

**HUMAN PAPILLOMAVIRUS (HPV) INFECTION OF SEXUAL PARTNERS OF
WOMEN PRESENTING CERVICAL INTRAEPITHELIAL NEOPLASIA**

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ABSTRACT

Human papillomavirus (HPV) infections of the genital tract are the most prevalent sexually transmitted viruses worldwide. Oncogenic HPV types cause pre-malignant lesions that can progress to cervical carcinoma. In the male genital tract, most HPV infections are sub-clinical and associated with a vicious circle [stet] of treatment-reinfection of women. Nevertheless, HPV pathogenic processes are still poorly understood. The literature suggests that different HPV types can be found among sexual partners. In our study, we aimed to verify HPV infections in female patients as well as in their sexual partners, to test this hypothesis. The HPV DNA prevalence in women with Cervical Intraepithelial Neoplasia (CIN) was 92.5% compared with 25% for normal women, with a statistically significant difference ($p < 0.001$). In male samples, the HPV DNA prevalence in partners from CIN women was 50% and for normal women partners, it was 17.5%. In the group of CIN women, we observed that 20 couples had HPV DNA in both partners. However, only 50% of the couples shared the same HPV type. In the group of normal women, only 6 couples were simultaneously infected by HPV, and from them only 33.3% had the same virus type. These results may be attributed to differences in local immunity and organization of the genital epithelia of each sex. On the other hand, female lesions would not be the result of re-infection by sexual partners, but rather a true recurrence of a latent infection. Finally, such 40% of agreement among all couples leads us to suggest a vicious circle [stet] of infectious processes, perpetuating HPV in the sexually active population.

INTRODUCTION

Human papillomaviruses (HPV) are small DNA viruses that infect human epithelia. Genital infection caused by HPV is a sexually transmitted disease that affects the worldwide population with a prevalence ranging from 7% to 70%, according to the group studied and the diagnostic methodology (Syrjanen & Syrjanen 1999).

In the last decade, the significantly increased incidence of genital HPV infections has been attributed to an early start of sexual activity, poor hygiene, promiscuous sexual life and inadequate preventive procedures (Rosenblatt et al. 2004). Such HPV infections can result in persistent processes leading to premalignant neoplasia of the cervix.

Cervical neoplasia is often described in women with a precocious beginning of sexual life as well as to those with multiple sex partners, being nearly absent in women without sexual activity. On the other hand, partners of men presenting penile cancer presented a risk eight times higher of developing cervical cancer (Gissman 1984).

Few studies focused on male infection. Usually, HPV infected men present subclinical lesions and are considered the epidemiological source of female infections that lead to cervical malignant transformation (Schneider et al. 1988). The expected transmission rate between partners is about 60%, according to Brown et al. (1999). Several studies described a high prevalence of HPV infections in the sexual partners of women with CIN or condyloma, compared to the expected 10% of prevalence in the general population (Schneider et al. 1988).

The present study aimed to determine the prevalence of HPV infection in sexual partners of women presenting CIN as well as in matched controls (normal cervix), and to verify HPV types prevailing in each sex.

MATERIAL AND METHODS

Study design.

This was a case-control study designed to evaluate the presence of HPV infection in female patients presenting CIN as well as in their male sexual partners. For each woman presenting CIN, a normal woman's cervical sample was matched with a sample from her corresponding sexual partner.

Sample.

Forty women presenting CIN at several grades (25 CIN I, 11 CIN II and 14 CIN III) and forty women without CIN (normal patients) that came to the Sexually Transmitted Disease Clinic of our University Hospital for cervical cancer screening (Pap smear), as well as their respective partners, were assessed. The study was conducted from February 1998 to July 2003. The study was approved by the UFF Ethics Committee. All subjects signed an informed consent form to participate in the study. All patients were examined and after applying 5% acetic acid, scrapings were collected using peniscopy/colposcopy. Directed biopsies were obtained from areas suggestive of HPV infection and confirmed CIN and normal tissues were selected to this study.

Peniscopy.

Peniscopic images were classified as condilomatous lesions (acuminated, pigmented or non-pigmented warts), lesions suggestive of HPV infection (aceto-white areas, erythematous or macular lesions, papillomas or pearly papules) or normal. Penile scrapes were done by using a Urotest brush in areas identified by peniscopic images as being of clinical or subclinical significance. All the brushes were kept in TE solution (10mM Tris hydrochloride pH 7.5, 1mM EDTA) at -20°C until DNA extraction.

Histopathology.

Cervical smears and biopsies from confirmed normal and CIN cases were collected, kept at -20°C and sent to the Virological Diagnostic Lab from UFF.

HPV Detection and typing - DNA extraction.

Samples were incubated for 4 hours at 56°C in digestion buffer (10 mM Trishydrochloric acid pH 8.3, 1 mM EDTA pH 8.0, 0.5% Tween 20, proteinase K (final concentration of 400 µg/ml). Later, they were extracted with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated with one-tenth volume of 0.3 M sodium acetate and three volumes of 100% ice-cold ethanol, washed with 70% ethanol, air-dried and suspended in 50 µl of sterile water.

PCR procedure.

MY09/11 consensus primers, which amplify 450-bp (base pair) DNA sequences within the L1 region of HPV, were used to detect generic HPV DNA. Amplification was carried out in 50 µl of reaction mixture (1 X polymerase chain reaction [PCR] buffer, 200 mM dNTPs, 1.5 mM MgCl₂, 50 pmol of each primer, 0.25 U unit of Taq polymerase, and 5

µl of sample) with 35 cycles of amplification. Each cycle included a denaturation step at 94°C for 1 minute, an annealing step at 55°C for 2 minutes, and a chain elongation step at 72°C for 2 minutes using DNA Thermal Cycler (Perkin Elmer, CETUS). The beta-actin primers Ac1 and Ac2 (0.1 pmol each), which amplify a 330-bp region of the human DNA, were used as a sample internal control. Negative controls for background contamination were added to the DNA template. Polymerase chain reaction products were analyzed on 1.3% agarose gel with ethidium bromide staining for visualization of DNA under ultraviolet light and their molecular weight was determined by comparison with a 100-bp DNA ladder (Oliveira et al. 2003).

HPV typing.

HPV typing was done by PCR amplification with primers from the E6 gene DNA sequences of HPV 6, 11,16, 18, 31, 33, 35, 45 and 58 (Cavalcanti et al. 2000).

Statistical analysis.

The Chi-square and the Fisher tests were used to compare the two independent groups (CIN X normal women) as well as their sexual partners. Confidence intervals of 95% were applied.

RESULTS

Our female study group was composed by eighty women with average age of 29.7 years old. Analyzes of age groups revealed that the CIN group presented older patients (32.1 years old) while normal women had average age of 27.2 years old, showing statistically a significant difference ($p<0.01$). The 80 men studied presented an average age

of 33.8 years. The CIN group partners had an average age of 34.6 and normal group partners of 33.1, with no significant difference ($p=0.562$).

Total HPV prevalence in women was 58.8% (47/80). HPV DNA incidence in women with CIN was 92.5% and 25% for normal women, with a statistically significant difference ($p<0.001$) (Table 1). In male samples, HPV prevalence was 33.8% (27/80). HPV DNA incidence in partners of CIN women was 50% and for partners of normal women, it was 17.5%, also significant by statistical analysis ($p<0.001$) (Table 2). Although rates of infection were statistically significantly different among normal and CIN women (25% x 92.5%, $p<0.001$), among the male group, there were no statistically significant differences (25% x 17.5%, $p>0.01$).

Table 1. Prevalence of HPV DNA detection by PCR in female patients according to histological diagnosis.

Diagnosis at Histo-pathology	Infection by HPV in female patients			
	n (%)			
	High risk HPV	Low-risk HPV	Multiple HPV Types	Total Prevalence
CIN	22/40 (55%)	7/40 (17.5%)	8/40 (20%)	37/40 (92.5%)
Normal	7/40 (17.5%)	3/40 (7.5%)	- (0%)	10/40 (25%)

High risk viruses prevailed in both CIN (70% x 37.5%) and normal women (17.5% x 7.5%, $p<0.001$) (Table 1). Nevertheless, for the male partners, no significant difference was found regarding HPV type frequencies for CIN women partners (30% x 25%, $p=0.186$) and for normal women partners (12.5% x (7.5%, $p=0.324$) (Table 2).

Table 2. Prevalence of HPV DNA detection by PCR in male partners from both normal and CIN women according to peniscopy

Diagnosis at Peniscopy	Infection by HPV in male partners			
	n (%)			Prevalence of HPV
	High risk HPV	Low-risk HPV	Multiple Infections	
Partners from CIN group with altered peniscopy	6 (15%)	6 (15%)	2 (5%)	14 /20
Partners from CIN group with normal peniscopy	4 (10%)	2 (5%)	-	6/20
Total CIN partners	10/40 (25%)	8/40 (20%)	2/40 (5%)	20/40 (50%)
Partners of Normal women	4/40 (10%)	2/40 (5%)	1/40 (2.5%)	7/40 (17.5%)

Table 3. Agreement rates of HPV infection among female patients and their partners.

HPV infection in both sexual partners among CIN women group				
n= 20				
Concordant HPV types				
n=10				
HPV types by PCR	4 HPV 6	4 HPV 16	1 HPV 18	1 HPV6/16

All the male lesions (14) found in peniscopy, cytology and biopsy were from partners of CIN women. Among this male group, only three had evident clinical lesions, characterized as acuminated condyloma. The other 11 were sub-clinical carriers of HPV DNA, presenting aceto-white lesions (8) or pearl papules (3). PCR revealed that all of them presented HPV infection (Table 2). All partners of normal women were also normal at peniscopy, as well as in cytology scrapes. Thus, biopsies were not performed.

In the group of CIN women, we observed that 20 couples had DNA in both partners. However, only 10 couples shared the same HPV type (50%) (Table 3). In the group of normal women, only 6 couples were simultaneously infected by HPV, and of them only 2 had the same virus (33.3%) (Table 4).

Table 4. Agreement rates of HPV infection among normal female patients and their partners.

HPV infection in both sexual partners among normal women group		
n= 6		
Concordant HPV types		
n=2		
HPV types by PCR	1 HPV 6	1 HPV 16

DISCUSSION

In the present study, we found that 50% of sexual partners of women with CIN had clinical or subclinical lesions infected by HPV. Rombaldi et al. (2006) described a rate of infection of 62.6% in male partners of CIN women, with an average age of 30.7 years old. In similar studies, Tabrizi et al. (1992) had already shown 38% of HPV infection. Recently, Bleeker et al. (2006) studying older male populations (age = 38) found a 40% infection rate. Our male group had an average age of 34 years, with similar intermediate prevalence rates. The world literature associates HPV infection with decreasing rates in older men.

Our data, in agreement with the literature showed that HPV infection was frequent in male sexual partners of CIN women, indicating that a systematic study of this population is necessary in order to avoid a vicious circle of infection-treatment-reinfection. Among normal couples, prevalence of HPV infection was 25% in women and 17.5% in their male

partners, with no statistically significant difference, similar to other studies obtained from Rio de Janeiro state population (Cavalcanti & Oliveira 2003).

In the group of women presenting CIN as well as in the group with normal cervix (controls), high risk HPV prevailed, accounting for 75% and 17.5% of infections respectively, a statistically significant differences ($p < 0.001$). Low risk types were detected in 17.5% of the CIN group and 7.5% of the normal group. In male partners of CIN women, we found nearly coincident prevalence rates for high and low risk viruses (30% and 25% respectively) while in partners of normal women the rates were 12.5% high risk and 7.5% low risk, with no statistical significance ($p > 0.1$). Paesi et al. (2003) carried out a prospective study with couples, among which, women presented CIN III and the prevalent HPV types were 16 and 18, similar to our data. On the other hand, Rombaldi et al. (2006) found types 6 and 11 prevailing in couples, although the population studied presented heterogeneous diagnoses in cytopathology, with both males and females presenting with lesions ranging from condylomatous to severe CIN.

In two reports, Castellsagué et al. (1997, 2002) studied couples infected by HPV but found little agreement between the types identified (31.8%). The group then suggested that this was due to the different levels of biological activity of the genital tract as well as the differences in local immunity and organization of the genital epithelia of each sex. Restriction to pathogenic processes may present differently for each sex, allowing diverse HPV types to establish infectious pathogenic processes. The degree of keratin expression, number of epithelia layers within each stratum as well as colonization by bacterial flora may be factors influencing diverse patterns of viral infection. Rosenblatt et al. (2004) found only 13% of agreement, and proposed a complete different panel, where recurrence of

lesions in CIN women might not be associated with reinfection from sexual partners but rather a true recurrence of a latent infection. Nevertheless, HPV infections do not have an experimental model to ensure the occurrence of true latency, and proposals are mainly theoretical, based on other DNA viruses that infect the genital tract, such as Herpes simplex virus. Hence, latency remains a possible outcome of HPV infection but needs to be elucidated.

Although our results showed that 50% of the infected couples presented the same HPV types in CIN group, and were, then, higher than the ones published, we believe that the 50% disagreement in infecting types is due to such differences in the genital tract of men and women (Table 3). On the other hand, 50% of agreement among sexual partners leads us to suggest a vicious circle of infections among couples, perpetuating HPV in this group.

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