Short Communication
Community Dental Health

Elevated prevalence of high-risk HPV in healthy oral mucosa of users and nonusers of drugs in Northeastern Brazil

Mariana Goveia Melo RIBEIRO[a]
Larissa Doddi MARCOLINO[b]
Bruna Ribeiro de Andrade RAMOS[b]
Cleverson Luciano TRENTO[c]
Karina Conceição Gomes Machado de ARAUJO[a]
José Rodrigo Santos SILVA[d]
Sona JAIN[a]
Ricardo Queiroz GURGEL[e]
Márcia Guimarães SILVA[b]
Silvio Santana DOLABELLA[a]

[a]Universidade Federal de Sergipe – UFS, Department of Morphology, Aracaju, SE, Brazil.

[b]Universidade Estadual Paulista – Unesp, Botucatu Medical School, Department of Pathology, Botucatu, SP, Brazil.

[c]Universidade Federal de Sergipe – UFS, Department of Dentistry, Aracaju, SE, Brazil.

[d]Universidade Federal de Sergipe – UFS, Department of Statistics and Actuarial Sciences, Aracaju, SE, Brazil.

[e]Universidade Federal de Sergipe – UFS, Department of Medicine, Aracaju, SE, Brazil.

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Corresponding Author:
Silvio Santana Dolabella
E-mail: dolabella@ufs.br

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Abstract: Variable rates of HPV infection have been reported in healthy oral mucosa worldwide. The main objective of this study was to detect and genotype HPV infection in users and nonusers of drugs with clinically healthy mucosa from the Northeast Brazil. Samples from 105 patients were amplified using the primers MY09/MY11 and GP5+/GP6+, and genotyping was performed by multiplex-PCR for HPV-6/11, 16 and 18. A total of 81.9% samples were positive. Among drug users, 84.5% presented the virus and 20.4% showed multiple infections. Among non-drug users, 78.7% were positive and 13.5% had multiple infections. Limited information is available on oral HPV in Brazilian population, especially for drug users, and our results showed higher HPV infection rates in both users and nonusers of drugs. More studies and researches focused on drug users including factors like sexual behavior, nutrition and cultural habits are necessary to enhance the comprehension of this relationship, and develop preventive strategies.

Keywords: Papillomavirus Infections; Epidemiology; Genotype; Risk Behavior.

Introduction

Human Papillomavirus (HPV) is one of the most prevalent sexually transmitted infections worldwide and can present itself in clinical, subclinical or latent forms. Most infected patients present the subclinical form where the immune system is able to efficiently eliminate the virus without the patients even realizing that they were infected.[1]

Approximately 200 HPV types have been identified and classified into high or low oncogenic risk groups.[2] In the oral cavity, benign lesions are mainly associated with the types 2, 4, 6, 7, 11, 13 and 32, which are of low oncogenic risk. Other types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66) of high oncogenic risk are associated with malignant lesions of the oral cavity, mainly squamous cells.[2,3]

In Brazil, 500,000 to 1 million cases of HPV infections are estimated annually.[1] Detection rates for HPV in healthy oral mucosa vary significantly around the globe, suggesting that this site may work as a reservoir for

the virus.[3,4] A population study conducted in the US between 2009 and 2010 with asymptomatic patients reported 6.9% prevalence of oral HPV, 3.7% of which was genotyped as high-risk HPV (HR-HPV) and the other 3.2% as low risk HPV (LR-HPV).[5] In São Paulo state, Brazil, Tristão et al.[1] described a prevalence of 23.2% HPV in healthy oral mucosa and associated the presence of the virus with regular oral sex. Similarly, in Pará state, Brazil, Araújo et al.[6] reported 24.1% positive oral samples for HPV-DNA.

Changes in male and female sexual behaviors may be associated with the increased prevalence of HPV infection in oral cavity recently reported.[6] However, HPV oral infection is also transmitted by autoinoculation of existing genital or skin lesions and may also be transmitted vertically from the mother to the fetus.[2,7]

The detection of the virus in the epithelium of clinically healthy oral mucosa does not yet allow the accurate inference of its role in oral carcinogenesis. In other words, it is still not conclusive, whether HPV is the main etiological agent of oral cancer, or if it plays a supportive role in carcinogenesis, or if it should be even considered a commensal agent that may become pathogenic under certain conditions.[2]

Despite previous evidence that drug users may be at increased risk for HPV infection,[8,9] until this moment we could find only one work done in Puerto Rico that analyzed oral HPV infection for this risk group.[10] Their study involving only drug users found that binge drinking, commercial sex and HIV-positivity were factors approximately 4 times more likely to be related to higher HPV infections.

Currently, the limited data available from the Northeast Brazil does not allow the epidemiological inference regarding non-cervical HPV infections, especially among drug users. Thus, the aim of this study was to determine the prevalence of HPV infection and common HPV genotypes in users and nonusers of drugs with clinically healthy oral mucosa from the Northeast Brazil.

Methodology

We conducted a prospective transversal study with convenience sampling. Samples from 105 patients (69 men and 36 women) visiting the Oral Diagnostic Care Unit of the Federal University of Sergipe, Brazil (58 patients) and the Drug and Alcohol Rehab Centers in Sergipe (47 patients) were analyzed. The samples were collected from January 2013 to March 2014. The study was approved by the Human Research Ethics Committee of the Federal University of Sergipe (protocol 76317) and all the participants provided written informed consent. Sociodemographic and clinical data were obtained from medical records. Oral mucosa was examined by dentists and only patients with clinically healthy oral mucosa were included in this study. No other sociodemographic or clinical factors were used for exclusion.

Samples were collected by exfoliation of oral mucosa throughout the soft and hard palates, floor of the mouth, buccal mucosa and dorsal and ventral surfaces of the tongue with individual sterile cytobrush to avoid cross-contamination between patients samples.[2] The material was stored in 70% ethanol at 4ºC up to 15 days until processing. Total DNA was extracted through enzymatic digestion and stored at -20ºC.[11]

DNA extraction was confirmed by amplification of the β-globin gene using the primers PCO3/PCO4. For HPV-DNA amplification, initially MY09/MY11 primers were used and then nested-PCR using GP5+/GP6+ primers was performed.[11] A multiplex-PCR assay was performed for genotyping HPV-6/11 (LR-HPV), 16 and 18 (HR-HPV) which are the most common HPV types associated with cancers of this anatomic region[6] using specific primers and parameters as described in Ribeiro et al.[11] HPV-16 DNA extracted from HeLa cells was used in all reactions as a positive control and sterile Milli-Q water was used as a negative control.

The descriptive analysis was performed in the form of tables and the observed data was expressed as absolute (n) and relative (%) frequency. Comparison of discrete variables such as gender, marital status, smoking habits, alcohol, illicit drugs consumption and HPV infection was performed by Chi-squared and Fisher Exact Test. Analyses were performed using R software version 3.3. and $p < 0.05$ was considered as significant.

Results

A total of 81.9% (86/105) samples were positive for HPV-DNA (Figure). HPV-6/11 were the most frequent types identified (41.8%), followed by HPV-18 (36.0%) and HPV-16 (4.6%). Multiple infections were detected in 15 individuals (17.4%). In 34.9% of positive samples, HPV type could not be identified (Table). Majority of patients were men (65.7%) and single (62.8%). Prevalence of HPV among patients aged 11–40 was 84,0% (58/69). Frequency for patients aged between 41 and 59 was 73.4% (22/30) and for those between 60 and 79 years was 100% (6/6), with no statistical differences among the different age groups (p = 0.220).

Smoking habits and/or alcohol consumption were reported by 62.0% (65/105) of patients and illicit drugs by 55.2% (58/105). The most illicit drugs used were marijuana and crack. Detection rate for patients that reported tobacco, alcohol and illicit drugs consumption was 85.4% (35/41) and 22.8% (8/35) of these showed multiple infections by HPV-6/11 and HPV-18. There was no statistical difference between these patients and those that did not consume any abusive substances (p = 0.550).

Among illicit drugs users 84.5% (49/58) were HPV-positive and HPV-6/11 were the most common type found (44.9%) including ten patients with multiple infection by HPV-6/11 and HPV-18, followed by HPV-18 (40.8%) and HPV-16 (4.0%). No HPV infection could be identified in 30.6% of users. Among non-drug users, 78.7% (37/47) presented HPV infection among which 37.8% were typed as HPV-6/11, 29.7% as HPV-18 and 5.4% as HPV-16. Multiple infections were observed in 13.5% of these samples and in 40.5% HPV genotypes could not be identified.

Discussion and Conclusion

Similar to other studies with oral lesions; variable rates of HPV have been detected and reported in clinically healthy oral mucosa.[5,12]

In the present study 81.9% of patients without oral lesions presented HPV-DNA. This rate is similar to the one reported by Terai et al.[13] in Japan (81%), but substantially different from those reported worldwide with a lower prevalence of the virus.[6,7,12]

Little information is available regarding the prevalence of oral HPV infection in the Brazilian population. A study conducted with undergraduate students from a University in the state of Rio de Janeiro did not detect any virus in samples of healthy oral mucosa.[2] However, authors from a different state found a prevalence of 24.1% in their population.[6] Another study reported the presence of HPV in 54% of women with clinically healthy oral mucosa but whose sexual partners presented HPV-associated genital lesions.[14]

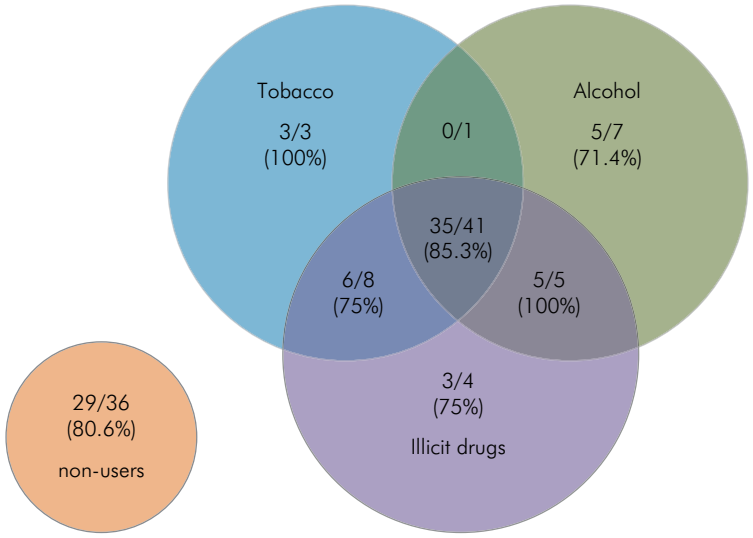

Figure. Venn diagram showing HPV positivity (86/105) in users and nonusers of drugs.

Table. Socio-demographic data and presence of HPV-DNA in the healthy oral samples analyzed in this study.

Characteristics	n (%)	HPV occurrence	HPV-6/11	HPV-16	HPV-18	HPV-6/18	HPV 6/16	Not identified
Gender								
Female	36 (34.3)	27 (75.0%)	7	1	5	3	0	11
Male	69 (65.7)	59 (85.5%)	14	2	12	11	1	19
Ethnic								
Black	23 (21.9)	20 (86.9%)	9	0	2	2	0	7
White	23 (21.9)	17 (73.9%)	1	1	3	5	0	7
Other	41 (39.0)	35 (85.3%)	9	2	7	5	1	11
Missing	18 (17.1)	14 (77.8%)	2	0	5	2	0	5
Education								
Literate	81 (77.1)	67 (82.7%)	15	3	10	10	1	28
Non-literate	14 (13.4)	11 (78.6%)	4	0	3	3	0	1
Missing	10 (9.5)	8 (80.0%)	2	0	4	1	0	1
Age								
≥14	7 (6.7)	6 (85.7%)	0	0	2	1	0	3
18 to 40	62 (59.0)	52 (83.9%)	12	1	10	10	1	18
41 to 59	30 (28.6)	22 (73.4%)	7	1	3	3	0	8
>60	6 (5.7)	6 (100%)	2	1	2	0	0	1
Marital status								
Single	66 (62.8)	54 (81.8%)	12	2	10	10	1	19
Married	23 (21.9)	18 (78.3%)	5	0	2	3	0	8
Divorced	3 (2.9)	3 (100%)	1	0	0	0	0	2
Widower	3 (2.9)	3 (100%)	1	1	1	0	0	0
Missing	10 (9.5)	8 (80.0%)	2	0	4	1	0	1
Family income								
< 1	24 (22.9)	18 (75.0%)	6	1	4	3	0	4
1 to 3	60 (57.1)	52 (86.7%)	13	1	11	7	1	19
> 3	14 (13.4)	13 (92.8%)	2	1	2	2	0	6
Missing	7 (6.7)	3 (42.8%)	0	0	0	2	0	1

According to Mbulawa et al.[7], risk for HPV infection is inversely proportional to age and Esquenazi et al.[2] reported higher prevalence of the virus among young individuals. Cason and Mant[15] reported the presence of high-risk HPV in the samples collected from children and teenagers in many countries. We also identified high-risk HPV in samples of three patients aged between 11 to 18 years. However, no statistical difference was found among different age groups for the presence of HPV in this study.

Geographic and/or population differences have been associated with the distinct rates of HPV infection described in the literature.[3] Methodological differences may also account for this variation. As suggested by Cason and Mant[15], rates of HPV detection vary depending on the methods of collection, transportation, DNA extraction and detection technique, and careful choice of these tasks is essential to limit false-positive and false-negative results. Also, collection of epithelial cells from oral mucosa by exfoliation presents several advantages such as low cost, easy transportation and processing, and high acceptability among the patients for its non-invasive characteristics.[16]

Several types of HPV have been reported in clinically healthy oral mucosa worldwide. In an African setting, 22 HPV types were identified in oral mucosa of heterosexual couples without lesions.[7] In the present study we genotyped HPV-6/11 in 41.8% of positive samples, HPV-18 in 36.0% and HPV-16 in 4.6%. HPV multiple infection comprised 17.4% of cases. In 34.9% of 91 positive samples, HPV type could not be identified. This could be due to infection by other HPV types that were not analyzed in this study.

These results are different from those described by Turner et al.[12], who detected HPV-16 in all positive samples, while HPV-18 was not identified in their population. In the study performed by Gillison et al.[5], HPV-16 was the most frequent virus (48 of 408 positive samples), while HPV-18 and HPV-6 were present in 12 and 21 cases, respectively. Our results are similar to those from Gichki et al.[4] that described 25% prevalence of HPV-18 and 9% for HPV-16 in a population with no oral lesions from Pakistan. In Brazil, a study conducted in Pará state reported HPV-18 to be the most frequent type with 12.5% prevalence.[6] High rates of HPV multiple infections in clinically healthy oral mucosa was also found by Terai et al.[13] and Machado et al.[3] with prevalence of 56.7% and 42.8%, respectively.

In our study, HPV detection rate for patients that reported tobacco, alcohol and illicit drugs consumption was 85.4% (35/41), and 22.8% of them were multiple infections by HPV-6/11 and HPV-18. Several studies have associated tobacco use with immunological suppression and consequent establishment of persistent HPV infection.[4,5] In a work by Gichki et al.[4], 72.8% of individuals with clinically healthy oral mucosa infected by HPV were smokers. Gillison et al.[5] found increased HPV prevalence among individuals who smoke and drink alcohol. The authors also reported increased risk for oral HPV infection among people that consume marijuana. Similar result was found by Muller et al.[17] in Italy. Marijuana was consumed by 82.7% of drug users analyzed in this study.

Besides that, drug use may be only one of the variables that affect prevalence of HPV. Other risk factors as sexual behavior, toothbrush sharing[18], poor oral health and deficient oral hygiene[19] have been also associated with higher oral HPV infection.

It is well established in the literature that people who use drugs present risky sexual behavior that raises the risk to sexually transmitted diseases. Changes in sexual behavior may be associated with the increased infection by HPV in oral cavity.[6,20] Several studies have linked multiple partners for oral sex and kissing with increased risk for acquisition of HPV.[3,7,14] Inconsistent use of preservatives can be a prevalent risk factor among sexually active young adults in Brazil. Barbosa and Koyama[21] described the Brazilian North and Northeast regions with the highest reports of men with more than one sexual partner/year. An increase in oral sex from 1998 to 2005 for both genders in these locations was also reported.

Bearing in mind the fact that HPV infection is the initial step before the development of HPV-related lesions, studies have suggested vaccination as a strategy to prevent HPV infection and oral lesions.[15] Prophylactic vaccination is thought to reduce the number of oral lesions by the protection against HR HPV-16 and HPV-18.[6] In Brazil, since 2014 girls are vaccinated against HPV and starting January 2017 boys between 12 to 13 years are also vaccinated, which raises the question about the effect of this extended vaccination in the reduction of cancer cases in the country.

This study found a high frequency of HPV infection in clinically healthy oral mucosa of both users and nonusers of drugs. This, along with variable rates of HPV detection in oral lesions described in the literature makes the role of HPV in the development of such lesions rather controversial. As mentioned before, drug consumption could just be one of the factors influencing HPV rates. More studies including factors like early exposure to sexual activity, nutritional facts and cultural backgrounds could give us a better insight of this relationship and help develop preventive strategies.

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