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ORIGINAL ARTICLE

No high-risk HPV detected in SCC of the oral tongue in the absolute absence of tobacco and alcohol—a case study of seven patients

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Abstract
Introduction In recent years, there has been an increase in the number of patients with squamous cell carcinoma (SCC) of the mobile tongue in the absence of tobacco and alcohol. The literature suggests that human papilloma virus (HPV) may be involved in the development of SCC of the head and neck, especially in non-smoking and non-drinking patients. In the oral cavity, however, the presence of the HPV genome has been reported with various percentages. This may be due to misclassification of the oropharyngeal tongue as oral cavity subsite and the use of various detection methods.
Materials and methods Therefore, we evaluated the role of HPV in seven consecutive non-smoking and non-drinking patients (2003–2006) with a SCC located at the oral, mobile tongue using in situ hybridization and SPF_{10}Line Blot 25 polymerase chain reaction assays.
Results No HPV was detected in these specimens. To further determine whether a relationship exists between HPV and SCC in the absence of tobacco and alcohol, subsequent studies at specific locations are necessary.

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Introduction

Oral cavity cancer is the eighth most common malignancy worldwide, with more than 300,000 new cases annually [1]. Although it is generally agreed that long-term tobacco, alcohol and betel quid consumption are the major environmental risk factors for the development of oral squamous cell carcinoma (SCC) [1, 2], in recent years, the Netherlands have seen an increase in the number of patients with an SCC of the mobile tongue in the absence of tobacco and alcohol. This rise is in line with studies suggesting that the incidence of tongue and tonsillar cancer is increasing in patients who have had no exposure to these traditional risk factors or with an exposure time not long enough for malignant transformation [2–4].

Nowadays, the question arises whether there is a possible association between this increased incidence of SCC of the tongue and tonsil and high-risk human papilloma virus (HR-HPV) infection. It is generally accepted that there is a strong association between tonsillar SCC and HR-HPV [5–8]. However, with regard to the oral cavity, no unambiguous conclusions can yet be drawn as to whether HR-HPV also plays a role in the development of oral SCC [2, 5, 9]. The International Agency for Research on Cancer multicentre study by Herrero et al. [10] detected HR-HPV DNA in 3.9% of the biopsy specimens of the oral cavity, whereas a large population-based review study conducted by Kreimer et al. [11] reported that the HR-HPV prevalence in SCC of the oral cavity was 23.5%.

The literature suggests that the variety of results can be attributed to geographical differences [2], different detection methods and their sensitivity [2, 12], but also to a

misclassification of the oropharyngeal tongue, which is considered part of Waldeyer's tonsillar ring, as oral cavity subsite [2, 7, 10]. It has been suggested that this ring might be particularly susceptible to HPV, and therefore, cancer site misclassification is considered to be a serious problem that may bias risk estimates in many epidemiological studies [2].

To evaluate the oncogenetic role of HR-HPV in an absolute non-smoking and non-drinking patient group with an oral SCC of the mobile tongue, a case study was performed using two different detection methods.

Materials and methods

Clinical data concerning consecutive Dutch patients with histologically confirmed primary SCC of the mobile tongue, treated between 2003 and 2006, were obtained from the medical charts of the Department of Oral and Maxillofacial Surgery, Radboud University Nijmegen Medical Centre (RUNMC), The Netherlands. Patients who had been exposed to radiotherapy and chemotherapy in their history because of another oncological problem were excluded. Only patients who have never smoked and consumed alcoholic beverages up to one unit a day were included. These patients were screened for tobacco and alcohol use in their history, with a detailed retrospective review of the charts of the hospitals, of general dental practitioners and of general practitioners. This was confirmed by direct communication with the patient or close family. In addition, other data concerning sex, age at diagnosis, tumor histology, and tumor–node–metastases classification (according to the International Union Against Cancer) were obtained from their medical charts.

Consequently, the paraffin-embedded tissue resections from seven consecutive patients were retrieved from the archives of the Department of Pathology, RUNMC. For each patient, a total of three stained microscopic sections were evaluated; one section was stained with haematoxylin and eosin, two stained for the detection of HPV. It was determined which sections of each tumour were most suitable for inclusion in our study, based on the presence of sufficient numbers of tumour cells, the absence of damaged malignant tissue and, where possible, the presence of normal and/or dysplastic epithelium for comparison purposes. These obtained specimens were tested by in situ hybridization (ISH) [13] (HPV 16/18 and 31/33) and a short polymerase chain reaction (PCR) Fragment-PCR (SPF_{10}Line Blot 25 PCR) [14] assay. This PCR assay amplifies a fragment of only 65 bp and is therefore considered a highly sensitive detection method for different HPV types (up to 43 individual HPV-types) on paraffin-embedded specimens in particular [14, 15]. Consequently, this combined detection–genotyping assay is used in the routine clinical practice of screening cervical scrapes and oral and cervical research applications [14, 16]. The integrity of the extracted DNA was also determined by amplification of the β-globin gene, which also served as an internal amplification check.

To avoid cross-contamination with HPV, all tissue specimens were processed separately. A tissue block from a confirmed HR-HPV-positive tonsil carcinoma was used as a positive control. Both assays were performed as previously described [13, 14].

Results

No HR-HPV was detected in the primary tumours of the seven consecutive non-smoking and non-drinking Dutch patients using ISH and SPF_{10}Line Blot 25 PCR assays (Table 1).

Discussion

We evaluated the oncogenetic role of HR-HPV in an absolute non-smoking and non-drinking patient group with an oral SCC of the mobile tongue using two different detection methods. According to the literature, there are two specific European studies that differentiate between SCC of the oral tongue and carcinomas originating from the base of the tongue [17, 19]. Kantola et al. [17] could not detect HR-HPV in 105 specimens of the oral tongue, whereas Dahlgren et al. [19] found only two of the 85 analysed mobile tongue cancers to be HR-HPV positive. However, it remains unclear if these studies included absolute non-smoking and non-drinking patients.

Table 1 Patient data

Age	Sex	Pathology stage	Recurrence	Survival	Follow-up interval
23	Female	$pT_1N_0\ M_0$	1	0	10
49	Male	$pT_1N_0\ M_0$	0	1	40
95	Female	$pT_3N_0\ M_0$	0	1	38
44	Male	$pT_3N_1\ M_0$	0	1	24
37	Male	$pT_1N_0\ M_0$	0	1	37
56	Female	$pT_1N_0\ M_0$	1	1	26
40	Female	$pT_2N_0\ M_0$	0	1	57

Follow-up interval: months
1 Yes, *0* no

Based on the literature, infection with HR-HPV has been suggested as a risk factor for the development of SCC of the head and neck (HNSCC) in non-smoking patients [2, 10, 12]. Yet almost all studies known in the absence of tobacco and alcohol were conducted in HNSCC in general [18, 21]. In a study by Dahlstrom et al. [18], 60 non-smoking patients with a developed HNSCC were evaluated for the presence of HR-HPV. Nevertheless, 17 of these patients appeared to have an SCC of the oral tongue, and only one appeared to be HR-HPV positive. These results seem to be in line with the results of our study. However, the results of the study by Dahlstrom et al. [18] were based on serological antibody testing which is not considered a standard method because of the low sensitivity and specificity of the assays [20].

According to the literature, the various reported HPV percentages in the oral cavity may be due to the different sampling and detection methods used [2, 12]. For instance, according to McKaig et al. [22], the HPV prevalence in HNSCC as detected by PCR was 34.5%, by ISH 15.8%, and by Southern blot 24.5%. According to Gillison [23], ISH assays may have limited sensitivity for some HPV types, and therefore, the validity of the prevalence data is unproven. It has been suggested that conventional PCR-based assays can be hampered in archival smears or paraffin-embedded materials [15, 24]. Therefore, we used SPF PCR primers that permit 100% detection of HPV DNA in paraffin-embedded materials [15, 25].

Based on the results found in our case study and the results found by Dahlstrom et al. [18], Kantola et al. [17] and Dahlgren et al. [19], it seems that HR-HPV is not involved in the development of SCC of the oral tongue. However, to further determine whether a relationship exists between HR-HPV and OSCC in the absence of tobacco and alcohol, further research is necessary. To ease comparability between different studies, it seems recommendable to take into account the type of detection assays and misclassification of anatomical tumour sites.

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