

Oral Human Papillomavirus Infection among Smokeless Tobacco-using Tribal Women in Mysuru, India

Kavitha Ravi¹, Tanvi Kaur¹, Anisa S. Khan¹, Benjamin Pope², Kim Y. Nguyen³, Kiranmayee Muralidhar¹, Karl Krupp^{1,2}, Brenda Y. Hernandez³, Angela Sy³, Purnima Madhivanan^{1,2*}, Vivek R. Nerurkar^{3*}

¹Public Health Research Institute of India, Mysuru, India, ²Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, ³John A. Burns School of Medicine, University of Hawaii, Honolulu, USA

*Joint last authors

Abstract

Smokeless tobacco (SLT) is consumed by more than 300 million people worldwide. Studies show high use among Indian indigenous women who are also at high risk for oral cancers. Both human papillomavirus infection (HPV) and SLT have been associated with oral cancer, this study examined the presence of high-risk HPV in oral samples collected from tribal smokeless tobacco users in Mysuru, India. Between June and August 2019, 100 tribal females (50 SLT-users and 50 non-users) from rural Mysuru District, Karnataka, were enrolled in a cross-sectional study. Following informed consent, demographic data and oral samples were collected and processed using a digene HC2 High-Risk HPV DNA test (Qiagen, USA). On average participants were 45.5 (SD: ±6.6) years. Chronic SLT users were mostly married (73%), Hindu (100%), illiterate (62%), and employed (90%). One woman was positive for high-risk HPV infection. Oral HPV infection was low in this sample and this is consistent with the literature from other low and middle-income countries. SLT use is high in this group so interventions to reduce tobacco use are warranted.

Keywords: HPV, human papillomavirus, indigenous peoples, oral cancer, smokeless, tobacco, women

INTRODUCTION

About 300 million people use smokeless tobacco (SLT) worldwide, and about 80% of those reside in India and Bangladesh.^[1,2] SLT is consumed in many forms that contain cured, unburnt, finely ground, or loose tobacco leaves with nicotine alkaloids, tobacco-specific nitrosamines (TSNAs), toxic metals, and other compounds and are ingested singly or in combination with a variety of flavorings and non-tobacco ingredients that are chewed, sucked, gargled, or inhaled.^[3,4] In India, the most popular form of SLT is called *Betel Quid* and contains ground tobacco, areca nut, lime, and catechu rolled in a betel leaf. Other popular forms include *Khaini*, a combination of tobacco and lime, and *Mishri*, which is roasted and powdered tobacco with molasses, clove oil, menthol, and camphor.^[5]

SLT use has been associated with both incident oral [OR: 1.48-27.4] and esophageal cancers [OR 2.06-12.8] and elevated cancer-related mortality.^[6] Moreover, SLT-use-related risk of

oral cancers has been found higher among women using chewed smokeless products compared to men (RRR, 1.75; 95%CI, 1.15–2.66; $P = 0.008$).^[7] In India, indigenous populations, classified as scheduled tribes (STs), have been found to consume high levels of SLT products, particularly loose-leaf tobacco.^[8] Surveys suggest that tobacco consumption of all kinds is significantly associated with poverty, low education, and being a member of a scheduled caste or ST population.^[9] A study in Karnataka has shown that tribal women have a

Address for correspondence: Dr. Purnima Madhivanan, Department of Health Promotion Sciences, Mel and Enid Zuckerman College of Public Health, University of Arizona, 1295 N Martin Avenue, PO Box 245209, Tucson, AZ 85724-5209, USA. E-mail: pmadhivanan@email.arizona.edu

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high prevalence of leukoplakia, lesions often associated with SLT use, and the development of malignant oral cancers.^[10] Another study in Kerala has observed the high prevalence of various tobacco habits and its related oral mucosal lesions like leukoplakia, oral submucous fibrosis (OSMF), and oral squamous cell carcinoma (OSCC) among the tribes of Kerala.^[11]

Some research suggests that one of the mechanisms for increased cancer risk may be an increased prevalence of oncogenic HPV infection among tobacco users. For instance, studies have reported specific viruses including Epstein–Barr virus (EBV) and human papillomavirus (HPV) appear to play a role; this has yet to be elucidated in the pathogenesis of oral and nasal cancers in the presence of tobacco-related behaviors.^[12] While studies have shown that HPV infection alone is not sufficient by itself to cause cancer, growing evidence suggests that cofactors like tobacco consumption may initiate a carcinogenic effect.^[13] It is possible that tobacco contributes to HPV infection by altering the oral microbiota facilitating HPV pathogenesis. Viruses may also instigate cellular signaling pathways by deactivating tumor suppressors and promoting pathogenesis.^[14] One study found, for instance, that oral HPV16 prevalence was greater in current tobacco users (2.0%, 95% CI = 1.3–3.1) compared with never/former tobacco users (0.6%, 95% CI = 0.4–0.9), $P = 0.004$.^[15] Whether HPV contributes to carcinogenesis among SLT users is currently unknown.

Since SLT use is high among tribal women in south India,^[16] this study examined whether it was associated with high-risk HPV infection in indigenous tribal women in Mysuru, India.

MATERIAL AND METHODS

Between June and August 2019, 50 regular SLT users and 50 non-users were recruited from two rural tribal villages in Mysuru District for an observational study. To be included, participants had to be female, belong to a government-recognized ST group, be aged 35–64 years, be willing to complete all study procedures, and be able to undergo an informed consent process. Following informed consent, participants underwent a standardized interviewer-conducted questionnaire collecting data on socio-demographic status, tobacco use, and alcohol consumption. The oral samples were collected using a protocol adapted from the NIH Human Microbiome Project and tested using the *digene* Hybrid Capture 2 HPV DNA Test according to manufacturer's instructions, (Qiagen, Gaithersburg, MD, USA).^[17–19] Digital photographs were taken of the participant's oral cavity and palms. The study protocol was approved by an institutional review committee at the institution - protocol # 2018-02-10-40.

Statistical analysis

Descriptive analysis for socio-demographic variables and alcohol and tobacco use variables was conducted. Counts and percentages were calculated if they were categorical/ordinal variables, while mean, standard deviation, and median with interquartile ranges were used if they were continuous variables. T-tests for continuous variables and Fisher's exact or

Chi-square test for categorical variables were also calculated. For ordinal variables (e.g. income), the nonparametric Wilcoxon rank sum test was considered. For variables that were exclusive to those using tobacco, only summary statistics for those using tobacco were included.

RESULTS

The sample of 100 women with 50 SLT users had a mean (Standard Deviation [SD]) age of 45.5 ± 6.6 years. The mean age of initiation of using SLT products was 24.4 ± 11.9 years. The majority of participants (67, 67%) had no formal education and worked as daily-wage laborers (62, 73%). A little over half the sample had a monthly income of between 3,000 and 10,000 Indian rupees or approximately 36 to 120 USD (57, 58%) [Table 1].

Nearly half of the SLT users (56%) cited chewing tobacco as a habitual practice; the other 44% mentioned that they used SLT products because of other reasons including dental pain (4%) chest pain (4%), or because they liked the taste (2%). The majority (72%) of the SLT users used betel leaves and nuts along with tobacco products, and the mean duration of use was 5.88 ± 1.58 days per week. About 54% of the participants reported that they used tobacco within five minutes of waking up, and nearly the same proportion (58%) found it difficult to refrain from chewing in forbidden areas. Consumption of alcoholic beverages occasionally or at some point in time was reported by 18 (18%) participants who were SLT users [Table 2].

Among SLT users, 62% of participants had not received any formal education as compared to 72% in the SLT non-users ($P = 0.324$). A majority of participants (20 SLT users, 42 non-users) were engaged in daily-wage labor activities ($P < 0.001$) [Table 1]. A greater number of SLT users (76%) reported being exposed to passive smoke as compared to non-users (70%) ($P = 0.499$). Almost all (99%) of the study participants reported having never seen a dentist for a regular dental check-up, with only one participant having seen a dentist in the prior three years, and she was an SLT non-user [Table 2].

HPV testing of the 100 samples showed one sample (1%) positive for high-risk HPV. The participant who tested positive for HPV was a 61-year-old Hindu illiterate widow employed as a daily-wage laborer. She was a SLT user who started using the product at the age of 25 and consumes it in its pure form without the use of any adjuvant like betel leaves, nuts, or lime. She used tobacco regularly even when reports being unwell [Figures of the palm and oral cavity of the woman shown in Figure 1]. She reported being exposed to passive smoking every day and never consumed alcohol.

DISCUSSION

Our study found the burden of oral HPV infection, reported to be a precursor HPV-associated oropharyngeal cancer, to be

Table 1: The socio-demographic characteristics of the study participants and their tobacco consumption patterns in Mysuru, India

Characteristics	n (%)	SLT users n (%)	SLT non-users n (%)	P
Age in years ¹	Mean: 45.5±6.6 Median (IQR): 45 (40,50)	Mean: 45.6±6.7 Median (IQR): 44 (40, 50)	Mean: 45.3±6.5 Median (IQR): 45 (40, 50)	0.868
Education ^{#2}				0.324
Graduate	1 (1.0%)	0 (0%)	1 (2%)	
Secondary School	5 (5.0%)	2 (4%)	3 (6%)	
High School	17 (17.0%)	12 (24%)	5 (10%)	
Primary School	10 (10.0%)	5 (10%)	5 (10%)	
No formal education	67 (67.0%)	31 (62%)	36 (72%)	
Occupation ²				<0.001
Agriculture	13 (15.3%)	11 (27.5%)	2 (4.4%)	
Business ⁵	2 (2.4%)	2 (5%)	0 (0%)	
Daily-wage Laborers	62 (72.9%)	20 (50%)	42 (93.3%)	
Caste occupation [†]	3 (3.5%)	3 (7.5%)	0 (0%)	
Service [*]	5 (5.9%)	4 (10%)	1 (2.2%)	
Monthly income (Indian Rupees) ³				0.017
<3,000	24 (24.2%)	18 (36%)	6 (12.2%)	
3,000-10,000	57 (57.6%)	23 (46%)	34 (69.4%)	
>10,000	18 (18.2%)	9 (18%)	9 (18.4%)	

[#]Education: Secondary School: 12 years of education; High School: 10 years of education; Primary School: up to 8 years of education. [†]Caste occupation: Artisans, Tailor, Blacksmith, Carpenter, Washerman, Potter, etc.; ^{*}salaried people; ⁵Business: Shops, trade activities. ¹P-value obtained using Student's *t*-test, ²P-value obtained using Fisher's exact test, ³P-value obtained using Chi-square test



Figure 1: The pictures show the palm and oral cavity of the participant who tested high-risk HPV positive

very low in this population.^[20] To our knowledge, our study is one of few studies in rural India examining the prevalence of oral HPV infection among indigenous SLT users in a community setting. While the prevalence of oral HPV infection

was extremely low in this study, the only positive HPV case was a chronic SLT user.

Our study aimed to investigate the presence of high-risk HPV in oral samples of SLT users among indigenous women, where the

Table 2: The reasons for chewing tobacco, exposure to tobacco and alcohol, and the oral care of the SLT users in Mysuru, India

Characteristics	n (%)	SLT users n (%)	SLT non-users n (%)	P
Reason for chewing tobacco			N/A	N/A
Out of Habit	28 (56%)	28 (56%)		
Other reasons*	22 (44%)	22 (44%)		
Are you exposed to tobacco smoke from others regularly? ¹				0.499
Yes	73 (73%)	38 (76%)	35 (70%)	
No	27 (27%)	12 (24%)	15 (30%)	
How often do you use alcoholic beverages?				N/A
Occasionally	16 (16%)	16 (32%)	0 (0%)	
Quit more than 6 months ago	2 (2%)	2 (4%)	0 (0%)	
Missing	82 (82%)	32 (64%)	50 (100%)	
Have you visited your dentist for a regular dental check-up in the past 3 years? ²				0.999
Yes	1 (1%)	0 (0%)	1 (2%)	
No	99 (99%)	50 (100%)	49 (98%)	
Are there other people in the house who smoke? ³				0.159
Yes	55 (55%)	31 (62%)	24 (48%)	
No	45 (45%)	19 (38%)	26 (52%)	
First start consuming tobacco Mean (± SD)	24.4±11.0	24.4±11.9	N/A	N/A
How soon after you wake up do you chew your first tobacco?			N/A	N/A
0-5 Minutes	27 (54%)	27 (54%)		
6-30 Minutes	12 (24%)	12 (24%)		
31-60 Minutes	5 (10%)	5 (10%)		
60 Minutes	6 (12%)	6 (12%)		
Consume tobacco with			N/A	N/A
Madhu	1 (2%)	1 (2%)		
Betel leaves, nuts; lime	8 (16%)	8 (16%)		
Only tobacco	2 (4%)	2 (4%)		
Panparag	1 (2%)	1 (2%)		
Betel leaves and nuts	36 (72%)	36 (72%)		
With lime only	1 (2%)	1 (2%)		
Vimal	1 (2%)	1 (2%)		
Chew more frequently during the first hours			N/A	N/A
Yes	23 (46%)	23 (46%)		
No	27 (54%)	27 (54%)		
Difficult to refrain from chewing tobacco in places where it is forbidden			N/A	N/A
Yes	29 (58%)	29 (58%)		
No	21 (42%)	21 (42%)		
How many days do you use tobacco in a week? ³ Mean (±SD)	5.89±1.58	6.05±1.39	5.71±1.78	0.371
How old were you when you had sex for the first time? ³ Mean (±SD)	15.94±3.74	15.4±2.13	16.48±4.81	0.151
Oral sex habit ²				0.999
Yes	2 (2%)	1 (2%)	1 (2%)	
No	98 (98%)	49 (98%)	49 (98%)	

*Pain relief, friend's influence, addicted to tobacco, avoid chest pain; relieve dental pain and cavity, for comfort, cost reason. ¹P-value obtained using Chi-square test, ²P-value obtained using Fisher's exact test, ³P-value obtained using Student's t-test

habit of chewing tobacco is high, based on the hypothesis that there is a possibility that tobacco consumption could contribute to HPV infection. But compared to what we anticipated, oral HPV prevalence among tribal women was much lower.

Our study with a single case of oral HPV in rural and tribal women is much lower than what is reported in other studies.^[21] Research from Taiwan reported a prevalence of 3% in individuals who were betel nut chewers or cigarette smokers.^[20] Our study only included female subjects to

examine the possible association between oral HPV infection and SLT, while other studies have mostly focused on the effect of smoking and sexual behaviors on oral HPV infection.^[21-23] In our study, the participant who tested HPV positive reported that she had never been involved in oral sex activities, while other studies report that such practices may increase the risk of being infected.^[24]

The tobacco addiction levels among rural women imply the importance of working on public health primary prevention

strategies which include health promotion and communication of risk factors along with awareness regarding HPV vaccines. While there is a lack of evidence on the actual efficacy of HPV vaccines in the prevention of oral HPV infection, one study conducted in an adolescent health clinic in the USA among sexually active females aged 13–21 years reported that although the prevalence of HPV infection was not uncommon, the detection of HPV vaccine strains among participants who had received at least one dose of the vaccine was significantly lower compared to those who were unvaccinated.^[25]

In our study, only one out of 100 participants reported having visited a dentist in the prior three years, highlighting the need for awareness, oral health promotion, and preventative actions. None of the studies that measured the association between behavioral risk factors and oral HPV focused on the importance of dental visits. A dentist plays a major role in screening and detection of early lesions, promotion of HPV vaccinations, and education of young adults regarding sexual activities and therefore could act as the first line of defense against the spread of oral HPV infection.^[26]

There are a few limitations to this study. A major limitation of this study was a small non-probability sample which could lead to selection bias. The findings from this study cannot be generalized to other populations. Repeating the study with a larger, randomized sample could lead to more definitive and generalizable results. There is a possibility of social desirability bias as many of the behaviors were self-reported. The testing kit we used had yet another drawback in that it was not intended to detect all high-risk oral HPV strains. This was a cross-sectional study which precludes our ability to determine causal association. Despite these limitations, there were several strengths to this study. Our study recruited women from tribal populations, so we were providing extensive education and awareness to sensitize the entire community about modifiable risk factors, which may motivate them to alter their lifestyles and behaviors to lower their risk for cancer. It also highlights the tobacco epidemic in the country, and the need for further studies to evaluate the role of SLT and oral HPV infection in the pathogenesis of oropharyngeal cancers.

CONCLUSION

Oral HPV infection in this study among a population of tribal women was extremely low. A low prevalence of oral HPV infection was reported in chronic SLT users and is consistent with the literature. The tobacco dependency level of the study subjects raises the need for preventative approaches.

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Author contributions

PM, KK, BH, and VN were responsible for the study concept and design. KR, KN, and AK were responsible for the

acquisition of the data. BP, KK, PM, BH, VN, AS, KR, TK, AK, KN, and KM analyzed and interpreted the data. KR, TK, BP, and KK drafted the manuscript. PM, KK, BH, and KM critically revised the manuscript for important intellectual content. All authors had full access to all the data including statistical reports and tables in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Ethical approval

The study protocol was approved by an institutional review committee at the Public Health Research Institute of India (protocol # 2018-02-10-40), Mysuru, India.

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Conflicts of interest

There are no conflicts of interest.

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