ORIGINAL ARTICLE

Vol. 38 (3): 411-418; May - June, 2012

Clinical and laboratorial study of HPV infection in men infected with HIV

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ABSTRACT

Objectives: To determine the prevalence of precursor lesions of penile cancer, to establish the concordance of diagnostic techniques (PCR, Hybrid Capture (HC) and peniscopy with acetic acid 5%) in the diagnosis of Human Papilloma Virus (HPV) of the penis of men infected with HIV and to evaluate the influence of the immune status.

Patients, Methods and Results: 276 men were studied, with a median age of 34.6 years. Prevalence of High Risk HPV, Low Risk HPV and infection with both, according to HC, was 43%, 32% and 22%, respectively. PCR showed 50% of positivity for HPV DNA. Peniscopy was positive in 27% of individuals. Peniscopy showed good specificity and low sensitivity for the detection of penile HPV, and low concordance with PCR. Men with white lesions had a 3.6 higher relative risk of positivity for HPV. The most common clinical lesion observed was vegetation, identified in 29% of patients. PCR and HC techniques showed high sensitivity for HPV DNA and there was an excellent correlation between them. Immunosuppressed individuals with CD4< 200 cells/mm3 had the highest prevalence of pre-malignant lesions that were observed in 10% of the studied individuals.

Conclusions: Peniscopy was important for identification and treatment of subclinical lesions. PCR and HC techniques were sensitive methods for the detection of HPV DNA with high concordance. Severely immunosuppressed individuals showed a higher prevalence of pre-malignant lesions of the penis.

ARTICLE INFO

Key words:
DNA Probes; HPV; Men; HIV

Int Braz J Urol. 2012; 38: 411-18

Submitted for publication:
January 01, 2011

Accepted after revision:
November 09, 2011

INTRODUCTION

In the literature, several studies point out that 10% to 20% of sexually active adults have HPV infection, although only 1% presents classic condyloma and 2% visible lesions after acetic acid application (1). According to world literature, it is rational to expect the existence of 3 to 6 million males infected with HPV (2).

The relationship between HPV infection and cervical cancer is well established and there are strong evidences that it may also be implicated in the etiology of anal and genital cancer (3).

The studies related to the determination of prevalence of HPV infection in males are very important, since they can present a subclinical and asymptomatic infection and become potential source of infection of HPV to their male or female sexual partners (4).

In view of all these facts, our study was designed to determine the prevalence of precursor lesions of penile cancer, to establish the concordance among different diagnostic techniques (PCR, Hybrid Capture (HC) and Peniscopy with acetic acid 5%) in the diagnosis of infection with the Human Papilloma Virus (HPV) of the penis of

HIV positive males and to evaluate the influence of the immunologic status on the occurrence of the lesions.

MATERIAIS AND METHODS

This is a cross-sectional descriptive study of men affected with HIV attended at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD). Data including demographic, epidemiologic and clinical characteristics of patients were collected. Physical examination (urological inspection and peniscopy with acetic acid 5%), molecular biological tests (PCR in house and Hybrid Capture II, Digene & Co®) and conventional histopathology study were also performed. Criteria for inclusion were: HIV positive males, with ≥ 18 years old, who provided written informed consent to join the study. Criteria for exclusion were: HIV negative males, Indians, psychiatric patients and those that didn't complete all steps of the study. Data were collected at Epi Info® version 6.04 platform and the statistical analysis was made through Statistical Package for Social Sciences® (SPSS) version 16.0 for Windows.

Patients lied in supine position in order to be submitted to the urological and scrotal inspection. A surgical brush with saline was rubbed against the foreskin, balanopreputial sulcus, glans and navicular fossa of the penis. The brush was immersed in an Eppendorf vial containing 1 mL of T1 buffer (commercial kit for nuclear extraction Spin Tissue-Macherey-Nagel®). The vial was tightly closed and sent to the laboratory, where was maintained at -70ºC until PCR analysis.

Another brush was used to Hybrid Capture (HC) for High and Low Risk HPV, and it was stored in an appropriate kit.

After the cytological collection, we proceeded with peniscopy and penile and scrotal inspection. A gauze soaked with acetic acid 5% was placed around the penis for 10 minutes. Positive lesions (white lesions) were biopsied, except in patients with previous histopathological or laboratorial diagnosis or that didn't allow the procedure. The samples were fixed in buffered formalin 10% and were sent to histopathological studies.

For statistical analysis, it was used Pearson's Chi-Square test with Yates correction whenever necessary; Fisher's exact test was used to categorical variables for values under 5 and significance analysis including Odds Ratio (OR) and 95% confidence intervals was performed. Significance was established for $p < 0.05$ (5%).

For concordance analysis, it was used the kappa (k) associative test.

RESULTS

Two hundred and seventy six HIV-positive male patients older than 18 years old were included. Median age was 34.6 years. Table-1 depicts socio-demographics characteristics of patients. Table-2 shows the variables related to sexual behavior, use of condoms and previous STDs and Table-3 those related to HIV virus.

Peniscopy was positive for white lesions in 27% of patients. The most frequent lesion was vegetation (29%) (Table-4). Biopsy was obtained in 22% of participants; some of them had more than one lesion and a total of 75 fragments of skin were collected for conventional histopathological study (Table-5). Pre-malignant lesions were observed in 10% of patients (Table-6), and most of them (59%) had CD4 < 200 cells/mm3.

According to HC, the prevalence for High Risk, Low Risk and both High and Low Risk HPV infection were 43%, 32% and 22%, respectively. PCR had 50% of positivity for HPV DNA.

The concordance between peniscopy and PCR was observed in 62% of samples, revealing a "weak concordance" according to kappa associative test (k = 0.2317). Patients with white lesions observed at peniscopy had a 3.6 higher risk of HPV infection.

Peniscopy was considered a diagnostic test with high specificity (86%) and low sensitivity (37%). Positive and negative predictive values were 73% and 58%, respectively. Exam accuracy was 62%.

When PCR and HC for High Risk and Low Risk HPV results were compared, 88% of samples showed similar results. There was an "excellent concordance" between the different techniques according to kappa associative test (k = 0.7522).

Table 1 - Socio-demographic variables of 276 men with HIV+/AIDS.

Demographic indices	N	%
Age		
18-29 years	101	36
30-49 years	151	55
≥ 50 years	24	9
Race/Colour		
Brown	207	75
White	47	17
Black	18	7
Yellow	4	1
Marital status		
Single	158	57
Married	53	19
Fixed partner	56	20
Divorced	7	3
Widow	2	2
Education		
Illiterate	2	1
Primary	97	35
Secondary	125	45
Terciary	52	19
Use of unlawfully drugs		
Yes	66	24
No	210	76
Smoking		
Yes	75	27
No	201	73

Table 2 - Distribution of sexual behavior variables, use of condoms and previous STDs of 276 HIV+/AIDS males.

Variables	N	%
Sexual Orientation		
Homosexual	101	36
Heterosexual	107	39
Bisexual	68	25
Beginning of sexual activity		
≤ 15 years	105	55
15-19 years	151	38
≥ 20 years	20	7
Nº of sexual partners during last year		
0	24	9
1	97	35
2-9	93	34
≥ 10	62	22
Use of condom prior to HIV+		
Occasionally	230	85
Always	7	3
Never	39	14
Use of condom after HIV+		
No sexual relations	35	13
Occasionally	28	10
Always	206	75
Never	7	2
Prior STDs*		
Yes	177	64
No	99	36

*sexually transmitted diseases.

Table 3 - Variables associated to the presence of HIV virus.

Variables	N	%
Time to HIV diagnosis		
≤ 3 years	198	72
4-6 years	38	14
7-9 years	15	5
≥ 10 years	25	9
Opportunistic Diseases Associated to HIV		
Yes	134	48
No	142	52
Use of ART*		
Yes	152	55
No	124	45
ART* time of use		
< 1 year	79	52
1-2 years	16	10
2-3 years	12	8
> 3 years	45	30
Lymphocyte count TCD4		
< 200	103	37
200-349	68	25
350-500	41	15
> 500	40	15
No exam	24	9
Viral load		
Undetectable	68	25
< 30.000	108	39
> 30.000	71	26
Superior limit (> 500.000)	4	1
No exam	25	9
HIV infection phase		
AIDS	179	65
Carrier	97	35

*antiretroviral therapy

Table 4 - Distribution of dermatological lesions observed in 75 positive peniscopies. After: Rook's 2010 (5).

Peniscopy Lesion	N	(%)
Vegetation	25	29
White lesion	13	15
Ulcer	10	12
Papule normochromic	13	15
Crust	1	1
Papule Hypochromic	4	5
Papule Hyperchromic	4	5
Macula hypochromic	9	10
Macula hyperchromic	3	4
Eritema	1	1
Hyperchromic Plate	2	2
Total	85	100

DISCUSSION

The prevalence of HPV in our study was higher than of the study of Goldstone et al. They evaluated 602 HIV negative males who were engaged in sex with other males and observed a prevalence of 18.2% of HPV infection of the penis using also PCR (6).

Peniscopy showed high specificity and low sensitivity. However, most studies showed a weak specificity of the exam and also a good sensitivity (7-9). We believe that our results were biased due to the high prevalence of HPV in the studied population (around 50%), explaining the good specificity, and that most of the patients had subclinical or latent infection with HPV, that impaired the identification through peniscopy, only with biomolecular techniques, explaining the low sensitivity.

Some risk characteristics for HPV infection of penis of HIV positive males were identified in our study, including heterosexual behavior (higher rate of penile HPV infection compared to bisexuals and homosexuals, probably due to the high rate of female infection in our population, demonstrated in several studies done in Manaus (10-12).

PCR and HC techniques had high concordance and sensitivity for the detection of HPV. Rodrigues et al. (13) demonstrated that HC and PCR techniques for the detection of HPV in clinical samples had a fair concordance, including conventional and real time techniques (k = 0.338). When they compared conventional PCR with real time PCR they observed an almost perfect concordance (k = 0.818).

There are very few studies related to intraepithelial neoplasms or penile cancer of HIV positive men. Kreuter et al. studied 263 HIV-positive homosexual men and found penile intraepithelial neoplasms of penis in 11 (4.2%) and of anus in 156 (59.3%) (14).

The limitations of our study included the small size of sample that prevented a strong association among the techniques. The study was conducted in an AIDS ambulatory. However, most

Table 5 - Distribution of histopathological findings of patients with clinical lesions detected.

Histopathological Findings	N	(%)
Angioceratoma	1	1
Chronic inespecifc balanitis	2	2
Inespecific ulcerated balanitis	1	1
Inespecific chronic balanopostitis	2	2
Condyloma Acuminata	19	27
Flat Condyloma	1	1
Epidermodysplasia	2	2
Nonspecific chronic eczema	1	1
Hypermelanose	1	1
High grade intra-epitelial lesion (HSIL)	4	6
Fungal infection	1	1
Scleroathrofic lichen	2	2
Lichen planus	1	1
Low grade Intra-epitelial lesion (LSIL)	18	25
Molluscum Contagiosum	6	9
Melanocytic nevi	1	1
Bowenoid papulosis	5	7
Chronic inespecific postitis	1	1
No significative alterations	6	9
Total	75	100

Table 6 – Distribution of histopathological findings of 27 pre-malignant lesions.

Pre-malignant lesions	
Epidermodysplasia Verruciformis Like	02
High grade intra-epitelial lesion (HSIL)	04
Low grade Intra-epitelial lesion (LSIL)	17
Bowenoid papulosis	03
Bowenoid papulosis+ LSIL+Bowenoid papulosis	01
Total	27

HIV+/AIDS patients from Amazonas are attended at FMT-HVD, which we believed allowed the study of a significant sample of patients.

We believe that the present results can be used to delineate preventive programs for early detection of penile cancer, in individuals with higher risk, including immunosuppressed patients. Diagnosis and treatment of male partners infected with HPV would also allow a reduction of sexually transmitted diseases.

CONCLUSIONS

Prevalence of DNA HPV was approximately 50%.

Peniscopy proved to be a high specific and low sensitive exam.

Concordance of peniscopy and PCR for the detection of HPV was low.

Concordance of PCR and HC for HPV detection was excellent.

We observed a prevalence of 10% of patients with pre-malignant lesions determined by histopathological studies and that most of them were severely immunosuppressed (TCD4 < 200 cells/mm^3).

CONFLICT OF INTEREST

None declared.

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