Human Papillomavirus infection in oral and anogenital sites: prevalence and rates of concordance

Infecção pelo Papilomavírus Humano em regiões orais e anogenitais: prevalência e taxa de concordância

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ABSTRACT

Introduction: HPV infection causes cancer at several anatomical sites. However, the infection's natural history in non-cervical sites is understudied. **Objective:** To evaluate oral and anogenital HPV infections, correlating HPV prevalence rates and genotypes with site of infection and risk factors. **Methods:** In the present study, 351 samples from oral, genital, and anal sites of 117 patients were investigated by using PCR MY09/11 detection, followed by genotyping with RFLP. **Results:** HPV DNA prevalence was 89.7% (105/117) in genital lesions, 53.8% (63/117) in oral samples, and 58.9% (69/117) in anal samples. Regarding the risk factors associated with HPV in genital lesions, statistically significant rates for oral (p=0.039) and anal sex practices (p=0.0000012) were found. For oral samples, a relevant correlation concerning oral contraceptive use (p=0.039), tobacco smoking (p=0.036), and alcohol use (p=0.0075) were observed; whereas in anal samples, higher risk for HPV infection in patients who reported non-exclusive sexual partners (p=0.013) were found. The presence of viral DNA in all the three sites concurrently was observed in 36.8% of the cases (43/117). Among them, 18% (21/117) presented concordant HPV genotypes, diverging from the literature, and thus corroborating that there is still much to learn about HPV natural history, since different biological behaviors are expected within different populations. Differences in anatomy and physiology of the studied sites can determine different prevalence rates of infection by diverse genotypes. **Conclusion:** Due to the high prevalence of HPV DNA in extragenital sites, further studies are required to define aspects of HPV natural history among different human anatomical sites.
Keywords: papillomaviridae; wounds and injuries; genitalia.

RESUMO

Introdução: As infecções causadas pelos papilomavírus humanos (HPV) são responsáveis pelo desenvolvimento de cânceres em diversos sítios anatômicos humanos. Entretanto, a história natural da infecção em sítios que não a cérvice uterina não é muito clara. **Objetivo:** Avaliar infecções orais, genitais e anais por HPV, correlacionando taxas de prevalência do vírus e seus genótipos aos sítios de infecção e a fatores de risco sócio-demográficos. **Métodos:** Em nosso estudo, investigamos 351 amostras coletadas dos sítios oral, genital e anal de 117 pacientes, por meio da técnica de PCR MY09/11, seguida de genotipagem por RFLP. Todos os pacientes apresentavam lesões genitais benignas. **Resultados:** A prevalência do HPV foi de 89,7% (105/117) nas lesões genitais, 53,8% (63/117) nas amostras orais e 58,9% (69/117) nas amostras anais. Em relação aos fatores de risco associados à infecção genital, encontramos diferenças estatísticas significativas para prática de sexo oral (p=0,039) e sexo anal (p=0,0000012). Já para as amostras orais, observamos importante correlação entre infecção e uso de contraceptivo oral (p=0,039), tabagismo (p=0,036) e uso de álcool (p=0,0075) enquanto nas amostras anais, alto risco de infecção pelo HPV foi associado a pacientes relatando parceiros sexuais não exclusivos (p=0,013). A presença do DNA viral simultaneamente nos três sítios estudados foi observada em 36,8% dos casos (43/117). Desses, 18% (21/117) apresentaram genótipos concordantes, diferindo da literatura, na qual há grande disparidade de descrições. **Conclusão:** Há a necessidade de novos estudos a fim de esclarecer a história natural do HPV em sítios extragenitais em diferentes populações, avaliando características anatômicas e fisiológicas com o intuito de esclarecer diferentes taxas de infecção por genótipos do HPV e diferentes processos de doença.
Palavras-chave: papillomaviridae; ferimentos e lesões; genitália.

INTRODUCTION

Human papillomavirus (HPV) is the most common viral infection of the reproductive tract, as well as the cause of a range of conditions in both females and males, including genital warts, precancerous lesions, and anogenital cancers[1]. Nowadays, there are more than 200 papillomaviruses that have been identified and completely sequenced[2]. Studies concerning HPV natural history focused on cervical infection and disease, but have scarcely described the pathogenesis and transmission of HPV in extragenital sites. Although HPV has already been accepted as the etiological agent for cervical and anal cancer, little is known about the etiology of oral and penile carcinoma[3]. Therefore, the natural history of HPV is still under construction. There is much to know about how the transmission of HPV occurs, the behavior of this virus in extra-genital sites, and the synergism of co-infections by high-risk and low-risk genotypes. Thus, the detection of the virus in extragenital sites could help in understanding the cycle of infection-spread and re-infection by HPV in the human genital tract. This would contribute to proper screening of the virus along with clinical diagnosis, especially in the initial period of the injury, thus providing a better prognosis.

OBJECTIVE

The objective of the present study was to contribute to the knowledge of the natural history of HPV infection, determining prevalence and concordance rates of HPV infection in oral, genital, and anal sites, besides evaluating risk factors that may contribute to the infection.

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METHODS

This is a cross-sectional study, comprising 117 patients, totaling 351 samples, conducted in the Virological Diagnostic Laboratory of Universidade Federal Fluminense (UFF). The study was approved by the Ethics Committee of UFF (CAAE:36683514.0.0000.5243). Samples were collected between January 2013 and December 2018 in the Sexually Transmitted Diseases (STD) Sector of UFF and in the STD Sector of Santa Casa de Misericórdia of Rio de Janeiro. In order to participate, patients needed to had genital warts clinically diagnosed by doctors. Three samples were concurrently taken from each patient: genital, oral, and anal. Genital samples were biopsies of benign lesions, whereas anal and oral samples were only healthy mucosa smears. Samples were collected with Tris-EDTA buffer (pH 7.2) and frozen at -20°C until their use. Oral and anal samples were collected with cytobrush, in which the brush is inserted in the anal canal and oral cavity, and rotated 360º three times. They were later transported to the Virological Diagnostic Laboratory of UFF and kept at -20°C.

Questionnaire

Epidemiological data were collected at the enrollment visit to the STD Sector of UFF and Santa Casa de Misericórdia do Rio de Janeiro. For this, the doctor who collected the samples performed the questionnaire. Information collected was as follows: sociodemographic characteristics (including sexual history and sexual practices), history of STI, oral contraceptive consumption, and drug consumption (tobacco, alcohol).

DNA extraction

Samples were incubated in digestion buffer for 4 h at 56°C [10 mM Tris hydrochloric acid (pH 8.3), 1mM EDTA (pH 8.0), 0.5% Tween 20, 400µg/mL proteinase K] and subsequently extracted with the use of phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated with one-tenth volume of 0.3 M sodium acetate and three volumes of ice-cold 100% ethanol, washed with 70% ethanol, air-dried, and suspended in 50 µL of sterile water.

HPV Generic Polymerase chain reaction (PCR)

For detecting HPV-MY09/11[4], consensus primers that amplify 450-bp DNA sequences within the L1 region of HPV were used to detect generic HPV DNA. Amplification was carried out in 50 µL of reaction mixture [1 x PCR buffer, 200 mM deoxynucleotide triphosphates (dNTPs), 1.5 mM MgCl2, 50pmoles of each primer, 0.25 units (U) of Taq polymerase, and 5 µL of DNA sample] with 35 cycles of amplification. Each cycle included a denaturation step at 94°C for 1 min, an annealing step at 55°C for 2 min, and a chain elongation step at 72°C for 2 min with the use of a Lifetech DNA thermal cycler (USA). Beta-actin primers Ac1 and Ac2 (0.1pmol each), which amplify a 330-bp region of human DNA, were used as an internal control. Negative controls for checking contamination were added to the DNA template. PCR products were analyzed on 1.3% agarose gel with ethidium bromide staining for visualization of DNA under UV light; their molecular weights were determined by comparison with a 100-bp DNA ladder[5].

HPV genotyping – Specific PCR

Typing was done by PCR amplification with primers for the E6 gene of HPVs 6, 11, 16, 18, 31, 33, 35, 45, and 58[4]. All the 351 studied samples were submitted to this reaction. Amplification was carried out in 50µL of reaction mixture (1 x PCR buffer, 200mM dNTPs, 1.5mM MgCl$_2$, 50pmoles of each primer, 0.25U of Taq polymerase, and 5µL of DNA sample) with 35 cycles of amplification. Each cycle included a denaturation step at 94°C for 30 sec, an annealing step at 55°C for 30 sec, and a chain elongation step at 72°C for 1 min with the use of a DNA thermal cycler. Negative controls for checking contamination were added to the DNA template. PCR products were analyzed on 1.3% agarose gel with ethidium bromide staining for visualization of DNA under UV light; their molecular weights were determined by comparison with a 100-bp DNA ladder[5].

HPV genotyping – PCR (RFLP) analysis

HPV typing was done with RFLP (Restriction fragment length polymorphism) analysis after PCR amplification. The 450-bp amplicons resulting from the MY09/11 PCR were submitted to digestion by a panel of six restriction endonucleases (*BamHI, DdeI, HaeIII, HinfI, PstI*, and *RsaI*) (Invitrogen, Brazil). The pattern of length polymorphism of each sample was analyzed on 1.3% agarose gel with ethidium bromide staining for visualization of DNA under UV light and compared with the RFLP patterns for mucosal virus types, as described by Bernard et al.[6] and modified by Melgaço et al.[7]

Statistical analysis

A data bank was generated and analyzed by using the Epi InfoTM 7 statistical software package (Center for Disease Control and Prevention, Atlanta, USA). The associations of qualitative factors with HPV infection in the different sites were done with the chi-square test; two-by-two tables were used to evaluate the association between a possible risk factor ('Exposure') and an outcome ('Disease'). P-values<0.05 were considered significant.

RESULTS

The present study was composed by 351 samples from 117 patients, among which 33 were female and 84 were male. HPV DNA prevalence was 89.7% (105/117) in genital lesions, 53.8% (63/117) in oral samples, and 58.9% (69/117) in anal samples. Nine patients (7.7%) did not present infection in none of the three studied sites. On the other hand, in 43 patients (36.8%), HPV-DNA was detected in the three sites. A total of 108 out of the 117 studied cases (92.3%) had HPV infection in at least one site.

Regarding the risk factors associated with HPV in the studied patients' genital lesions, statistically significant rates for oral (0.039) and anal sex practices (p=0.0000012) were found (**Table 1**). Receptive anal sex was related with an HPV prevalence of 82.6% in genital site, whereas those patients relating non-receptive anal sex showed a prevalence rate of only 17.4% of HPV, as shown in **Table 1**. For oral samples, there was a relevant correlation concerning oral contraceptive use (p=0.039), tobacco smoking (p=0.036), and alcohol use (p=0.007) (**Table 2**). In anal samples, there were

statistically significant differences analyzing the presence of HPV in patients relating exclusive or non-exclusive sexual partners (p=0.013) (**Table 3**).

Regarding data according to sex, among the 33 female patients, 32 (96.9%) had HPV in at least one site. HPV was shown in 31 (93.9%) genital lesions, 14 (42.5%) oral samples, and 25 (75.7%) anal samples. For male patients, 78 (92.8%) were positive for HPV DNA, and analyzing by sample site, 74 (88.1%) genital lesions, 49 (58.3%) oral samples, and 44 (52.3%) anal samples infected by one or more genotypes were detected. No statistical differences were found for HPV genital, or oral infection and sex (p=1). Nevertheless, for anal tract, female samples presented statistically relevant HPV prevalence rates comparing to samples of males (p=0.016) (**Table 4**).

HPV genotyping showed that 89.9% of the studied samples presented low-risk types, whereas 10.1% presented high-risk HPV. Multiple infections were observed in 19.3% of samples, among which 85.2% were HPV 6/11 infections, with no relevant differences between sites. In nine samples (2.8%), defining the infecting genotype was not possible. The most frequent genotype in all HPV-positive samples was HPV 11 (46%), followed by HPV 6 (42.8%), HPV 18 (4.1%), HPV 16 (1.9%), and HPV 45 (1.6%). HPV genotypes 31, 33, 35, and 58 were not found in any of tested samples

(**Table 5**). HPV 11 was the most prevalent genotype in both genital lesions and oral samples. HPV 6 prevailed over HPV 11 only in the anal site, but with no statistical significance (p>0.05) (**Table 5**).

When analyzing genotypes by sex, HPV 6 was the most frequent in women, whereas HPV 11 prevailed in men. There were no statistical relevant differences concerning HPV types and sex (**Table 6**). On the other hand, in female oral tract, along with HPV 6 and 11, HPV45 was found in 7.1% of the samples. For male oral tract, besides HPV 6 and 11, HPV 16, 18, and 45 had 2% of prevalence each. Considering the female anal site, there were relevant differences in prevalence rates of HPV 6 and HPV 11 (28 *versus* 36%, p=0.02). Nevertheless, on male anal site, HPV 6 was detected in 31.8% of cases; HPV 11, in 25%; and HPV 18, in 6.8%.

The presence of viral DNA in all the three sites was observed in 36.8% of cases (43/117). Among them, 18% (21/117) presented concordant HPV genotypes: 12% (14/117) had at least one concordant type and 6% (7/117) had complete concordance among the three sites.

DISCUSSION

In our study, genital samples were obtained from benign clinical infections and presented 89.7% of HPV prevalence, as expected[1].

Table 1 – HPV DNA Prevalence in genital lesions and potential risk of factors in patients from Sexually Transmitted Diseases clinics of Universidade Federal Fluminense and Sexually Transmitted Diseases Clinics of Santa Casa de Misericórdia of Rio de Janeiro.

Factor	Positive genital HPV (%)	Negative genital HPV (%)	OR (95%CI)	p-value
Age (years old)				
15–25	52 (50)	3 (25)	3 (0.76–11.71)	0.089
>25	52 (50)	9 (75)		
Age of first sexual intercourse (years old)				
Up to 17	84 (84)	11 (91.6)	-	0.160
>17	16 (16)	0		
Sexual orientation				
Heterosexual	102 (97.1)	11 (91.6)	3.09 (0.29–32.31)	0.350
Homosexual (MSM and WSW)	3 (2.8)	1 (8.4)		
Oral sex				
Receptive	83 (79)	12 (100)	-	0.039
Non-receptive	22 (21)	0		
Anal sex				
Receptive	86 (82.6)	2 (16.6)	23.80 (4.81–118.9)	0.000
Non-receptive	18 (17.4)	10 (83.4)		
Partners				
Exclusive	64 (62.1)	6 (50)	1.64 (0.49–5.44)	0.300
Non-exclusive	39 (37.8)	6 (50)		
Use of condoms				
Regularly	13 (13.5)	2 (16)	0.62 (0.11–3.2)	0.42
Not regularly	83 (86.5)	8 (66.6)		
Oral contraceptive				
Uses	4 (20)	2 (100)	-	0.75
Does not use	25 (83.3)	0		
Alcohol consumption				
Yes	59 (56.2)	6 (50)	1.2 (0.38–4.2)	0.45
No	46 (43.8)	6 (50)		
Current smoker				
Yes	35 (33.3)	3 (0.25)	1.5 (0.38–5.89)	0.41
No	70 (66.7)	9 (75)		

OR reference value=1.0; MSM: men who have sex with men; WSW: woman who have sex with woman.

Patients describing anal sex practices had a higher risk for HPV detection, especially those presenting receptive anal practices (p<0.001). The same elevated risk was observed for patients with oral sex practices (p=0.039) (**Table 1**), although it remains as an undefined and poorly studied risk factor among those related to sexual behavior[8].

Out of clinically healthy oral samples, 53.8% presented HPV DNA. Studies have suggested that 0.6 to 14% of the population harbor HPV in their oral mucosa[9-12]. Our high prevalence rate suggests that the presence of genital lesion favors oral infection, by autoinoculation or by oral sex practices. Termine et al.[13] found higher prevalence rates of oral HPV infection in women presenting cervical HPV infection, which suggest that genital HPV infection might represent a predisposing condition to HPV in oral mucosa (14 *versus* 18%). A similar study conducted by Kofoed et al. to analyze genital lesions and oral HPV showed only 10.4% of HPV in oral samples[14]. Giraldo et al. and Badaracco et al. found 37.1% oral HPV in women showing cervical lesion, and 50% prevalence in Brazilian and Italian women, respectively[15,16]. However, Kreimer et al. when reviewing 4,070 subjects, found 4.5% oral prevalence of HPV (7.5% in developing countries)[17]. Therefore, there is huge disagreement about oral HPV prevalence and further studies are needed. Related risk factors, probably associated with sexual behavior, can explain such discrepancies along with different sample collection and diagnostic methods. To the best of our knowledge, there are no guidelines or protocols to study oral and anal samples collection, processing, transporting, or purification, in opposition to studies evaluating cervical samples.

When analyzing risk factors for oral infection, tobacco smoking (p=0.036) and alcohol use (p=0.007) were statistically related to HPV infection (**Table 2**). Alcohol use is already known to be associated with a poor effectiveness of the local immune system; and dehydration compromises immune cells migration, especially dendritic cells[18]. Besides that, alcohol is also associated with an altered sexual behavior, which can result in higher risk of exposition[19]. As to tobacco smoking, tobacco and nicotine are known for inducing not just changes of cervical cells, morphologically, but also in oral cells[20,21]. An unexpected correlation was found concerning oral contraceptive use, and a protection effect against HPV (p=0.039) was shown, maybe due to a periodical attendance in medical care and guidance on safe sex practices. Despite the suggestion about the use of oral contraceptives increasing the chances of cervical carcinogenesis associated with HPV infection, like Moreno et al. pointed out, t, but no studies linking risk of infection, hormones, and cancer were

Table 2 – HPV DNA Prevalence in oral cavity and potential risk of factors in patients from Sexually Transmitted Diseases clinics of Universidade Federal Fluminense and Sexually Transmitted Diseases Clinics of Santa Casa de Misericórdia of Rio de Janeiro.

Factor	Positive oral HPV (%)	Negative oral HPV (%)	OR (95%CI)	p-value
Age (years old)				
15–25	26 (41.2)	28 (52.8)	0.62 (0.30–1.31)	0.145
>25	52 (58.7)	25 (47.2)		
Age of first sexual intercourse (years old)				
Up to 17	47 (82.4)	41 (87.3)	2.29 (0.76–6.80)	0.106
>17	10 (17.5)	6 (12.7)		
Sexual orientation				
Heterosexual	61 (96.8)	51 (96.2)	1.19 (0.16–8.79)	0.620
Homosexual (MSM and WSW)	2 (3.2)	2 (3.7)		
Oral sex				
Receptive	53 (84.1)	41 (77.3)	1.5 (0.61–3.9)	0.240
Non-receptive	10 (15.8)	12 (22.7)		
Anal sex				
Receptive	10 (16.1)	6 (11.5)	1.47 (0.49–4.37)	0.330
Non-receptive	52 (83.8)	46 (88.5)		
Partners				
Exclusive	38 (61.2)	29 (54.7)	1.21 (0.57–2.50)	0.370
Not exclusive	26 (41.9)	24 (45.2)		
Use of condoms				
Regularly	10 (17.2)	6 (12.2)	1.49 (0.5–4.45)	0.320
Not regularly	48 (82.7)	43 (87.8)		
Oral contraceptive				
Uses	0	4 (23.5)	-	0.039
Does not use	12 (100)	13 (76.4)		
Use of alcohol				
Yes	42 (66.7)	23 (42.5)	2.96 (1.27–5.71)	0.007
No	21 (33.3)	31 (57.5)		
Current smoker				
Yes	25 (39.6)	13 (24)	2.075 (0.930–4.62)	0.036
No	38 (60.6)	41 (76)		

OR reference value=1.0; MSM: men who have sex with men; WSW: woman who have sex with woman.

conducted[22]. For Kreimer and colleagues, a new oral HPV infection was significantly higher in men who were single, separated, or widowed; for tobacco smokers and bisexual people the same was true, but was not related to oral sex practices[23].

Regarding the anal samples studied, there was a prevalence of HPV of 59% (**Table 3**). There are divergent results from few studies, and prevalence rates can vary from 1 to 28% in the immune competent population[14,24,25]. The studied patients presented genital HPV lesions that could favor HPV spread by either sex practices or auto inoculation. In a study with men who have sex with men (MSM), HPV rate in a healthy anal site reached 70%[26]. In fact, a significant association between infection and non-exclusive sexual partners was found, highlighting that an exclusive sexual partner protects against anal HPV infection (p=0.013) (**Table 4**).

In fact, when comparing both extragenital sites studied, no differences in rates of HPV infection were observed in healthy oral and anal sites (p>0.05), probably due to the anatomy-physiological similitude of these mucosal sites.

In the present study, the male population prevailed, and no statistical differences were found between genera regarding HPV infection for both anal and genital sites (**Table 4**). For oral infections, HPV DNA were more frequent in male than in female, but without statistical relevance. Our results partially agree with the available literature, which described oral HPV in men as three times more common than in women, and with higher risk of malignant conversion[27]. Some authors have also showed oral transmission related to sexual behavior and suggested a discrepant rate of HPV between sexes due to hormonal effects, anatomy-physiological differences between sites, as well as to sexual behavior. As to HPV infection in the anal site, the opposite of what was observed in oral mucosa was found here, with HPV prevalence in healthy anal mucosa higher in women than in men (p=0.016) (**Table 4**). Although anal mucosa can be infected by self-inoculation, and act as a reservoir which can

Table 4 – HPV DNA prevalence separated by sex and sites of infection.

Site of infection	Female (n=33)	Male (n=84)	p-value
	HPV (+) (%)	HPV (+) (%)	
Genital lesions	31 (93.9)	74 (88.1)	0.470
Oral sample	14 (42.5)	49 (58.3)	0.240
Anal sample	25 (75.5)	44 (52.3)	0.016
Total	70 (70.7)	167 (66.2)	0.390

Table 3 – HPV DNA Prevalence in anal site and potential risk of factors in patients from Sexually Transmitted Diseases clinics of Universidade Federal Fluminense and Sexually Transmitted Diseases Clinics of Santa Casa de Misericórdia do Rio de Janeiro.

Factor	Positive anal HPV (%)	Negative anal HPV (%)	OR (95%CI)	p-value
Age (years old)				
15–25	32 (47)	23 (4.8)	0.96 (0.46–2.05)	0.530
>25	36 (53)	25 (52)		
Age of first sexual intercourse (years old)				
Up to 17	57 (83.8)	38 (88.3)	0.68 (0.21–2.1)	0.350
>17	11 (16.2)	5 (11.7)		
Sexual orientation				
Heterosexual	66 (97)	46 (97.8)	0.710 (0.063–8.140)	0.630
Homosexual (MSM and WSW)	2 (3)	1 (2.2)		
Oral sex				
Receptive	54 (78.3)	40 (85.1)	0.63 (0.23–1.6)	0.240
Non-receptive	15 (21.7)	7 (17.5)		
Anal sex				
Receptive	11 (16.4)	5 (10.6)	0.23 (0.57–5.5)	0.230
Non-receptive	52 (77.6)	42 (89.4)		
Partners				
Exclusive	10 (16.3)	16 (34)	0.330 (0.135–0.823)	0.013
Not exclusive	51 (85.2)	31 (66)		
Use of condoms				
Regularly	10 (16.3)	6 (13.6)	1.24 (0.41–3.71)	0.450
Not regularly	51 (85.2)	38 (86.3)		
Oral contraceptive				
Uses	2 (8.4)	2 (28.5)	0.21 (0.02–2.02)	0.210
Does not use	22 (91.6)	5 (71.5)		
Use of alcohol				
Yes	25 (36.2)	21 (43.8)	0.73 (0.34–1.55)	0.260
No	44 (63.7)	27 (56.2)		
Current smoker				
Yes	25 (36.2)	13 (27)	1.53 (0.68–3.41)	0.200
No	44 (63.7)	35 (73)		

OR reference value=1.0; MSM: men who have sex with men; WSW: woman who have sex with woman.

eventually result in injury[27] our results suggest that the acquisition of infection at this site, though asymptomatic, is more frequently associated with sexual behavior, as suggested by King et al.[8]

The efficacy of the vaccine against oral HPV infection is under study[28]. High prevalence, found by the present study for both sexes, indicated the relevance of analyzing HPV vaccine for oral infections, as the oral mucosa has several factors that contribute to virus infection and mainly act as a source of viral transmission. A study in Costa Rica with women who were vaccinated showed a decreased of oral genotypes prevalence in the vaccine after four years of vaccination[29]. For men, further studies are needed to better report on the benefits of HPV vaccination in this population, due to the higher prevalence of oral HPV infection, already shown[27].

Regarding the viral genotypes evaluated in the present study, the most prevalent low-risk genotypes were detected worldwide: HPV6 and 11 (**Table 5**)[30-33]. Certain prevalence discrepancies between the

Table 5 – HPV genotypes Prevalence found in the 117 patients from Sexually Transmitted Diseases Clinics of Universidade Federal Fluminense and Sexually Transmitted Diseases Clinics of Santa Casa de Misericórdia do Rio de Janeiro.

Site of infection	Prevalence of HPV genotypes						
	HPV6 (%)	HPV11 (%)	HPV16 (%)	HPV 18 (%)	HPV45 (%)	HPVx* (%)	HPV mixed** (%)
Genital lesions	34 (29)	37 (31.6)	2 (1.7)	1 (0.9)	1 (0.9)	-	30 (25.6)
Oral sample	14 (11.9)	21 (17.9)	1 (0.9)	1 (0.9)	3 (2.5)	4 (3.4)	19 (16.2)
Anal sample	21 (17.9)	20 (17.1)	-	4 (3.4)	-	5 (4.2)	19 (16.2)
Total***	69 (59)	78 (66.6)	3 (2.5)	6 (5.1)	4 (3.4)	9 (7.6)	68 (58.1)

*HPVx: Positive samples for HPV generic PCR (MY06/MY11) but negative for Specific PCR (for all six different primers tested); **HPV mixed: The presence of two or more different genotypes; ***n=117 genital, 117 oral, and 117 anal samples.

Table 6 – Prevalence of HPV genotypes in different sites, separated by sex.

Female genital lesion (n=31)*		Male genital lesion (n=74)*	
HPV genotype	Samples (%)	HPV genotype	Samples (%)
HPV6	12 (38.7)	HPV6	22 (29.7)
HPV11	11 (35.5)	HPV11	26 (35.1)
HPV6 and HPV11	6 (19.3)	HPV6 and HPV11	21 (28.3)
HPV11 and HPV18	1 (3.2)	HPV16	2 (2.7)
HPV6 and HPV11 HPV18	1 (3.2)	HPV18	1 (1.4)
-	-	HPV45	1 (1.4)
-	-	HPV6 and HPV16	1 (1.4)

Female oral samples (n=14)**		Male oral samples (n=49)**	
HPV genotype	Samples (%)	HPV genotype	Samples (%)
HPV6	5 (35.7)	HPV6	9 (18.3)
HPV11	4 (28.5)	HPV11	17 (34.7)
HPV6 and HPV11	4 (28.5)	HPV6 and HPV11	12 (24.5)
-	-	HPV16	1 (2)
-	-	HPV18	1 (2)
HPV45	1 (7.1)	HPV45	2 (4)
-	-	HPV6 and HPV18	1 (2)
-	-	HPVV11 and HPV18	1 (2)
-	-	HPV6 and HPV11 HPV16 and HPV18	1 (2)
		HPVX	4 (8.1)

Female anal samples (n=25)***		Male anal samples (n=44)***	
HPV genotype	Samples (%)	HPV genotype	Samples (%)
HPV6	7 (28)	HPV6	14 (31.8)
HPV11	9 (36)	HPV11	11 (25)
HPV6 and HPV11	6 (24)	HPV6 and HPV11	9 (20.4)
HPV18	1 (4)	HPV18	3 (6.8)
HPV6 and HPV11 HPV18	2 (8)	HPV11 and HPV18	1 (2.2)
-	-	HPV6 and HPV11 HPV16 and HPV18 HPV45	1 (2.2)
-	-	HPVX	5 (11.3)

*Total of 31 female genital positive samples and 74 male genital samples positive for the virus; **total of 14 female oral positive samples and 49 male oral positive samples for the virus; ***total of 25 female anal positive samples and 44 positive male anal samples for the virus.

different sites of infection separately by sex were also observed. Primarily, observing the same anatomical site in different sexes, HPV6 was more prevalent in female oral mucosa than in male oral mucosa. This finding is interesting, since a similar percentage was expected for both sexes. However, similar HPV11 oral mucosa's prevalence in both sexes was observed (**Table 6**, p>0.05). Indeed, a statistical difference of HPV 11 prevalence comparing the anal sites could be noted, with a higher prevalence in women (p=0.003). To summarize, differences in anatomy and physiology between male and female hosts could explain such discrepancies in prevalence.

As shown in **Table 6**, comparing different anatomical sites and same sex, HPV11 obtained a higher prevalence compared to the male anal mucosa in which HPV 6 prevailed. This discrepancy may be explained by site restriction factors, allowing HPV11 to adapt to male mucosa due to a higher plasticity compared to HPV6, and hence a wide spread in different host sites. Moreover, among the 12 cases negative for the virus in genital lesions, there were three cases in which HPV DNA was exclusively detected in oral mucosa, all with HPV11. In the present study, HPV 11 in different sites of infection was observed, especially in the oral mucosa, reinforcing that this genotype may have undergone evolutionary changes leading to better adaptation to different sites. It is also possible that some intrinsic factor of the host, such as the body's defense system that favors some front sites of infection to other HPV genotypes, could be responsible for doing that.

Although healthy tissue samples with high prevalence of low-risk oncogenic genotypes were studied, it is of utmost importance remembering that the presence of high-risk types can determine the malignant conversion in these tissues. Throughout the entire study, 2.5% HPV 16; 5% HPV18; and 3.4% HPV45 in single infection were found. The Global prevalence of HPV 16 found in reviews by Kreimer et al. was 1.3%, and men and women had the same prevalence[17], reinforced by Bruni et al. HPV 45 was also detected in both sexes, in healthy oral mucosa. As it is described as an insidious HPV genotype that can be found in malignant lesions but not frequently in premalignant ones, it can represent a silent carcinogen. The considered low-risk genotypes may be involved in the malignant transformation of all the three studied sites[34,35].

There are few studies that assess the concurrent infections in the genital, oral, and anal sites; and even more scarce is the work studying heterosexual populations. When the presence of HPV in the different sites were analyzed, the presence of viral DNA in the three sites (genital, oral, and anal) were found simultaneously in 36.8% of cases (43 total cases, 13 females and 30 male). For Videla and colleagues, and King and colleagues, the prevalence of HPV in the three sites reached 6 and 9% in asymptomatic men who have sex with men (MSM), HIV-positive and HIV-negative, respectively[8,36]. Our results are not in agreement with what was mentioned by the authors but we studied a population with genital lesions already diagnosed, which increases the chances of finding the viral DNA in other sites of infection.

Scientific literature has shown several works with infections in paired sites data. Seen that, we also evaluated paired sites. Our study found the presence of viral DNA in the genital and anal sites, simultaneously, in 57.2% of patients (67/117 OR=8.80, 95%CI=1.83–42.35, p=0.002; RR=3.82). This high prevalence can be possibly explained by anatomical proximity of the two sites of infection, and the ratio risk of genital infection extending itself to the anal tract was almost four times. Simultaneous DNA viral presence in the oral and genital sites was observed in 49.5% of patients (58/117 OR=1.70, 95%CI=0.51–5.79; p=0.27, RR=1.32), which can also be explained by the sexual practices of the population studied, since only 13% of them did not practice oral sex (**Table 1**). Finally, the concurrent oral and anal infections were found in 38.4% of studied patients (45/117 OR=3.12, 95%CI=1.45–6.72; p=0.002; RR=1.61), with lower risk, but still relevant. Kofoed et al. found concomitant anal/genital infections in 78.1% of studied patients, oral/anal infections in 21.7% of them, and oral/genital infections in 60.9% of the studied subjects[14]. Videla and colleagues found that percentage of simultaneous infection in paired sites was lower than in other studies: 21% in the anal/genital sites, 14% in oral/anal, and 7% in oral/genital[36]. This wide divergence of results confirms that there is still much to learn about the natural history of the virus, since HPV may have a different biological behavior in different populations and we may be dealing with important socio-epidemiological differences among the populations studied.

One of the study limitations was the reduced sample size. Besides that, there are no guidelines or protocols to study oral and anal samples collection, processing, transporting, or purification, in opposition to studies evaluating cervical samples, which reinforces the need for subsequent studies. Nevertheless, the high prevalence of HPV in multiple sites of patients showing genital infection was described, thus contributing to the knowledge of the natural history of HPV.

Our results support that genital HPV infections do not predispose an oral infection, or the inverse: an oral infection does not indicate the viral presence in the genital site for both sexes, as well as the results described in the Rautava and Syrjänen meta-analysis show[37]. However, there are high rates of concordant types, different from what was described by these authors. When studying the 43 patients who had infections simultaneously in the three sites, 14 (12%) achieved an agreement of at least one viral type and seven (6%) cases obtained the full agreement of the types in the three sites, totaling 21 (18%) concordant cases. Termine and colleagues stated that concordant types are most prevalent (24%) than expected by chance, suggesting a dependent relation between sites.

Strengths

The high prevalence of HPV DNA found by the present study, in both sexes, indicates the relevance of analyzing HPV vaccine for oral infections, as the oral mucosa has several factors that contribute to virus infection and mainly act as a source of viral transmission. Data herein contribute to the knowledge of HPV's natural history.

Limitations

One of the study limitations was its reduced sample size. Besides that, there are no guidelines or protocols to study oral and anal samples collection, processing, transporting or purification, in opposition to studies evaluating cervical samples, which reinforces the need for subsequent studies to evaluate guidelines for diagnosing HPV.

CONCLUSION

In the present study, the relation among HPV genotypes and site of infection were described. It seems to be an important feature regarding the natural history of infection with great spread of efficiency in the host.

Participation of each author

Methods: TIC, KCS, WMR, DCL. Paper writing: TIC, MRLP, CMK, SMBC. Analysis: WMR, SMBC. Sample collection: MRLP, TDG. P Supervisor on methods: SMBC.

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Conflict of interests

There is no conflict of interests to be declared.

Approval by the Human Research Ethics Committee

The present study was approved by the Ethics Committee of UFF (CAAE: 36683514.0.0000.5243).

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