PREVALENCE OF HPV INFECTION IN CROATIAN MEN DURING A 12-YEAR PERIOD: A COMPARATIVE STUDY OF EXTERNAL GENITAL AND URETHRAL SWABS

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SUMMARY

Aim: The aim of the study was to compare the prevalence and distribution of both low-risk and high-risk HPV infection of the urethra and outer genital region in Croatian men. Thus far there is a consensus that sampling the coronal sulcus and glans of the penis is essential for adequately assessing HPV status in men but less agreement is noted for urethral sampling.

Methods: External genital brushing and urethral swabs were taken from 1,342 men during a 12-year period and tested with the hc2 HPV DNA Test using Hybrid Capture 2 technology.

Results: The overall prevalence of male HPV infection in this study was 36.66%. Infection with high-risk HPV types (44.72%) was significantly more frequent than infection with low-risk HPV types (28.86%) or co-infection with both low-risk and high-risk HPV types (26.42%). HPV was more frequently demonstrated in the outer genital area (58.33%) when compared to the sole infection of the urethra (17.89%) or infection of both genital sites (23.78%).

Conclusions: Results from this study indicate high prevalence of HPV infection in men and suggest that optimal sampling method for the testing of men is the combination of external genital and urethral swabs. Further research about the proper collection of biological samples and testing methods for HPV detection in men is necessary since our future end-goal is to implement standardized guidelines on sampling and diagnostic testing of males.

INTRODUCTION

Human papillomavirus (HPV) infections are regarded as the most common sexually transmitted infections worldwide (1). They are a large family of double strand DNA viruses encompassing more than 180 types, with more than 40 HPV types that can infect the anogenital region (2). Many genital HPVs regarded as low-risk (such as HPV 6 and 11) produce warts; on the other hand, high-risk viruses (such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) have the ability to induce tumors (2). In 2008, an estimated 610,000 cancers in men and women were attributed to HPV infection globally (3).

First studies in men were focused on HPV infection and subsequent risk of cancer in homosexual population, but more recently research has been extended to the heterosexual male population (4). Many men can be classified as healthy carriers who can unknowingly serve as an asymptomatic reservoir, thus contributing to the development of HPV-related diseases in their partners (5, 6). Therefore, screening of men is thought to be a relevant practice in the prevention of cervical cancer in women.

Still, although men do not carry the same burden as women for high-risk HPV-related lesions, impact of the disease is regarded as significant – especially considering low-risk HPV-related genital warts (4). On the other hand, early studies of both low and high-risk HPV infection in men used acetowhitening of the penis as a diagnostic marker (7). Although HPV is significantly associated with this phenomenon (8), a myriad of other conditions can be also related with such lesions, subsequently resulting in poor specificity for HPV detection (9, 10).

Hence in the absence of clinical lesions, the most trustworthy diagnostic approach for men is testing for HPV DNA (4, 11). The overall prevalence of HPV in men is quite variable depending on the study and it ranges from 1.3% to 72.9% (12). Such wide range of rates, alongside different profiles of the patients and different HPV assays employed, may be ascribed to the variation in the clinical material analysed due to a lack of agreement on the anatomical sites that should be sampled (13, 14).

Epidemiology of HPV infection in men is well established. However, little is known about the prevalence of HPV infection in men at different anatomical sites. Thus far there is a consensus

that sampling the coronal sulcus and glans of the penis is essential for adequately assessing HPV status in men because of the direct contact with the cervix, but less agreement is noted for urethral sampling (notably among asymptomatic men) (14).

Therefore, the objective of this study was not to extensively evaluate all epidemiological factors in the study population, but to concentrate on the comparison of the prevalence and distribution of both low-risk and high-risk HPV infection of the urethra and outer genital region in Croatian men visiting outpatient clinic.

MATERIALS AND METHODS

Sampling

This study included a total of 1,342 men aged between 18 and 65 years visiting outpatient clinic that were tested in a 12-year period (between January 2002 and December 2013). Two types of genital specimens were collected. The first type was collected by penile brushing with a saline-prewetted cytobrush consisting of collecting the cells from dorsal and ventral surfaces of the penile shaft as well as from the inner part of the foreskin, frenulum, coronal sulcus, and glans. Six to eight forward and backward brush movements were performed at each site. The second type of genital specimens were urethral swabs, collected by inserting and rotating a dry rayon swab with an aluminum shaft (Copan Diagnostics, Italy). The swab was inserted 2 cm into the urethra and rotated for 360 degrees whilst removing it. Both samples were placed in 1 ml of Digene collection and preservation medium provided by the manufacturer.

hc2 HPV DNA Test Procedure

The hc2 HPV DNA Test using Hybrid Capture 2 technology is a signal amplified hybridization antibody capture assay that utilizes microplate chemiluminescent detection. In this test, specimens containing the target DNA hybridize with a specific HPV RNA probe. It distinguishes between two groups of HPV types: HPV 6/11/42/43/44 (low risk types) and 16/18/31/33/35/39/45/51/52/ 56/58/59/68 (high risk types). The testing of samples was carried out according to the manufacturer's instructions.

Calibrators, quality controls and all the specimens tested were stored at −20 ℃ for up to one month before testing each batch of samples. After hybridization step, the resultant RNA:DNA hybrids were captured onto the surface of a microplate well coated with antibodies specific for those type of hybrids. Upon their immobilization they were reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and subsequently detected with a chemiluminescent substrate. Multiple conjugated antibodies bound to each captured hybrid, and as several alkaline phosphatase molecules were conjugated to each antibody, it resulted in substantial signal amplification. As the substrate was cleaved by the bound alkaline phosphatase, the emitted light was measured as relative light units (RLU) on a luminometer. The intensity of such emitted light denoted the presence or absence of target DNA in the specimens.

RLU measurements equal to or greater than the cut-off value indicated the presence of HPV DNA sequences in the tested specimens. RLU measurements less than the cut-off value indicated the absence of specific HPV DNA sequences in the tested specimens or HPV DNA levels below the detection limit of the assay.

Statistical Analysis

A chi-square test for independence was employed to determine whether there was a significant association between the different paired variables from the studied population. The one-way analysis of variance (ANOVA) was used to determine whether there are any significant differences between the means of three or more independent groups of HPV types (F-test), which was followed by Tukey's HSD test to find which pairwise group means are statistically unequal.

RESULTS

Samples of external genital region and the urethra were taken from 1,342 men aged between 18 and 65 years. HPV was found in 492 (36.66%) of the tested individuals: 28.86% were rated as the low-risk HPV group, 44.72% as the high-risk HPV group, and in 26.42% both high and low risk HPV were found (Table 1). In HPV-positive men, the detection of virus per anatomic site was 58.33% in the outer genital area, 17.89% in the urethra and 23.78% in both of those locations (Table 2).

In 117 of the tested individuals, HPV-infection was found both in the external genital region and the urethra. The occurrence of an identical HPV type pattern in both of those locations was found in 63 of them (53.85%) (Table 3). Statistical analysis of the distribution of low-risk and high-risk HPV types found only in the outer genital region or the urethra revealed that the association between HPV types and the aforementioned anatomic locations was not significant (chi-square = 5.26, p value = 0.072) (Table 4).

The F-test for testing whether the group means of HPV types are equal was significant at 1% level, F = 6.02, p value < 0.01. Therefore, at least two group means of HPV types in this study were not statistically equal. Tukey's HSD test confirmed that the mean for high-risk HPV group (18.33) is significantly higher than means of low-risk HPV group (11.83) and both groups (10.83).

Table 1. Prevalence of low-risk and high-risk human papillomavirus (HPV) in all positive patients by year

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
Low-risk HPV	14	12	13	14	16	21	8	14	10	6	5	9	142
High-risk HPV	24	21	17	19	28	33	19	18	10	11	11	9	220
Low-risk and high-risk HPV	11	13	10	12	11	18	16	16	10	7	2	4	130
Total positive	49	46	40	45	55	72	43	48	30	24	18	22	492
Total tested	155	143	145	138	155	167	115	97	73	56	50	48	1,342

Table 2. *Anatomic location of the detected human papillomavirus (HPV) infection in all positive patients by year*

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
Outer genital area	26	25	28	24	31	48	24	25	15	15	9	17	287
Urethra	12	15	6	7	10	13	5	7	3	4	3	3	88
Both locations	11	6	6	14	14	11	14	16	12	5	6	2	117
Total positive	49	46	40	45	55	72	43	48	30	24	18	22	492
Total tested	155	143	145	138	155	167	115	97	73	56	50	48	1,342

Table 3. *Co-infection pattern of the outer genital region and the urethra by low-risk (A) and high-risk (B) HPV types.*

Outer genital region	Urethra	Total (2002–2013)
A+B+	A+B+	11
A+B+	A+B-	18
A+B+	A-B+	16
A+B−	A+B+	5
A+B−	A+B-	30
A+B−	A−B+	6
A−B+	A+B+	1
A−B+	A+B-	8
A−B+	A−B+	22
		117

Plus sign (+) denotes presence of low-risk and/or high-risk HPV type(s), whereas minus sign (−) denotes absence of low-risk and/or high-risk HPV type(s).

No statistical difference between the group means corresponding to low risk HPV and both HPV types has been found.

The F-test for testing whether the group means of anatomic locations are equal is significant at 1% level, $F = 21.13$, p value < 0.01. Therefore, at least two group means of anatomic locations were statistically different. Tukey's HSD test confirmed that the mean for outer genital area (23.92) is significantly higher than other two group means corresponding to urethral swabs (7.33) and both locations (9.75). No statistical difference between the group means corresponding to the latter two anatomic locations has been found.

Prevalence rates of HPV positivity showed an increasing trend from 2002 to 2013 and ranged from 27.59% in 2004 to 49.48% in 2009 (Fig. 1). The mean prevalence rate for the first six years of the study (2002–2007) was 33.76%, and for the subsequent six years (2008–2013) was 42.11%.
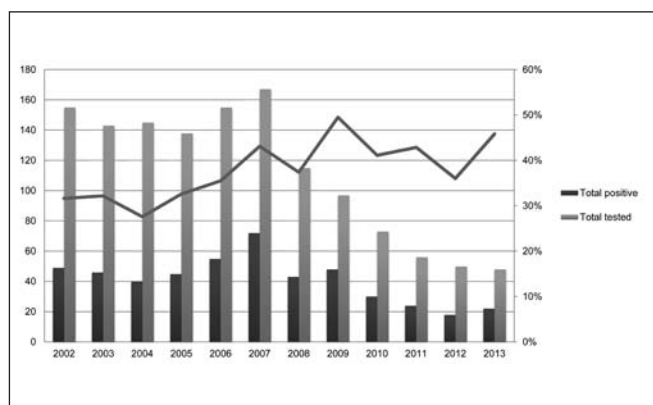
Fig. 1. *Graph showing prevalence rates for HPV-positive individuals by year.*

A cross tabulation followed by chi-square test of independence was performed to test whether the two variables (HPV types and years) are independent or not. The same was done for anatomic locations and years. The tests were not significant at 5% level for both pairs of variables (chi-square = 13.63, p value = 0.914 for HPV types and years; chi-square = 30.54, p value = 0.106 for anatomic locations and years). In other words, no association between the tested variables and time (years) has been found. Therefore, HPV types and anatomic locations in our study are independent of time.

DISCUSSION AND CONCLUSION

The overall prevalence of male HPV infection in our study was 36.66%. Infection with high-risk HPV types was significantly more frequent than infection with low-risk HPV types or co-infection with low-risk and high-risk HPV types. HPV was more frequently demonstrated in the outer genital area when compared to the sole infection of the urethra or infection of both genital sites. There was an increasing trend in the prevalence rates during the years, with the peak prevalence noted in 2009 (49.48%). There was no statistically significant association between the HPV types, anatomic location and time.

The strengths of this study are a large sample size, long study period of 12 years, thorough sampling of two different sites, the use of consistent methods and analysis of samples in one laboratory. The main weakness of the study is that the hc2 HPV DNA test only differentiates low and high risk groups of HPV types; hence it does not distinguish among the viral types within these groups. In addition, the manufacturer states that there is a possibility of cross-reactivity between the hc2 HPV DNA Test probe and the plasmid pBR322. Sequences homologous to pBR322 are present in human genital specimens, which might be erroneously interpreted as indicating the presence of viral sequences when this specific plasmid is present in high levels (15). Sexual history of the tested individuals is also unknown, which would help to establish a broader picture from an epidemiological aspect.

There is a significant variability in HPV prevalence estimates for males in the medical literature (12). Some of that variability can be attributed to the differences in tested populations, but much of it is likely due to incomplete sampling of men. The anatomic sites with the highest published prevalence of HPV are the penile shaft, prepuce, glans penis, coronal sulcus and scrotum, while urethral swabs most often result in a low detection rate (12, 14). Testing of semen and urine specimens for HPV is not efficacious (14, 16). The continuing challenge for researchers and clinicians alike is to select the smallest combination of anatomic sites for sampling that will yield reliable prevalence estimates, but at the same time be practical and acceptable to men (14).

Table 4. *Distribution of low-risk and high-risk HPV types found only in the outer genital region or in the urethra*

	Only outer genital region	Only urethra
Low-risk	85 (29.62%)	28 (31.82%)
High-risk	146 (50.87%)	52 (59.09%)
Low-risk + High-risk	56 (19.51%)	8 (9.09%)
Total	287 (100%)	88 (100%)

Like stated previously, overall prevalence of HPV in men demonstrated in the literature ranges from 1.3% to 72.9% and is highly dependent on the research methodology (12). In a cross-sectional study of HPV infection conducted in Arizona on 463 men, Giuliano et al. found the overall prevalence of 65.4% (14). The HPV detection in that study was highest at the penile shaft, followed by the glans penis and scrotum, and lowest in the urethra and semen. Detection of HPV was also highest in the penile shaft in the study by Hernandez et al. on a cohort of students in Hawaii (17). Prevalence of HPV in the outer genital region was approximately 50% amongst young men in Kenya; that study also found that urethral sampling for HPV detection added no sensitivity for HPV detection over that found from sampling the glans, coronal sulcus and penile shaft (18).

Conversely, some authors advocate testing both outer genital region and the urethra as the best approach to the problem. In a study on 50 partners of HPV-positive women in Italy, Giovannelli et al. found that parallel testing by penile brushing and urethral brushing yielded 100% rates of HPV detection (13). That finding is consistent with the study from Nicolau et al. who also used hc2 HPV DNA test and found that the HPV detection rate increased from 58% to 70% when urethral sampling was analysed concomitantly with the penile brushings (11). In our population HPV prevalence would be 30.01% if only external genital samples were used, compared to 36.66% prevalence rate when both sites were analysed.

Several studies have looked at HPV prevalence in Croatia. Between 2006 and 2008, Grahovac et al. showed an overall prevalence of 27.4% for both low-risk and high-risk HPV types (19). Although the swabs were taken from both external genital region and the urethra (akin to our study), they were combined into one specimen. 21% of 100 men from Zagreb were shown to be infected in a study conducted between 1996 and 2000 (20). Bošnjak et al. studied prevalence and genotype distribution of high-risk HPV in male genital samples in the eastern part of the country (21). The overall prevalence of HPV infection in their study was 32.12%, but more comprehensive analysis of their results is hindered by the fact that only high-risk HPV types were thoroughly addressed and that the half of all the samples were collected from urethral canals only.

Some recent studies also employed the hc2 HPV DNA Test using Hybrid Capture 2 technology in order to study the prevalence and behaviour of HPV infection in men. In a study from Poland on 826 male participants, Walczak et al. found a prevalence rate of 30% for low-risk HPV and 14.3% for high-risk HPV types (22). HPV DNA Hybrid Capture was also a method of choice in a cytologic study of Eleutério et al. from Brazil; the authors concluded that assessing the presence of non-nucleated and nucleated squamous cells on cytologic smears prior to performing a test with this method represents a useful tool for quality control of penile samples (23).

Using the same nucleic acid hybridization assay with signal amplification, Hadjivassiliou et al. demonstrated that male partners of infected females and males with genital warts are predominantly infected by low-risk HPV types, albeit a substantial proportion is concomitantly or only affected by high-risk virus types (24). This is in accordance with our results where we have also shown the occurrence of both HPV types in the urethra and demonstrated different co-infection patterns (Table 3). The evident contrariety in the test results of some participants where low-risk HPV has been found in the external genital region and high-risk HPV in the urethra (or vice-versa) should also be emphasized.

Although this kind of testing is regarded as an "off-label" use, it must be noted that HPV testing is appropriately employed in a wide range of clinical settings that were not part of the original FDA intended use, as outlined in the CETC statement (25). "Off-label" use of well-established test is much less risky than using an unproven test for clinical decision-making. Proper usage of HPV testing beyond the intended-use claims has been established through a consensus guideline development process with a stringent evaluation of peer-reviewed studies, and the fundamental clinical performance of the HPV test itself (based on the optimized positive cut point) remains unchanged (26).

The justification for such use of HPV testing is also reinforced by the fact that there is no generally accepted and validated test for HPV screening in males in the clinical practice, but the general consensus endorses testing in cases when the patient has the HPV positive partner, when HPV-related clinical manifestations are present and when the patient has sex with men (27–29). Lenzi et al. accentuate that test which can identify both high-risk and low-risk HPV is clinically most useful as it enables differential diagnosis between benign and malignant lesions as well as those related or not related to HPV (e.g. molluscum contagiosum) (4).

Randomized control trials with the quadrivalent HPV virus-like particles (VLP) vaccine have shown robust antibody responses and high efficacy against genital warts anal precanceroses in men (30). Still, few countries have recommended male vaccination on the basis that this is not cost effective. Therefore, education and preventive HPV testing before engaging in sexual relationship still represent the cornerstone of all preventive endeavours.

In conclusion, results from this study indicate high prevalence of HPV-infection in men and suggest that optimal sampling method for the testing of men is the combination of external genital and urethral swabs. Prevalence studies in males should be further conducted as more epidemiological data is needed in order to appropriately evaluate disease burden. Likewise, more studies about the proper collection of biological samples and testing methods for HPV detection in men are needed since our future end-goal is to implement standardized guidelines in sampling and diagnostic testing of males.

Conflict of Interests
None declared

REFERENCES

1. Al Moustafa AE, Al-Awadhi R, Missaoui N, Adam I, Durusoy R, Ghabreau L, et al. Human papillomaviruses-related cancers. Presence and prevention strategies in the Middle east and north African regions. Hum Vaccin Immunother. 2014;10(7):1812-21.

2. Rosales R, Rosales C. Immune therapy for human papillomaviruses-related cancers. World J Clin Oncol. 2014 Dec 10;5(5):1002-19.

3. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012 Nov 20;30 Suppl 5:F12-23.

4. Lenzi A, Mirone V, Gentile V, Bartoletti R, Ficarra V, Foresta C, et al. Rome Consensus Conference - statement; human papilloma virus diseases in males. BMC Public Health. 2013 Feb 7;13:117. doi: 10.1186/1471-2458-13-117.

5. Bosch FX, Castellsagué X, Muñoz N, de Sanjosé S, Ghaffari AM, González LC, et al. Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst. 1996 Aug 7;88(15):1060-7.

6. Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walbomers JM, et al. High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse). Cancer Epidemiol Biomarkers Prev. 2001 Feb;10(2):101-6.

7. Krebs HB, Schneider V. Human papillomavirus-associated lesions of the penis: colposcopy, cytology, and histology. Obstet Gynecol. 1987 Sep;70(3 Pt 1):299-304.

8. Voog E, Ricksten A, Olofsson S, Ternesten A, Ryd W, Kjellström C, et al. Demonstration of Epstein-Barr virus DNA and human papillomavirus DNA in acetowhite lesions of the penile skin and the oral mucosa. Int J STD AIDS. 1997 Dec;8(12):772-5.

9. Pinto PA, Mellinger BC. HPV in the male patient. Urol Clin North Am. 1999 Nov;26(4):797-807, ix.

10. Richart RM, Barrasso R, Ferenczy A. Examining male partners of women who have abnormal smears. Contemp Ob Gyn. 1988;31:157-72.

11. Nicolau SM, Camargo CG, Stávale JN, Castelo A, Dôres GB, Lörincz A, et al. Human papillomavirus DNA detection in male sexual partners of women with genital human papillomavirus infection. Urology. 2005 Feb;65(2):251-5.

12. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: A systematic review of the literature. J Infect Dis. 2006 Oct 15;194(8):1044-57.

13. Giovannelli L, Migliore MC, Capra G, Caleca MP, Bellavia C, Perino A, et al. Penile, urethral, and seminal sampling for diagnosis of human papillomavirus infection in men. J Clin Microbiol. 2007 Jan;45(1):248-51.

14. Giuliano AR, Nielson CM, Flores R, Dunne EF, Abrahamsen M, Papenfuss MR, et al. The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. J Infect Dis. 2007 Oct 15;196(8):1146-52.

15. Ambinder RF, Charache P, Staal S, Wright P, Forman M, Hayward SD, et al. The vector homology problem in diagnostic nucleic acid hybridization of clinical specimens. J Clin Microbiol. 1986 Jul;24(1):16-20.

16. Lazcano-Ponce E, Herrero R, Muñoz N, Hernandez-Avila M, Salmerón J, Leyva A, et al. High prevalence of human papillomavirus infection in Mexican males: comparative study of penile-urethral swabs and urine samples. Sex Transm Dis. 2001 May;28(5):277-80.

17. Hernandez BY, McDuffie K, Goodman MT, Wilkens LR, Thompson P, Zhu X, et al. Comparison of physician- and self-collected genital specimens for detection of human papillomavirus in men. J Clin Microbiol. 2006 Feb;44(2):513-7.

18. Smith JS, Moses S, Hudgens MG, Agot K, Franceschi S, Maclean IW, et al. Human papillomavirus detection by penile site in young men from Kenya. Sex Transm Dis. 2007 Nov;34(11):928-34.

19. Grahovac B, Dorić A, Hruškar Ž, Hadžisejdić I, Grahovac M. Human papillomavirus infection in Croatian men: prevalence and HPV type distribution. In: Malla N, editor. Sexually transmitted infections [Internet]. InTech; 2012 [cited 2016 Jan 22]. p. 127-40. Available from: http://www.intechopen.com/books/sexually-transmitted-infections/human-papillomavirus-infection-in-croatian-men-prevalence-and-hpv-type-distribution.

20. Skerlev M, Grce M, Sirotkoviae-Skerlev M, Husnjak K, Lipozencić J. Human papillomavirus male genital infections: clinical variations and the significance of DNA typing. Clin Dermatol. 2002 Mar-Apr;20(2):173-8.

21. Bosnjak Z, Perić M, Krizan IR, Dzijan S, Ruzman N, Pastuović T, et al. Prevalence and genotype distribution of high-risk human papillomavirus (HR HPV) in male genital samples of Osijek-Baranja County. Coll Antropol. 2013 Dec;37(4):1203-8.

22. Walczak L, Dutkiewicz S, Marszałek A. Incidence and prevalence of multiple types of genital human papillomavirus (HPV) infection in men: a study in Poland. Ginekol Pol. 2013 Feb;84(2):112-5.

23. Eleutério J Jr, Barros IC, Cavalcante DI, Eleutério RM, Giraldo PC. HPV-DNA hybrid capture test: influence of cellularity in penile samples. Acta Cytol. 2010 Jul-Aug;54(4):546-50.

24. Hadjivassiliou M, Stefanaki C, Nicolaidou E, Bethimoutis G, Anyfantakis V, Caroni C, et al. Human papillomavirus assay in genital warts - correlation with symptoms. Int J STD AIDS. 2007 May;18(5):329-34.

25. Davey DD, Goulart R, Nayar R; Cytopathology Education and Technology Consortium (CETC). 2013 statement on human papillomavirus DNA test utilization. Am J Clin Pathol. 2014 Apr;141(4):459-61.

26. Lee SH. HPV DNA test utilization. Am J Clin Pathol. 2010 Feb;133(2):339; author reply 240. doi: 10.1309/AJCPGVKG8DMI1XIB.

27. Nyitray AG, Smith D, Villa L, Lazcano-Ponce E, Abrahamsen M, Papenfuss M, et al. Prevalence of and risk factors for anal human papillomavirus infection in men who have sex with women: a cross-national study. J Infect Dis. 2010 May 15;201(10):1498-508.

28. Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. J Adolesc Health. 2010 Apr;46(4 Suppl):S12-9.

29. Giraldo PC, Eleutério J Jr, Cavalcante DI, Gonçalves AK, Romão JA, Eleutério RM. The role of high-risk HPV-DNA testing in the male sexual partners of women with HPV-induced lesions. Eur J Obstet Gynecol Reprod Biol. 2008 Mar;137(1):88-91.

30. Stanley M. HPV vaccination in boys and men. Hum Vaccin Immunother. 2014;10(7):2109-11.