The Brazilian Journal of
INFECTIOUS DISEASES
www.elsevier.com/locate/bjid

Original article

Presence of highly oncogenic human papillomavirus in the oral mucosa of asymptomatic men

Ana Paula Machado[a], *Flávia Gatto de Almeida*[a], *Camila Mareti Bonin*[a], *Thiago Theodoro Martins Prata*[a], *Leandro Sobrinho Ávilla*[b], *Cacilda Tezelli Junqueira Padovani*[b], *Alda Maria Teixeira Ferreira*[b], *Carlos Eurico dos Santos Fernandes*[b], *Inês Aparecida Tozetti*[a,c,*]

[a] *Postgraduate Program of Infectious and Parasitary Diseases from Medicine School, Universidade Federal de Mato Grosso do Sul/UFMS, Cidade Universitária, Campo Grande, Mato Grosso do Sul, Brazil*
[b] *Biological and Health Center from Universidade Federal de Mato Grosso do Sul Cidade Universitária, Campo Grande, Mato Grosso do Sul, Brazil*
[c] *Medicine School from Universidade Federal de Mato Grosso do Sul/UFMS, Cidade Universitária, Campo Grande, Mato Grosso do Sul, Brazil*

ARTICLE INFO

Article history:
Received 29 April 2013
Accepted 24 July 2013
Available online 3 January 2014

Keywords:
Human papilomavírus
Men
Viral type
Oral mucosal

ABSTRACT

Objectives: The aim of this study was to identify highly oncogenic forms of human papillomavirus in the oral mucosa of asymptomatic men.
Methods: In this study, we analyzed samples of exfoliated cells from the oral cavity of 559 asymptomatic men. DNA-human papillomavirus was detected using the consensus primers PGMY09/11; viral genotyping was performed using type-specific PCR and restriction fragment length polymorphism.
Results: DNA-human papillomavirus was detected in 1.3% of the study participants and of those 42.8% were infected by more than one type of virus. Viral types included HPV6, 11, 89 (low oncogenic risk), and HPV52, 53 (high oncogenic risk). Increased vulnerability to human papillomavirus infection was observed in individuals aged over 26 years, among those who reported oral sex practices, and in those who have had more than 16 sexual partners since first engaging in sexual intercourse.
Conclusions: There was a low prevalence of human papillomavirus detection in the oral mucosa of asymptomatic men. Highly oncogenic human papillomavirus types and infection by more than one viral type was observed. Oral sex practices and a large number of sexual partners may increase the risk of acquiring human papillomavirus infection.

* *Corresponding author at*: Faculdade de Medicina, UFMS, Campo Grande, Brazil.
E-mail address: inestozetti1408@hotmail.com (I.A. Tozetti).
1413-8670
http://dx.doi.org/10.1016/j.bjid.2013.07.013

Introduction

The human papillomavirus (HPV) is a primary risk factor for cervical neoplasia and is associated with 25% of cancers affecting the head and neck region,[1] and may induce development of oropharynx carcinoma.[2]

There are two forms of HPV, including high oncogenic risk (HR) and low oncogenic risk (LR) types. Low-risk types are associated with benign lesions in the host characterized as ordinary or condylomatous warts and include HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81. High-risk types have carcinogenic potential and include HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 59, 66, 68, 72, and 81.[3]

The more an individual engages in risky behaviors such as oral sex and has high number of sexual partners, the greater the risk of acquiring the virus, with subsequent development of cellular differentiation and progression to neoplasia.[4,5] However, some individuals develop oropharynx carcinoma without prior exposure to these risk factors, suggesting that potentially oncogenic viruses may lead to changes in control and cell proliferation mechanisms.[6]

Previous studies have examined the prevalence of HPV in the oral mucosa of asymptomatic men and suggested its association with neoplastic development; however, the assessment methods have shown disparate frequencies of infection, ranging from 0% to 100%.[7] Thus, the aim of this study was to assess the frequency of highly oncogenic forms of HPV in the oral mucosa of asymptomatic men.

Methods and subjects

Exfoliated cells from the oral cavity of 559 asymptomatic men were analyzed. Ethical approval was granted by the Ethics Committee in Research of UFMS, protocol CAAE 0251.0.049.000-11. All participants completed a questionnaire containing information regarding risk behaviors that may predispose them to HPV infection.

Collection of specimens and detection of HPV-DNA

Samples were collected through 5–10 brushings in regions of pre-established oral mucosa, including the right buccal mucosa (position from top to bottom); left buccal mucosa (position from top to bottom); right, left and dorsal side of the tongue; and inner regions of upper and lower lips. After collection, DNA was extracted from the samples using the Wizard Genomic DNA Purification kit (Promega, Fitchburg, WI, USA) and quantified using a NanoDrop (Thermo Scientific, Waltham, MA, USA) (180–260 nm).

HPV-DNA detection was performed using PCR with a pool of consensus *primers* that amplify PGMY09/11 450-bp DNA sequences within the L1 region of HPV, as described previously.[8] An endogenous control was used to verify DNA integrity using primers for the β-globin gene, PC04 and GH20, which amplify a 286-bp region of human DNA. Negative controls for background contamination were added to the DNA template. PCR products were analyzed using 1.5% agarose gel electrophoresis with ethidium bromide staining to visualize the DNA under ultraviolet (UV) light. Molecular weights were determined by comparison with a 100-bp DNA ladder.

Genotyping using type-specific PCR (TS-PCR) and restriction fragment length polymorphism (RFLP)

HPV-DNA positive samples were genotyped by PCR using TS-PCR for the *E6* and *E7* gene DNA sequences of HPV 6, 11, 16, 18, 31, 33, and 45.[9] PCR products were analyzed on a 2.5% agarose gel with ethidium bromide staining to visualize DNA under UV light. Molecular weights were determined by comparison with 100-bp and 50-bp DNA ladders. The same samples were analyzed using RFLP. Next, the PGMY 09/11 PCR product of these samples was purified from the agarose gel using the QIAEX II Gel Purification Kit Qiagen (Hilden, Germany) according to the manufacturer's protocol. The concentration of extracted materials was determined using a NanoDrop (180–260 nm); samples containing the PCR product were subjected to enzymatic digestion for one hour at 37 °C. The enzymes used for reaction included *Bam*HI, *Dde*I, *Hae*III, *Hinf*I, *Rsa*I, *Pst*I, and *Sau*3A. The digestion pattern was analyzed on a 3% agarose gel with ethidium bromide under UV light and interpreted using an algorithm described previously.[10]

Statistical analysis

The distribution of positivity for HPV-DNA was investigated according to age range ($\leq$25 years and $\geq$26 years), marital status, report of oral sex practices, and estimated number of sexual partners. Statistical analysis was performed using SPSS, version 10.0[11] of Pearson χ^2 test for contingency tables, adjusted for *Phi Cramer's* V. To compare frequencies between age subgroups, when significant, the G test corrected by Yates index was used. The proportion of positive findings within the group was analyzed using the binomial test for two independent samples.

Results

Samples of asymptomatic oral mucosa from men aged 18–68 years (mean, 23 years) was analyzed. Of the 559 samples collected, 514 (91.9%) were positive for the β-globin gene and thus included in the study. HPV-DNA was detected in seven samples, which accounted for 1.3% of the study participants.

Detected viral types included HPV6, 11, and 89 in the LR group and HPV52 and 53 in the HR group (Figs. 1 and 2). Highly oncogenic infection was detected in two out of the seven samples (28.5%), and infection by more than one viral type was found in three of the seven samples (42.8%).

Among the participants, 71.7% reported being single and 27.2% married, with a higher HPV-DNA positivity among married men. The binomial test showed that individuals older than 26 years of age were more vulnerable to infection ($p < 0.01$). Oral sex practices were reported by 71.9% of the respondents (Table 1), particularly among those over 26 years of age ($p < 0.01$), where HPV-DNA positivity was higher.

Among individuals who reported having had more than 16 sexual partners since their first sexual intercourse, HPV-DNA positivity was 2.8%.