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Infection with human papillomaviruses of sexual partners of women having cervical intraepithelial neoplasia

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Abstract

Epidemiological studies show that human papillomaviruses (HPV) are strongly related to cervical cancer and cervical intraepithelial neoplasias (CIN). Unlike the case for women, there are no consistent data on the natural history of HPV in the male population even though these viruses are prevalent in males. We carried out a prospective study to assess the prevalence of HPV in males as well as the factors that determine such infections in 99 male sexual partners of women with CIN. The genitalia of the males were physically examined and subjected to peniscopy for the collection of scrapings which were subjected to the polymerase chain reaction and restriction fragment length polymorphism to detect HPV. Of the 99 males sampled, 54 (54.5%) were positive for HPV DNA, 24% of whom presented normal peniscopy, 28% presented evident clinical lesions and 48% isolated lesions consistent with subclinical infection. In the HPV-negative group, 53% showed normal peniscopy, 4% presented evident clinical lesions and 42% isolated lesions consistent with subclinical infection. The study detected a statistically significant association ($P < 0.02$, Pearson chi-square test) between HPV infection and both the mean number of sexual partners which a male had during his life and the mean number of sexual partners in the year prior to testing. Viral types 6 and 11 were most frequently encountered. The study shows that infection with HPV was frequent in male sexual partners of women with CIN.

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Introduction

Infection with human papillomavirus (HPV), a non-culturable DNA virus of the family Papillomaviridae (1) has gained great importance during the last decade due to the recognition that this virus participates in the genesis of cervical cancer (2) and has high worldwide prevalence (3). More than 118 different HPV genotypes have been identified, of which about 40 were detected in the anal-genital area (4). It is known that HPV is mainly transmitted sexually (5,6) and that the expected transmission rate between partners is about 60% (7). Genital HPV infection in males is less well studied than in females and there are few consistent data on the prevalence of this virus and the natural his-

tory of HPV infection in males. A previous study (8) found that the prevalence of HPV in males ranged from 3.6 to 84% depending on the population and the methodology used, while it is also known (9-11) that the highest incidence (63 to 84%) occurs in men attending clinics for the treatment of sexually transmitted diseases. There is a high prevalence (10-85%) (12-17) of HPV lesions in the sexual partners of women with genital condyloma or cervical intraepithelial neoplasias (CIN) as compared with the 10% prevalence found in the general male population (18).

The present study determined the prevalence and the factors of HPV infection in an epidemiological survey of 99 male partners of women known to have CIN. Genital scrapings were submitted to the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) to identify HPV carriers.

Material and Methods

Population

A prospective study was carried out at the University of Caxias do Sul (UCS, Caxias do Sul, RS, Brazil) between February 2003 and July 2004 on 99 male sexual partners of women with CIN. The study was approved by the UCS Ethics Research Committee. There was no conflict of interest for any of the authors. All subjects signed an informed consent form to participate in the study. The external genitalia of each male were examined physically before applying 5% acetic acid for 5 min and examining the genital area and collecting scrapings using peniscopy. Peniscopic biopsies were obtained from areas suggestive of HPV infection.

Epidemiological survey

In the epidemiological survey we considered the following variables: age, age at first intercourse, circumcision, condom use with female sex workers, condom use with sexual partners, educational level, marital status, marital stability (number of years married), race, sexual intercourse with female sex workers, sexual partners up to the date of the survey, sexual partners in the year preceding the survey, sexually transmitted diseases, and tobacco smoking.

Peniscopy

Peniscopic images were classified as condylomatous lesions (acuminated, pigmented or non-pigmented warts), lesions suggestive of HPV infection (aceto-white areas, erythematous or macular lesions, papillomas or pearled papules) or normal.

Sampling methods

For each of the males studied a Urotest® brush was used to scrape the genitalia in the following sequence: urethral canal, areas identified by peniscopic images as being of clinical or subclinical significance regarding HPV, dorsal and ventral pre-glans region, preputial mucosa, and penile shaft. The brush was kept in TE solution (10 mM Tris hydrochloride, pH 7.5, plus 1 mM EDTA) at 2-4ºC until the DNA was extracted. The scraping of the genitalia was performed after the peniscopy. Biopsies were taken from areas identified by peniscopic images as being of clinical or subclinical significance regarding HPV infection, a small piece of tissue being excised using a 'rat-tooth' forceps and an 'iris-curve' scissors. The excised material was fixed in 10% neutral formalin, embedded in paraffin and evaluated histologically, samples being considered HPV-positive when they exhibited cyto-architectural signs suggesting koilocytotic lesion, HPV-negative when the histological evaluation was normal or showed non-specific chronic inflammation, and suggestive of HPV infection when histological examination showed alterations characteristic of

hyper-keratinosis, hyperplasia, keratinization, or papillomatosis. Biopsy samples were checked by submitting them to a second evaluation by the same pathologist, who was aware of the result of the first diagnosis.

Detection of HPV DNA

Total viral DNA was extracted from the samples using a commercial kit (Puregene™ from buccal cells; Gentra Systems Inc., Minneapolis, MN, USA) and tested for HPV DNA using a PCR protocol which amplified a 450-bp segment of a conserved region of the L1 viral gene delineated by the MY9 and MY11 primers (19,20). The reaction mixture contained 2 µL of non-quantified extracted sample DNA in a final volume of 51 µL containing 10 mM Tris-HCl, pH 8.3, 5 mM $MgCl_2$, 50 µM of each dNTP, 0.4 µg of each primer, and 1.75 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). Before amplifying the sample DNA the quality of the host-cell DNA was evaluated using the PCO_4 and GH20 primers (21) to amplify a 268-bp segment of the human ß-globulin gene. Amplification was carried out in a PTC100 thermocycler (MJ Research, Watertown, MA, USA) with 5 min for denaturation followed by 40 annealing cycles of 94ºC for 30 s, 55ºC for 1 min and 72ºC for 1 min, and a final extension at 72ºC for 5 min. All amplifications included a positive control consisting of HPV 16 DNA extracted from *SiHa* cells (Ludwig Cancer Research Institute, São Paulo, SP, Brazil) and a negative control consisting of a reaction mixture minus the genomic sample DNA. Amplification products were separated on 1% (w/v) agarose gel using Tris/boric acid buffer (TBE, containing 50 mM Tris, 50 mM boric acid, and 2.5 mM EDTA), pH 8.4, and a constant volt (3 V/cm), with a molecular mass marker (4 µg ϕX174 restriction fragment cleaved with *Hae*III) being run simultaneously with the samples. After electrophoresis the gels were stained with ethidium bromide (4.62 ng/µL) and HPV or ß-globulin fragments visualized under ultraviolet light.

Viral typing

HPV-positive samples were typed using RFLP according to the method described by Bernard et al. (22). The product of the MY9/MY11 amplifications was digested with a mixture containing 50 mM Tris-HCl, 10 mM $MgCl_2$, and 10 units/µL of a restriction enzyme in a separate reaction (*Bam*HI, *Dde*I, *Hae*III, *Hinf*I, *Pst*I, *Rsa*I, or *Sal*3AI (GibcoBRL, Gaithersburg, MD, USA). Fragments of different molecular masses were separated on 7% polyacrylamide gel (20.3% acrylamide, 0.7% bis-acrylamide, 0.07% ammonium persulfate and 0.7 µL/mL TBE 10X TEMED (GibcoBR). A sample of undigested amplified sample DNA and 2.5 µg standard ϕX174 DNA were run simultaneously in a vertical miniVE electrophoresis cell (Hoefer, San Francisco, CA, USA). After silver staining the fragments were compared with those described by Bernard et al. (22).

Statistical analysis

Data were analyzed statistically with the Statistical Package for the Social Science (SPSS) version 12.0, using descriptive statistics and the conventional Pearson chi-square test at a pre-fixed α level of 0.05.

Results

Of the 99 men studied, 54 (54.5%) were positive for HPV DNA, and 24.1% of them presented normal peniscopy, 27.8% evident clinical lesions (acuminated, pigmented or non-pigmented warts) and 48.1% isolated lesions consistent with subclinical infection (aceto-white areas, erythematous or macular lesions, papillomas or pearled papules). In the group negative for HPV DNA, 53.3% presented normal peniscopy, 4.4% showed

evident clinical lesions and 42.2% isolated lesions consistent with subclinical infection (Table 1). The following areas were found to suffer HPV attack: the frenular delta (31.4%), the preputial mucosa (29.6%), the glans-preputial sulcus (20.4%), the glans-penis (9.3%), the penile shaft (7.4%), and the urethral canal (1.9%).

Fifty-four of the 74 biopsies examined histologically were from the men shown to be positive for HPV DNA, koilocytosis being found in 27.8% of these men and constituting the most common histological finding (Table 2). Koilocytosis and other morphological symptoms such as hyperplasia, keratinization or papillomatosis attributed to HPV infection were present in both the HPV DNA-positive and -negative groups. Histological examination identified *in situ* carcinoma of the penis in only one tissue sample.

Table 1. Number and percentage (in parentheses) of men positive or negative for human papillomavirus (HPV) DNA and the type of their lesions as detected by peniscopy.

Type of lesion	Men positive or negative for HPV/DNA		
	Positive	Negative	Total
Condylomatous lesions[1]	15 (28%)	2 (4.5%)	17 (17%)
Lesions suggestive of infection[2]	26 (48%)	19 (42%)	45 (46%)
Normal	13 (24%)	24 (53.5%)	37 (37%)
Total	54 (100%)	45 (100%)	99 (100%)

[1]Acuminated, pigmented or non-pigmented warts. [2]Aceto-white areas, erythematous or macular lesions, papillomas or pearlized papules.

Table 2. Number and percentage (in parentheses) of men positive or negative for human papillomavirus (HPV) DNA and the histological type of their lesions when examined by biopsy.

Histology	Men positive or negative for HPV/DNA		
	Positive	Negative	Total
Koilocytosis (HPV positive)	15 (28%)	5 (11%)	20 (20%)
Lesions suggestive of HPV infection[1]	14 (26%)	13 (29%)	27 (27%)
HPV negative[2]	13 (24%)	12 (27%)	25 (26%)
Intraepithelial penile lesions	1 (2%)	-	1 (1%)
No biopsy obtained	11 (20%)	15 (33%)	26 (26%)
Total	54 (100%)	45 (100%)	99 (100%)

[1]Papillomatosis, hyperplasia, keratinization, or hyperkeratosis. [2]Normal, or chronic non-specific inflammatory process.

Analysis of the personal data of the 54 men positive for HPV DNA revealed that their mean age was 30.7 ± 10.3 years (range: 18-56 years; Table 3). There was no significant relationship between the presence of HPV DNA and the following variables: age at first sexual encounter, educational level, frequency of condom use with sexual partners, frequency of condom use with sex workers, marital status, marital stability expressed in years, circumcision, race, sex relationships with sex workers, tobacco use, and previous history of sexually transmitted diseases (Tables 2 and 3). As shown in Table 3, there was a significant positive correlation between the presence of HPV DNA and number of sexual partners up to the date of the examination ($P = 0.0014$) and number of sexual partners in the year preceding the survey ($P = 0.021$). The HPV types detected in the study were: HPV 6 (56.1%), 11 (36.8), 16 (3.5%), 40 (0.7%), 61 (0.7%), and 84 (0.7%). The DNA from two HPV types, 11 and 84, was detected in one man.

The most frequently cited sexually transmitted diseases in both the HPV DNA-negative and -positive groups were gonorrhea, acuminated condyloma, syphilis, and genital herpes.

Discussion

The present study demonstrated that 62.6% of the sexual partners of women with CIN had condylomatous, macular or papular genital lesions upon peniscopic examination after application of acetic acid (Table 1). Tabrizi et al. (23) studied the sexual partners of women with CIN and found that 38% of them had clinical lesions and/or lesions identifiable by peniscopy. In our study we found that the areas most affected by genital HPV were the frenular delta and the preputial mucosa, probably because these are the most moist areas and are most subject to micro-

Table 3. Epidemiological variables investigated in the present study.

Epidemiological variable	Men positive or negative for HPV/DNA	
	Positive (N = 54)	Negative (N = 45)
Age		
≤19	5 (9.3%)	2 (4.4%)
≥20 to ≤29	23 (42.6%)	19 (42.2%)
≥30 to ≤39	16 (29.6%)	9 (20.0%)
≥40 to ≤49	6 (11.1%)	14 (31.1%)
≥50 to ≤59	4 (7.4%)	1 (2.2%)
Mean age for HPV-positive men (30.7 ± 10.3 years)	-	-
Mean age for HPV-negative men (33.0 ± 9.5 years)	-	-
Age at first intercourse		
≤16	43 (79.6%)	26 (57.8%)
≥17 to ≤20	10 (18.5%)	17 (37.8%)
≥21 to ≤30	1 (1.9%)	2 (4.4%)
Mean age for HPV-positive men (15.5 ± 2.8 years)	-	-
Mean age for HPV-negative men (16.2 ± 2.6 years)	-	-
Circumcision		
Circumcised	7 (13.0%)	3 (6.7%)
Uncircumcised	47 (87.0%)	42 (93.3%)
Condom use with female sex workers		
Never	4 (16.0%)	6 (28.6%)
Sometimes	5 (20.0%)	7 (33.3%)
Frequently	5 (20.0%)	2 (9.5%)
Always	11 (44.0%)	6 (28.6%)
Condom use with sexual partner		
Never	31 (57.4%)	28 (62.2%)
Sometimes	15 (27.8%)	15 (33.3%)
Frequently	4 (7.4%)	1 (2.2%)
Always	4 (7.4%)	1 (2.2%)
Educational level		
Illiterate	1 (1.9%)	1 (2.2%)
Primary school (complete or incomplete)	32 (59.2%)	1 (2.2%)
Secondary school (complete or incomplete)	18 (33.4%)	13 (28.9%)
University (complete or incomplete)	3 (5.6%)	5 (11.1%)
Marital status		
Single	9 (16.7%)	8 (17.8%)
Married	28 (51.9%)	27 (60%)
Cohabiting	17 (31.5%)	10 (22.2%)
Marital stability (number of years married)		
≥1 to ≤2	23 (42.6%)	16 (35.6%)
≥3 to ≤5	10 (18.5%)	7 (15.6%)
≥6	22 (40.7%)	22 (48.9%)
Mean for HPV-positive men (6.7 ± 7.4 years)	-	-
Mean for HPV-negative men (9.1 ± 8.2 years)	-	-

Continued on next page

Table 3 continued.

Epidemiological variable	Men positive or negative for HPV/DNA	
	Positive (N = 54)	Negative (N = 45)
Race		
White	46 (85.2%)	42 (93.3%)
Non-white	8 (14.8%)	3 (6.7%)
Sexual intercourse with female sex workers		
Yes	25 46.3%)	21 (46.7%)
No	29 (53.7%)	24 (53.3%)
Sexual partners up to the date of the survey		
≥1 to ≤10	22 (40.7%)	27 (60.0%)
≥11 to ≤50	27 (50.0%)	18 (40.0%)
≥51*	5 (9.3%)	-
Mean for HPV-positive men (23.5 ± 22.5)*		
Mean for HPV-negative men (14.1 ± 12.1)*		
Sexual partners in the year preceding the survey		
1	33 (61.1%)	35 (77.8%)
≥2	21 (38.9%)	10 (22.2%)
Mean for HPV-positive men (2.2 ± 1.4)	-	-
Mean for HPV-negative men (2.1 ± 0.9)	-	-
Sexually transmitted diseases[1]		
None	36 (66.7%)	31 (68.9%)
Gonorrhea	10 (18.5%)	8 (17.8%)
Condyloma	5 (9.3%)	4 (8.9%)
Syphilis	2 (3.7%)	-
Hepatitis	1 (1.9%)	1 (2.2%)
Genital herpes	1 (1.9%)	3 (6.7%)
Soft chancre	1 (1.9%)	-
Chlamydial urethritis	1 (1.9%)	-
Smoking		
Non-smokers	32 (59.3%)	29 (64.4%)
<10 cigarettes per day	7 (13.0%)	4 (8.9%)
≥10 cigarettes per day	15 (27.8%)	12 (26.7%)

Data are reported as number and percentage (in parentheses) of men positive or negative for human papillomavirus (HPV). [1]The same individual sometimes gave more than one response to this question. *P < 0.05 indicates a statistically significant difference between the positive and negative groups by Pearson's chi-square test.

traumas during sexual activity.

The collection of material followed by peniscopy was justified by the possibility of working with minimum amounts of viral DNA in the penile samples obtained through scrapings, which might imply false-negative results for DNA/HPV, although the collection covers the areas that are most affected by HPV (6,24,25). The study cannot infer the percentage of false-negative results for DNA/HPV, but false-negative results could be explained by 1) the limitations of brush use to obtain the sample (coverage of scraped areas, pressure applied to perform the scraping, and the limitation of the brush itself in obtaining the sample); 2) by the limited

amount of human cells contained in the sample; 3) by the amplification technique used for the b-globin, and 4) the presence of inhibitors or the technique used to extract DNA. Studies comparing different sampling methods must be performed and the method used to extract human and viral genetic material should be improved.

Koilocytosis was the most frequent histological finding (27.8%) in the men who were positive for HPV DNA (Table 2). Hippeläinen et al. (24) found that 53.7% of a group of HPV DNA-positive men exhibited koilocytosis, while Nicolau et al. (26) using the same methodology, found a 50% frequency of koilocytosis. The results of our study did not allow us to infer that koilocytosis is a pathognomonic characteristic of HPV infection of the male urinogenital area because there were as many false-positives as false-negatives, a fact which may lead to false information being given to the patient.

The mean age of our group of men who showed HPV DNA was 30.7 years (Table 3), as compared to the mean of 38 years in the study by Bleeker et al. (27) and 26.5 years in the study by Baken et al. (28), although the case-controlled study carried out in different countries by Franceschi et al. (29) showed no association between age and HPV infection.

With respect to educational level (Table 3), our sample may not have been representative of all HPV-contaminated males in the city of Caxias do Sul and it is quite possible that men with completed secondary or higher education would have better access to more reliable information on the disease they were infected with and were also able to attend private clinics for treatment. All the men in the group positive for HPV DNA had only basic or secondary education and belonged to the segment of society that has least access to information and whose only access to medical assistance is via the public health service. These data suggest that health education is very precarious and that an epidemiological survey should be conducted to ascertain what level of sex education this group of men receives in school and at home.

There was no association between marital status of the men and the presence of HPV DNA (Table 3). The study of the association between marital stability ≥6 years and marital status revealed the presence of HPV DNA in 12.5% of single men, in 23.5% of cohabiting men and in 60.7% of married men. There was a statistically significant ($P = 0.025$) association between the presence of HPV DNA in married males and in males with marital stability ≥6 years.

The age at first sexual encounter (Table 3) showed that 79.6% of HPV DNA-positive men had initiated sexual activity when they were ≤16 years old, but this was not statistically significant. In a 2002 study of men in the US, Castellsagué et al. (30) found that 22.9% of uncircumcised men had their first sexual experience when they were less than 16 years old while only 4.2% of circumcised men had their first sexual experience at this age. Colombian and Spanish males were the subjects of a study published in 1997 by Castellsagué et al. (31), who found that 20.1% of males had their first sexual encounter when they were less than 15 years old, while Franceschi et al. (29) found that 16% of males had their first sexual encounter at ≤16 ($P = 0.67$). In our study, men in the HPV DNA-positive group had their first sexual encounter at a mean age of 15.5 years but there was no statistically significant association between the presence of HPV DNA and age at first sexual encounter. Hippeläinen et al. (25) published an epidemiological study which showed that Finnish males had their first sexual encounter when they were 16.2 ± 1.5 years old. Rotola et al. (5) showed that the mean age at which Italian males in the city of Bologna had their first sexual encounter was 18 years.

Our data indicate that in the group of men studied the greatest risk factor ($P = 0.038$) for acquiring HPV DNA was intimately re-

lated to the total number of sexual partners up to the date of the survey, with men who had the highest number of sexual partners also having the highest risk (P = 0.038) of being positive for HPV DNA (Table 3). Castellsagué et al. (30) studied uncircumcised men who had had less than five sexual partners up to the time of the study and found that 12.5% of them were positive for HPV DNA, while among men who had had more than five sexual partners up to the time of the study the percentage of HPV DNA-positive subjects increased to 44.7%. Franceschi et al. (29) found a highly significant association (P < 0.01) between the presence of HPV DNA and the number of sexual partners up to the date of the study, with 21.1% of men having less than 10 sexual partners being positive for HPV DNA, as opposed to 43.3% of men having more than 10 sexual partners.

The number of sexual partners in the year preceding our study (Table 3) was a highly significant (P = 0.021) variable concerning the presence of HPV DNA; however, this variable has not been previously evaluated by researchers in this area. The results obtained show us that the higher number of sexual partners is correlated to higher risks of being HPV DNA positive.

We found that for the shortest period of marital stability (≥1 to ≤2 years) 42.6% of subjects were positive for HPV DNA and for the highest period of marital stability (≥6 years) 40.7% of the subjects were positive. Both the minimum and maximum marital stability showed the highest percentages of HPV DNA-positive men (Table 3). In their 1993 study, Hippeläinen et al. (25) found that 68.2% of men who had had stable relationships for up to 2 years were positive for HPV DNA, while only 31.7% of men who had had stable relationships for more than two years were HPV DNA positive. These results were also not statistically significant.

The analysis showed that 59.3% of HPV DNA-positive males smoked tobacco (Table 3). However, as previously observed by Rotola et al. (5), Hippeläinen et al. (24,25), Franceschi et al. (29), and Wikstrom et al. (32), no significant correlation was observed between smoking and HPV positiveness in males. The absence of correlation between smoking and HPV infection in males, and the positive correlation observed in females (33) can be considered as an indicative that tobacco smoking, local immunity and the different structure of epithelial tissue of the female genital tract favor infection by HPV in females and the genesis of precursor and invasive lesions in the neck of the uterus.

Of the men in our HPV DNA group, 13% were circumcised, but there was no statistically significant association between circumcision and HPV DNA infection (Table 3). Castellsagué et al. (30) studied 292 circumcised and 847 uncircumcised men in different countries and found that 5.5% of circumcised and 19.6% of uncircumcised men were infected with HPV, and found an association between male circumcision and a reduced risk of genital infection with HPV. There is a reduced risk of cervical cancer in women with circumcised sexual partners, but with a high risk of HPV infection for the women.

We found that 46.3% (not significant) of the HPV DNA-positive group of males in our study had sexual intercourse with female sex workers (Table 3). Franceschi et al. (29) found that 57.6% (not significant) of their sample of males had sexual contact with female sex workers, while Castellsagué et al. (30) reported that 20.4% of their sample of males had such relationships and Castellsagué et al. (31) cited that 41.6% of Spanish and 61.4% of Colombian men had sexual contact with female sex workers. In our study, the lack of association between previous contact with female sex workers and HPV infection may be explained by the fact that our sample was relatively small. During the epidemiological interviews with the men it was noted that the majority of the 65 men who admitted to having had contact with

female sex workers mentioned that their contact was casual and in the past. It is known that female sex workers have a higher risk of acquiring and disseminating sexually transmitted diseases because they have a greater number of sexual partners. To clarify this question, there is the need for a more elaborate epidemiological study in which this variable and other aspects of sexual behavior are surveyed in more detail.

About 84% of the HPV DNA-positive group used condoms (sometimes, frequently or always) during sexual activity with female sex workers but this was not statistically significant (Table 3). Franceschi et al. (29), in a similar Brazilian study, found that condom use was 86% and that 37% of the men studied in different countries regularly used condoms during sexual intercourse with female sex workers and demonstrated that the HPV-negative group made better use of condoms in sexual relationships with female sex workers. Although our study did not confirm the important protective role of condoms, this factor in itself indicates the possible existence of other unidentified epidemiological or behavioral risk factors in the male population infected with HPV and that new epidemiological studies on condom use are needed to better understand our results. In our study, 57% of the HPV DNA-positive men never used condoms with their regular female (non-sex worker) partner while 62.2% of HPV DNA-negative men never used a condom under the same circumstances (Table 3). Hippeläinen et al. (25) pointed out the importance of condoms as a protective factor against HPV infection.

We found that a previous history of sexually transmitted disease in the HPV DNA-positive group (33.3%) was not statistically significant (Table 3). Wikstrom et al. (32) found that 23% (not significant) of males studied had previously had a sexually transmitted disease, while Hippeläinen et al. (24) reported an incidence of 15.8%. In our study the sexually transmitted diseases most frequently reported by both groups were (in decreasing order) gonorrhea, acuminated condyloma, syphilis, and genital herpes but there was no statistically significant association between a history of sexually transmitted disease and the presence or absence of HPV DNA. Hippeläinen et al. (24) detected *Chlamydia* infection and gonorrhea in their study.

Our study demonstrated for the first time that there is an association between the mean number of female sexual partners in the year preceding the study and the presence of HPV DNA, strengthening the belief that the higher the number of sexual partners the greater the chance of acquiring and transmitting HPV.

The percentage of the different types of HPV identified in our study was: HPV 6 = 56.1%; HPV 11 = 36%; HPV 16 = 3.5%; HPV 40 = 0.7%; HPV 61 = 0.7%, and HPV 84 = 0.7%, with one man being doubly infected. Paesi et al. (34) carried out a prospective study of women carrying CIN from August to December 2000 at the same institution as surveyed in the present study. They found that the most prevalent HPV types were 16 and 18 compared with 6 and 11 in the present study. Franceschi et al. (29) conducted a case-controlled study of the sexual partners of women with *in situ* carcinomas and invasive carcinomas of the neck of the uterus and found that the most prevalent HPV types were 16, 18, 31, and 33 which are at high risk of being oncogenic. Castellsagué et al. (31) studied couples infected with HPV but found little agreement between the types of HPV virus identified (31.8%). These conflicting results are probably related to the different diagnostic methodology used in these studies. Tabrizi et al. (23) studied benign histological samples from HPV-infected tissues from the sexual partners of women who had cervical tissue with cytopathological changes suggestive of HPV infection or who had lesions which could be considered to be precursors of cervical cancer and found that

73% of the viruses encountered were types 6 and 11.

The results of the present study are consistent with the epidemiological data available in the literature, except for our data on the number of female sexual partners up to the time of the study ('lifetime sexual partners') or the number of female sexual partners in the year preceding the study and HPV infection. HPV infection was frequent in the male sexual partners of women with CIN, indicating that a systematic study of this population is needed so that these couples can be better informed.

Our results, taken together with literature data, show that there is little agreement between the prevalence of infection with HPV in men at high risk of HPV infection and women carrying high and low grade lesions of the neck of the uterus. This may be explained by the different levels of biological activity and differences in local immunity and organization of the genital epithelia of each sex. These factors can favor viral transmission and multiplication and predispose both sexes towards infection with one or more viruses. The different levels of biological activity and differences in local immunity and organization of the genital epithelia of each sex indicate that a prospective study is needed using samples of genital epithelia collected from a reasonable number of sexual partners. Thus, a more detailed epidemiological study can be carried out to gather more information on the biological behavior of infection by human papillomavirus.

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