Acta Oto-Laryngologica, 2005; 125: 1337–1344

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ORIGINAL ARTICLE

Strong association between infection with human papillomavirus and oral and oropharyngeal squamous cell carcinoma: A population-based case-control study in southern Sweden

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Abstract

Conclusions. The results of this study demonstrate a strong association between infection with high-risk types of human papillomavirus (HPV) and oral and oropharyngeal squamous cell carcinoma (OOSCC), suggesting that high-risk HPV types play a key role in carcinogenesis. The estimated proportion of OOSCC cases attributable to HPV infection was 35%. *Objective*. HPV appears to have an aetiological role in OOSCC, despite the fact that the reported prevalences of HPV in both OOSCC patients and healthy individuals have varied widely. We aimed to investigate the presence and spectrum of both high- and low-risk HPVs in all consecutive cases of OOSCC in a Swedish healthcare region over a 3-year period and in population-based, matched healthy controls. *Material and methods*. A total of 131 patients with OOSCC were studied. Samples taken from the surface of the tumour and from the tonsillar fossa using cotton-tipped swabs were investigated, together with exfoliated cells collected using a mouthwash. Tonsillar fossa and mouthwash specimens were collected in the same way from 320 matched controls. All samples were tested for HPV DNA by nested polymerase chain reaction using the primer pairs MY09/MY11 and GP5+/GP6+, and in positive cases the HPV type was determined by DNA sequencing. *Results*. Infection with high-risk HPV was shown to be a strong risk factor for OOSCC (OR = 63; 95% CI 14–480). Forty-seven (36%) of the cancer patients had ≥ 1 specimen that was positive for a high-risk HPV type (81% of which were HPV 16), while only 3 (0.94%) of the controls were positive for a high-risk HPV type. Seven (5.3%) of the cancer patients and 13 (4.1%) of the controls were positive for any of the mucosal, mucocutaneous or cutaneous low-risk HPV types.

Keywords: *Attributable proportion, human papillomavirus DNA, International Classification of Diseases code, mouthwash, oral and oropharyngeal squamous cell carcinoma, risk factor*

Introduction

The incidence of oral cancer varies widely in different parts of the world. In Southeast Asia, and particularly in India, oral carcinoma is the commonest form of cancer, accounting for up to 50% of malignant tumours [1]. Although oral and oropharyngeal squamous cell carcinoma (OOSCC) is unusual in Sweden, representing only $\approx 1\%$ of cases of malignant tumours, the disease is becoming more frequent and an increasing incidence of tongue cancer, especially in young individuals, has been reported in many parts of Europe [2–4].

Human papillomavirus (HPV) has been identified as the major cause of cervical cancer, and certain high-risk HPV types have been detected in virtually all cases of cervical cancer [5], as well as in $>50\%$ of other forms of anogenital cancer [6]. It has also been

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(Received 10 March 2005; accepted 2 June 2005)

ISSN 0001-6489 print/ISSN 1651-2551 online
DOI: 10.1080/00016480510043945

indicated in a number of studies [7–12] that HPV infections play a role in oral carcinogenesis, although interpretation of these studies is complicated by the extreme variation in the prevalence of HPV infection found in both patients and healthy individuals. The great variation in HPV prevalence found in tumours of the oral cavity may be due to differences between the populations analysed, but also due to differences in the materials used for testing (i.e. formalin-fixed or fresh biopsies, exfoliated fresh cells), the methods of DNA extraction and, probably most importantly, the HPV detection methods used.

The aim of this study was to investigate the presence of HPV DNA of both high- and low-risk mucosal/genital types in patients diagnosed consecutively with OOSCC and in population-based, matched healthy controls. The method used was a nested polymerase chain reaction (PCR) with high sensitivity and specificity, and the material used for HPV DNA analysis included both exfoliated tumour cells and cells from the oral cavity as a whole.

Material and methods

Cases

Initially, 132 patients were enrolled for investigation, representing $\approx 80\%$ of all consecutive cases of OOSCC in the Southern healthcare region of Sweden during the study period (September 2000 to January 2004). The inclusion criteria were as follows: patients with tumours with the International Classification of Diseases—seventh revision (ICD-7) codes 141 (tongue; $n=29$), 143 (floor of the mouth; $n=25$), 144 (oral cavity, not otherwise specified; $n=32$) and 145 (oropharynx; $n=46$). As the base of the tongue is a subsite of the oropharynx, tumours in this location ($n=10$) were grouped together with the ICD-7 code 145. Only patients with no previous cancer diagnosis (with the exception of skin cancer) who were born in Sweden were eligible. Ninety-one of the OOSCC patients were male (median age at diagnosis 59 years; range 36–87 years) and 41 were female (median age at diagnosis 69 years; range 33–87 years).

Controls

By means of stratified random sampling, 320 healthy controls [215 males (median age 60 years; range 36–89 years) and 105 females (median age 66 years; range 33–89 years)] were drawn from the Swedish Population Registry using the following criteria: no previous cancer diagnosis (with the exception of skin cancer); same age ± 3 years; same sex; born in Sweden; and matched region of residence.

A detailed description of the recruitment procedure and drop-outs will be published in a forthcoming paper. Informed consent was obtained from all patients and controls.

Samples

All specimens were collected by the same person (K. R.), a Registered Nurse and dental surgeon. From the patients, samples were collected from two sites, the tumour and tonsillar fossa, using cotton-tipped swabs. These were drawn back and forth over the sampling site and the exfoliated cells were suspended in separate tubes containing 1 ml of 0.9% NaCl solution. In addition, a mouthwash sample was obtained from each individual by rinsing the mouth for 30 s with 7 ml of 0.9% NaCl solution, which was then collected in a tube. A swab sample from the tonsillar fossa and a mouthwash sample were collected from each of the healthy controls using the same technique. All specimens were kept refrigerated until they were frozen at -20°C, always within 8 h of collection.

Before analysis, the samples were thawed and vortexed and a 200 µl portion from each specimen was subjected to automated nucleic acid purification in a MagNA Pure instrument using the Total Nucleic Acid Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany).

PCR

All specimens were tested for HPV DNA by nested PCR using outer primers MY09 and MY11 [13] and inner primers GP5+ and GP6+ [14]. The final volume of PCR solution (25 µl) contained 0.5 µM concentrations of the MY09 and MY11 primers in the first PCR run and of the GP5+ and GP6+ primers in the second PCR run, dNTPs at concentrations of 0.2 mM, 1 U of AmpliTaq DNA polymerase, reaction buffer and 3.5 mM $MgCl_2$ (Applied Biosystems, Warrington, UK). Five µl of extracted sample DNA was added to the MY09/MY11 PCR mixture, and 2 µl of the resulting PCR product was used as input for the second PCR with the GP5+/GP6+ primers.

The first PCR consisted of an initial 4-min denaturation step at 94°C, followed by 40 identical cycles at 94°C for 1.5 min, 48°C for 1.5 min and 72°C for 2 min, plus a final elongation step at 72°C for 4 min. The second PCR started with a 4-min denaturation step at 94°C, followed by 40 cycles at 94°C for 1.5 min, 40°C for 1.5 min and 72°C for 2 min, plus a final elongation step at 72°C for 4 min.

By testing an HPV 16 plasmid standard in a dilution series, the method used was shown to have

the sensitivity to detect 1–10 HPV 16 genome copies per assay.

As a PCR amplification control, all samples were subjected to a PCR test for the human β-globin gene, using PCO3 and PCO5 primers [15]. The β-globin PCR was performed under the same conditions as the GP5+/GP6+ PCR, but with the $MgCl_2$ concentration adjusted to 1.5 mM and at an annealing temperature of 48°C.

In each test run, H_2O (without template) was included as a negative control, and for the nested PCR a clinical sample containing HPV 11 served as a positive control. Proteinase K-treated human embryonic lung fibroblasts were used as a positive control in the β-globin PCR.

Five μl of the amplified material was analysed by electrophoresis in a 2% agarose gel (Sea Kem; FMC Bioproducts, Rockland, ME) in Tris–borate–EDTA buffer containing ethidium bromide (0.02 μg/ml), and the amplicons were identified by size determination under UV light.

HPV type determination

All samples that proved positive in the nested HPV PCR assay were retested, and the PCR product of the retest was purified on a MicroSpin S-300 column (Amersham Biosciences) before it was subjected to DNA sequencing (Big Dye Terminator cycle sequencing kit 3.1; Perkin-Elmer, Foster City, CA) and analysed on a Perkin-Elmer 373A automated sequencer. For most of the samples only one of the DNA strands was sequenced, but in cases where the results from the first strand to be analysed were unclear the other strand was also sequenced. The sequences obtained were compared to all DNA sequences available in GenBank using the BLAST server (www.ncbi.nlm.nih.gov/blast.cgi). The HPV type of an individual sample was identified when it had >90% DNA sequence homology with a known HPV prototype. The HPV types were grouped as either high- or low-risk HPVs according to the scheme of de Villiers et al. [16].

Statistical analysis

The association between the factors studied and the presence of OOSCC were analysed using standard statistical methodology. HPV DNAs of high- and low-risk types in mouthwash samples were evaluated separately by univariate analysis. In addition, multivariate logistic regression methods were used, with adjustment for potential confounders. As only 2/320 mouthwash samples of the controls were high-risk HPV DNA-positive, the total control group was used in the OR calculations for each of the tumour site groups. Unless otherwise stated, adjusted ORs are presented.

Associations between factors were analysed in cross-tables by means of Pearson's χ^2 statistics using the hypothesis that the rows and columns in a two-way table were independent, or using Fisher's exact test when appropriate. In the following, CIs refer to 95% CIs.

Population-attributable proportions were calculated as $S[1-(1/RR)]$, where S is the proportion of OOSCC cases with high-risk HPV DNA positivity identified in at least one sample and RR is the relative risk. The RR was approximated by the OR based on the findings of high-risk HPV DNA in the mouthwash samples in this population-based case-control study. All analyses were performed using the statistical software package STATA (version 8.2; StataCorp, College Station, TX).

The project was approved by the Ethics Committee of Lund University (approval No. LU 315-00).

Results

A total of 132 cases and 320 controls were recruited and investigated.

The result of the quality control for sample collection and DNA extraction, as judged by the outcome of the β-globin PCR, was very good. Only 1/132 patients and none of the 320 controls had to be excluded due to a negative β-globin test result. For the female patient (ICD-7 code 144) who had to be excluded, all of the samples, i.e. tumour, mouthwash and tonsillar fossa samples, were negative, possibly due to a strongly inhibitory substance in the saliva. Thus, 131 patients and 320 controls were fully eligible for the study and were included for the evaluation of HPV infection.

For 18 of the patients and 12 of the controls, a tonsillar fossa sample was not available for HPV analysis due to loss for technical reasons ($n=14$) or because collection was not possible due to a strong gag reflex during the sampling procedure ($n=16$), which reduced the number of tonsillar fossa samples to 113 from patients and 308 from controls. The tumour sample from 1 of the patients in the ICD-7 145 group had been lost before the DNA extraction, reducing the number of tumour samples analysed to 130.

Taking all samples into account, 52/131 (40%) OOSCC patients were found to be positive for HPV DNA in 1, 2 or all 3 of the samples collected from each patient. Forty-seven (36%) of the 131 OOSCC patients tested positive for HPV DNA of a high-risk type, and 7 (5.3%) tested positive for a low-risk HPV type (Table I). Two of the patients were found to be positive for both high- and low-risk HPV DNA

Table I. Prevalence of HPV DNA of high- or low-risk types in 3 different samples from 131 patients with OOSCC at different sites (according to ICD-7 code).

Tumour site (ICD-7 code)	Type of HPV DNA	No. of HPV DNA-positive samples/ no. of samples tested (%)			No. of HPV DNA-positive patients/no. of patients tested (%)
		Tumour	Mouthwash	Tonsillar fossa	
Tongue (141)[a]	High risk	4/29 (14)	3/29 (10)	0/26	7/29 (24)
	Low risk	0/29	2/29 (6.9)	1/26 (3.8)	2/29 (6.9)
Floor of the mouth (143)	High risk	8/25 (32)	5/25 (20)	2/21 (9.5)	10/25 (40)
	Low risk	2/25 (8.0)	3/25 (12)	0/21	3/25 (12)
Oral cavity, not otherwise specified (144)	High risk	1/31 (3.2)	4/31 (13)	1/26 (3.8)	5/31 (16)
	Low risk	0/31	0/31	0/26	0/31
Oropharynx (145)[a]	High risk	19/45 (42)	21/46 (46)	7/40 (18)	25/46 (54)
	Low risk	0/45	1/46 (2.2)	1/40 (2.5)	2/46 (4.3)
All sites	High risk	32/130 (25)	33/131 (25)	10/113 (8.8)	47/131 (36)
	Low risk	2/130 (1.5)	6/131 (4.6)	2/113 (1.8)	7/131 (5.3)

[a]Cases of SCC of the base of the tongue were moved from ICD-7 code 141 to 145.

types. From one of these patients two of the samples contained HPV 16 and the third contained HPV 25, and from the second patient one sample was positive for HPV 16 and another was positive for HPV 10 (Table IV). The samples taken from the surface of the tumours and the mouthwash samples were found to be positive for a high-risk HPV type with equal frequency (25%), while the prevalence of high-risk HPV DNA in the samples taken from the tonsillar fossa was lower (8.8%) ($p < 0.01$). Samples from patients with OOSCC of the oropharynx (ICD-7 code 145), including the base of the tongue, had the highest prevalence of high-risk HPV infections (54%), followed by carcinoma of the floor of the mouth (ICD-7 code 143; 40%), tongue (ICD-7 code 141; 24%) and oral cavity, not otherwise specified (ICD-7 code 144; 16%) (Table I).

Of the 320 healthy controls, only 3 (0.94%) were positive for a high-risk HPV type, and 13 (4.1%) were positive for a low-risk HPV type. Two of the three high-risk HPV-positive samples and 12/13 low-risk HPV-positive samples were mouthwash samples (Table II).

Table III shows the spectrum of HPV types found in the patients and their matched controls. Seven different high-risk HPV types were identified in the samples from the patients. Eighty-one percent of the high-risk HPV-positive samples (61/75) contained HPV 16 DNA, and these 61 HPV 16-positive samples came from a total of 38 patients. The second commonest HPV type was HPV 33 (seven samples from three patients). In the control group, three different high-risk HPV types were identified: HPV 16 and HPV 67 were found in two mouthwash samples, and one sample from the tonsillar fossa was positive for HPV 68. In total, 11 different low-risk mucosal, mucocutaneous or skin HPV types were found in the patients and controls. HPV 13 was seen in both groups (two samples in each), as were HPV 25 and HPV 62 (one positive sample in each group for both) and HPV 76 (three samples in each group).

Seven of the patients were HPV 16-positive at all three sampling sites, and in another patient HPV 33 was found in all three samples. Table IV shows the various combinations of HPV findings in the patient samples.

The sensitivity of identification of high-risk HPV infection among the OOSCC patients was the same (25%) whether only the samples taken with cotton-tipped swabs from the tumour surface or the mouthwash samples were considered: in both instances there were 32/46 patients (70%) in whom 1 or both of these samples was positive. The sensitivity of identification of high-risk HPV

Table II. Prevalence of HPV DNA in 2 different samples from 320 healthy controls.

Type of HPV DNA	No. of HPV DNA-positive samples/ no. of samples tested (%)		No. of HPV DNA-positive controls/ no. of controls tested (%)
	Mouthwash	Tonsillar fossa	
High risk	2/320 (0.63)	1/308 (0.32)	3/320 (0.94)
Low risk	12/320 (3.8)	1/308 (0.32)	13/320 (4.1)

Table III. Distribution of HPV types found in patients with OOSCC ($n=131$) and matched healthy controls ($n=320$). The values shown represent the numbers of positive samples.

	Mucosal high-risk types									Mucosal low-risk types						Mucocutaneous low-risk types		Benign cutaneous types		Cutaneous EV[a] type
	16	18	33	45	58	59	67	68	70	13	32	54	55	62	87	10	25	75	76	RTRX9
Patients																				
Tumour	27		3			1			1		1								1	
Mouthwash	26	2	2	1	1	1				1	1			1		1			2	
Tonsillar fossa	8		2							1							1			
Healthy controls																				
Mouthwash	1						1			2		1	1	1	1		1	1	3	1
Tonsillar fossa								1										1		

[a]Epidermodysplasia Verruciformis.

infection was much lower for the tonsillar fossa samples (Tables I and IV).

The prevalence of HPV infection among the male patients, 39/91 (43%), did not differ significantly from that found for the female patients, 12/40 (30%).

As fewer tonsillar fossa than mouthwash samples were analysed, and as the prevalence of HPV DNA was found to be highest in the mouthwash samples, calculations of HPV infection as a risk factor for OOSCC were based on the results from the mouthwash samples only. HPV DNA of defined high-risk types in mouthwash samples was found to be a risk factor for OOSCC for all of the tumour sites in this investigation. The OR was highest, 230 (CI 45–1200), for cancer of the oropharynx and base of the tongue, and lowest, 22 (2.8–170), for SCC of the oral cavity, not otherwise specified. For all of the OOSCC patients, regardless of the site of the tumour, the OR was 63 (CI 14–280). HPV DNA of defined low-risk types did not constitute a risk factor for OOSCC (Table V). A multivariate analysis including other potential risk factors for OOSCC is presented in two forthcoming reports. The population-based proportion of OOSCC cases attributable to infection with a high-risk HPV type, and thus potentially preventable by HPV vaccination, was calculated to be 35% (Table V). For SCC of the oropharynx and base of the tongue, the proportion attributable to high-risk HPV was 54%.

Table IV. HPV findings in tumour, mouthwash and tonsillar fossa samples from 131 patients with OOSCC.

	HPV DNA type found[a]		
No. of patients	Tumour	Mouthwash	Tonsillar fossa
7	**16**	**16**	**16**
1	**16**	**16**	25
1	**33**	**33**	**33**
7	**16**	**16**	
1	**16**	10	
1	32	32	
1	**33**	**33**	
1	**33**	**45**	
1	**59**	**59**	
1	76	76	
11	**16**		
1	**70**		
1		**16**	**16**
1		13	13
10		**16**	
2		**18**	
1		**58**	
1		62	
1		76	
1			**33**

[a]High-risk HPV types are shown in bold type.

Discussion

This population-based case-control study clearly demonstrates that infection with HPV of the so-called high-risk genotypes is a major risk determinant of oral cancer (OR 63; CI 14–280). An association between HPV and tonsillar cancer has been described in many previous studies [10–12]. Likewise, in the present study, the strongest association was seen between high-risk HPV infection and SSC of the oropharynx and base of the tongue (OR 230; CI 45–1200). Even for OOSCC at other sites, the presence of high-risk HPV infection represented a significantly increased risk (Table V).

Overall, 36% of the OOSCC patients were positive for HPV DNA of a high-risk type in at least 1 of 3 different samples, while only 0.94% of the matched healthy controls were positive for HPV DNA of a high-risk type. In addition to the potentially oncogenic mucosal HPV types, a total of 11 different low-risk mucosal, mucocutaneous or cutaneous HPV types were found scattered among both the cases and controls with approximately the same prevalence: 7/131 (5.3%) and 13/320 (4.1%), respectively.

Table V. HPV DNA findings in mouthwash samples from patients with OOSCC and population-based healthy controls.

Tumour site (ICD-7 code)	Type of HPV DNA	No. of positive samples/total no. of samples	Univariate analysis OR (95% CI)	Univariate analysis p	Multivariate analysis[b] OR (95% CI)	Multivariate analysis[b] p	Attributable proportion (%)
Healthy controls	High risk	2/320	Reference		Reference		
	Low risk	12/320	Reference		Reference		
Tongue (141)[a]	High risk	3/29	18 (2.9–120)	0.002	24 (3.2–180)	0.002	23
	Low risk	2/29	1.9 (0.4–8.9)	0.42	2.4 (0.5–11)	0.28	
Floor of the mouth (143)	High risk	5/25	40 (7.3–220)	<0.001	51 (3.2–810)	0.005	49
	Low risk	3/25	3.5 (0.9–13)	0.066	3.3 (0.7–17)	0.15	
Oral cavity, not otherwise specified (144)	High risk	4/31	24 (4.1–140)	<0.001	22 (2.8–170)	0.003	15
	Low risk	0/31					
Oropharynx (145)[a]	High risk	21/46	130 (29–600)	<0.001	230 (44–1200)	<0.001	54
	Low risk	1/46	0.57 (0.1–4.5)	0.59	0.8 (0.1–6.9)	0.86	
All sites	High risk	33/131	54 (13–230)	<0.001	63 (14–280)	<0.001	35
	Low risk	6/131	1.2 (0.37–3.6)	0.68	1.4 (0.5–4.3)	0.50	

[a]Cases of SCC of the base of the tongue were moved from ICD-7 code 141 to 145.
[b]Adjusted for alcohol and tobacco smoking.

Seven different high-risk HPV types were detected in the cancer patients. Consistent with other reports [10,11], we found that the vast majority of the HPV DNA-positive patients harboured HPV 16 (71% of the total number of HPV DNA-positive patients and 79% of those positive for a high-risk HPV type).

There have been many reports on HPV infections in OOSCC patients and also in healthy individuals, and the consensus picture which emerges from them is kaleidoscopic. Even in recent publications, the prevalence of HPV found in OOSCC patients has varied from a few percent up to 100%, and similar results have been reported for oral samples from healthy individuals. However, despite the extraordinarily diverse findings regarding HPV levels, evidence of an association between HPV infections and at least a subgroup of oral cancers is accumulating. HPV 16 has consistently been found to be the most prevalent HPV type in oral tumours, although there are some exceptions. Remmerbach et al. [17] found that 65% of brush samples collected from patients with untreated cancerous oral lesions were HPV DNA-positive. HPV 6, a non-oncogenic genital HPV type, represented the majority (64%) of these HPV types. In a study from Sicily [18], 62% of patients with OOSCC and 5.5% of control subjects were found to be HPV DNA-positive. Eighty-six percent of the HPV-positive lesions and 80% of the HPV-positive samples from the healthy controls contained HPV 18. HPV 18 has also been most frequently seen in OOSCC patients from Greece [19], India [20] and Taiwan [21] which, interestingly, is in accordance with the predominance of HPV 18 found in cervical cancer among Indonesian women [22]. Remarkable results came from a study [23] of Japanese children aged 3 and 5 years, in which 48% of oral squamous cell samples collected from swabs of normal buccal mucosa were found to be HPV DNA-positive. HPV 16 was the predominant HPV type and was found in 30% of all children. Thus, the results of the present report should be weighed against the motley background of previously published investigations of HPV in oral SCC and of HPV findings in oral samples from healthy individuals.

In our opinion, the strengths of the present study are as follows:

1. The study material was homogenous, representing most (consecutive) new cases of OOSCC in a defined geographical region over a given time period. Each case was followed by means of two to three matched population-based healthy controls.
2. All patients and controls were examined by the same person, who was also responsible for the collection of exfoliated cells from both cases and controls.
3. The same experienced technician handled and tested all of the samples for HPV.
4. To minimize the risk of PCR-inhibitory substances from the samples and to obtain a high, even quality of DNA, all samples were subjected to automated DNA extraction.
5. In order to obtain maximal sensitivity, we chose to use a nested PCR test, with the most frequently used and (for mucosal and mucocutaneous HPV types) extensively evaluated primer pairs, MY09/MY11 and GP5+/GP6+.

6. DNA sequencing was chosen as the optimal method for confirmation of suspected PCR-positive findings and for HPV type determination.

Our results constitute strong evidence of a link between infection with oncogenic (high-risk) HPV types—the same types that have been proven to be the cause of most anogenital cancers—and at least a fraction of cases of OOSCC. As with cervical cancer, co-factors are probably also involved in the carcinogenesis.

In cervical cancer, HPV DNA is generally integrated into the genome of the tumour cells. This is probably a less common event in oral tumours [24]. Also, the HPV DNA copy numbers in oral tumours are generally low. These observations may be interpreted as evidence that the HPV infection functions as an initiator, possibly together with one or more co-factors, in the process of tumorigenesis. Once the tumour has developed, the HPV infection may not be needed for maintenance of the malignant state. It may even be the case that the established tumour does not support further HPV replication. This hypothesis is supported by the observation that cell lines derived from oral tumour tissue, even if initially HPV-positive, often lose the virus after some passages [25]. This contrasts with the situation in cell lines originating from cervical tumours.

If established oral tumours can indeed lose their HPV infections, this could explain the seemingly low copy number of HPV DNA found in most tumours, and in turn serve as an explanation for some of the great differences in HPV prevalence found in different studies. It would also raise the question of whether a much greater proportion of oral tumours may have been caused by HPV infections but that when samples from these patients were collected, the virus was no longer present. If so, the proportion of OOSCC cases attributable to HPV infections and preventable by vaccination would be even higher than the figure of 35% suggested in this study. Studies of HPV antibodies in patients and control subjects may provide deeper insight into this question. Further knowledge about the aetiological importance of HPV infections in various forms of cancer will come from ongoing and future HPV vaccination studies, which will also eventually reveal how much of the human cancer burden can be prevented by HPV vaccination. OOSCC will certainly be on that list.

Acknowledgements

We thank Helen Emilsson for excellent technical help with the HPV analysis. This work was supported by the Cancer Foundation of Malmö University Hospital, the Alfred Österlund Foundation, the Swedish Cancer Society, the King Gustaf V Jubilee Fund, the Gunnar Nilsson Cancer Foundation, the Mrs. Berta Kamprad Foundation, the Swedish Dental Society and the Faculty of Odontology, Malmö University.

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