCC individuals the prevalence of HPV in the pharynx was 9.7%, while various high-risk subtypes were identified (16,18, 31,33,39,45,51,56,58 and 59). In the USA, an oral HPV prevalence study conducted as part of the US National Health and Nutrition Examination Survey (NHANES) found that 6.9% of men and women between 14–69 years of age were HPV (+) [38]. Since HPV-16 predominate in HNSCC samples, only one sample was found to be positive in non-HNSCC group.

In conclusion, 42.2% of patients with HNSCC were HPV (+), whereas, HPV- 16 was the most common subtype detected. Given the rapidly increasing incidence of oropharyngeal cancer and its association with HPV infection, more research in this area would assist in the formulation of appropriate disease surveillance interventions. Furthermore, our data emphasizes the need for a wide vaccination of Greek people by the 9-valent HPV vaccine that includes the subtypes 6, 11, 16, 18, 31, 33, 45, 52, and 58 [38].

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