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Human papillomavirus detection in matched oral rinses, oropharyngeal and oral brushings of cancer-free high-risk individuals

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ABSTRACT

Objectives: The detection of oral Human Papillomavirus (HPV) may be of clinical utility because of the major role HPV plays in the etiology of oropharyngeal cancer. However, oral HPV testing is not standardized and the best sampling method has yet to be identified. We aimed to compare HPV findings in matched oral rinse-and-gargles (rinses), oropharyngeal brushings and oral brushings.

Materials and methods: HPV-DNA was investigated using Linear Array in samples collected from cancer-free individuals at increased risk for oral HPV.

Results: 163 oral rinses already tested for HPV were selected. The matched oropharyngeal (n = 163) and oral brushings (n = 100) were analyzed. The detection rate for any HPV, high-risk (HR)-HPVs and HPV16 was significantly higher in rinses than brushings. The overall agreement for any HPV between rinses and oropharyngeal brushings was 51.2% (Cohen K: 0.14, 95% CI: 0.07–0.21). The proportion of positive agreement was 16.8%. The overall agreement for HR-HPVs was 74.1% (Cohen K: 0.20, 95% CI: 0.07–0.33). The genotype-specific profile of rinses and brushings which were concomitantly HPV-positive only partially overlapped in cases with multiple infections, with more genotypes detected in the rinse, which were not isolated in the corresponding brushings.

Conclusion: The agreement for HPV status between rinses and brushings is poor, particularly for the HPV-positive findings. Despite the fact that the origin of the HPV-infected cells present in the oral rinse is unclear, since they could not be traced back to the oropharynx or oral cavity, oral rinses provided the highest detection rate for HR-HPVs and HPV16.

Introduction

There is now strong and sufficient evidence that persistent oral infection by high-risk Human Papillomaviruses (HR-HPVs) can lead to head and neck cancer (HNC), with a main role in oropharyngeal cancer (OPC) [1] and only a minor association with oral cancer [1,2]. Oral infection by HPV16, which is by far the most frequent type in HPV-driven OPC [2,3], is strongly associated with OPC in case-control studies [4] and with a 22-fold increased risk of developing OPC in a prospective study [5]. In recent decades, increasing incidence rates of HPV-associated OPC have been observed, particularly in the USA and Northern European countries [6–9], probably as a consequence of reduced tobacco consumption and modified sexual habits. Oral sex, which plays a key role in acquisition of oral HPV infection, has been shown as a major predictor of oral HPV16 infection [10]. These observations have fostered interest in possible HPV-based tools for screening and/or early detection of HPV-driven OPC. Although the accuracy indicators of HPV detection in oral rinse-and-gargles (hereafter defined as rinses) or swabs do not seem to support its use as a tool for the identification of HPV-driven HNC [11], more research is needed to better investigate the possible clinical utility of an oral HPV test, also for other clinical applications. In fact, evaluation of oral HPV may also provide prognostic indications in post-treatment follow-up of HPV-positive cancer patients [12–15].

In this context, it is important to consider that oral HPV testing is not standardized. The best sampling method has yet to be identified and

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neither are there any tests validated for the evaluation of oral HPV in clinical settings. Oral HPV test performance varies considerably in response to the sampling technique (device, collection medium, protocol), as well as the DNA extraction and purification method [16]. A variety of sampling methods have been used both in healthy controls and cancer patients, such as oral rinses [17–23], swabs [18,24,25], brushings [21,26], as well as a variety of collection media for oral rinses, such as saline [17,25,27], mouthwash [16,18–21], water [23], and media from commercial kit for saliva collection [22]. While it has been extensively proven that oral rinses represent suitable samples for epidemiologic studies since they provide the highest HPV detection rate [28,29], further investigation is necessary to clarify whether or not they have the desirable characteristics for clinical applications. Since the most relevant site of infection from a clinical point of view is represented by the oropharynx, it appears of importance to well distinguish oropharyngeal infections, significant in terms of risk for OPC, from clinically irrelevant oral infections. However, it is not possible to establish the origin of the HPV-positive cells detected in oral rinses, whether they are from the oropharynx or oral cavity. Thus, the clinical significance of the infections detected in oral rinses of cancer-free individuals remains unclear. Interestingly, a study on oral rinses of subjects undergoing tonsillectomy evidenced HPV-positivity in 10.3% of these samples, but none of the resected tonsils was HPV-positive [30]. Clarification of the origin of the infections revealed by testing oral rinses might help cast light on whether this type of specimen is suitable to identify oropharyngeal infections or rather reveals oral infections that are not causally associated with malignant transformation at the oropharynx. A few studies analyzed different types of oral specimens, but they compared the oral rinses only to another type of sample [21,28–30]. In addition, those that investigated samples collected from the general population lacked a sufficient number of HPV-positive cases to estimate reliably the proportion of positive agreement, because of the low prevalence of oral HPV in the study samples [21,30]. In this study, we compared HPV findings in three types of specimens, i.e., oral rinses and site-specific oropharyngeal and oral brushings from cancer-free males with expected high prevalence of oral HPV infection, including HIV-infected individuals. This allowed us to select a large number of HPV-positive cases, and thus to obtain reliable results for the concordance between the different clinical specimens also in terms of positive agreement.

Materials and methods

Case series

Study samples were obtained from men who have sex with men (MSM) attending the STI/HIV centre of the San Gallicano Dermatological Institute (Rome, Italy) and participating in a longitudinal study for the assessment of oral HPV infection (OHMAR study). Criteria for enrollment in the OHMAR study and classification of risk factors have been previously detailed [20]. Briefly, consenting MSM aged ≥18 years with no history of HNC and no clinically evident lesions suspicious for HNC were recruited, irrespective of HIV status. To exclude the presence of clinically evident lesions suspicious for HNC, each MSM underwent a full otolaryngology examination conducted by qualified otolaryngologists with a long-lasting experience in a cancer center. Three types of specimens were collected from each eligible participant, i.e., oral rinse, oropharyngeal brushing and oral brushing. A study case was defined as a set of three matched samples. All the individuals enrolled in the OHMAR study were tested for HPV on the baseline and follow-up oral rinses. For the purposes of the present investigation, a subset of cases were selected from the electronic archive of the OHMAR study and the corresponding oropharyngeal and oral brushings were tested for HPV.

All procedures were performed in accordance with the Helsinki Declaration. The OHMAR study was cleared by the institutional Ethics Committee (CE/417/14). Participants provided written informed consent.

Sample collection

Specimens were collected as follows: (i) a 30 s oral rinse was first obtained using 15 ml of Listerine® mouthwash, and immediately processed as previously described [20]; (ii) a cytobrush was then used to sample the oral cavity (hard palate, gums, front two-thirds of the tongue and floor of the mouth below the tongue); (iii) finally, a second cytobrush was used to sample the oropharynx (soft palate, base of the tongue and tonsils or tonsillar region in cases of tonsillectomy or atrophic tonsils). The cytobrushes were swirled vigorously in two different PreservCyt vials (Hologic, Pomezia, Italy). Brushings were obtained by two otolaryngologists expert in the collection of these samples. PreservCyt samples were stored at 4 °C and tested for HPV within 1 week. A thin-layer slide was also obtained from each sample, and evaluated for the presence of malignant cells as previously described [26].

HPV testing and genotyping

Samples were tested for the presence of HPV-DNA using the Linear Array HPV Genotyping test (Roche Diagnostics, Milan, Italy), which detects 37 mucosal HPVs, and provides genotype-specific information. Total nucleic acids were extracted from 250 µl of each type of specimen following the manufacturer's instructions, and 50 µl of the eluate was used for amplification. Hybridization and detection steps were performed automatically using a Profiblot T48 (Tecan, Männedorf, Switzerland). Results were interpreted as indicated by the producer: samples with presence of one or more HPV hybridization bands, independently of the presence of the β-globin internal control, were considered positive.

Carcinogenic risk was assigned to each genotype as indicated by the International Agency for Research on Cancer [1]: HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68 were considered as HR types.

Data analysis

To compare HPV test results in oral rinses and brushings with a sensitivity of 90%, a specificity of 90%, a precision level of 10% and a desired opportunistic rate of HPV-positivity of 50%, a sample size of 151 cases was calculated as necessary. To obtain the final study group, this estimated sample size was increased by around 5% to take into account the possibility of invalid results for the brushings.

Descriptive statistics were computed for all the variables of interest in order to provide summarized descriptions of the study group. Whenever at least one of the 37 types detectable by the Linear Array was found, the sample was considered as HPV-positive (any HPV). A sample was considered as HR-HPV positive when at least one of the HR types was detected. HPV (any type) and HR-HPV detection rates and corresponding 95% confidence interval (CI) were computed for each type of specimen.

To compare HPV status (negative vs. positive) in the different types of specimens, the overall proportion agreement was computed for each pair of samples: oral rinse vs. oropharyngeal brushing, oral rinse vs. oral brushing, oropharyngeal vs. oral brushing. Additionally, the proportion of positive agreement was calculated (n. of HPV-positive cases in both samples being compared/n. of HPV-positive cases in either sample). Kappa statistics was also used to compare HPV findings in paired samples. Genotyping results for the HPV-positive specimens were also compared and were defined as: concordant when an identical HPV type-specific profile was observed; compatible when the HPV type-specific profile was not identical but one or more genotypes were in common; discordant when the samples compared had none of the genotypes in common. Analyses were performed using MedCalc

Statistical Software version 18.9 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Results

Between November 2014 and February 2018, 310 MSM were enrolled in the OHMAR study. A total of 827 cases (matched oral rinse, oropharyngeal brushing and oral brushing collected at enrollment or during follow-up) were available. Based on the sample-size calculation, 163 cases were selected (69, 42.3%, from HIV-infected MSM). Sixty-nine subjects (42.3%) were current smokers, and 60 (36.8%) referred moderate/heavy alcohol consumption.

Of the 163 study cases, 96 (58.9%) were HPV-positive (any HPV), 50 (30.7%) HR-HPV positive, and 29 (17.8%) positive for > 1 HPV type in the oral rinse. Moreover, 17 of the 163 rinses (10.4%) were positive for HPV16.

All the oropharyngeal brushings corresponding to these 163 oral rinses and a subset of 100 matched oral brushings (61.3%) were evaluated for morphology and tested for HPV (not all oral brushings could be tested for HPV due to funding constraints). In none of the samples did the morphologic evaluation evidence the presence of malignant cells.

One of the 163 oropharyngeal samples (0.6%) gave an invalid HPV test result (no β-globin control and no HPV-specific hybridization bands were detected), whereas all the oral brushings were successfully tested. Excluding the only oropharyngeal specimen with an invalid result, the rate of any HPV detection was 9.9% (95% CI: 5.6–16.0) in the oropharyngeal and 8.0% (95% CI: 3.4–15.8) in the oral brushings. These rates were significantly lower than that in the oral rinses ($p < 0.0001$ in both cases, data not shown). Comparison of HPV findings for any HPV and HR-HPVs of the paired brushings and rinses is shown in Table 1. All the 67 patients with an HPV-negative oral rinse were HPV-negative in the corresponding oropharyngeal brushing (Table 1A). Only 16 of the 95 (16.8%) cases that were HPV-positive in the oral rinse tested HPV-positive in the corresponding oropharyngeal brushing. This was also the proportion of positive agreement. The overall agreement was 51.2%, and Cohen K was 0.14 (95% CI: 0.07–0.21).

All the 44 MSM with an HPV-negative oral rinse were HPV-negative in the corresponding oral brushing, whereas only 8 of the 56 (14.3%) MSM with an HPV-positive oral rinse tested HPV-positive also in the respective oral brushing (Table 1A). The proportion of positive agreement was 14.3%. The overall agreement was 52.0%, and Cohen K was 0.13 (95% CI: 0.04–0.21).

The overall agreement for any HPV between oropharyngeal and oral brushings was 90.0%, with a Cohen K of 0.39 (95% CI: 0.03–0.75) (data not shown).

HR-HPVs were detected less frequently in oropharyngeal brushings (5.5%, 95% CI: 2.5–10.5) compared to rinses (30.2%, 95% CI: 22.4–40.0), $p < 0.0001$ (Table 1B). Similarly, HPV16 was detected in 5/162 oropharyngeal brushings (3.1%, 95% CI: 1.0–7.2) and 16/162 rinses (9.9%, 95% CI: 5.6–16.0), $p = 0.013$ (data not shown). The overall agreement for HR-HPVs between oropharyngeal brushings and oral rinses was 74.1% with a Cohen K of 0.20 (95% CI: 0.07–0.33). Only in one case was the oropharyngeal brushing HR-HPV positive (specifically HPV16-positive) while the matched oral rinse was HR-HPV negative (Table 1B). The overall agreement for HR-HPVs between oral brushings and rinses was 76.0% with a Cohen K of 0.23 (95% CI: 0.06–0.40).

The agreement between brushings and rinses was higher for the HIV-infected subjects than the HIV-uninfected counterparts, both for the infection by any HPV and HR-HPVs. Cohen K varied between 0.14 and 0.34 for the HIV-infected MSM, and between 0.08 and 0.17 for the HIV-uninfected MSM (data not shown). Additionally, the proportion of positive agreement was higher for the HIV-infected than HIV-uninfected individuals, ranging between 20.7 and 26.3% for the former group and between 7.4 and 12.5% for the latter one (data not shown).

The HPV status for the 100 cases that had an HPV test result for all three types of specimens is shown in Table 2. Only in four cases (4.0%) did all the three specimens test positive. The oral rinse and either the oropharyngeal or oral brushing were concurrently positive in six (6.0%) and four cases (4.0%), respectively. In none of the cases, did the oral rinse test HPV-negative while either or both the corresponding brushings were HPV-positive.

The genotype-specific profile was compared for the cases that tested concomitantly positive in the brushing(s) and rinse, as shown in Table 3. No discordant cases were observed. Eight of the 16 cases (50.0%) that were HPV-positive in both the oral rinse and oropharyngeal brushing showed a concordant result. All these cases had a single HPV genotype. In the remaining cases (50.0%), the genotypes detected in the oropharyngeal brushing only partially overlapped those found in the oral rinse (compatible cases, with 31 infections detected in the rinse vs. 18 in the oropharyngeal brushing). Except for two cases (ID 89 and 116), in which the oropharyngeal brushing was positive for HPV genotypes not detected in the oral rinse, in all the other compatible cases, genotypes found in the oral rinse were not detected in the oropharyngeal brushing.

Of the eight cases that were simultaneously positive in the oral rinse and oral brushing, four (50.0%) showed a concordant result (they all harbored single infections) and four (50.0%) showed compatible results. The HPV genotypes that were not in common were detected either in the oral rinse (ID 79, 88 and 113) or in the oral brushing (ID 71 and 113). Overall, the number of infections detected in the compatible cases

Table 1
Comparison of the HPV status for any HPV (A) and HR-HPVs (B) in the oral rinses and corresponding oropharyngeal (n = 162) and oral brushings (n = 100).

	Oral rinse n (%)		
Negative	Positive	Total	
A. Any HPV			
Oropharyngeal brushing			
Negative	67 (41.3)	79 (48.8)	146 (90.1)
Positive	0 (0.0)	16 (9.9)	16 (9.9)
Total	67 (41.3)	95 (58.7)	162 (100.0)
Oral brushing			
Negative	44 (44.0)	48 (48.0)	92 (92.0)
Positive	0 (0.0)	8 (8.0)	8 (8.0)
Total	44 (44.0)	56 (56.0)	100 (100.0)
B. HR-HPVs			
Oropharyngeal brushing			
Negative	112 (69.2)	41 (25.3)	153 (94.5)
Positive	1 (0.6)	8 (4.9)	9 (5.5)
Total	113 (69.8)	49 (30.2)	162 (100.0)
Oral brushing			
Negative	71 (71.0)	24 (24.0)	95 (95.0)
Positive	0 (0.0)	5 (5.0)	5 (5.0)
Total	71 (71.0)	29 (29.0)	100 (100.0)

Table 2
HPV status for any HPV in the 100 cases with a valid HPV test result for the matched oral rinse and site-specific brushings.

Any HPV			
Oral rinse	Oropharyngeal brushing	Oral brushing	n (%)
Neg	Neg	Neg	44 (44.0)
Pos	Pos	Pos	4 (4.0)
Pos	Pos	Neg	6 (6.0)
Pos	Neg	Pos	4 (4.0)
Pos	Neg	Neg	42 (42.0)
Total			100 (100.0)

Table 3
Comparison of the HPV genotype-specific profile in the matched oral rinses, oropharyngeal and oral brushings that simultaneously tested HPV-positive. Genotypes which were detected in all the samples being compared are underlined.

ID	HPV genotypes			Genotype-specific comparison of HPV-positive samples	
	Oral rinse HPV-positive	Oropharyngeal brushing HPV-positive	Oral brushing HPV-positive	Oral rinse vs. oropharyngeal brushing	Oral rinse vs. oral brushing
70	62	62	62	Concordant	Concordant
71	69	69	6, 69	Concordant	*Compatible*
79	11, 16, 18, 59, 81	16, 18, 81	16, 18, 81	*Compatible*	*Compatible*
113	16, 59, 61, CP6108, IS39	16, 61, CP6108, IS39	16, 61, 66, CP6108	*Compatible*	*Compatible*
	HPV-positive	HPV-positive	HPV-negative		
2	6, 51, 72	72	–	*Compatible*	n.a.
22	16	16	–	Concordant	n.a.
34	66	66	–	Concordant	n.a.
109	68	68	–	Concordant	n.a.
112	16, 33, 59, 61, CP6108, IS39	16, 59, CP6108, IS39	–	*Compatible*	n.a.
116	55	16, 55	–	*Compatible*	n.a.
	HPV-positive	HPV-negative	HPV-positive		
36	33	–	33	n.a.	Concordant
88	16, 45, 55, 82	–	16	n.a.	*Compatible*
91	62	–	62	n.a.	Concordant
94	39	–	39	n.a.	Concordant
	HPV-positive	HPV-positive	Not tested		
13	68	68	Not tested	Concordant	n.e.
89	33, 51, 55, 82	45, 82	Not tested	*Compatible*	n.e.
131	16, 53, 70, 72	72	Not tested	*Compatible*	n.e.
135	55	55	Not tested	Concordant	n.e.
139	16, 51, IS39	IS39	Not tested	*Compatible*	n.e.
145	73	73	Not tested	Concordant	n.e.

n.a = not applicable; n.e. = not estimable.

was higher in the oral rinse than in the corresponding oral brushing (15 vs. 10).

Discussion

The best sampling method to evaluate oral HPV infection for possible clinical applications has yet to be identified. Since collection of oral rinses is simple, inexpensive, not invasive, and may be self-performed, these specimens are appealing tools. However, the source of the HPV-DNA detected in these samples remains unclear. In order to investigate whether evaluation of HPV in oral rinses and corresponding site-specific specimens may provide insights in this respect, we compared HPV status and genotype-specific profile of matched oral rinses, oropharyngeal and oral brushings.

Compared to the oral rinses, a significantly lower rate of positivity for any HPV, HR-HPVs and HPV16 was observed both in the oropharyngeal and oral brushings, in agreement with other investigations. Previously, a four-fold lower rate of HPV-positivity was observed in tonsillar brush biopsies compared to oral rinses [28]. Similarly, Combes et al. observed in two studies a three-fold lower positivity in tonsil brushings than oral rinses [21,31]. Our observations are also similar to those obtained on 100 matched oral rinses, oral brushings and tonsil brushings, which showed an HPV-positivity of 39.0%, 13.0% and 12.5%, respectively [17]. In line with the findings by Steinau et al. [17], in all the cases that either or both brushings were HPV-positive, the oral rinse was also positive.

We observed a poor agreement between oral rinses and brushings both for any HPV and HR-HPVs. A similar poor correlation between tonsil brushings and rinses was found by others (K = 0.16 in the study by Kreimer et al. [28]; K = 0.12 in that by Combes et al. [21]). In the French study, a low proportion of positive agreement (9.5%) was also observed [21]. This proportion was higher, but still poor, in our investigation (16.8%). It must be noted, however, that the French study analyzed tonsil brushings, whereas in our study the entire oropharynx was sampled, and this might have increased the proportion of positive agreement. Other studies have shown a higher, but still moderate agreement between oral rinses and oropharyngeal swabs (K = 0.49 in the investigation by Chikandiwa et al. [25], although only three HPV-positive oral rinses were found; K = 0.42 in that by Read et al. [29], although this compared oral rinses with self-collected throat-and-mouth swabs).

Considering the two study groups, the agreement between brushings and rinses for the infection by any HPV was poor both for the HIV-infected and uninfected subjects. However, Cohen K was higher for the former group. As far as concerns the infection by HR-HPVs, a fair agreement was observed for the HIV-infected MSM, whereas the agreement was poor for the HIV-uninfected counterparts. These findings are due to a higher agreement on the positive results for the samples collected from the HIV-infected MSM. This might be explained by a higher viral load in the specimens collected from these subjects compared to those obtained from the HIV-uninfected MSM.

Regarding the HPV type-specific profile in paired oral rinses and brushings that were concurrently HPV-positive, concordant results were only observed for single HPV infections. Whenever multiple genotypes were detected in the oral rinse, these were only partially found in the corresponding brushings. The number of infections was in fact higher in the rinses than in the brushings.

The significantly lower HPV detection rate in the oropharyngeal brushings compared to oral rinses, together with the low positive agreement as well as the lower number of infections detected in the former samples, may be due to several reasons, linked to the features of oropharyngeal infection or technical motives. HPV in tonsillar crypts may be not easily detected in cells brushed from the tonsil surface, although a recent investigation has shown that HPV detection is even lower in frozen tonsillar tissues than in tonsil brushings, i.e., 2.3% vs. 4.1% [31]. Yet, it seems also unlikely that oral rinse testing makes it possible to detect infections established deep within the tonsillar crypts, although in cases of active oropharyngeal infections viral particles may be released in the saliva. It may be also hypothesized that infections detected in the oral rinses are passengers and clinically irrelevant

infections of the oral cavity. However, we observed a low agreement also between rinses and oral brushings, and their positive agreement was even lower than that between rinses and oropharyngeal brushings (14.3% vs. 16.8%). Low viral loads may also decrease the possibility to detect HPV in the brushings, causing false-negative results. It is noteworthy that recent findings suggest that reticulated epithelium of tonsillar crypts, where HPV-driven OPC mostly arise, is not permissive for HPV infection, and for this reason is more prone to transformation than squamous stratified epithelium of the oral cavity and other head and neck subsites [32]. Therefore, transforming oropharyngeal infections likely do not produce high viral titers.

It seems unlikely that the findings outlined above are due to a higher extent of degradation of nucleic acids in the brushings. In fact, these samples were collected in a medium which is optimal for the preservation of nucleic acids. On the contrary, this might have posed a problem for oral rinses which were collected using a mouthwash, since this medium does not preserve nucleic acids, and the samples have to be kept on ice and processed rapidly in order to avoid DNA breaking by saliva enzymes. Our observations might be partly explained by the lower amount of material in the brushing compared to the rinses. Although oropharyngeal brushings were obtained by two otolaryngologists experienced in collecting this type of specimen, oropharynx is not easy to be sampled by brushing and we estimated that the amount of epithelial cells in the brushings was roughly 4 times lower than in the oral rinses (data not shown). Similarly, a previous study estimated that the median number of cell input for HPV analysis was 10-fold lower for the tonsillar brush biopsies than oral rinses [28]. However, we observed no differences in the HPV results obtained when we used for amplification 10 times more input brushing material compared to the described protocol (data not shown).

This study has a few limitations. Firstly, DNA obtained from each sample type was not quantified, thus it is unknown whether the same amount of material was used for amplification. Secondly, the viral load was not estimated and compared between specimens. Nonetheless, this investigation has also several strengths. Case studies were selected from individuals with a high prevalence of oral HPV infection that also provided longitudinal samples, so that a large number of HPV-positive oral rinses were available. By using conservative assumptions for sample-size calculation and by including a relevant number of HPV-positive cases, we were able to obtain reliable estimates also for the positive agreement between the specimens analyzed. In addition, testing of oral brushings allowed us to obtain more comprehensive data to interpret the significance of HPV detection in oral rinses regarding site-specific HPV-positivity. Differently, in previous studies either only two types of specimens were compared [21,28], or oral HPV prevalence in the analyzed populations was too low to evaluate with precision the agreement for positivity [17,21]. Case selection from a population with high prevalence of multiple HPV infections and possibly high viral load, being that also HIV-infected individuals were included, increased the likelihood to observe differences between sampling methods, avoiding the risk of misclassification of samples as false-negative due to low or fluctuating viral loads. Importantly, the Linear Array that was used to analyze in parallel all the specimens is a very robust and sensitive assay, which detects more multiple infections than other HPV-DNA tests [33–35].

Conclusion

HPV detection rate in both oropharyngeal and oral brushings was lower than in matched oral rinses. We also observed a poor agreement between brushing and oral rinse HPV status. The agreement was particularly poor for the HPV-positive findings. More HPV types were detected in the oral rinse, which were not isolated in the corresponding brushings. Although the high HPV detection rate in oral rinses makes this type of specimen suitable for epidemiologic studies, the origin of HPV infections revealed in oral rinses remains unclear, since they could not be traced back to the oropharynx or oral cavity in this study. The clinical utility of HPV-DNA detection in oral rinses to identify subjects at risk for OPC needs to be further explored. Evaluation of HR-HPV mRNA in oral rinses should be taken into account, as it might make it possible to identify active, transforming, thus clinically significant infections.

Conflict of interest

None declared.

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Appendix A. Supplementary material

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