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Brief Communication

Papillomavirus infections in the oral and genital mucosa of asymptomatic women



Ledy Horto Santos Oliveira*, Larissa Silva Santos, Carolina Oliveira Silva, Everton Faccini Augusto, Felipe Piedade Gonçalves Neves

Universidade Federal Fluminense (UFF), Departamento de Microbiologia e Parasitologia, Niterói, RJ, Brazil

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ABSTRACT

Human papillomavirus (HPV) has been found in several regions of the body, including the oral cavity. Recently, this virus has been associated with oropharyngeal cancer, but little is known about HPV transmission to the oral cavity. We carried out a study to investigate concurrent oral and cervical infections in 76 asymptomatic women attending a healthcare program. Demographic and behavior data were obtained through a structured questionnaire. Oral and cervical mucosa scrapings were collected and stored for DNA extraction. HPV DNA amplification was performed by polymerase chain reaction assay (PCR) using both primers My09/My11 and FAP59/64, followed by HPV typing with restriction fragment length polymorphism analysis (RFLP) and sequencing. The data collected revealed no risk factors for HPV infection in these 76 women. HPV prevalence of 9.2 and 5.3% was found in cervical and oral mucosa, respectively. Concurrent infections by discordant types were detected in one case only. Sequencing procedures allowed us to detect a new putative HPV 17 subtype from the *Betapapillomavirus* genus. Our results support the view that cervical and oral HPV infections are independent events. The observed low prevalence of both oral and cervical HPV infections could be associated with attendance in a healthcare program.

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Human papillomavirus species from *Alpha*, *Beta*, and *Gamma* genera have been found in oral cavities, but their transmission mechanism remains unclear. Acquisition could be through oral–genital or mouth-to-mouth contact, autoinoculation, or an independent event.¹ This matter is controversial, and Rautava and Syrjänen² concluded that oral infection can be transmitted sexually, through kissing or by household contact, or even by vertical transmission from mother to child during childbirth. Vidotti et al.³ reported a significant

association between oral and genital HPV. On the other hand, genital infection could not predispose to oral infection, even if both sexual partners practiced oral sex.⁴ Oral HPV infections are frequently asymptomatic, but in the last decade some types were also associated with oropharyngeal cancer.⁵

Currently, three anti-HPV vaccines are commercially available. Although clinical trials have shown high efficacy in the prevention of malignancies in uninfected women, the impact of these vaccines on non-genital infections have not been

* Corresponding author.

E-mail address: virologia.uff@gmail.com (L.H. Oliveira).

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determined yet.⁶ However, Herrero et al.⁷ stated that a bivalent vaccine is effective in preventing oropharyngeal cancer. If vaccination is effective for some HPV types of the genital area, it is quite possible that it also acts in other parts of the body. The investigation of HPV genotypes in the cervical and oral tract of asymptomatic women who regularly attended a health program may contribute to the knowledge of oral HPV transmission.

We conducted a randomized, cross-sectional survey, initially investigating 351 sexually active women who were referred to the Family Health Program in Niterói, Rio de Janeiro State, from 2009 to 2010. Data about this study have been published elsewhere.⁸

Out of the 351 participants, 84 asymptomatic women without oral lesions agreed to participate in oral HPV tests. After undergoing a routine gynecological examination, two cervical specimens were collected from each participant for Papanicolaou staining and HPV testing. None of the women had been vaccinated against HPV. A scraping of the oral cavity from the borders of the tongue and cheeks was also performed using a sterile brush. Demographic data were obtained through a structured questionnaire. The Ethics Committee of the College of Medicine of the University provided ethical clearance for the protocol and informed consent (CEP CMM/HUAP 052/2010, CAAE 0037.0.258.000-10).

DNA was extracted from samples (High Pure PCR Template Preparation Kit, Roche, São Paulo, Brazil) and quantified (Thermo Scientific NanoDrop 2000 spectrophotometer, Wilmington, North Carolina, USA). Due to insufficient DNA, eight scrapings were discarded, remaining 76 samples. The quality of DNA extracted was assessed by polymerase chain reaction assay (PCR) amplification of a 260 bp DNA fragment for β -globin using the primers PC04/GH20.⁹ Amplification of HPV DNA was done with MY09/11 consensus primers (Síntese Biotecnologia, Belo Horizonte, MG), which amplify a 450 bp in the L1 region of HPV DNA.¹⁰ To improve the sensitivity of the assay to detect oral HPV, a second PCR system using the primers FAP59/64¹¹ (Síntese Biotecnologia, Belo Horizonte, MG) was added. These primers amplify a 480 bp within the same L1. All oral samples were submitted to MY09/11 and FAP/64 systems. Although MY09/11 degenerate primers are routinely and largely used to detect mucosal HPV (Alpha species), especially from genital mucosa, the FAP 59/64 system has been applied in the detection of cutaneous HPV (Beta and Gamma species) and has been used to improve detection of oral HPV.¹² The combination of these systems tracks a broad range of types, even types not yet identified.

HPV typing was performed by restriction fragment length polymorphism analysis (RFLP) or sequencing. For RFLP analysis, the resultant 450 base pairs PCR products were digested by six restriction enzymes (BamHI, DdeI, HaeIII, HinfI, PstI, RsaI; Invitrogen, São Paulo, Brazil). The RFLP pattern of each sample was analyzed by agar gel electrophoresis under ultraviolet light and compared with the RFLP patterns for mucosal HPV.¹³ The smears that could not be typified by RFLP assay were submitted to sequencing. The amplified PCR product was purified and directly sequenced in an ABI DNA 3130 sequencer (Applied Biosystems, California, USA) with the same primers as those used for PCR amplification. The forward and reverse sequences were aligned and analyzed using

Table 1 – Distribution of HPV genital and oral genotypes.

Genital		Oral	
Types	n	Types	n
31	1	6	2
52	1	17	1
61	1	Not typified	1
62	2		
84	1		
Not typified	1		
	7		4

ClustalW in BioEdit Sequence Alignment Editor (North Carolina State University, Raleigh, NC).¹⁴ Similarity analysis was performed using BLAST (Alignment Search Tool Analysis).¹⁵ Types that could not be classified by any methods were considered to be unidentified. A databank was generated in the SPSS-18 statistical package. Chi-Square (χ^2) or Fisher's exact test were applied to compare categorical variables. t-Tests were used for comparing age means among groups. To identify associations between possible risk factors and the presence of HPV, odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. For all tests, the level of significance was set at 0.05.

The participants who enrolled in this study were aged 14 through 70 years, with a mean age of 37.26 years and standard deviation of 14.59; 60.5% of the women were over 30 years old. Most of them received a monthly family income of over U\$ 600, and 63.2% had attended only elementary school or reported being illiterate. Non-white people were the most prevalent ethnic group (69.7%). Most of the women maintained a stable sexual partner and had up to three lifetime sexual partners, with one partner during the last three months. Nearly half had begun their sexual activity before the age of 17. Sixty-six percent and 52.50% of the women had never used cigarettes or alcohol, respectively. Condoms were not used by 78.9%, but other contraceptive methods were employed by 84.2% of them. Parity up to two children was reported by 69.2% of the women, of whom 32.8% reported abortion episodes.

At the time of the interview, most of the participants (81.6%) had no history of any sexually transmitted disease. The remaining people reported past vulvar condyloma (7), syphilis seropositivity (4), candidiasis (2), and chlamydia (1). No women reported being HIV positive. Most of these women were submitted to regular gynecological examinations (93.4%). The cervical oncologic cytology smear from 94.7% of the participants was normal or inflammatory. The results show that a predominantly healthy population concerning cervical lesions, risk factors for HPV infection, and sexually transmitted diseases was screened. Therefore, educational support from basic healthcare staff could be associated with the profile of this population.

Genital and oral HPV were present in 9.2 and 5.3% of the women, respectively. The cervical and oral types are shown in Table 1. Each site harbored types totally discordant and the only woman HPV positive for both sites harbored different HPV types. In line with our findings, a German research reported that HPV oral autoinoculation and transmission to sexual partners were uncommon events in 60 cervical HPV-positive women.¹⁶ Similarly, Kero et al.¹⁷ concluded that although oral

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