Short Report

No role for human papillomavirus infection in oral cancers in a region in Southern India

Claudie Laprise[1,2], Sreenath Arekunnath Mathadil[2,6], Paul Allison[1,2], Priya Abraham[3], Anantharam Raghavendran[3], Shahul Hameed Kumamangalam Puthiyannal[2], Akhil Soman ThekkePurakkal[2], Geneviève Castonguay[2], François Coutlée[4], Nicolas F. Schlecht[5], Marie-Claude Rousseau[6,2], Eduardo L. Franco[1,2], Belinda Nicolau[1,2]

[1]Department of Oncology, Division of Cancer Epidemiology, McGill University, Montreal, Quebec, Canada; [2]Oral Health and Society Division, Faculty of Dentistry, McGill University, Montreal, Canada; [3]Department of Clinical Virology, Christian Medical College, Vellore, India; [4]Department of Microbiology and Infectious Diseases, Hôpital Notre-Dame du Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada; [5]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York, United States of America; [6]Epidemiology and Biostatistics Unit, INRS-Institut Armand-Frappier, Laval, Quebec, Canada

Corresponding author: Dr. Belinda Nicolau

Division of Oral Health and Society - Faculty of Dentistry - McGill University

2001 McGill College, suite 527

Montréal, QC, Canada H3A 1G1

Tel: 514-3987203 ext. 094655 Fax: 514-3987220

Email: belinda.nicolau@mcgill.ca

Short title: HPV in oral cancer in India

Keywords: Human papillomavirus, HPV, India, oral cancer, oral squamous cell carcinoma.

Abbreviations used: CI, Confidence interval; DNA, Deoxyribonucleic acid; H&E, hematoxylin and eosin; HNC, Head and neck cancer; HPV, human papillomavirus; ICD, International Classification Diseases; OR, Odds ratio; PCR, Polymerase chain reaction; TNM, Tumor Node Metastasis.

Abstract word count: 248

Manuscript word count: 2514

Reference count: 33

Table count: 2

Novelty and Impact Statements: No oral human papillomavirus infection was detected among 350 oral cancer and 371 control subjects from Kerala, India. In addition, we did not find an association between sexual behavior and oral cancer. These do not appear to be major risk factors for oral cancer in South India.

ABSTRACT

Oral cancer is a major public health issue in India with approximately 77,000 new cases and 52,000 deaths yearly. Paan chewing, tobacco and alcohol use are strong risk factors for this cancer in India. Human papillomaviruses (HPV) are also related to a subset of head and neck cancers. We examined the association between oral HPV and oral cancer in a sample of Indian subjects participating in a hospital-based case-control study. We recruited incident oral cancer cases (N=350) and controls frequency-matched by age and sex (N=371) from two main referral hospitals in Kerala, South India. Socio-demographic and behavioral data were collected by interviews. Epithelial cells were sampled using Oral CDx® brushes from the oral cancer site and the normal mucosa. Detection and genotyping of 36 HPV genotypes were done using a polymerase chain reaction protocol. Data collection procedures were performed by qualified dentists via a detailed protocol with strict quality control, including independent HPV testing in India and Canada. HPV DNA was detected in none of the cases or controls. Associations between oral cancer and risk factors usually associated with HPV infection, such as oral sex and number of lifetime sexual partners, were examined by logistic regression and were not associated with oral cancer. Lack of a role for HPV infection in this study may reflect cultural or religious characteristics specific to this region in India that are not conducive to oral HPV transmission. A nationwide representative prevalence study is needed to investigate HPV prevalence variability among Indian regions.

INTRODUCTION

Worldwide, nearly 300,000 new cases of oral cancers are diagnosed each year, accounting for 145,000 deaths. Approximately 77,000 new cases and 52,000 oral cancer-related deaths occur yearly in India, representing a quarter of all incident cases in the world.[1] Known risk factors include tobacco smoking and chewing, high alcohol consumption and in a country such as India, paan chewing.[2] Oral human papillomavirus (HPV) infection has recently been recognized as another important risk factor for head and neck cancer (HNC), mainly for the oropharyngeal cancer sub-site. In fact, HPV has changed HNC epidemiology and some authors describe the rising incidence of HPV-related HNC as an epidemic.[3, 4] Transmission of the virus, which occurs through oro-genital contact or oral-oral contact, and other sexual behavior[5-7], may explain the presence of HPV in the oral cavity and its possible relation with HNC.

Although HPV has been associated particularly with the oropharyngeal sub-site, it is also involved in other oral cancers.[8, 9] In a recent systematic review of 72 studies of oral cavity cancers worldwide, HPV prevalence was found to be 24.2% on average, compared to 45.8% in the oropharynx, with HPV-16 being present in 14.9% of the oral cavity cancer cases.[10] This review also indicated that 16.3% of oral cavity cancers may be attributable to HPV infection.[10]

Oral cancer is a major health issue in India and the possible role of HPV in the aetiology of this disease needs to be addressed. This is especially important in the new era of HPV vaccine availability. We therefore studied the association between oral HPV infection and oral cancers in Kerala, India.

MATERIAL AND METHODS

Patient and data collection

We conducted a hospital-based case-control study from September 2008 to October 2012 at two major teaching hospitals in Kerala, South India. All participants provided a signed informed consent, and the study protocol was approved by the Research Ethics Offices at the Government Dental and Medical Colleges, India, and McGill University, Montreal, Canada.

Cases were patients with newly diagnosed, histologically confirmed oral squamous cell carcinoma, which included malignant neoplasms of the oral cavity and tonsils based on the International Classification of Diseases (ICD)-10: codes C00.3-C06.9 and C09.[11] A total of 409 eligible cases were identified, out of which 350 (85.6%) participated in the study. Cancer site and TNM stage (I to IV) were classified according to the Union for International Cancer Control classification of malignant tumors.

Control subjects, frequency matched by age (5-year categories) and sex, were selected from 8 outpatient clinics in the same hospitals as the cases from a list of diseases unrelated to tobacco or alcohol consumption. The general eligibility criteria were to: (i) be born in India; (ii) be at least 18 years old; (iii) have no history of cancer, immunosuppressive conditions, or mental disorders; and (iv) live within 150 km of the hospital area. A total of 371 controls out of 837 eligible patients agreed to take part in the study (44.3%). The distribution of the main socio-demographic characteristics in the control sample was similar to the results of a recent survey from the source population (data not shown)[12].

One-on-one semi-structured interviews using a questionnaire and life grid technique, which has been shown to improve recall,[13] were conducted by three professionally trained dentists. Data collected included socio-demographic information, religious beliefs, environmental variables and

sexual behavior including age at first sexual intercourse, number of sexual partners and oral sex practice frequency during three different life stages (≤16 years, 17-30 years and > 30 years).

Oral cell sample collection

The dentists collected biological specimens for HPV detection and genotyping from both cases and controls. Oral CDx® brushes (CDx Diagnostics, USA) were used to collect epithelial cells from various sites in the oral cavity and, for the cases, from the tumor site as well. The patients were seated in a dental chair and the brushes were firmly pressed against the lesion or normal mucosa and rotated until pinpoint bleeding was observed. This procedure ensured that a complete transepithelial brush biopsy specimen was collected. The brushes were then carefully inserted into a PreservCyt® buffer bottle (Hologic, USA) and twirled vigorously to release the exfoliated cells into the solution. The samples were kept in a refrigerator and shipped monthly to our laboratory in Vellore, India, where they were stored at -20 °C until time of testing.

DNA was extracted according to the manufacturers' protocol for the Master Pure™ DNA purification kit (Epicentre, USA). DNA was pelleted by centrifuging the tubes at 14,000 x g for 10 minutes, air dryed for 15 minutes, and then resuspended in 35 µl of TE buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA) as per manufacturer's instructions. Purified DNA was then stored at -70°C until the polymerase chain reaction (PCR) testing.

HPV DNA detection and genotyping

HPV detection and genotyping was done using Linear Array (Roche Molecular Diagnostics). Presence of the human β-globin gene was assessed in 10 µl of extracted DNA for each sample by performing PCR using PC04 and GH20 and subsequent agarose gel electrophoresis, to check for DNA integrity, absence of inhibitors, and to ensure that there was sufficient cellularity for PCR

analysis. β-globin-negative specimens were considered inadequate for analysis. β-globin-positive DNA extracts were amplified with PGMY09-PGMY11 primers for HPV. Typing was done by reverse hybridization by using biotin-labelled probes for the following HPV genotypes: 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89. The co-amplification of β-globin DNA sequence was also done to ensure sample adequacy and cellularity. Because we did not detect HPV in the first 350 participants recruited for the study, we carried out a histopathological analysis of brush biopsy specimens on a randomly chosen 10% of participants (n=70) using hematoxylin and eosin (H&E) staining in the Oral Pathology Department at the Government Dental College. The results of this analysis confirmed that the Oral CDx® brush sampling method provided sufficient cellular material.

Statistical analysis

Univariate and multivariate unconditional logistic regression was used to calculate odds ratios (OR) and respective 95% confidence intervals (CIs) for the associations between HPV status and oral cancer. Practice of oral sex and lifetime number of sexual partners were also evaluated for a possible association with oral cancer using the same analytical method. Because of the different oral cancer risk profiles between men and women, we fitted models stratified by sex. Potential empirical confounders were examined from several socio-demographic, behavior, and oral health behavior variables including age, sex, religion, lifetime years of education, bidi smoking, cigarette smoking, paan chewing and alcohol drinking. In each model, potential confounders were kept if their addition to the models changed the ORs for the independent variables by 10 percent or more. Stata 13.1 (Stata Corp., USA) was used for statistical analysis.

Canada-India independent HPV testing and genotyping

As part of the study quality control procedures, we carried out blinded independent HPV and β-globin testing in the Indian and Canadian laboratories. Forty randomly selected blinded samples were sent to Montreal for re-testing in our collaborating laboratory (FC). In addition, 16 blinded samples with known results for HPV DNA positivity were sent from the Montreal laboratory to the Indian laboratory (PA). HPV detection and genotyping were performed by each laboratory using the procedures described above for the main study. Both laboratories also conducted amplification of β-globin to assess sample quality. The percentage of observed agreement between laboratories and Cohen's kappa coefficient were calculated for HPV positivity and for HPV accounting for β-globin positivity.

RESULTS

Baseline characteristics of study participants are shown in Table 1. There was a slight preponderance of men in the study sample: 56% and 55% of cases and controls, respectively. The mean age was 60.3 years for male cases and 61.4 years for female cases. The majority of the participants were Hindu with 62.2% and 69.5% in men and women, respectively. Cases had lower levels of educational attainment than controls and higher mean cumulative histories of paan chewing. Irrespective of sex, the mean pack-years of cigarette smoking was somewhat lower for cases relative to controls. Alcohol consumption levels were higher in male cases than controls, but did not differ between female cases and controls. More than ninety-five percent of females participating in the study never smoked or drank alcohol. Among female cases, a lower proportion of cases (16.2%) compared to controls never chewed (80.2%). Among male subjects, a high proportion of controls never smoked bidi, drank alcohol and chewed paan (29.4%). Cases, however, were more likely to have never smoked cigarettes (47.5%) compared to controls

(38.2%). The vast majority of cases and controls had 0 or 1 lifetime sexual partner (89.9% of study sample) and about half of all participants had ever practiced oral sex on their partner(s).Thirty-nine percent of the cancers were in the buccal mucosa and a majority of cancers were classified as TNM stage III or IV at diagnosis.

HPV-DNA was detected in none of the cases or controls. Independent HPV testing and genotyping was carried out in Canada and India to determine if the lack of HPV DNA in Indian subjects represented false-negative results due to technical artefacts in HPV testing. Every one of the random samples chosen to check sample adequacy showed nuclear material under H&E staining, indicating adequacy of specimens. The percentage of observed agreement between the two laboratories was 100% for HPV positivity when restricting to beta-globin positive specimens. However, 17.5% of the samples shipped from India (7 out of 40) were beta-globin negative in Canada laboratory only. Thus, our agreement among all the samples was 87.5%, with a Cohen's Kappa of 0.71 (95% CI: 0.54-0.86).

Table 2 shows the ORs for the association between sexual behavior and oral cancer, stratified by sex and for all participants. Sexual behavior usually associated with HPV infection, such as increased number of sexual partners or practice of oral sex, was not significantly associated with oral cancer risk in our study.

DISCUSSION

We investigated the association between HPV and oral cancer in a case-control study in South India. The absence of HPV DNA in all oral cancer cases and controls implies that HPV infection is not an influential risk factor on the burden of oral cancer in this study population from Kerala,

South India. We conducted complementary analyses to confirm our results by validating the methodology on-site, and organized HPV validation testing and genotyping in the context of an India-Canada collaboration. The Linear Array is among the most common in molecular epidemiologic studies of HPV-associated cancers. The independent India-Canada inter-laboratory comparison of HPV testing performance yielded high agreement between results in samples positive and negative for HPV and/or beta-globin. In addition, the Indian laboratory had participated and was judged proficient in an international HPV DNA proficiency study led by the World Health Organization. In light of the established proficiency of the laboratory, we raised the possibility that a problem could have occurred at the sample collection, transportation or storage stages. In fact, there was a slightly disagreement between the Indian and Canadian laboratories on beta-globin detection. However, we could not identify any flaw in our protocol or its application that could explain the results obtained. Data collection in this multicenter international study followed a comprehensive quality control protocol including: (i) a detailed study protocol was followed; (ii) qualified dentists collected the samples in a dental surgery clinic; (iii) the international principal investigator (PI, BN) monitored the study by visiting the sites several times.

We reviewed studies on HPV detection and genotyping conducted in India from 1995 to 2013 to better understand the prevalence of HPV considering the use of different sample collection and HPV testing methodologies. The reported HPV prevalence in oral cancers in India varied from 0% to 100% in studies using biopsies in cases and PCR assay for HPV detection and genotyping.[14-30] For the studies using a methodology similar to ours (oral brushing for collecting epithelial cells instead of biopsy, combined with PCR for detection and genotyping), the prevalence reported for oral cancer cases were 31.5%[26] and 32.4%.[15] Our results are in agreement

with two studies conducted in Gujarat and Mumbai in West India, which reported a null prevalence of HPV in oral cancer using biopsies (in one of the studies, only HPV-16 and 18 were tested).[27, 28] However, considering the fact that only two previous studies showed a null prevalence in oral cancer in India, we explored other explanations for these results.

As complementary analyses, we decided to further investigate risk factors such as oral sex and lifetime number of sexual partners, known to be associated with oral HPV infection.[5, 31, 32] The population characteristics show that 46.6% of all the participants had ever had oral sex but at the same time, the proportion of cases and controls that reported having one lifetime sexual partner is 89.9%, suggesting that risky sexual behavior is low. A relatively high proportion of subjects had missing values for oral sex, and this proportion was somewhat higher among the cases. However, adjusted estimates of associations for subjects who did not provide this information (prefer not to say/don't know) as compared to those who reported not having performed oral sex did not suggest an increased risk of oral cancer in this subgroup. The acquisition of oral HPV infection can be limited by safer sexual behavior that may stem from religious norms, which would help explain our null results for HPV infection in both cases and controls.

133

One important risk factor for oral cancer identified in India is paan chewing.[2] Paan is a colloquial name of a mixture composed of betel leaf, areca nut, slaked lime (paste of calcium hydroxide) with or without tobacco. According to the International Agency for Research on Cancer, paan chewing, independent of the presence of tobacco, is carcinogenic to humans.[34] In addition, it is well known that paan chewing also causes leukoplakia of the buccal mucosa. Hence it is conceivable that using an oral brush biopsy instead of a standard biopsy could have influenced the HPV detection results. However, our histopathological validation of the presence of nuclear

material confirmed that the brush biopsy indeed collected tumor cells. Moreover, if the lack of oral HPV was simply related to paan chewing, we would presumably have detected some HPV in the exfoliated cells of controls, since they are less exposed to paan chewing in this study (72.3% of cases vs. 17.5% of controls reported ever chewing paan).

In summary, our results seem to indicate that HPV is not a major risk factor for the occurrence of oral cancers in a sample of subjects living in Kerala, India. The gradual westernization of India, which begun in the last few decades, may eventually change the epidemiological profile of oral cancer in this region to a more prominent role of HPV infection in explaining the burden of disease. On the other hand, should sexual mores remain what they are today, HPV vaccination would not have an appreciable impact in reducing the burden of oral cancer incidence in this region of India.

ACKNOWLEDGMENTS

We would like to thank our collaborators at the Indian site I.V. Varghese, Shameena Shiraz and the HeNCe Life Study team. *Funding:* This work was supported by the Canadian Institutes of Health Research [MOP 81172, MOP111207]; Ministère du Développement économique, de l'Innovation et de l'Exportation du Québec: Programme de soutien à la recherche (PSR), volet: Soutien à des initiatives internationales de recherche et d'innovation (SIIRI). B. Nicolau holds a Canada Research Chair in Life Course Oral Epidemiology. C. Laprise received a postdoctoral fellowship from the Fonds de Recherche Santé-Québec-Network for Oral Bone Health Research and Canadian Health Research Institutes (FRN:135506). S. Madathil is a recipient of a scholarship from the Fondation Universitaire Armand-Frappier de l'INRS and a Psychosocial Oncology Research Training (PORT) top-up award.

FOOTNOTE

EL. Franco has no conflicts of interest but wishes to disclose that he has served as occasional consultant to companies that make products related to HPV vaccination (Merck, GSK) and HPV diagnostics (BD, Roche). His university has received unconditional grants from Merck and Roche to assist projects initiated by his unit. Other authors have no conflicts of interest to declare.

REFERENCES

1. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr, accessed on 26/06/2015. 2013.

2. Gupta B, Ariyawardana A, Johnson NW. Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. *International dental journal* 2013;**63**: 12-25.

3. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *The lancet oncology* 2010;**11**: 781-9.

4. Friedman JM, Stavas MJ, Cmelak AJ. Clinical and scientific impact of human papillomavirus on head and neck cancer. *World journal of clinical oncology* 2014;**5**: 781-91.

5. D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *The Journal of infectious diseases* 2009;**199**: 1263-9.

6. Kreimer AR, Villa A, Nyitray AG, Abrahamsen M, Papenfuss M, Smith D, Hildesheim A, Villa LL, Lazcano-Ponce E, Giuliano AR. The epidemiology of oral HPV infection among a multinational sample of healthy men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011;**20**: 172-82.

7. Dahlstrom KR, Burchell AN, Ramanakumar AV, Rodrigues A, Tellier PP, Hanley J, Coutlee F, Franco EL. Sexual transmission of oral human papillomavirus infection among men. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2014;**23**: 2959-64.

8. Chaturvedi AK. Epidemiology and clinical aspects of HPV in head and neck cancers. *Head and neck pathology* 2012;**6 Suppl 1**: S16-24.

9. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernandez L, Idris A, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *Journal of the National Cancer Institute* 2003;**95**: 1772-83.

10. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsague X, Laporte L, Bosch FX, de Sanjose S, Trottier H. HPV DNA, E6/E7 mRNA, and p16(INK4a) detection in head and neck cancers: a systematic review and meta-analysis. *The lancet oncology* 2014;**15**: 1319-31.

11. WHO. World Healh Organization. Malignant neoplasms of lip, oral cavity and pharynx (C00-C14) ICD-10. Available at http://apps.who.int/classifications/icd10/browse/2010/en#/C00-C14, accessed on 10/12/2014. 2010.

12. IIPS. International Institute for population sciences (2010). Global Adult Tobacco Survey India 2009–2010. Available at: http://www.who.int/tobacco/surveillance/survey/gats/gats_india_report.pdf. Accessed on February 27, 2015. 2010.

13. Blane DB. Collecting retrospective data: development of a reliable method and a pilot study of its use. *Soc Sci Med* 1996;**42**: 751-7.

14. Balaram P, Nalinakumari KR, Abraham E, Balan A, Hareendran NK, Bernard HU, Chan SY. Human papillomaviruses in 91 oral cancers from Indian betel quid chewers--high prevalence and multiplicity of infections. *International journal of cancer Journal international du cancer* 1995;**61**: 450-4.

15. Chaudhary AK, Pandya S, Mehrotra R, Bharti AC, Singh M, Singh M. Comparative study between the Hybrid Capture II test and PCR based assay for the detection of human papillomavirus DNA in oral submucous fibrosis and oral squamous cell carcinoma. *Virology journal* 2010;**7**: 253.

16. D'Costa J, Saranath D, Dedhia P, Sanghvi V, Mehta AR. Detection of HPV-16 genome in human oral cancers and potentially malignant lesions from India. *Oral oncology* 1998;**34**: 413-20.

17. Elango KJ, Suresh A, Erode EM, Subhadradevi L, Ravindran HK, Iyer SK, Iyer SK, Kuriakose MA. Role of human papilloma virus in oral tongue squamous cell carcinoma. *Asian Pacific journal of cancer prevention : APJCP* 2011;**12**: 889-96.

18. Gheit T, Vaccarella S, Schmitt M, Pawlita M, Franceschi S, Sankaranarayanan R, Sylla BS, Tommasino M, Gangane N. Prevalence of human papillomavirus types in cervical and oral cancers in central India. *Vaccine* 2009;**27**: 636-9.

19. Jalouli J, Ibrahim SO, Mehrotra R, Jalouli MM, Sapkota D, Larsson PA, Hirsch JM. Prevalence of viral (HPV, EBV, HSV) infections in oral submucous fibrosis and oral cancer from India. *Acta oto-laryngologica* 2010;**130**: 1306-11.

20. Jalouli J, Jalouli MM, Sapkota D, Ibrahim SO, Larsson PA, Sand L. Human papilloma virus, herpes simplex virus and epstein barr virus in oral squamous cell carcinoma from eight different countries. *Anticancer research* 2012;**32**: 571-80.

21. Koppikar P, deVilliers EM, Mulherkar R. Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. *International journal of cancer Journal international du cancer* 2005;**113**: 946-50.

22. Mishra A, Bharti AC, Varghese P, Saluja D, Das BC. Differential expression and activation of NF-kappaB family proteins during oral carcinogenesis: Role of high risk human papillomavirus infection. *International journal of cancer Journal international du cancer* 2006;**119**: 2840-50.

23. Mitra S, Banerjee S, Misra C, Singh RK, Roy A, Sengupta A, Panda CK, Roychoudhury S. Interplay between human papilloma virus infection and p53 gene

alterations in head and neck squamous cell carcinoma of an Indian patient population. *Journal of clinical pathology* 2007;**60**: 1040-7.

24. Mondal R, Ghosh SK, Choudhury JH, Seram A, Sinha K, Hussain M, Laskar RS, Rabha B, Dey P, Ganguli S, Nathchoudhury M, Talukdar FR, et al. Mitochondrial DNA copy number and risk of oral cancer: a report from Northeast India. *PloS one* 2013;**8**: e57771.

25. Nagpal JK, Patnaik S, Das BR. Prevalence of high-risk human papilloma virus types and its association with P53 codon 72 polymorphism in tobacco addicted oral squamous cell carcinoma (OSCC) patients of Eastern India. *International journal of cancer Journal international du cancer* 2002;**97**: 649-53.

26. Pal D, Banerjee S, Indra D, Mandal S, Dum A, Bhowmik A, Panda CK, Das S. Influence of regular black tea consumption on tobacco associated DNA damage and HPV prevalence in human oral mucosa. *Asian Pacific journal of cancer prevention : APJCP* 2007;**8**: 263-6.

27. Patel KR, Vajaria BN, Begum R, Desai A, Patel JB, Shah FD, Shukla SN, Patel PS. Prevalence of high-risk human papillomavirus type 16 and 18 in oral and cervical cancers in population from Gujarat, West India. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 2013.

28. Pathare SM, Gerstung M, Beerenwinkel N, Schaffer AA, Kannan S, Pai P, Pathak KA, Borges AM, Mahimkar MB. Clinicopathological and prognostic implications of genetic alterations in oral cancers. *Oncology letters* 2011;**2**: 445-51.

29. Kulkarni SS, Kulkarni SS, Vastrad PP, Kulkarni BB, Markande AR, Kadakol GS, Hiremath SV, Kaliwal S, Patil BR, Gai PB. Prevalence and distribution of high risk human papillomavirus (HPV) Types 16 and 18 in Carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. *Asian Pacific journal of cancer prevention : APJCP* 2011;**12**: 645-8.

30. Saranath D, Tandle AT, Teni TR, Dedhia PM, Borges AM, Parikh D, Sanghavi V, Mehta AR. p53 inactivation in chewing tobacco-induced oral cancers and leukoplakias from India. *Oral oncology* 1999;**35**: 242-50.

31. Edelstein ZR, Schwartz SM, Hawes S, Hughes JP, Feng Q, Stern ME, O'Reilly S, Lee SK, Fu Xi L, Koutsky LA. Rates and determinants of oral human papillomavirus infection in young men. *Sexually transmitted diseases* 2012;**39**: 860-7.

32. Cook RL, Thompson EL, Kelso NE, Friary J, Hosford J, Barkley P, Dodd VJ, Abrahamsen M, Ajinkya S, Obesso PD, Rashid MH, Giuliano AR. Sexual behaviors and other risk factors for oral human papillomavirus infections in young women. *Sexually transmitted diseases* 2014;**41**: 486-92.

33. Bhatla N, Lal N, Bao YP, Ng T, Qiao YL. A meta-analysis of human papillomavirus type-distribution in women from South Asia: implications for vaccination. *Vaccine* 2008;**26**: 2811-7.

34. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans; v.85. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. 2003.

Table 1. Socio-demographic, lifestyle, and tumor characteristics of study participants, HeNCe Life study Kerala site, India

Variables	Male				Female			
	Cases (N=196)		Controls (N=204)		Cases (N=154)		Controls (N=167)	
	N	%	N	%	N	%	N	%
Age								
Mean (sd)	60.3 (10.7)		60.2 (11.3)		61.4 (12.0)		60.9 (12.1)	
Religion								
Hindu	122	62.2	118	57.8	107	69.5	112	67.1
Muslim	60	30.6	78	38.2	46	29.9	52	31.1
Christian	14	7.1	8	3.9	1	0.7	3	1.8
Educational level								
Low	126	64.3	91	44.6	141	91.6	91	54.5
High	70	35.7	113	55.4	13	8.4	76	45.5
Smoking bidi								
Mean pack/year (sd)	15.1 (20.5)		8.4 (19.8)		0.10 (1.0)		0.09 (0.9)	
Smoking cigarette								
Mean pack/year (sd)	11.7 (21.6)		18.3 (31.6)		0.02 (0.2)		0.7 (9.0)	
Alcohol drinking								
Mean drink/week (sd)	12.0 (44.9)		6.0 (50.3)		0.05 (0.6)		0.1 (0.8)	
Paan chewing								
Mean cumulative chew-year (sd)	157.6 (200.1)		21.6 (81.2)		238.8 (197.8)		52.7 (145.9)	
HPV status								
% positivity	0		0		0		0	
Lifetime number of sexual partners								
0 or 1	158	80.6	178	87.3	150	97.4	162	97.0
2 or more	36	18.4	26	12.7	3	2.0	4	2.4
PNTS/DK	2	1.0	0	0	1	0.7	1	0.6
Ever had oral sex								
No	66	33.7	74	36.3	43	27.9	47	28.1
Yes	97	49.5	106	51.9	49	31.8	84	50.3
PNTS/DK	33	16.8	24	11.8	62	40.3	36	21.6
Tumor site (ICD-10 code)								
Tongue (C02)	42	21.4			20	13.0		
Gum (C03)	45	23.0			56	36.4		
Floor of the mouth (C04)	16	8.2			2	1.3		
Palate (C05)	8	4.1			7	4.6		
Buccal mucosa (C06)	69	35.2			65	42.2		
Tonsils (C09)	6	3.1			0	0		
Other[a]	10	5.1			4	2.6		
Global TNM stage								
Stage I	19	9.7			15	9.7		
Stage II	16	8.2			19	12.3		
Stage III	102	52.0			71	46.1		

Stage IV	59	30.1	49	31.8

*Abbreviations: Sd, standard deviation; PNTS/DK, prefer not say/don't know.
[a] Oral cancer lesions that span more than one anatomical subsite.

Table 2. Odds ratios (OR) for the associations of sexual behavior indicators and oral cancer, HeNCe Life study, Kerala site, India

	OR (95% CI)					
	Male		Female		All	
	Crude	Multivariate	Crude	Multivariate	Crude	Multivariate
Oral sex						
Never	1.00	1.00	1.00	1.00	1.00	1.00
Ever	1.03 (0.67-1.58)	1.29[a] (0.75-2.24)	0.64 (0.37-1.10)	0.64[c] (0.30-1.35)	0.85 (0.61-1.20)	1.01[e] (0.65-1.55)
PNTS/DK	1.54 (0.83-2.87)	0.93[a] (0.43-2.03)	1.88 (1.05-3.37)	1.20[c] (0.55-2.64)	1.76 (1.16-2.66)	1.24[e] (0.73-2.09)
Lifetime number of sexual partners						
0-1	1.00	1.00	1.00	1.00	1.00	1.00
2 or more	1.56 (0.90-2.70)	1.12[b] (0.56-2.22)	0.81 (0.18-3.68)	0.45[d] (0.06-3.31)	1.44 (0.87-2.37)	0.97[f] (0.48-1.92)
PNTS/DK	-	-	1.08 (0.07-17.4)	0.17[d] (0.01-2.89)	3.31 (0.34-32.0)	0.55[f] (0.05-5.83)

*Abbreviations: PNTS/DK, prefer not to say/don't know.
[a] Adjusted for age, smoking bidi and paan chewing (196 cases and 204 controls).
[b] Adjusted for smoking bidi, paan chewing and alcohol drinking (196 cases and 204 controls).
[c] Adjusted for age, educational level and paan chewing (154 cases and 167 controls).
[d] Adjusted for oral sex, educational level and paan chewing (154 cases and 167 controls).
[e] Adjusted for age, educational level, paan chewing and alcohol drinking (350 cases and 371 controls).
[f] Adjusted for oral sex, educational level, smoking bidi, smoking cigarette, paan chewing, alcohol drinking (350 cases and 371 controls).