The role of HPV DNA in the evaluation and follow-up of asymptomatic male sexual partners of females with CIN3

A. Bar-Am, *M.D.*; **J. Niv,** *M.D.*

Cervical Pathology Unit, Department of Obstetrics and Gynecology, Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv (Israel)

Summary

Purpose: To determine whether and how asymptomatic sexual partners of females with high-grade cervical intraepithelial neoplasia (CIN3) lesions should be examined. *Methods:* Sexual partners of females with CIN3 were evaluated for HPV-related lesions by scraping samples for high-risk HPV DNA and androscopy (colposcopic inspection of the penis, scrotum and peri-anal area). Abnormal androscopically detected lesions were sampled for cytology by Pap smears. *Results:* 74 partners of 87 females were studied and underwent androscopy, and 17 (22.9%) had abnormal findings: 11/74 had clinical genital condyloma acuminata and 6/74 had aceto-white lesions on the penile shaft or scrotum. Cytology of the 17 abnormal androscopies showed that six smears were normal and 11 had atypia and koilocytosis. Positive high-risk HPV DNA indicated that 13/74 (17.5 %) were infected with HPV. Two partners (2/74, 2.8%) had concomitant HPV DNA 16. *Conclusions:* Male sexual partners of females with CIN3 should undergo androscopy and cytology of colposcopically detected abnormal areas.

Key words: Male sexual partners of females with CIN3; Androscopy, HPV DNA testing in males.

Introduction

Human papilloma virus (HPV) is the most common sexually transmitted infection. About 80% of newly sexual couples will develop HPV-related lesions within three years from commencing sexual activity, most of whom will spontaneously regress within one to two years or until the age of 30-35 years [1]. HPV is the primary cause of cervical, anal and other anogenital cancers [2]. The main cancer risk factors are infection by high-risk (HR) HPV type (the majority by HPV 16 and 18), persistence of the viral DNA, and high load of HR HPV DNA. Secondary factors that increase the risk include smoking, long-term use of oral contraception and, for men as well, sexual promiscuity and history of previous sexually transmitted diseases (STDs), such as chlamydia trachomatis, gonorrhea and herpes simplex virus (HSV) type II [1].

The prevalence of genital HPV infection in the general male population is unknown [3], and the male sexual partner's role, if any, in his partner's genital warts or high-grade cervical intraepithelial neoplasia (CIN2-3) lesions is also undefined. The issues involve his potential contribution as the source of her present infection as well as of future recurrences or, alternatively, the likelihood of HPV being transmitted to him by his infected sexual partner, and they remain unresolved.

The objective of the present work was to address the perennial issue of whether an asymptomatic male sexual partner of a woman with CIN3 lesions should also be examined and, if so, by whom, how and for how long.

Materials and Methods

Eighty-seven females with CIN3 underwent cone biopsy at our unit during 2004. HPV DNA was obtained by the Hybrid Capture type II (HCII - Digene, USA) before the conization procedure. Results of the HPV tests did not influence our decisions regarding indications for performing the cone procedures, since the reports of the HPV tests were not yet available. The purpose of obtaining the information on the HPV DNA and its load was for their use as baseline data for the follow-up. To reduce costs, the test consisted of a cocktail of HR HPV DNA only (including types 16, 18, 31, 33, 35, 45, 52, 56) which covers most of the active high-risk genotypes. The information on the HPV DNA load was obtained from the relative light unit (RLU) method of the Digene type II system which provides a semi-quantitative measure of the amount of HPV DNA in the sample, based on the intensity of light omitted in a reaction against a standard tube. The computerized calibrated value is the cutoff point, expressed as an RLU score. The DNA load is estimated by comparing each test result against the RLU value. A score below or above that point indicates a negative, low, moderate or high load. Specific HPV genotype was tested only when HPV was detected concomitantly in both partners.

The male's evaluation was carried out in two steps as follows: androscopy was performed at the first visit. In this setting, "androscopy" consisted of a colposcopic examination of the male penile shaft, scrotum and peri-anal area after the application of 5% acetic acid. Any suspicious finding, such as aceto-white (AW) lesions suspected as being foci of HPV lesions or condyloma acuminata foci, were marked by black dye. These patients were recalled for HPV DNA testing and conventional Pap smear a few days later. The samples were obtained by scraping the marked areas with a cotton swab for the HPV tests and by a wooden spatula for the conventional Pap smear.

Results

Out of the 87 women who underwent cone biopsy due to CIN3, the sexual partners of 74 of them (85%) com-

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prised the study cohort. The mean age of the 87 women was 31 years (range 16-54) and that of the 74 sexual partners was 39 years (range 19-62). Forty-three males (58.1%) were married, five (6.7%) divorced and 26 (35.1%) single. The mean duration of the sexual relationship with the present partner was 72 months (range 10-94) for the married group, 11 months (range 8-19) for the divorced group and 16 months (range 6-31) for the single group. All the males were circumcized.

Source of referral

Thirty-six of the 74 males (48.6%) had been seen by dermatologists at least once during the three years prior to the present evaluation. They reported having been examined either by the physician's unaided eye or with a magnification loop. The ones who had genital warts were treated with cautery by an electric needle, liquid nitrogen and various medications (e.g., trichloroacetic acid), and only those who failed to respond or had recurrences were referred to a gynecologist. Twelve (12/74, 16.2%) had visited urologists who examined the penile shaft without any magnification: most of them had only urine analyses and culture tests, and none of them had been referred by the urologist to a gynecologist for a second opinion. Twenty-six (35.1%) had received no previous medical attention for their association with a sexual partner who had a CIN lesion and were seen by us for the first time.

Androscopy and Pap smear findings

Fifty-seven (57/74, 77.0%) men had normal androscopic findings, 11 (14.9%) had clinical genital warts and six (8.1%) had AW lesions on the penile shaft and scrotum. Out of the 17 abnormal androscopic cases, Pap smears were abnormal in 12/17 (70.5%) and normal in 5/17 (29.4%). The abnormal Pap reports were atypical in seven cases and atypical with koilocytosis in the other five.

HPV DNA findings

All the 87 females were HR HPV positive: seven (9.4%) had high HPV DNA load, 51 (68.9%) had moderate load and 16 (21.6%) had borderline and low loads.

Thirteen males (13/74, 17.6%) had positive HR HPV DNA. They included six of the 57 men with normal androscopic tests: one had a moderate HPV load and five had low loads. Of the 11 males with clinical genital warts, six were HR HPV DNA negative and five were HR HPV positive. Of the six patients with AW lesions, two were HR HPV DNA positive (one had a low HPV load and the other had a moderate HPV load) and four were HR HPV DNA negative.

Concomitant HPV DNA 16 was found in two couples (2/74, 2.8%), one with a moderate HPV load and one with a low HPV load.

Discussion

Given the facts that HPV is the most common sexually transmitted infection for both males and females and that HR HPV DNA is a primary etiologic factor for the development of CIN lesions, the time is long past due to establish the risk of disease transmission among the sexual partners of infected individuals of both sexes. The present study attempted to provide some answers for males by analyzing the medical literature and our own clinical data.

Who is at risk?

The population at risk has been well defined in the medical literature as the men who meet the primary and secondary risk factors [1-3]. In spite of having identified their risk characteristics, medical authorities conduct various screening programs to identify high-risk females, but very little is done to screen high-risk males. It is beyond the scope of the present paper to discuss male screening projects in depth. Noteworthy, our experience and certainly that of other gynecological oncologists is that a woman diagnosed as having genital warts or CIN *invariably* asks if her sexual partner should be examined.

Who should do the evaluation?

Various medical specialists, such as general practitioners, dermatologists, urologists, STD units and gynecologists are involved in the evaluation of the male sexual partner of females with genital warts or CIN lesions. Unfortunately, the medical literature provides little help in choosing the most appropriate medical specialty for this undertaking. Urologists who evaluated asymptomatic healthy young Japanese men for STD [4] showed an 8% incidence of STD with HPV-related lesions, but the authors did not identify latent foci of HR HPV infections. One group of dermatologists reported on cancer of the penis as an outcome of HPV infection [5], but they studied only symptomatic males. Dermatologists, urologists and STD specialists very often see patients with genital warts (condyloma acuminata), but it is easy to miss small clinical lesions and, certainly, latent HPV lesions. We consider that a gynecologist who works in a colposcopic unit is possibly more likely to have the necessary level of awareness, expertise and the facilities for diagnosis.

Which anatomical site should be examined?

Several studies have dealt with the question of which part of the male anatomy should be tested in search of HPV infection. Aguilar *et al.* [6] examined 582 Mexican soldiers and sampled the deep distal urethra, the urethral meatus, as well as the penile shaft and anal region for HPV by polymerase chain reaction (PCR) for 27 HPV types. They report that HPV DNA was detected most frequently in the external genitalia (46.4%) compared to the urethra and the meatus together (20.8%). They concluded that "...the addition of cell samples from the meatus and anus to those from external genitalia contributed negligibly to the evaluation of the prevalence of HPV in men". Nicolau *et al.* [7] investigated the most affected sites in the genitalia of 50 males who were stable sexual partners of females with positive HPV DNA infection. HPV

detection per anatomical site was 24% around the glans penis, 68% on both the internal and external sides of the prepuce, 12% in the scrotum and 8% in the anus. In the present study, samples were taken from the penile shaft, scrotum and peri-anal area: all the abnormal findings were on the penile shaft and scrotum.

Which method should be used?

The currently available methods for evaluating male sexual partners of women post cone biopsy due to CIN2-3 are cytology, HPV DNA testing, androscopy and biopsy of abnormal androscopic findings. There is no doubt that histology is the gold standard for diagnosis of pre-malignant, malignant or any other skin abnormality. In the present study, the aim of evaluating the male sexual partners was not to find the prevalence of pre-malignant or malignant genital or penile lesions. A positive HPV or finding of condyle lesions on the genitalia of a male does not necessarily mean that he is the source of his sexual partner's CIN lesion. The same applies when the HPV report is negative: it does not mean that the male will not be infected in the future. The aim of this work, rather, was to assess the actual HPV status of the currently asymptomatic male sexual partners since it can impact the HPV status of the couple in the future. Nicolau *et al.* [7] examined that issue and concluded that histological examination was an inaccurate method to diagnose HPV DNA infection in the 50 males they studied, while cytology samples detected HPV DNA in 92.1% of the infected men. Convinced by the above observation, androscopic-guided cytology and HPV sampling from the penile AW lesions – and not histology – became our preferred method of assessment. Although biopsies had not been taken, all six cases of AW lesions in our study population were detected by both cytology and HPV testing. Androscopy failed, however, to detect the six patients (6 of the 57 normal androscopies) who had positive HR HPV DNA tests (i.e., false-negative results). The "reflex" test was not used due to financial restraints. Even had we carried out the "reflex" test, however, the patient would have had to return for a second visit.

For the most part, examining a new male patient in the colposcopic unit usually starts with an interview, followed by androscopy and one of two alternative measures: 1) obtaining cells for cytology and/or HPV DNA tests randomly, followed immediately by androscopy, or 2) performing an androscopic examination and taking samples for cytology and/or HPV testing from the most suspicious colposcopic findings, if present. The advantage of the first option is that all tests take place in one visit. Its disadvantage is that the specificity and sensitivity of samples taken randomly are lower than those obtained under direct colposcopic view. The only procedure that can be conducted after the application of acetic acid is biopsy of the penile skin, but the indication for biopsy is not clearcut. As mentioned earlier, Nicolau *et al.* [7] concluded that histological examination was an inaccurate method to diagnose HPV DNA infection in males. The specificity of the Pap and HPV tests was higher than that for randomly obtained samples because they were taken under direct colposcopic vision. The disadvantage of this approach is that it is impossible to take samples for Pap smear (and/or HPV testing by the liquid base cytology (LBC) method known as a "reflex" test) after the application of 5% acetic acid, and the patient must return for another visit.

In the present study, the second option was chosen in order to obtain more accurate samples for the HPV DNA and cytology testing, excluding the "reflex" test. The six cases of AW lesions were not biopsied, but detected by both cytology and HPV testing. The six patients (6 of the 57 normal androscopies) who had positive HR HPV DNA tests (i.e., false-negative results) were not picked up by androscopy.

The role of HPV DNA testing

The prevalence of HPV in the general male population depends on the nature of their sexual activities [7, 8], and the presence of genital warts and CIN lesions varies among the female partners of various male populations as well [9, 10]. Regardless of the prevalence in any given group, all authors agree that the best method to identify the presence of HPV infection is by HPV DNA testing.

In the present study HR HPV DNA tests detected 13, (17.6%) cases; five of the 11 men with clinical condyloma acuminata, two of the six cases of AW lesions and six out of the 57 with normal androscopic tests. It is highly probable that the other negative seven males with condyloma and the four with AW lesions harbored LR HPV DNA types. The fact that LR HPV DNA had not been tested would not appear to have a negative effect on the patient's prognosis, because LR HPV types are not etiologic agents for CIN lesions [11].

Concomitant HPV 16 was found in only two couples (2.8%), suggesting a common source. Although a positive HR HPV DNA result does not mean that the male is the source of the CIN lesion of his sexual partner or vice versa, the question of responsibility is sometimes of major concern to the couple. Awareness of the correct information can put the issue of blame to rest. Knowing the extent of HPV DNA load does not contribute much at the initial stage of the study. The values of DNA positivity and load will, however, certainly play an important role in predicting the prognosis of a particular case by repeated HPV DNA tests taken during the follow-up period. Indeed, follow-up should be continued as long as HPV is found to be positive in the periodic tests since persistent positive HPV test results in males can be the reason for potential recurrent CIN of his sexual partner [9].

In conclusion, our results indicate that 1) male sexual partners of females with CIN lesions should be referred for evaluation in colposcopic units, 2) combined androscopy and HPV and cytology testing (possible by reflex method) will ensure accurate detection of HPV status among males and 3) long-term follow-up will contribute to the knowledge about the influence of HPV positivity and HPV load on the prognosis.

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Address reprint requests to:
A. BAR-AM, M.D.
Cervical Pathology Unit
Department of Obstetrics and Gynecology
Lis Maternity Hospital
Tel-Aviv Sourasky Medical Center
Tel Aviv, 64239 (Israel)