doi: 10.5216/rpt.v44i4.39241

ORIGINAL ARTICLE

EVALUATION OF ANOGENITAL HUMAN PAPILLOMAVIRUS INFECTION IN ASYMPTOMATIC MEN FROM RIO DE JANEIRO, BRAZIL

Willker Menezes da Rocha[1], Larissa Alves Afonso[1], Elisabete Dobao[2], Tegnus Depes Gouvea[3], Fernanda Nahoum Carestiato[1] and Silvia Maria Baeta Cavalcanti[1]

ABSTRACT

Infection by human papillomavirus (HPV) is among the most prevalent sexually transmitted diseases worldwide. However, there are still gaps in the knowledge regarding the natural history of HPV infection in men. This study aimed to determine the prevalence of HPV infection in penile swab samples, derived from a clinically asymptomatic male population. For this purpose, 261 samples were collected between January 2011 and July 2013 in different institutions in the city of Rio de Janeiro. These samples were collected from the glans, corona, frenulum and coronal sulcus of the penis. Viral identification was made through generic and type-specific Polymerase Chain Reaction, and Restriction Fragment Length Polymorphism techniques. Prevalence of HPV infection was 16.5% (43 subjects). The most prevalent HPV type was HPV6 (34.9%), followed by HPV16 (23.3%), HPV11 (16.3%), HPV45 (9.3%) and HPV58 (2.3%). Hence, infection was associated with low-risk oncogenic types in 53.7% of the studied individuals, while high-risk oncogenic types were detected in 46.3%. Statistically significant results were found for the group of men who have sex with men, the group who have active anal intercourse, and subjects that lacked circumcision. After adjustments, sexual behavior and lack of circumcision remained as independent risk factors for HPV infection. We believe that these results may contribute to a clearer view about the circulation of HPV in the general male population, as well as to the identification of risk factors associated with the epidemiology of HPV infection in our state.

KEY WORDS: Papillomavirus infections; sexually transmitted diseases; men, asymptomatic infections; polymerase chain reaction.

1. Department of Microbiology and Parasitology, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil.
2. Department of Dermatology, Santa Casa de Misericórdia do Rio de Janeiro, Brazil.
3. Sexually Transmitted Diseases Clinics, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil.

Corresponding author: Dr. Silvia Maria Baeta Cavalcanti. Dept. de Microbiologia e Parasitologia/UFF. Rua Prof. Ernani Melo 101, CEP 24210-130 Niterói, RJ, Brazil. E-mail: silviacavalcanti@vm.uff.br

Received for publication: 6/5/2015. Reviewed: 9/9/2015. Accepted: 15/9/2015.

RESUMO

Avaliação da infecção anogenital por papilomavírus humanos em homens assintomáticos do Rio de Janeiro, Brasil.

A infecção pelo papilomavírus humano (HPV) está entre as doenças sexualmente transmissíveis (DST) mais prevalentes no mundo. No entanto, ainda existem lacunas no conhecimento sobre a história natural da infecção por HPV em homens. Este estudo teve como objetivo determinar a prevalência de infecção por HPV em amostras de esfregaços penianos derivadas de uma população masculina clinicamente assintomática. Para tanto, 261 amostras foram coletadas entre janeiro de 2011 e julho de 2013 em diferentes instituições da cidade do Rio de Janeiro. As amostras foram coletadas da glande, corona, frênulo e sulco coronal. A identificação viral foi feita por meio das técnicas de Reação em Cadeia da Polimerase genérica e tipo-específica e de Polimorfismo do Comprimento do Fragmento de Restrição. A prevalência da infecção por HPV foi de 16,5% (43 indivíduos). O tipo de HPV mais prevalente foi o HPV6 (34,9%), seguido pelo HPV16 (23,3%), HPV11 (16,3%), HPV45 (9,3%) e HPV58 (2,3%). Assim, a infecção foi associada a tipos de baixo risco oncogênico em 53,7% dos indivíduos estudados e a tipos de alto risco oncogênico em 46,3% destes indivíduos. Resultados estatisticamente significativos foram encontrados para o grupo de homens que fazem sexo com homens, o grupo que afirma manter relações sexuais anais e indivíduos não circuncidados. Após ajustes estatísticos, o comportamento sexual e a não circuncisão permaneceram como fatores de risco independentes para a infecção por HPV. Acreditamos que estes resultados possam contribuir para uma visão mais clara a respeito da circulação do HPV na população masculina em geral, bem como para a identificação dos fatores de risco associados com a epidemiologia da infecção por HPV em nosso estado.

DESCRITORES: Infecções por papilomavírus; doenças sexualmente transmissíveis; homens; infecções assintomáticas; reação em cadeia da polimerase.

INTRODUCTION

Human papillomavirus (HPV) infection causes one of the most prevalent sexually transmitted diseases (STDs) worldwide. The pathological and epidemiological features of HPV infection have been studied extensively in women due to the high prevalence of this disease and its well-established link to cervical cancer (CC) (WHO, 2012).

Because most HPV infections in men are asymptomatic and the male population is not routinely screened for HPV, men may act as reservoirs of HPV infection, resulting in continuous transmission of both high-risk and low-risk HPV types to women (Castellsagué et al., 2002). In addition to being vectors of anogenital HPV infection, men have recently been recognized to manifest the pathological features of this disease, mainly through anogenital warts and their consequences; anal intraepithelial neoplasia (AIN); penile intraepithelial neoplasia (PIN), and invasive carcinoma (Palefsky, 2010; Garland, 2010).

Now that prophylactic HPV vaccines are known to be efficacious in men, understanding the factors associated with HPV acquisition in men

is critical to the development of comprehensive preventive programs to control HPV infection (Giuliano et al., 2009). Few studies have examined the epidemiology and risk factors associated with HPV infection in the male population. In a systematic review, Dunne et al. (2006) reported that half of the published papers concerning HPV prevalence among healthy subjects pointed out rates of infection of approximately 20%, although it can vary between different populations, sampling methods and diagnostic methodologies.

The aim of our study was to describe the prevalence of HPV DNA among asymptomatic male subjects resident in Rio de Janeiro State in order to evaluate its circulation and the risk factors associated with HPV infection.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study evaluated HPV infection in asymptomatic men, recruited from several institutions of the city of Rio de Janeiro, namely: the STD clinic of the Universidade Federal Fluminense, the dermatology clinic of Santa Casa da Misericórdia, a private university, and a metallurgical factory near to Rio de Janeiro (MAENFE). The study was carried out between January 2011 and July 2013, and aimed to evaluate the prevalence of HPV infection among asymptomatic men and the occurrence of possible socio-epidemiological risk factors associated with the infection. The clinical sample size calculation determined that a minimum of 139 subjects were required for this study (for a 95% confidence interval), based on the estimated prevalence of asymptomatic HPV infection among the Brazilian male population, according to data released by the Brazilian Urology Society (Sociedade Brasileira de Urologia, 2013). The queried data mentions that the incidence of new cases among the Brazilian male population is approximately 60,000 cases/year, and that the total estimated prevalence is around 600,000 cases over the country, of which 540,000 are subclinical infections. Furthermore, it is mentioned that the estimated prevalence for the metropolitan region of Rio de Janeiro is 10% of this value, totaling approximately 54,000. Therefore, to assess the representativeness of the entire population of Rio de Janeiro, accounting for 10 million inhabitants, 139 individuals would be necessary.

The participants did not present any clinical anogenital lesions related to clinical features of HPV infection. The exclusion criteria were: age under 18 years, and presence of anogenital lesions histopathologically compatible with HPV.

The Ethics Committee of Instituto Oswaldo Cruz from Fundação Oswaldo Cruz approved this study (CEP IOC FIOCRUZ 567/2010), and all the

subjects signed an informed consent form. The socio-epidemiological information (number of sexual partners, sexual behavior, circumcision, hygiene habits, tobacco use, history of STD, use of condom and anal intercourse) were collected from all participants by a structured questionnaire.

Sampling

The volunteers were clinically evaluated for the occurrence of genital symptoms related to HPV. Individuals considered asymptomatic exclusively for this type of infection were invited to join this study. A total of 261 samples were collected from the glans and balanopreputial sulcus with a swab used for cytological examinations, which was twisted clockwise three times at the anatomical site of sampling and, after that, kept in TE solution [10 mM Tris hydrochloride (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA)] (Invitrogen, USA) at –20 °C until DNA extraction. Then, the participants went through the informed consent form, and the socio-epidemiological questionnaire.

DNA extraction, PCR amplification and genotyping

Samples were incubated for 4 hrs at 56 °C in 1 mL digestion buffer [10 mM Tris hydrochloric acid (pH 8.3), 1 mM EDTA (pH 8.0), 0.5% Tween 20, 400 µg/mL proteinase K (Invitrogen, USA)], then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) (Invitrogen, USA). DNA was precipitated with 300 µL 0.3 M sodium acetate plus 900 µL of ice-cold ethanol, washed with 70% ethanol, air dried, and suspended in 50 µL of sterile water. MY09/11 consensual primers for HPV detection, which amplify 450 bp DNA sequences at the L1 region, were used to detect generic HPV DNA via polymerase chain reaction (PCR). Thirty-five amplification cycles were carried out in 50 µl of reaction mixture (1x PCR buffer, 200 mM deoxyribonucleoside triphosphates, 1.5 mM $MgCl_2$, 50 pmol of each primer, 0.25 U Platinum®Taq DNA polymerase [Life Technologies®], 5 µL sample) using a DNA thermal cycler (Life Technologies, USA). Each cycle comprised denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and chain elongation at 72 °C for 2 min. The beta-actin primers Ac1 and Ac2 (0.1 pmol each), which amplify a 330 bp region of human DNA, were used as internal sample controls. Genotyping was performed by PCR amplification with type-specific primers targeting the E6 gene sequences of low-risk (LR) HPVs 6, 11 and 53, and high-risk (HR) HPVs 16, 18, 31, 33, 35, 45, 56, and 58, as previously described (Carestiato et al., 2006). Thirty-five amplification cycles were carried out in 50 µL reaction mixture with denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and chain elongation at 72 °C for 1 min.

For generic and specific genotyping, negative controls for background contamination were added to DNA templates. PCR products were analyzed on 1.3% agarose gel with ethidium bromide staining to visualize DNA under ultraviolet light, and their molecular weights were determined by comparison with a 100 bp DNA ladder (Afonso et al., 2012).

Restriction fragment length polymorphism (RFLP) analysis for HPV genotyping

Some samples which were positive for the generic HPV DNA detection could not be identified by the 11 primers used in the type-specific reaction. Therefore, for the further genotyping of these samples, the RFLP technique was performed.

RFLP was performed following PCR amplification using the 450 bp amplicons from the MY09/11 PCR. The samples untyped by the type-specific PCR were submitted to digestion by a panel of six restriction endonucleases (BamHI, DdeI, HaeIII, HinfI, PstI, RsaI) (Invitrogen, Brazil). The pattern of length polymorphism of each sample was analyzed under ultraviolet (UV) light and compared with RFLP patterns for mucosal virus types, as described by Melgaço et al. (2011).

Statistical analysis

A database was generated and analyzed using EpiInfo 8.0 (CDC). Biological data were compared using Fisher's exact test ($p<0.1$). Risk factors, HPV genotypes and socio-demographic features were evaluated. Associations of LR and HR HPV infections with socio-epidemiological variables were examined.

RESULTS

The studied group was composed of 261 asymptomatic men, showing no clinically detectable HPV lesions. The average age of participants was 26.3 years, ranging from 18-65 years. The HPV infected group had an average age of 29.8 years old; for the HPV-negative subjects this was 25.3. No statistical differences were detected between them ($p>0.05$) (Table 1).

Table 1. HPV DNA prevalence according to socio-demographic characteristics of the studied population.

Variables	HPV+ (positive/total)	HPV- (negative/total)	n	p-value
Age				
Less than or equal to 24 years	19	65	84	p>0.05
25 years or more	24	153	177	
Sexual behavior				
MSM	8	15	23	p=0.047
MSW	38	121	159	
Number of sexual partners				
1-2	7	29	36	p>0.05
>2	36	110	146	
Condom use				
Yes	2	21	23	p>0.05
No	41	118	159	
Anal intercourse				
Yes	21	37	58	p=0.049
No	31	93	124	
Previous history of STD				
Yes	1	7	8	p>0.05
No	42	132	174	
Hygiene habits (baths/week)				
Six or less	-	13	13	p>0.05
Seven or more	43	126	169	
Tobacco smoking				
Yes	13	85	98	p>0.05
No	30	54	84	
Circumcision				
Yes	-	6	6	p=0.002
No	43	133	176	
Total	43	139	182	

Regarding HPV infection, HPV DNA was detected in 16.5% of the patients (43/261). HPV 6 was the most prevalent type (34.8%, 15/43), followed by HPV 16 (23.3%, 10/43), HPV 11 (16.3%, 7/43), HPV45 (9.3%, 4/43) and HPV 58 (2.3%, 1/43). The HR types, 16, 18, 45 and 58, were found in 43.4% of the cases. LR types 6 and 11 were the predominant types (53.7%). Multiple infections were found in 9.3% (4/43) of the samples (namely: HPV types 16 and 45, 11 and 58, 45 and 35 and 6 and 16). All these multiple infections were detected by the RFLP technique. Two samples (4.7%) presented HPV

DNA according to MY09/11 PCR, but typing by both PCR specific primers and RFLP was inconclusive, thus they are referred to as HPV X (Table 2).

Table 2. Prevalence of HPV genotypes according to PCR and RFLP in male smears (n=43).

HPV type	n (%)
HPV 6	15 (34.9%)
HPV 11	7 (16.3%)
HPV 16 *	10 (23.3%)
HPV 45 *	4 (9.3%)
HPV 58 *	1 (2.3%)
HPV X[a]	2 (4.7%)
Co-infections[b] *	4 (9.3%)
Total	43 (100%)

[a] - MY(+)PCR untyped by type-specific PCR or RFLP techniques. [b] - Individuals infected by more than one type of HPV (HPV types, namely: 16 and 45, 11 and 58, 45 and 35 and 6 and 16). * - High oncogenic risk HPV types.

It is interesting to notice that older men presented exclusively oncogenic HPV genotypes, compared with younger subjects ($p<0.0001$).

Socio-demographic factors that could be associated with risk of infection were analyzed: mean age, sexual behavior, number of sexual partners, hygiene habits, circumcision, history of previous STD, tobacco smoking, active anal intercourse and condom use. Analysis revealed that there were statistically significant differences regarding: sexual behavior ($p=0.047$), active anal intercourse ($p=0.049$) and lack of circumcision ($p=0.026$) (Table 1). After adjustment, both sexual behavior (AOR=11.0, $p=0.02$) and lack of circumcision (AOR+, $p=0.001$) remained as independent risk factors for HPV infection. Anal intercourse lost its statistical significance as a risk factor after adjustment (AOR=1.2, $p=0.4$).

DISCUSSION

The prevalence of human papillomavirus (HPV) anogenital infections among healthy subjects ranges from 1.3% to 72% depending on the population studied and the diagnostic method used, and it accounts for 5% of all cancers worldwide (Dunne et al., 2006). Within these rates there is the cervical carcinoma, which serves until now as a paradigm for understanding the carcinogenesis caused by high-risk HPV infections (WHO, 2012).

As penile carcinoma is a rare tumor in developed countries, and its etiology still remains under discussion, little is known about HPV infection in men (Kreuter et al., 2008). Recent studies have provided considerable evidence about the oncogenic potential of some high-risk HPV types in the male anogenital tract (Hoots et al., 2009; Palefsky, 2010). Hence, HPV infection in men has become an important object of research and discussion, instead of being considered solely a source of transmission to the feminine population (Fox, 2009). Although studies of HPV prevalence in male anogenital lesions have yielded highly variable results, we have found a prevalence rate of 16.7%, which is similar to several studies from Brazil and other countries (Vardas et al., 2011; Carestiato et al., 2006). Nevertheless, an important study conducted in different countries (the HIM [HPV in men] study) (Giuliano et al., 2008) described HPV DNA rates higher than 70%. This high rate of HPV infection could be explained, in part, by the fact that the HIM study was conducted with subjects in the general population, not excluding individuals with clinical symptoms like warts. Besides that, differences in HPV prevalence may reflect sociocultural and demographic peculiarities (Gravitt, 2011).

Among the positive results for the detection and typing of the viral genetic material, we found a higher prevalence of HPV 6, followed by HPV 11, HPV 16, HPV 45 and HPV 58 (Table 2). Dobao et al. (2015), studying a similar population from Rio de Janeiro, also observed low variability in detected HPV genotypes (predominantly HPVs 6, 11 and 16). In agreement with the meta analysis performed by Dunne et al. (2006), these are the most prevalent HPV genomes; but the review pointed out that undetermined types were also commonly described. Different from other studies, multiple infections were not frequent in our sample (9.30%) and its role in the establishment and progression of the disease remains inconclusive (Table 2) (Ferlay et al., 2014).

It is also interesting to observe that although there are no differences concerning the rates of infection, there was a statistical upward trend regarding HPV infection and age, revealing that in older men only high-risk types were detected. It is interesting to note the absence of HPV 18 in these results, since this is considered one of the most prevalent types in the female population (Chen et al., 2009). The absence of HPV 18 was consistent with other recent studies of male subjects (Afonso et al., 2012; Vardas et al., 2011). On the other hand, the presence of HPV 45 was remarkable, and recent studies have shown

an increasing prevalence among the female population (WHO, 2012). HPV 45 is currently considered to be the second most prevalent type in cases of cervical cancer in Brazil, being associated with insidious cases with difficult early detection (Fernandes et al., 2009). These results draw attention to the increased circulation of this type in the world, including the male population, which makes it relevant to understand its inclusion as well as other emerging viral types in the recently approved nonavalent prophylactic vaccine in order to extend the immunization coverage for the types with major clinical relevance (Ferlay et al., 2014).

It has been demonstrated that sampling and methodological strategies used in prevalence studies may influence diagnostic failure, due to the characteristics of HPV infection sites in men and to the lack of a routine regarding preventive screening to detect penile/anal lesions in at-risk men (Palefsky, 2009). The samples used in our study were taken from the glans, corona, frenulum and coronal sulcus of the penis, locations which are known to have higher prevalence of HPV infection when compared to other anatomical sites of the male genitalia (Vardas et al., 2011). Even with this methodological approach, it is possible that individuals with HPV-related lesions in other sites of the penis might have been diagnosed as false negatives.

In our study, among the evaluated risk factors shown to increase the risk of anogenital HPV infection in men, we identified statistically relevant correlation between HPV DNA detection and sexual behavior, anal intercourse and lack of circumcision (Table 1). After adjustment, both sexual behavior and lack of circumcision remained as independent risk factors for HPV infection. Other authors have described that sexual behavior such as the number of lifetime sexual partners and men who have sex with men, is associated with viral infection, particularly when it relates to HIV status (Giuliano et al., 2008; Partridge & Koutsky, 2006). Corroborating our data, some studies have shown male circumcision as a protective practice and associated with a reduced risk of penile HPV infection and cancer (Castellsagué et al., 2002; Nielson et al., 2009).

The quadrivalent HPV vaccine is highly efficacious for the prevention of anogenital warts and precancerous cervical, vulvar, and vaginal lesions, prompting efforts to define its role in the prevention of male genital disease (Palefsky, 2010). Although the protective efficacy of HPV vaccination in men has not been fully established, pending the outcomes of public policy discussions and cost-efficacy studies, a strong rationale may underlie the vaccination of boys, like girls, at an early age, when they have engaged in limited or no sexual activity. In our study, nearly 75% of the studied subjects presented infection by genotypes covered by the quadrivalent vaccine, reinforcing the suggestion that this vaccine might be highly effective in reducing external genital lesions in young men (Palefsky et al., 2011).

In conclusion, we suggest that for high-risk groups, an appropriate

method of screening should be established as soon as possible, but we believe that in the short-term, clinical knowledge of this pathology should be encouraged by physicians who give assistance to this population, to be alert and think of this pathology among the diagnostic hypothesis even in the absence of anogenital warts. Education has a relatively low cost, quick results and will be as or more effective than laboratorial methods for early diagnosis. Finally, it is important to eliminate the idea, that HPV infection in men does not deserve great concern, without recognition of its importance.

REFERENCES

1. Afonso LA, Moyses N, Alves G, Ornellas AA, Passos MR, Oliveira LH, Cavalcanti SM. Prevalence of human papillomavirus and Epstein-Barr virus DNA in penile cancer cases from Brazil. *Mem Inst Oswaldo Cruz 107*: 18-23, 2012.
2. Castellsagué X, Bosch XF, Munõz, Meijer CJLM, Shah KV, Sanjose S, Eluf-Neto J, Ngelangel CA, Chichareon S, Smith JS, Herrero R, Moreno V, Franceschi S. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. *Engl J Med 346*: 1105-1112, 2002.
3. Chen Z, DeSalle R, Schiffman M, Herrero R, Burk RD. Evolutionary Dynamics of Variant Genomes of Human Papillomavirus types 18, 45 and 97. *J Virol 8*: 1443-1455, 2009.
4. Carestiato FN, Silva KC, Dimetz T, Oliveira LHS, Cavalcanti SMB. Prevalence of human papillomavirus infection in the genital tract determined by hybrid capture assay. *Braz J Infect Dis 10*: 331-336, 2006.
5. Dobao EAR, Afonso LA, Menezes W, Pires C, Kawa Kac B, Pesca LF, Fonseca LD, Nery JA, Nicol AF, Cavalcanti SMB. *Evaluation of anogenital human papillomavirus infection in men attending a dermatology clinic.* Virus Rev Res, 2015. Disponível em: http://157.86.113.86/index.php/vrrjournal/article/viewFile/96/131. Acesso em 05/10/2015.
6. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: a systematic review of the literature. *J Infec Dis 194*: 1094-1057, 2006.
7. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer 136*: E359-E386, 2014.
8. Fernandes V, Meissner RV, Carvalho MGF, Fernandes TAAM, Azevedo PRM, Villa LL. Prevalence of HPV infection by cervical cytologic status in Brazil. *Int J Gynaecol Obstet 105:* 21-24, 2009.
8. Fox P. Anal cancer screening in men who have sex with men. *Curr Opin HIV AIDS 4*: 64–67, 2009.
9. Garland SM. Strategies against human papillomavirus in males. *Gynecologic Oncology 117*: 20-25, 2010.
10. Giuliano AR, Lu B, Nielson C. Age Specific Prevalence, Incidence and Duration of Human Papillomavirus Infections Among a Cohort of 290 US Men. *J Infect Dis 17*: 234-239, 2008.
11. Giuliano AR, Lazcano E, Villa LL, Flores R, Salmeron J, Lee JH.Circumcision and sexual behavior: factors independently associated with human papillomavirus detection among men in the HIM study. *Int J Cancer 124*:1251-1257, 2009.
12. Gravitt PE. The known unknowns of HPV natural history. *J Clin Invest 121*: 4593-4599, 2011.

13. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer 124*: 2375-2383, 2009.

14. Kreuter A, Brockmeyer NH, Weissnborn SJ, Gambichler T, Stücker M, Altmeyer P, Pfister H, Wieland U. Penile intraepithelial neoplasia is frequent in HIV-positive men with anal dysplasia. *J Invest Dermatol 128*: 2316-2324, 2008.

15. Melgaço FG, Rosa MLG, Augusto EF, Haimuri JGS, Jacinto C, Santos LS, Cavalcanti SMB, Oliveira LHS. Human papillomavirus genotypes distribution in cervical samples from women living with immunodeficiency virus. *Arch Gynecol Obstet 283*: 809-817, 2011.

16. Nielson CM, Schiaffino MK, Dunne EF, Salemi JL, Giuliano AR. Associations between male anogenital human papillomavirus infection and circumcision by anatomic site sampled and lifetime number of female sex partners. *J Infect Dis 199*: 7-13, 2009.

17. Palefsky JM. Anal cancer prevention in HIV-positive men and women. *Curr Opin Oncol 21:* 433-438, 2009.

18. Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. *J Adolesc Health 13*: S12-S19, 2010.

19. Partridge JM, Koutsky LA. Genital human papillomavirus infection in men. *Lancet Inf Dis 6*: 21-31, 2006.

20. Sociedade Brasileira de Urologia. *Estimativa da Incidência de lesões anogenitais no Brasil - 2010*. Soc Bras Urol, 2010. Disponível em: http://www.sbu.org.br. Acesso em 05/10/2015.

21. Vardas E, Giuliano AR, Goldstone S, Palefsky JM, Moreira Jr ED, Moreira ED, Penny ME, Aranda C, Jessen H, Moi H, Ferris DG, Liaw KL, Marshall JB, Vuocolo S, Barr E, Haupt RM, Garner EIO, Guris D. External Genital Human Papillomavirus Prevalence and Associated Factors Among Heterosexual Men on 5 Continents. *J Inf Dis 203*: 58-65, 2011.

22. WHO/IARC Screening Group. The Bethesda system. Disponível em: http://screening.iarc.fr/atlasclassifbethesda.php. Acesso em 20/11/2012.