

Urethral Swab use in Diagnosis of Human Papilloma Virus Infection among Men Seeking Routine Evaluation

Abstract

More than 100 human papilloma virus (HPV) types have been identified so far. These oncoviruses can infect either mucosal or cutaneous sites. Mucosal HPV types predominantly infect the genital tract and are sexually transmitted. Several epidemiologic studies of genital HPV infection in women have been conducted lately but data on HPV prevalence and type distribution among men are lacking. It has been shown that in men HPV can be detected in urethral secretions. We aimed to verify that by the use of urethral swab we are able to diagnose HPV infection in men.

The investigation started with 113 patients (37 men, seeking routine investigation) who were referred to the Institute of Virology "Stefan S. Nicolau" laboratory on a 12 month period (2007-2008), prior the National Vaccination Campaign against HPV. For HPV diagnosis, samples were collected from lesions presumed HPV infected. In all men we collected urethral swab by scraping the urinary canal. We found a high presence of the HPV infection in the initial studied population, 59.3% patients were HPV positive. The following HPV genotypes were founded: 6; 11; 16; 18; 31; 33; 40; 42; 45; 51; 53; 54; 58; 59; 61; 66; 68; 70.

The men tested from the urethral swab turned out to be HPV positive in a percent of 40.5%, and from them 40% were infected with oncogenic HPV genotypes. In this study the urethral swab was used to detect HPV DNA even in asymptomatic HPV carriers and by collecting urethral swab we were able to diagnose HPV infection. Taking into account the overall presence of HPV, the high diversity of HPV types and the high proportion of oncogenic types, the epidemiologic characteristics of HPV infection in men seem different to that observed in women.

Keywords: Urethral swab; HPV infection; Men; Study inclusion; DNA; Patient; Vaccination

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Introduction

More than 100 human papillomavirus (HPV) types have been identified of which at least 42 are associated with infections of the genital tract [1]. HPV infection is commonly occurring as a sexually transmitted infection in Romania [2]. HPV infection with oncogenic high-risk types may be found in all cases of cancers in the anogenital tract in humans, for example cervical, penile and anal cancers. On the other hand, infection with low-risk HPV types is associated with anogenital warts or lesions present in men and women as well. Several epidemiologic studies of genital HPV infection in women have been conducted lately [3,4], but data on HPV prevalence and type distribution among men are lacking [5,6]. In women, the Papanicolaou (Pap) test is used for the detection of possible lesions of the cervix and for cervical cancer screening and management [7]. In contrast, there is no current approved test to detect HPV in men and the prevalence of male HPV infection is understudied [8].

In men, HPV infection may develop no symptoms, thus we encounter many asymptomatic carriers [9]. Therefore, a man may easily pass on the HPV infection to his female partner. It has been shown that in men HPV can be detected in urethral secretions samples [10]. We have therefore investigated HPV shedding

in urethral secretions samples from men seeking routine investigation in the Institute of Virology "St. S. Nicolau.

It is very important to prevent HPV infection with high risk genotypes; this is what the HPV vaccine campaign aimed in Romania. Also preventing transmission of HPV by sexual contact from men to women is important and if the man is an asymptomatic carrier of HPV, it may never know that he passed the HPV by sexual contact. This is why more about HPV prevalence in men is needed. In order to diagnose and study this infection we first need to have a good method to collect the samples for testing HPV in men, even for the asymptomatic HPV carriers. The goal of this study is to verify that by the use of urethral swab we are able to diagnose HPV infection in men.

Materials and Methods

Subject and samples

The investigation started from the 113 patients who were referred to the Institute of Virology "Stefan S. Nicolau" laboratory on a 12 month period (2007-2008), prior the National Vaccination Campaign against HPV. The patients presented with cervical lesions, vulvar, vaginal, or penian warts, conjunctival papillomas, oral papillomas, and as sexual partners of women diagnosed with

HPV. 37 men enrolled in our study (age 18-53, mean age 31.57). Six men presented genital lesions, nine had oral lesion and the rest were asymptomatic. Admission criteria included:

- a) Age between 18 and 55 years old.
- b) Sexual intercourse within a month prior to study inclusion.
- c) No urethral discharge or no documented sexually transmitted infection at time of study inclusion.

Sampling was done by collection of swabs. Men were asked to submit personal sexual behavior data, as they all were accompanied by their sexual partner. Samples were collected to all male patients by scraping the urinary canal with a cotton swab. The collection was performed in the morning, before urination, a thin cotton swab was inserted along the urethra about 1.5-2 cm, then the cotton swab was slowly rotated in order to allow the urethral cells to detach. The cotton swab was pulled out without touching anything. The procedure must be done without harming the urethral tissue and without any bleeding to occur. In order to avoid this only one sample was collected for each patient. After collection, the swab was placed in COPAN media (Italy). All specimens were stored at 4°C until use for HPV analysis (maximum 24 hours).

In order to maximize cellular material from the collected genital sample, when used, the swabs were squeezed and rotated against the side of the collection tube to release as much liquid as possible and then they were spun at 3200 rpm for 3 minutes to release the additional liquid that was trapped in the swab. The result, an aliquot of 200µl, was placed in a 1,5ml pop-top conical tube for HPV DNA analysis.

In order to avoid erroneous results, as well as infection of the personnel responsible for these processes, the collection and processing of biological samples involved the following rules:

1. Involved staff must wear protective suit, gloves (no talc) laboratory, goggles, and avoid using sharp or cutting devices.
2. Staff involved should be vaccinated against HBV and HAV, and training in the biological risk and safety rules recommended by CDC.
3. All containers with biological samples must be handled in accordance with the biological and biological safety risk (Biological Safety Level 2) recommended by the CDC.
4. Sampling and processing residues from biological samples must be collected, delivered and destroyed in accordance with procedures and legislation on chemical and biological residues arising from clinical and research laboratories.

DNA isolation

DNA was extracted from 200 µl COPAN media with biological sample, using the QIAmp DNA minikit (QIAGEN, France) commercial test, according to the manufacturer's instructions. The concentration and purity of each DNA were evaluated using NanoDrop spectrophotometer (NanoDrop Technologies, Montchanin, DE) while the integrity was confirmed in PCR for a 110 bp β-globin gene fragment using PC03/ PC04 primers.

HPV detection and genotyping

For HPV detection and typing commercially available INNOLIPA (INNOGENETICS NV, Gent, Belgium) kit based on the reverse hybridization principle was used. This assay amplifies a broad spectrum of HPV types but allows detection of 16 different genotypes (The 16, 18, 31, 33, 45, 51, 58, 59, 68 /73, 53, 66, 70, 6, 11, 40, 54). The undetermined types were noted HPV x. The kit is designed for the identification of different HPV genotypes by detection of specific sequences in the L1 region of the HPV genome. Part of the L1 region of the human papillomavirus (HPV) genome is amplified, and the resulting biotinylated amplicons are then denatured and hybridized with specific oligonucleotide probes. These probes are immobilized as parallel lines on membrane strips. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase is added, which binds to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen yields a purple precipitate and the results can be interpreted with according to the positive and negative controls.

Samples were classified as positive for "any HPV" if at least one HPV type was detected by genotyping. Samples were considered infected with oncogenic HPV types if they were positive by genotyping for at least one of the following types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66. Samples were considered non-oncogenic, if they turned out positive for at least one of the other HPV types, other than oncogenic ones. If a sample presented more HPV types and at least one was oncogenic, then the sample was considered oncogenic.

Results and Discussion

We found a high presence of the HPV infection in the initial studied population, 59.3% patients were HPV positive. The following HPV genotypes were founded: 6; 11; 16; 18; 31; 33; 40; 42; 45; 51; 53; 54; 58; 59; 61; 66; 68; 70.

From the male subjects, 37, enrolled in this study we harvested: urethral secretions swabs in all patients (37 cases), penile lesion swabs (6 cases) and oral swabs (9 cases). Viral testing confirmed HPV DNA presence in 17 cases as single and co-infections. The men tested from the urethral canal turned out to be positive in 40.5%, there were 15 positive men and from them 40%, (6 men), were infected with oncogenic genotypes and 13.4% were infected with HPV x genotype. Although HPV DNA was detected, in two cases (13.4%), the HPV genotype could not be determined because of the limitation of the commercial HPV genotyping kit used and these genotypes were noted as HPV x. Site-specific anatomic HPV prevalence is presented in Table 1.

HPV genotyping confirmed the presence of low and high risk HPV genotypes in urethral secretions. In 7 out of 17 cases only high risk types were detected. On the other hand, in penile lesions only co-infections were found (one case with 4 different types). HPV type distribution in single infection versus co-infection is presented in Table 2.

It is to be mentioned that in co-infections at least one oncogenic HPV type was detected. As mentioned, the subjects also completed a self-administered questionnaire. Data regarding HPV status of subjects, woman-partner showed that in 8 cases both partners were positive. HPV genotyping revealed that in all cases

both partners presented the same types and in one case the man presented one more different oncogenic genotype. HPV status of

both investigated subjects and their women partner are shown in Table 3.

Table 1: Site-specific anatomic HPV prevalence.

Anatomic Site	Cases	HPV Positives	Single Infection	Co Infections	HPV x
Penile	6	2/6	-	2	-
Urethral	37	15/37	10	3	2

Table 2: HPV type distribution in single infection vs co-infections; (infection with one genotype/Infection with at least two genotypes).

HPV type	6	16	18	31	33	39	40	45	53	54	59	61	68
Urethral swabs infected with one genotype/ Urethral swabs infected with at least two genotypes	4/1	1/2		2/1	1/0	0/1			1	1	0/1	0/1	0/1
Penile lesion infected with one genotype/ Penile lesion infected with at least two genotypes	0/1		0/1				0/1	0/2					

Table 3: HPV status of both investigated subjects and their women partner.

		Urethral Secretion		Penile Lesion	
		HPV Positive	HPV Negative	HPV Positive	HPV Negative
Woman partner	HPV positive	7	3	1	1
	HPV negative	4	2	-	2
	Unknown HPV status	4	2	1	1

This study aimed to verify the use of urethral swab as a sample when collected for HPV diagnosis. It is important to reveal the protocol of the sample collection as well as the steps encountered in the testing method used in each laboratory. In this study, HPV detection was evaluated according to the type of sample used. Previous studies reported HPV prevalence ranged from 15% to 32% [11], in our 37 men group 40.5% were HPV infected. Even if the men group studied is little, the fact that more than half was asymptomatic and turned out HPV positive, shows the need of a reliable collecting sample from which one can detect HPV DNA, if it is there.

From 37 men tested by collecting samples from urethral canal, 22 men presented no symptoms and out of them 40% were infected with oncogenic genotypes, and 9% presented more than two HPV genotypes. Taking together, these data indicate that HPV shedding occurs in a high proportion of apparently healthy men. In the studied population there were different HPV types and more than half of the studied subjects presented oncogenic HPV types. Taking into account the overall presence of HPV, the high diversity of HPV types and the high proportion of oncogenic types, the epidemiologic characteristics of HPV infection in men seem different to that observed in women.

The role of infected cells from the urethral canal in HPV sexual transmission is not yet completely understood [12]. International studies have showed that HPV have been found in seminal plasma and sperm cells, and expression of certain HPV genes in sperm cells has been observed [13]. However, to our knowledge, the infectious potential of semen has not been documented; this potential remains difficult to establish in the absence of cell culture or animal models. These findings need further confirmation and

the mechanisms involved in a possible impairment of sperm quality by HPV infection merit to be investigated.

The result of HPV genotyping depends by the correct identification of HPV type. This is important when the natural history of this infection is studied but also matters if there are studies which involve patients that are treated and need monitoring and evaluation or subjects that were vaccinated. In this study all oncogenic HPV types were correctly identified as well as the non-oncogenic types. The agreement based on oncogenic characters was excellent and even there were patients with multiple HPV infections the agreement was high as well as in single HPV infections encountered at the same patient. The present study is limited by the little number of studied subjects.

Conclusion

There are several studies that have revealed the predictive value of HPV testing in monitoring persistent HPV infection from cervical specimens [14]. In contrast there are fewer studies that address the study of HPV infection at asymptomatic HPV genitally infected men. Also the method of collecting samples at this level was not sufficiently evaluated in relation with the final outcome. At this level the HPV DNA is scarce and the good collection of the sample is essential for the final validity of HPV test. In this study the urethral swab was used to detect HPV DNA even in asymptomatic HPV carriers and by collecting urethral swab we were able to diagnose HPV infection. Taking into account the overall presence of HPV, the high diversity of HPV types and the high proportion of oncogenic types, the epidemiologic characteristics of HPV infection in men seem different to that observed in women.

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