Human papillomavirus infection in healthy men from Rio de Janeiro, Brazil

Infecções por papilomavírus humanos em homens saudáveis do Rio de Janeiro, Brasil

Willker Menezes[1], Daniele Ceperuelo Lisboa[2], Thaissa Isaias Cordeiro[3], Elisabete Dobao[4], Tegnus Vinicius Depes de Gouvêa[5], Fernanda Nahoum Carestiato[6], Silvia Maria Baeta Cavalcanti[7]

ABSTRACT

Introduction: Genital infections by human papillomavirus (HPV) are the most prevalent sexually transmitted viral diseases worldwide. Although the natural history of cervical cancer is better understood, there are still scarce information regarding the etiology of penile cancer, and the natural history of HPV infection in men is not yet fully elucidated. **Objective:** This study aimed to determine the prevalence of HPV infection in penile samples, from a clinically asymptomatic male population. **Methods:** A total of 550 samples were collected between January 2011 and July 2014 in different institutions in the State of Rio de Janeiro, including a dermatology clinic and a metallurgical company. The samples were collected from the anatomical regions of the glans and balanopreputial sulcus. HPV identification was made through the generic and type-specific Polymerase Chain Reaction (PCR), and Restriction Fragment Length Polymorphism (RFLP) techniques. **Results:** An overall prevalence of HPV infection was observed in 21.8% (120 subjects). The most prevalent HPV type was HPV 6 (35%), followed by HPV 16 (20.8%), HPV 11 (19.1%), HPV 31 (6.7%), HPV 33 (6.7%), HPV 45 (8.3%) and HPV 58 (3.3%). Hence, infection was associated with low-risk oncogenic types in 54.1% of the studied individuals, while high-risk oncogenic types were detected in 45.9% of them. The age of the studied subjects ranged from 18 to 65 years with a mean age of 28.4 years. **Conclusion:** According to our findings, we can infer that the prevalence of HPV infection among asymptomatic male population was considerably lower than the described in the literature, although in agreement with results reported in some recently published studies. We believe that the results may contribute to understand the features of circulation of HPV in male population, in order to evaluate risk-benefits and strategies of disease prevention.

Keywords: HPV; STD; men; asymptomatic; PCR.

RESUMO

Introdução: As infecções genitais pelo Papilomavírus humano (HPV) são hoje as mais prevalentes viroses de transmissão sexual em todo o mundo. Embora a história natural do câncer cervical seja melhor compreendida, pouco se sabe sobre a etiologia do câncer de pênis e a história natural do HPV no homem não está completamente elucidada. **Objetivo:** Este estudo teve como objetivo, determinar a prevalência do HPV em amostras penianas de uma população masculina assintomática. **Métodos:** Foram coletadas 550 amostras entre janeiro de 2011 e julho de 2014 em diferentes instituições do Estado do Rio de Janeiro, dentre elas: uma clínica de dermatologia e uma indústria metalúrgica. As amostras foram coletadas de sítios anatômicos como sulco balanoprepucional. A identificação do HPV foi feita pela Reação em Cadeia da Polimerase (PCR) genérica e tipo-específica, bem como pelo Polimorfismo do padrão de comprimento de fragmentos de restrição (RFLP). **Resultados:** A prevalência total da infecção pelo HPV foi de 21,8% (120 indivíduos). O tipo viral de maior prevalência foi o HPV 6 (35%), seguido pelo HPV 16 (20,8%), HPV 11 (19,1%), HPV 31 (6,7%), HPV 33 (6,7%), HPV 45 (8,3%) e HPV 58 (3,3%). A infecção foi majoritariamente associada a tipos de baixo risco oncogênico (54,1%), enquanto os genótipos oncogênicos foram detectados em 45,9% dos pacientes. A idade dos pacientes variou de 18 a 65 anos com média de 28,4. **Conclusão:** De acordo com nossos achados, podemos sugerir que a prevalência do HPV na população masculina assintomática foi consideravelmente menor do que a descrita em alguns estudos da literatura, mas em acordo com o reportado recentemente por vários autores. Acreditamos que estes resultados podem contribuir para a compreensão dos aspectos epidemiológicos associados à infecção no trato genital masculino, a fim de avaliar estratégias de prevenção de doenças associadas e avaliar o risco-benefício das diferentes abordagens aplicadas em Saúde-Pública.

Palavras-chave: HPV; DST; homens; assintomático; PCR.

INTRODUCTION

Although Human papillomavirus (HPV) infection causes the most prevalent sexually transmitted viral disease worldwide, the natural history of HPV infection have only been extensively studied in women, due to the prevalence of this disease and its well-established link to cervical cancer[1].

Most HPV infections in men are asymptomatic, and the male population is not routinely screened for HPV, so men may act as reservoirs of HPV infection, resulting in continuous transmission of both high-risk and low-risk HPV types to women[2]. Nevertheless, men have recently been recognized to manifest the pathological features of this disease, mainly through anogenital warts and neoplasias: anal intraepithelial neoplasia (AIN), penile intraepithelial neoplasia (PIN), and invasive carcinoma[3,4].

We believe that, since prophylactic HPV vaccines are recognized as effective in men, understanding the factors associated with HPV acquisition in men is critical to the development of public health strategies and preventive programs to control HPV infection[5]. Few studies have examined the epidemiology and risk factors associated with HPV infection in male population. In a systematic review, Dunne et al.[6] reported that half of the published studies concerning HPV prevalence among healthy subjects pointed out rates of infection of

[1]Doctoral student, Graduate Program in Applied Microbiology and Parasitology, Universidade Federal Fluminense (UFF) – Niterói (RJ), Brazil.
[2]Master in Applied Microbiology and Parasitology, UFF – Niterói (RJ), Brazil. Clinic phisician, Department of Dermatology, Santa Casa de Misericórdia do Rio de Janeiro – Rio de Janeiro (RJ), Brazil.
[3]Master's student, Graduate Program in Applied Microbiology and Parasitology, UFF – Niterói (RJ), Brazil.
[4]Master in Applied Microbiology and Parasitology, UFF Niterói (RJ), Brazil.
[5]Coordinator of the Sexually Transmitted Diseases Clinics, UFF – Niterói (RJ), Brazil.
[6]Doctoral student, Graduate Program in Medical Sciences, UFF – Niterói (RJ), Brazil.
[7]Supervisor and responsible for the Virological Diagnosis Lab, Department of Microbiology and Parasitology, UFF – Niterói (RJ), Brazil.

approximately 20%, although they may vary among different populations, sampling methods and diagnostic methodologies.

OBJECTIVE

This study aimed to describe the prevalence of HPV DNA among asymptomatic male subjects living in the State of Rio de Janeiro in order to evaluate the circulation of HPV infection among the studied population.

MATERIALS AND METHODS

Study design and participants

This cross-sectional study evaluated HPV infection in 550 asymptomatic men, treated in and recruited from several institutions of the city of Rio de Janeiro, namely: the STD clinic of Universidade Federal Fluminense, the dermatology clinic of Santa Casa da Misericórdia and a metallurgical factory from the Metropolitan region of Rio de Janeiro (MAENFE). The study was carried between January 2011 and July 2014, and aimed to evaluate the prevalence of HPV infection among asymptomatic men.

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

This study was approved by the Ethics Committee of Instituto Oswaldo Cruz from Fundação Oswaldo Cruz (CEP/IOC/FIOCRUZ, protocol no. 567/2010), and all subjects signed an informed consent. The social and epidemiological information (number of sexual partners, sexual behavior, circumcision, hygiene habits, tobacco use, history of STD, use of condom and anal intercourse) was collected from all participants using a structured questionnaire.

Samples

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 550 samples were collected from anogenital sites with a swab used for cytological exams, which was twisted clockwise three times in the anatomical site of sampling and, after that, kept in TE solution (10 mM Tris hydrochloride at pH 7.5, 1 mM ethylenediaminetetraacetic acid EDTA) (Invitrogen, USA) at -20°C until DNA extraction. Then, the participants signed the informed consent term and completed the social and epidemiological questionnaire.

DNA extraction, PCR amplification and genotyping

Samples were incubated for 4 hours at 56°C in 1mL digestion buffer (10 mM Tris hydrochloric acid at pH 8.3, 1 mM EDTA at 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 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 reaction mixture (1 X PCR buffer, 200 mM deoxyribonucleoside triphosphates, 1.5 mM $MgCl_2$, 50 pmol 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[7]. Thirty-five amplification cycles were carried out in 50 µL reaction mixture with denaturation at 94°C for 30 s, annealing at 55°C for 30 s, 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[8].

Restriction fragment length polymorphism (RFLP) analysis for HPV genotyping

RFLP was performed following PCR amplification using the 450-bp amplicons from the MY09/11 PCR. 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.[9].

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 sociodemographic features were evaluated. Associations of LR and HR HPV infections with social and epidemiological variables were examined.

RESULTS

The studied group was composed of 550 asymptomatic subjects, showing no clinically detectable HPV lesions. The average age of participants was 28.4 years, ranging from 18 to 65 years. The HPV-infected group presented an average age of 30.8, and HPV-negative subjects were 26.3 years old. No statistical differences were detected among them ($p>0.05$).

Regarding HPV infection, HPV DNA was detected in 21.8% of the patients (120/550). HPV 6 was the most prevalent type (35%, 42/120), followed by HPV 16 (20.8%, 25/120), HPV 11 (19.1%,

23/120), HPV31 (6.7%, 8/120), HPV33 (6.7%, 8/120), HPV45 (8.3%, 10/120) and HPV 58 (3.3%, 4/120). The HR types, 16, 31, 33, 45 and 58, were found in 45.9% of the cases. LR types 6 and 11 were the predominant types (54.1%). Multiple infections (e.g., HPV types 16 and 45, 11 and 58, 45 and 35 and 6 and 16) were found in 10.8% (13/120) of the samples. All the multiple infections were detected by the RFLP technique. Five samples (4.1%) presented HPV DNA according to MY09/11 PCR, but typing by both PCR specific primers and RFLP was inconclusive and are referred as HPV X (**Table 1**).

Age was the only socio-demographic factor that associated with risk of infection that could be analyzed, but no significant differences were found between infected and uninfected subjects (p>0.05).

DISCUSSION

Human papillomavirus (HPV) anogenital infections among healthy subjects presents prevalence rates ranging from 1.3% to 72% depending on the population studied and the diagnostic method used, but it is associated to 5% of all cancers worldwide[6]. Within these rates there is the cervical infection, which serves until now as a paradigm for understanding the carcinogenesis caused by high-risk HPV[1].

As penile carcinoma is a rare tumor and its etiology is still being discussed, little is known about HPV infection in men[10]. Recent studies have provided considerable evidence of the oncogenic potential of some HR-HPV types in the male anogenital tract[11,12]. Hence, HPV infection in men has increasingly become the object of research and discussion instead of being considered solely a source of transmission to women[13]. Although studies on HPV prevalence in male anogenital lesions have yielded highly variable results, we found a prevalence rate of 21.8%, which is similar to several studies from Brazil and other countries[7,14]. Nevertheless, an important study conducted in different countries (the HIM HPV in men study)[15] described HPV DNA rates greater than 70%. This high rate of HPV infection could be explained, in part, by the fact that the HIM study was conducted in subjects in the general population, not excluding individuals with clinical symptoms like warts.

Among the positive results for detection and typing of viral genetic material, we found a higher prevalence of HPV 6, followed by HPV 11, HPV 16, HPV 45, HPV31, HPV 33 and HPV 58 (Table 1). Dobao et al.[16], 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 from Dunne et al.[6], 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 (10.8%) and its role in disease establishment and progression remains inconclusive (Table 1), but recent studies point out that multiple-HPV infection can result in higher viral protein expression levels that can lead to genital disease. Prospective cohort studies linking sequential loss or gain of HPV types with cytological analysis are required to assess the impact of multiple HR-HPV infections on neoplastic progression[17].

Table 1 – Prevalence of HPV genotypes according to PCR and RFLP in male smears (n=120).

	n (%)
HPV 6	42 (35)
HPV 11	23 (19.1)
HPV 16	25 (20.8)
HPV 31	8 (6.7)
HPV 33	8 (6.7)
HPV 45	10 (8.3)
HPV 58	4 (3.3)
HPV X*	5 (4.1)
Co-infections**	**13 (10.8)**

*MY(+)PCR untyped by type-specific PCR and RFLP techniques.
**Individuals infected by more than one type of HPV.

We also observe statistical significance regarding HPV in older men, among which only high-risk types were detected (p>0.05).

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[18]. The absence of HPV 18 was consistent with other recent studies in male subjects[8,14]. On the other hand, the presence of HPV 45, whose prevalence has been shown to be increasing among the female population[1], was remarkable. HPV 45 is currently considered to be the second most prevalent type in cervical cancer cases in Brazil, being associated with insidious cases with difficult early detection[19]. These results draw attention to the increased circulation of this type of HPV in the world, including the male population, which highlights the relevance in considering its inclusion as well as other emerging viral types in future prophylactic vaccines, in order to extend the immunization coverage for the types with major clinical relevance.

It has been demonstrated that sampling and methodological strategies used in prevalence studies may have influence in diagnostic failure due to the characteristics of HPV infection sites in men and the lack of routine preventive screening to detect penile/anal lesions in men at risk for cancer development[20]. 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[14]. 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.

The HPV prophylactic vaccines are 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[12]. Although the protective efficacy of HPV vaccination in men has not been fully established, public policy discussions and cost-efficacy studies are necessary to support vaccination of boys, as of 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[20].

CONCLUSION

We suggest that, for high-risk groups, an appropriate method of screening should be established as soon as possible, but we believe that the clinical knowledge of this pathology by physicians who gives assistance to this population should be encouraged in the short-term,

so that they can be alert and consider this pathology among the diagnostic hypotheses, even in the absence of anogenital warts. Education has relatively low cost, quick results and will be as or more effective than laboratorial methods for early diagnosis. At last, it is important to eliminate the idea, although deep-rooted, that HPV infection in men is not worthy of great concern, without recognition of its importance.

Conflict of interests

The authors declared no conflict of interest.

REFERENCES

1. WHO/IARC Screening Group. The Bethesda system. Available at: http://screening.iarc.fr/atlasclassifbethesda.php Accessed in: 20 Nov, 2012.
2. Castellsagué X, Bosch XF, Munõz, Meijer CJLM, Shah KV, Franceschi S, et al. Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. Engl J Med. 2002;346(15):1105-12.
3. Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. J Adolesc Health. 2010;13(4 Suppl):S12-9.
4. Garland SM. Prevention strategies against human papillomavirus in males. Gynecol Oncol. 2010;117(2 Suppl):S20-5.
5. Giuliano AR, Lazcano E, Villa LL, Flores R, Salmeron J, Lee JH, et al. Circumcision and sexual behavior: factors independently associated with human papillomavirus detection among men in the HIM study. Int J Cancer. 2009;124(6):1251-7.
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. 2006;194(8):1044-57.
7. Carestiato FN, Silva KC, Dimetz T, Oliveira LH, Cavalcanti SM. Prevalence of human papillomavirus infection in the genital tract determined by hybrid capture assay. Braz J Infect Dis. 2006;10(5): 331-6.
8. Afonso LA, Moyses N, Alves G, Ornellas AA, Passos MR, Oliveira Ldo H, et al. Prevalence of human papillomavirus and Epstein-Barr virus DNA in penile cancer cases from Brazil. Mem Inst Oswaldo Cruz. 2012;107(1):18-23.
9. Melgaço FG, Rosa MLG, Augusto EF, Haimuri JG, Jacinto C, Santos LS, et al. Human papillomavirus genotypes distribution in cervical samples from women living with immunodeficiency virus. Arch Gynecol Obstet. 2011;283(4):809-17.
10. Kreuter A, Brockmeyer NH, Weissenborn SJ, Gambichler T, Stücker M, Altmeyer P, et al. German Competence Network HIV/AIDS. Penile intraepithelial neoplasia is frequent in HIV-positive men with anal dysplasia. J Invest Dermatol. 2008;128(9):2316-24.
11. Hoots BE, Palefsky JM, Pimenta JM, Smith JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int J Cancer. 2009;124(10): 2375-83.
12. Palefsky JM. Anal cancer prevention in HIV-positive men and women. Curr Opin Oncol. 2009;21(5):433-8.
13. Fox P. Anal cancer screening in men who have sex with men. Curr Opin HIV AIDS. 2009;4(1): 64-7.
14. Vardas E, Giuliano AR, Goldstone S, Palefsky JM, Moreira ED Jr, Penny ME, et al. External genital human papillomavirus prevalence and associated factors among heterosexual men on 5 contnents. J Inf Dis. 2011;203(1):58-65.
15. Giuliano AR, Lu B, Nielson CM, Flores R, Papenfuss MR, Lee JH, et al. Age-specific prevalence, incidence and duration of human papillomavirus infections among a cohort of 290 US men. J Infect Dis. 2008;198(6):827-35.
16. Dobao EAR, Afonso LA, Menezes W, Pires C, Kawa Kac B, Pesca LF, et al. Evaluation of anogenital human papillomavirus infection in men attending a dermatology clinic. Virus Rev Res, 2015. Available at: http://157.86.113.86/index.php/vrrjournal/article/viewFile/96/131
17. Cuschieri KS, Cubie HA, Whitley MW, Seagar AL, Arends MJ, Moore Cet al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. J Clin Pathol. 2004;57(1):68-72.
18. 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. 2009;83(3):1443-55.
19. Fernandes JV, Meissner RV, Carvalho MG, Fernandes TA, Azevedo PR, Villa LL. Prevalence of HPV infection by cervical cytologic status in Brazil. Int J Gynaecol Obstet. 2009;105(1):21-4.
20. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED Jr, Aranda C, Jessen H, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. N Engl J Med. 2011;365:1576-85.

Address for correspondence:
SILVIA MARIA BAETA CAVALCANTI
Dept. de Microbiologia e Parasitologia/UFF
Rua Professor Ernani Melo, 101
Niterói (RJ), Brazil
CEP: 24210-130
Phone: +55 (21) 2629-2431; Fax: +55 (21) 2629-2433
E-mail: silviacavalcanti@vm.uff.br

Received on: 01.20.2015
Approved on: 03.17.2015