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Importance of peniscopy, oncologic cytology and histopathology in the diagnosis of penile infection by human papillomavirus

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Introduction: Male genital infection by human papillomavirus is of particular importance since it is often asymptomatic. The patient generally presents no clinical lesion. Therefore, men represent an important reservoir of virus, playing a special role in the transmission and perpetuation of the disease. **Patients and methods:** In the present prospective clinical trial study, 190 sex partners of women with genital infection by human papillomavirus, associated or not with cervical intraepithelial neoplasia, were investigated. All patients were unaware of or denied the presence of a genital lesion. **Results:** Cytologic examination revealed koilocytosis in 9 cases (4.7%) in the urethra and in 3 cases (1.6%) in the corona of the glans and the distal prepuce. Peniscopy with the previous use of 5% acetic acid revealed white lesions in 97.9% of the patients. Toluidine blue stained most of the lesions. At least one fragment revealed koilocytosis in the histopathologic study of 97 cases (51.05%). **Conclusion:** The three methods complement one another, allowing a more precise diagnosis of this infection in men.

UNITERMS: Peniscopy, papillomavirus, HPV, colposcopy

INTRODUCTION

Genital warts have been known since ancient times, being referred to by several Greek and Roman writers. They were considered to be a venereal disease transmitted among male homosexuals. They were then called condyloma, a term of Greek origin that is still used.

The role of papillomavirus in the etiology of condyloma is well defined. Molecular biology studies have led to a better understanding of the infection. Genital infection may be caused by more than 20 of the 70 types of papillomavirus known up to the present. Based on their properties and on the identification of the virus in both malignant and premalignant condylomatous lesions, they are classified into low risk (6 and 11) and high risk (16 and 18) types as proposed by Syrjänen (1989).

After the studies of Meisels and Fortin (1976) and Meisels et al. (1977), the interest in the infection was renewed. The authors described the cytological, colposcopic and histopathologic features of the female genital infetion by the human papillomavirus.

The real interest in papillomavirus in the genesis of cervical cancer seems to have emerged from the identification of morphological aspects of viral cervical infection compatible with intraepithelial neoplasia both in terms of oncologic cytology and histopathology (Meisels and Fortin, 1976).

The high incidence rates of the viral infection and the relation to cervical neoplasia, in addition to the fact that the men had no visible penile lesion, led the investigators to make use of other diagnostic methods for an earlier identification of the infection.

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Levine et al. (1984) were the first investigators to use magnification to examine the penis of the partners of the women with condyloma.

The aim of the present study was to ascertain the incidence of the infection and the value of magnified penile examination, cytology and histopathology in the diagnosis of papillomavirus lesions in the penis of men whose sex partners presented the same disease.

PATIENTS AND METHODS

A total of 190 sex partners of women with genital infection by human papillomavirus, associated or not with intraepithelial cervical neoplasia, diagnosed by colposcopic, histopathologic and cervicovaginal cytologic examinations, were studied between April 1988 and March 1991.

All patients were submitted to careful anamnesis. Patients with genital lesions caused by human papillomavirus and visible to the naked eye were excluded from the study.

The age of the patients ranged from 18 to 71 years, with a predominance of 20-to 39-year range (126 cases or 66.3%) (Table I). White patients were greater in number (144 or 75.8%). Forty-two were black (22.1%) and four were asian (2.1%). Sexual activity had started more frequently between 15 and 20 years (106 cases or 55.8%), as shown in Table II. One hundred and fifteen (60.5%) patients were married and 82 (43.2%) had had sexual intercourse with more than one partner. One hundred and forty-four patients (75.8%) did not use condoms.

Table III shows the history of sexually transmitted diseases, which was positive in 87 (45.8%) patients. The most frequently reported disease was gonococcal urethritis (46 patients or 52.9%). In 7.4% of cases, the patients reported urogenital surgeries such as posthectomy, circumcision or correction of hypospadias.

Table I
Patient distribution by age

Age	Number	%
10 - 19	3	1.58
20 - 29	59	31.05
30 - 39	67	35.26
40 - 49	31	16.32
50 - 59	25	13.16
60 - 69	3	1.58
70 - 79	2	1.05
Total	190	100.0

Table II
Patient distribution by age at initiation of sexual activity

Age	Number	%
< 10	2	1.05
10 - 15	69	36.32
15 - 20	106	55.79
20 - 25	9	4.74
25 - 30	3	1.58
> 30	1	0.53
Total	190	100.0

Table III
Patient distribution by previous sexually transmissible disease

STD	number	%
Gonorrhea	46	52.9
Condyloma	14	16.1
Non-gonococcal urethritis	11	12.6
Syphilis	8	9.2
Concroid	4	4.6
Gonorrhea and Syphilis	1	1.1
Lynphogranuloma Venereum	1	1.1
Genital Herpes	1	1.1
Pediculosis	1	1.1
Total	87	100.0

Peniscopy

After anamnesis, the penis, distal urethra and scrotum were examined with a colposcope (D.F. Vasconcelos) under 4- and 6-fold magnification. The examination strictly required the following steps:

I - Examination of the entire penis and scrotum with the patient in the sitting position.

II - Examination of the 2 distal centimeters of the urethra, using a urethral speculum specially designed for this purpose.

III - Collecting of material for cytologic examination from the glans, from the distal prepuce and from the distal urethra.

IV - Wrapping of the penis and scrotum with gauzes soaked in 5% acetic acid. Placement of a cotton tip soaked in the same solution into the distal urethra. The acid was in contact with the structures for 5 minutes.

V - Repeated examination of the penis and distal urethra, after removing the gauzes and cotton tip. The characteristics and sites of the images revealed were then identified.

VI - Soaking of the penis and scrotum with 1% toluidine blue, removing the excess with 2% acetic acid after 3 minutes.

In the present study, we define peniscopy performed before and after the use of acetic acid and toluidine blue as widened peniscopy.

Cytology

Immediately after the first examination, material for cytology was collected from the distal urethra, the distal prepuce and the corona of the glans with a brush commonly used for endocervical smears (Terreiro et al., 1990).

Histopathology

Direct biopsies were collected in order to obtain representative fragments of each type of lesion, identified by peniscopy with and without acetic acid and toluidine blue. The biopsies were obtained under anesthesia, using a 2-3-mm wide dermatome. Hemostasis was obtained with a cotton tip soaked in concentrated methacresolsulfonic acid applied onto the raw area.

In cases in which urethral lesion could be easily reached, biopsies were eventually obtained using the forceps for the uterine cervix of the Gaylor type, modified by Medina (Medina et al., 1977).

RESULTS

The diagnosis of human papillomavirus infection was based on the cytologic and histopathologic alterations compatible with kolocytosis, i.e., presence of large, round and vacuolized squamous cells, which may be located at any level of the stratified epithelium, though hardly in the basal or parabasal layers.

The cell shows a light perinuclear halo and a hyperchromatic and retracted nucleus which seems to be suspended in empty space. Binucleated forms are common. Koss and Durfee (1956) called this alteration "koilocytotic atypia", a term originating from the Greek *koilos*, which means hole or cavity.

The initial examination with the peniscope revealed suspect lesions in 72 patients (37.9%).

The leucoacetic lesions were found in 186 cases (97.9%), with macules, micropapules, microspicules, annular microerosions, and hypertrophic papillae in the corona of the glans with a leucoacetic surface (Fig.1).

Toluidine blue stained most of the lesions described above, mainly the central area of the micropapules and of the annular lesions (Fig. 2). In some instances, it stained areas in which no leucoacetic lesions had been previously found. Similarly, some leucoacetic lesions were not stained by toluidine blue. Papillomatous urethral lesions were observed in 4 cases (2.1%); koilocytosis was not detected in only one of these cases.

Cytology revealed koilocytosis in 9 cases (4.7%) in the urethra, and in 3 cases (1.6%) in the corona of the glans and distal prepuce. Koilocytosis was detected in the biopsy specimens in these 3 cases. Only two (22.2%) of the nine cases of urethral cytology with koilocytosis presented a papillomatous lesion upon examination of the distal urethra. These lesions were submitted to biopsy and koilocytosis was detected by histopathologic examination.

Biopsies were collected from all patients. In the 4 patients in whom the acetic acid test proved negative, some epithelial areas were stained with toulidine blue and were biopsied. In one patient, small lesions were observed after the initial examination with magnification.

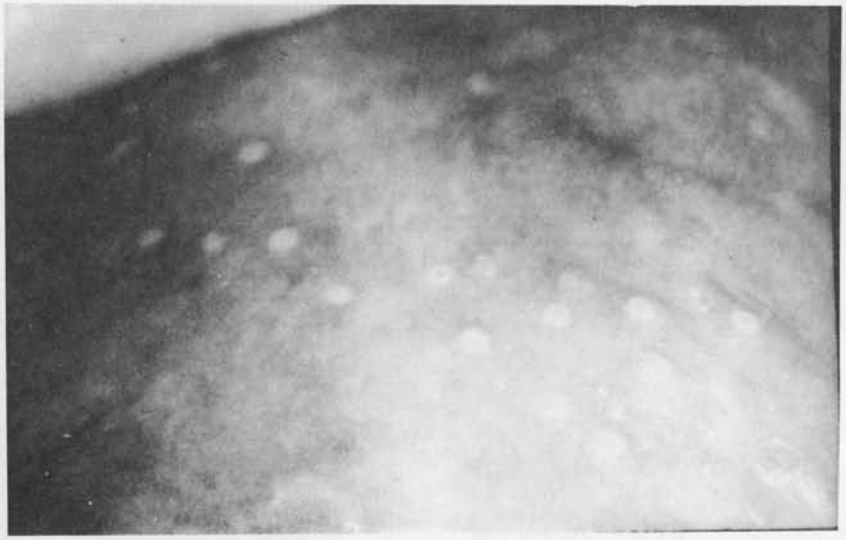

Figure 1 - Leucoacetic lesions with micropapules and annular microerosions on the distal prepuce.

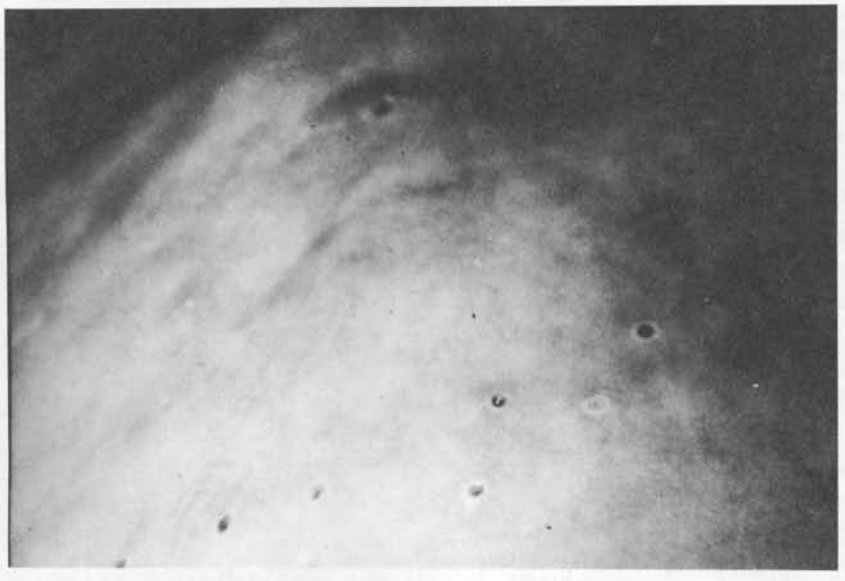

Figure 2 - Leucoacetic lesions on the distal prepuce stained with toluidine blue.

Koilocytosis was detected in at least one fragment in 97 (51.05%) of the 190 patients.

Histopathologic examination also detected molluscum contagiosum in 3 patients, and genital herpes in another.

DISCUSSION

Several investigators have recommended examination of the penis with the aid of an optical instrument, the colposcope, with the aim of diagnosing genital infections, specially when these are subclinical or not visible (Levine et al., 1984; Rosemberg, 1985; Barrasso et al., 1986; Krebs and Schneider, 1987; Rosemberg and Reid, 1987).

Although some authors use the term colposcopy of the penis (Barrasso et al., 1986; Krebs and Schneider, 1987), we prefer to call it peniscopy. Other words such as androscopy or genitoscopy are also used.

The criterion used in the present study for the cytologic and histopathologic diagnosis of this viral infection was the presence of koilocytosis.

Considering the criteria described in the cytology, with 100% results proving positive in the presence of cytoplasmic and nuclear alterations, we reached a cytologic diagnosis of the penile and urethral infection in 1.6% and 4.7% of the cases, respectivelly.

In spite of the few positive cases detected, urethral cytology was more effective than cytology of the distal prepuce and the corona of the glans. Only 2 patients presented papillomata in the distal urethra and the others did not have alteration of the aspects when under magnfication. Cytologic findings were important for the diagnosis.

In the distal part of the penis, cytology was positive in cases where histopathology showed koilocytosis. The specimens had many unnucleated keratinized cells, thus being inappropriate for the identification of koilocytosis.

The ideal time for collecting material for cytology with a brush should not be the same as for performing peniscopy. The brush injures the glans and prepuce epithelium and the urethral mucosa, possibly leading to false-positive peniscopic findings.

Peniscopy detected lesions in all cases, and was considered to be positive. In 97.9% it showed alterations with acetic acid and in the others only with toluidine blue.

We used a 5% acetic acid concentration. The main action of the acid is to coagulate cytoplasmic and nuclear proteins of the epithelial cells, turning them white. It is a progressive, superficial, and reversible process. In women, it occurs between 10 and 30 seconds and disappears after 30-40 seconds (Cartier, 1986). In men, not only the latency time but also its effect lasts longer.

The urethra should be equally examined with acetic acid, for sometimes it is possible to detect small lesions not visible under simple magnification. In fact, Wosnitzer (1988) recommends examining the urethra with a colposcope, both before and after the use of acetic acid in order to detect small lesions not visible to the naked eye.

The effect of 1% toluidine blue in aqueous solution is noted in the nuclei. Since this is a cell dye that behaves as a base, it has affinity for, and binds to, acid structures such as DNA and RNA, similarly to methylene blue (Junqueira and Carneiro, 1977).

Nicolau et al. (1991) compared the effects of the 2 solutions (acetic acid and toluidine) in 863 biopsies and concluded that acetic acid reacted slightly more frequently with lesions with koilocytotic cells.

In the present study, we concluded that the two tests complement each other, since some lesions showed reaction with only one of the two solutions. Therefore, both tests should be applied. The action of the dye has the

advantage of lasting much longer, which permits to decide about the area to be biopsied and to anesthetize it before the lesion disappears.

Many lesions revealed by peniscopy do not present koilocytosis. A large part of them were detected because of inflammatory or infectious processes causing balanoposthitis. Usually patients are not circumcised (in the present study, only 7.4% were). This hampers appropriate hygiene and facilitates the growth of infectious agents such as *Candida albicans, Gardnerella vaginalis*, herpes simplex, *Trichomonas vaginalis,* bacteroids and other anaerobic organisms (Peutherer et al., 1979; Cree et al., 1982; Kinghorn et al.,1982).

We preferred to examine the patient while seated because this position is more comfortable for both examiner and patient.

In medical literature there are reports of macroscopic urethral lesions in 0.5-5% of cases (Oriel, 1971; Cetti, 1984). In the present study, distal urethroscopy revealed lesions in 2.1% but there were cytologic alterations in 4.7%. This is the reason for performing both procedures.

Histopathologic examination was performed in all patients, several lesions being biopsied in each case. The great majority of the lesions were flat. We could note no correlation between the clinical aspect of the lesion and the anatomopathologic alterations; thus, it is necessary to biopsy all lesions, especially the flat ones.

In the present study, intraepithelial neoplasia was found to be associated with the viral infection in 1.58% of cases. These lesions were unremarkable when examined by peniscopy.

The biopsies were well tolerated. The most difficult step was anesthesia, which is somewhat uncomfortable.

The specimen is small, which allows performing of more than one biopsy, with a better representation of the lesions. In addition, due to the small size of the biopsy, the healing period is short, approximately 2 weeks.

About 50% of the cases did not present koilocytosis. According to the most recent reports, it is possible that these patients are carriers of a virus in the penis, urethra and semen, especially viruses of the types associated with premalignant and malignant lesions (Grussendorf-Conen et al., 1986; McCance et al., 1986; Ostrow et al., 1986; Villa et al., 1986; Del Mistro et al., 1987; O'Brien et al., 1989; Selvey et al., 1989).

Based upon these facts, it becomes clear that, from a clinical point of view, the examination of the penis with magnification is insufficient; performing of peniscopy after applying acetic acid and toluidine blue is necessary.

CONCLUSIONS

1. Peniscopy allowed the diagnosis of papillomavirus infection, especially with the use of acetic acid and toluidine blue; the white lesions were present in 97.9% of the cases and in the others the lesions were stained with toluidine blue.
2. Although a papillomatous lesion was rare (2.1%), the examination of the distal urethra, preferentially with a speculum, was important for the diagnosis.
3. Nine patients (4.7%) presented positive cytology findings in the distal urethra, but only 2 of them had macroscopic lesions.
4. Cytology of the corona of the glans and of the distal prepuce revealed a low frequency of koilocytosis, but histopathology was positive in all these cases.
5. In 97 patients (51.05%), histopathology demonstrated koilocytosis in at least one of the several biopsy specimens.
6. Intraepithelial neoplasia was found in association with the viral infection in 1.58% of the patients.

RESUMO

Introdução: A infecção genital masculina pelo papilomavírus humano tem especial importância, pois é, freqüentemente, assintomática. Na maioria dos casos, o indivíduo não apresenta lesão clínica. Por isso, os homens passam a ser encarados como importante reservatório do vírus, exercendo papel especial na transmissão e perpetuação da doença. **Pacientes e métodos:** No presente estudo clínico do tipo prospectivo, foram avaliados 190 parceiros de mulheres com infecção genital por papilomavírus humano, associada ou não à neoplasia intra-epitelial cervical. Todos os pacientes desconheciam ou negavam a presença de lesão genital. **Resultados:** A citologia uretral exibiu coilocitose em nove casos (4,7%) e três (1,6%) na coroa da glande e prepúcio distal. A peniscopia, após a aplicação de ácido acético a 5%, revelou lesões brancas em 97,9% dos pacientes. A solução do azul de toluidina corou a maior parte das lesões observadas. Pelo menos um fragmento revelou coilocitose quando do estudo histopatológico em 97 casos (51,05%). **Conclusão:** Os três métodos se complementam, permitindo diagnóstico mais preciso dessa infecção no homem.

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