CYTOLOGIC AND COLPOSCOPIC EXAMINATION IN THE DIAGNOSIS OF PENILE HPV INFECTION

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Summary: In this study, the Authors take into consideration 70 male partners of women who showed (after medical and/or surgical therapy) cervical and/or vaginal relapsing and/or persistent lesions associated with HPV infections.

Fifty-two (74%) of the male partners resulted "positive" for HPV genital infection by the colposcipc and/or cytologic examination. The histological examination of target biopsy performed on o macular lesion revealed slight-moderate dysplasia associated with HPV atypical alterations.

The Authors further underline the importance of the role played by the male partner in persistent and/or recurrent cervical-vaginal HPV infection.

For the therapy to be successful, we cannot overlook a diganostic and therapeutic course that fits the couple.

INTRODUCTION

The tendency to malignant degeneration of some of HPV induced lesions has long been established in humans and animals especially in experimental conditions [1].

The attention has especially been focused on some types of Human Papilloma Virus (HPV) infections and their relationship with the host's immunitary system [2]. HPV type 16 and, less frequently, HPV types 18, 33, 39, 42 have been found associated with cervical, vulvar and penile intraepithelial neoplasia (CIN, VIN and PIN) and to cervical, vulvar and penile invasive carcinoma [3, 4, 5, 6, 7, 8, 9, 10, 11].

In last few years, a great deal of statistic and epidemiologic studies on the cervix uteri carcinoma have revealed that this neoplasia has some features of a sexually transmitted disease and that the sexual partner («risk male») plays a role in the transmission of primary factors involved in the origin of pre- or neoplastic lesions of the cervix uteri [12, 13, 14, 15, 16, 17, 18].

In 1982, dealing with relapsing vaginal and cervical condylomata, Baggish noted that 82% of male partners of women with recurrent HPV infection were found to have penile condylomata. In 1984, Levine evaluated a series of male partners of women with different degrees of CIN during a retrospective study and he found a 64% incidence for penile condylomata in that subset of patients. Cates stated that in 1985 HPV infections ranked 3rd among the sexually transmitted diseases (STD) just behind Chlamydia trachomatis and Neisseria gonorrhoeae infections which holds the first place among viral STD [19, 20, 21].

In this study, we took into consideration 70 partners of women who, after medical or/and surgical therapy, showed cervical and/or vaginal relapsing and/or persistent lesions associated with HPV infections and evidenced by cyto-histologic and colposcopic examinations. These male partners underwent cytologic and colposcopic examinations.

MATERIAL AND METHODS

During the period ranging from September 1985 to December 1987, at the Gynaecologic Oncology Service - Obstetric and Gynaecologic Institute - Padua University, we selected 70 women with cervical and/or vaginal lesions, associated to HPV infections, that have relapsed

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Table 1. – *Prevalence of systemic diseases in the "positive" and "negative" male patients for genital HPV infection.*

	"Positive" 52 (74%)	"Negative" 18 (26%)
Virus infections:		
hepatitis B	4 (6%)	—
herpes labialis	24 (34%)	4 (6%)
cytomegalovirus	1 (1%)	—
mononucleosis	—	—
warts	18 (26%)	2 (3%)
condylomata	2 (3%)	—
Hypertension	2 (3%)	2 (3%)
Diabetes	—	—
Obesity	2 (3%)	—
Neoplasia	—	—
Auto-Immunitary diseases	—	—
Drugs	3 (4%)	—
Chemiotherapy	—	—
Radiotherapy	—	—
Immunotherapy	—	—
Others:		
psoriasis	11 (16%)	—
hypersens. type I	7 (10%)	—

Note: " Positive ", patients with colposcopic and/or cytologic alterations for HPV infection. "Negative", patients with neither colposcopic nor with cytologic alterations for HPV infection.

or persisted after the appropriate medical and/or surgical therapy. For at least six months, the respective male partners underwent cytologic and colposcopic examinations in order to detect probable HPV genital infections.

Dealing with the 70 male partners, we considered personal statistic and epidemiologic data: physiologic anamnesis, anamnesis for general diseases, such as virus infections, diabetes, obesity, hypertension, neoplasia, immunologic diseases, habitually used drugs, chemiotherapy and radiotherapy (Table 1). Moreover, we gathered information about previous genital diseases (Table 2) and current genital symptomatology (Table 3). Andrologic anamnesis included information about various aspects of the patient's sexual life (Table 4).

In all the 70 patients the following was carried out: an external examination of the genital and anus-perianal region; cytologic examinations on urethral meatus, balano-preputial fold, and if present, "suspicious areas" detectable with the naked-eyes. Target biopsies were performed when cytologic and/or colposcopic examinations were "positive" and colposcopic evaluation was carried out at first of the genital region directly and then, after having used 5% acetic acid and iodine stain (Lugòl solution). Finally, a cytologic examination of the centrifuged morning urine was performed every day for three days.

RESULTS

The result of the colposcopic and cytologic examinations revealed that 18 (26%) of the 70 male partners of women with HPV cervical and/or vaginal relapsing and/or persisting lesions resulted

Table 2. – *Preceding genital diseases in the "Positive" and in the "Negative" male patients for genital HPV infection.*

	"Positive" 52 (74%)	"Negative" 18 (26%)
Balano-posthitis	—	5 (7%)
Urethritis	10 (14%)	—
Cystitis	3 (4%)	—
Prostatitis	—	—
Epididymitis	—	—
Orchitis	—	—
Syphilis	—	—
Gonorrhea	—	—
Ven. lymphogranul.	—	—
Condylomata	—	—

Note: "Positive" patients with colposcopic and/or cytologic HPV alterations. "Negative": patients with neither colposcopic nor with cytologic HPV alterations.

Table 3. – *Symptomatology in male partners.*

	"Positive" 52 (74%)	"Negative" 18 (26%)
Itching	3 (4%)	—
Burning	4 (5%)	—
Pelvic pain	2 (3%)	—
Urethral secretion	—	—
Hematuria	—	—
Others	—	—

Note: "Positive" patients with colposcopic and/or cytologic HPV alterations. "Negative": patients with neither colposcopic nor with cytologic HPV alterations.

Table 4. – *Sexual anamnestic aspects in the "Positive" and "Negative" male patients for HPV genital infections.*

	"Positive" 52 (74%)	"Negative" 18 (26%)
Age	27-30	27-30
Number of sexual partners	8-9	2-3
First coitus age	16-17	18-19
Beginning of a regular sexual activity (years)	17-19	19-20
Weekly frequency of sexual activity	2-4	2-4
Pre-coitus care	78%	98%
Post-coitus care	88%	100%
Condom use	2%	81%

Note: "Positive" patients with colposcopic and/or cytologic HPV alterations. "Negative": patients with neither colposcopic nor with cytologic HPV alterations.

" negative " whereas 52 (74%) of them showed " specific " and " aspecific " signs of the viral infection (Table 5).

The mean age both of the " negative " patients and the " positive " ones was 27-30 years (Table 4).

As far as the marital status is concerned, there were more married than unmarried patients. The data from the cytology did not show any relationship between blood-group and positive or negative results to the cytologic and/or colposcopic examinations.

Moreover, none of these patients could be considered a heavy smoker or drinker. Dealing with the anamnesis for general diseases, we noted that 24 (34%) of the 52 " positive " patients showed relapsing episodes for Herpes Simplex Virus (HSV) type I infections, 18 (26%) had cutaneous warts and 4 (6%) hepatitis B virus infection in the years before the examination (Table 1). Statistics showed that there was no significant correlation between the other general diseases and the positive or negative results to the colposcopic and/or cytologic examinations whereas among the 46 patients found to be " positive " by the examinations 11 had (16%) psoriasis and 7 (10%) hypersensibility type I.

These diseases were not found among the " negative " patients.

There is no significant correlation in the statistics between the previous genital diseases and the positive or negative results of the above-cited diagnostic procedures.

On their first visit, whether positive or negative, most patients, were not aware of any symptoms.

The 70 patients we examined were divided into three groups by means of colposcopic examination (Tables 6-7).

— Group I was composed of 7 (10%) patients who had lesions already detected by an external examination. Using the direct colposcopic observation, we described these lesions as prominent areas, We then compared them macroscopically to

Table 5. – *Relationship between cytologic and colposcopic examinations in the diagnosis of HPV genital infections in male partners.*

	Cytology		Total
	"positivity"	"negativity"	
Colposcopic "positivity"	41 (59%)	8 (11%)	49 (70%)
Examination "negativity"	3 (4%)	18 (26%)	21 (30%)
Total	44 (63%)	26 (37%)	70 (100%)

Note: $\chi^2 = 6.53$; $\alpha = 0.01$

Table 6. – *Direct and colposcopic examinations in 70 male partners of women with relapsing HPV infection.*

	Number cases	Direct examination	Colposcopic examination
Group 1	7 (10%)	+	+
Group 2	42 (60%)	–	+
Group 3	21 (30%)	–	–
Total	70 (100%)		

Table 7. – *A proposal for classification for the main HPV colposcopic features in the three groups of male partners.*

		Colposcopic Examination		
		Direct	After Acetic acid	After Lugol sol.
Group 1 7 (10%)	4 (6%)	Flat condylomata (flat lesions)	idem	Iodine-negative or yellow chamois coloured areas
	3 (4%)	Condyloma acuminata (elevated lesions)	idem	
Group 2 42 (60%)	28 (40%)	Papules (elevated lesions)	Papules	
	7 (10%)	Negative	Micropapules 3 (4.3%)	Iodino-unsteady or yellow chamois coloured areas
			White macules 4 (5.7%)	
	7 (10%)	Macules (flat lesions)	Macules	
Group 3 21 (30%)	21 (30%)	Negative	Negative	Iodine-even stained surface

Note: 3 (4.3%) of patients show both flat (Macules) and elevated (Papules) lesions.

the flat and acuminatum condylomata of the cervix uteri. The features described by direct colposcopic examination were not modified by the application of 5% acetic acid and successively Lugol solution.

— Group II was composed of 42 (60%) patients who were negative with the external examinations but positive with direct colposcopic observation and showed elevated lesions described as " papules " and/or flat ones called " macules " (the features of such lesions became more evident after the application of 5% acetic acid whereas the application of Lugol solution revealed such an area described as jodine negative or a yellow chamois coloured one).

After the application of 5% acetic acid in areas of the genital mucosa, 7 patients of this second group, resulted negative to the direct colposcopic examination. We detected the presence of slightly protruding and translucent areas (micropapules) and/or flat, more or less white and opalescent areas defined as " white macules ". After application of Lugol solution, the micropapules and white macules appeared as iodine-unstead of yellow chamois coloured areas in the background of an uniformly coloured balano-preputial mucosa.

— Group III was composed of 21 (30%) patients who did not reveal any pathological features eiter by external examination or by direct colposcopic ob-

Table 8. – *Results of the cytologic examinations for HPV genital infection in male partners.*

Cytological findings	Sampling site		Urinary cytology
	Balano-preputial shaft	Urethral meatus	
Koilocytosis	3 (4%)	5 (7%)	3 (4%)
Dyskeratosis	5 (7%)	3 (4%)	2 (3%)
Hyperkeratosis	5 (7%)	5 (7%)	3 (4%)
Parakeratosis	3 (4%)	3 (4%)	2 (3%)
Mild dyskariosis	9 (13%)	16 (23%)	7 (10%)
Dyskaryotic immature cells	2 (3%)	4 (6%)	2 (3%)
Bacteria	7 (10%)	4 (6%)	9 (13%)
Yeasts	2 (3%)	3 (4%)	—
Polymorphonuclear leukocytes	—	—	4 (6%)
Negative	43 (61%)	49 (69%)	18 (26%)

Note: Association of two or more cytological findings is possible in the same patient ($\chi^2 = 34.81$; $\alpha < 0.01$).

Table 9. – *Relationship between cytological findings for HPV genital infections and sampling site in male partners.*

Sampling site	Cytological findings	
	positive	negative
Balano-preputial shaft	27 (39%)	43 (61%)
Urethral meatus	36 (51%)	34 (49%)
Urinary cytology	10 (23%)	54 (77%)

Note: Association of the cytological findings in the three samplings is possible in the same patient ($\chi^2 = 9.21$; $\alpha < 0.01$).

servation, or after the application of 5% acetic and Lugol solution (iodine even stained surface).

Considering the results of the cytologic examinations, some data emerged (Tables 8, 9, 10):

— 45 (64%) patients were defined as " positive » because they were affected by those cytologic alterations that are nowadays considered pathognomonic for HPV infection (koilocytosis and/or dyskeratosis, that is " specific positiveness ") and/or cytologic features that could be indicative of the HPV infection even if not pathognomonic (hyperkeratosis, parakeratosis, mild dyskaryosis, dyskaryotic immature cells, i.e., " aspecific positiveness ");

— 25 (36%) patients did not reveal either "specific" or "aspecific" cytologic abnormalities for HPV infection ("total negativeness ") (findings such as the presence of yeasts, bacteria and/or polymorphonuclear leukocytes in the cytologic samples could be revealed only in some of these patients) (table 10).

One of these 70 patients whose female partner had the histological diagnosis for CIN II - III associated to atypical viral alterations and whose urinary cytology was characterized by the presence of koilocytic and dyscaryotic cells (fig. 1 a, b, c), was found to have some macular lesions (fig. 1 d, e, f) and a spiked condyloma (fig. 1 g) by the colposcopic examination; they were located on the glans and on the frenum.

The other cytologic samples revealed a remarkable dyskeratosis and few cytologic koilocytotic cells. We performed a target biopsy of the macular lesion (fig. 1 h) and spiked condyloma: the histologic examination diagnosed slight-moderate dysplasia associated with HPV atypical alterations as far as the macular lesion was concerned (fig. 1 i, l) and spiked condylomata associated with inflammatory infiltration as far as the frenum biopsy was concerned (fig. 1 m, n). In the same patient, the la-

Table 10. – *The cytologic findigns for HPV genital infection in the male partners.*

Koilocytosis	11 (16%)	Positivity Specific 11 (16%) ↘		
Diskeratosis	10 (14%)		Total positivity 45 (64%) ↘	
Hyperkeratosis	13 (18%)	Aspecific ↗		
Parakeratosis	13 (18%)	positvity		
Mild diskaryosis	32 (46%)	39 (56%)		Total patients 70 (100%)
Dyskaryotic immature cells	11 (16%)			
No alteration	11 (16%)	Negativity 11 (16%) ↘	Total ↗	
Bacteria	20 (29%)		Negativity	
Yeasts	5 (7%)	Other findings	25 (36%)	
Polymorphonuclear leukocytes	4 (6%)	24 (34%) ↗		

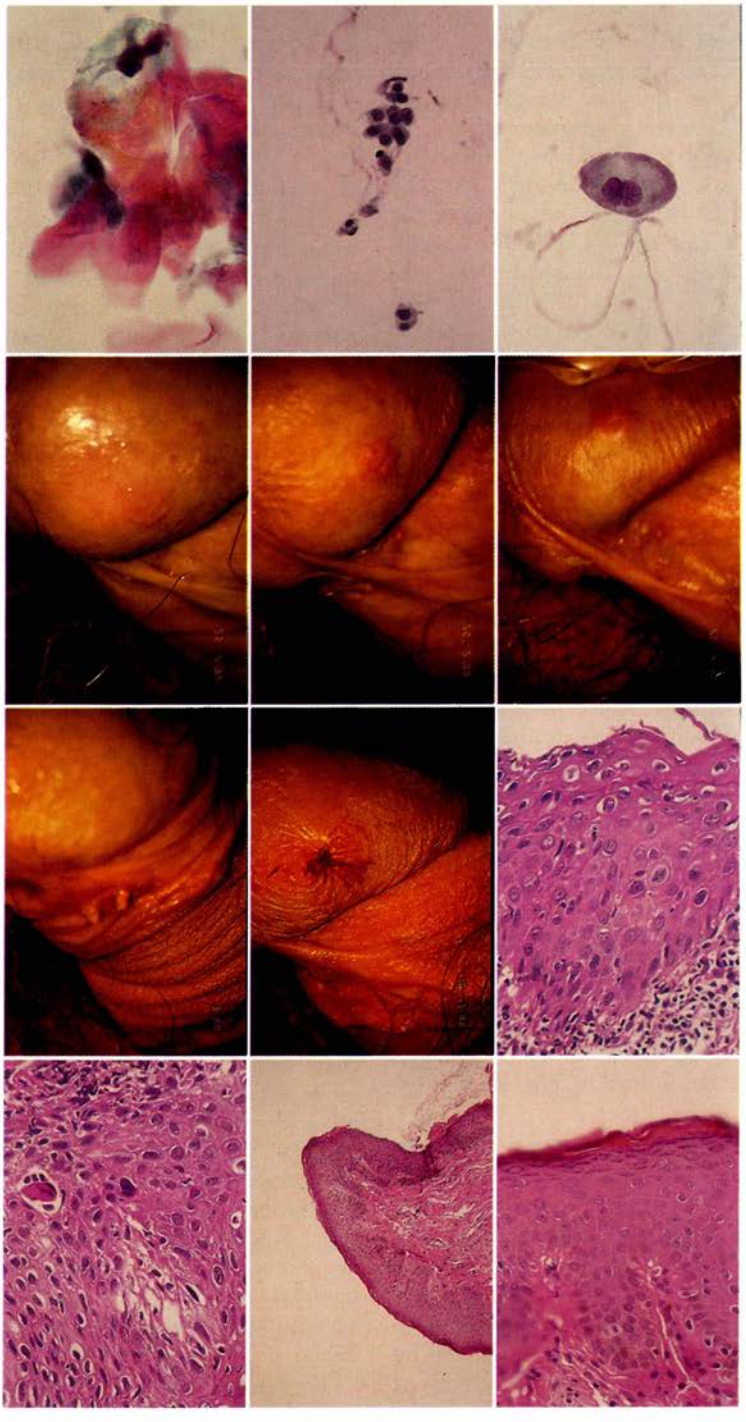

Fig. 1. — *a, b, c*) Cytologic findings of penile HPV infections; *d, e, f*) Colposcopic examination: macular lesions; *g*) Spiked condyloma; *h*) Target biopsy of the macular lesion; *i, l*) Histology of a penile macular lesion: slight-moderate dysplasia associated with HPV atypical alterations; *m, n*) Histology of a spiked condylomata.

boratory research for anti-herpes simplex virus antibodies, varicella-zoster virus and cytomegalovirus showed negative results as far as IgG were concerned with EID method; anti-Epstein-Barr virus antibodies were positive for anti-VCA IgG (1:256) and for anti-EBNA IgG and negative for anti-EA IgM and anti-VCA IgM. Moreover, the study of T-lymphocytes subgroups by monoclonal antibodies revealed T3 (peripheral = 65.8%; normal value = 69.9+6.9), Ta (T helper, inducer) = 36.9% (n.v. 44.20+6.7), T8 (T suppressor, cytotoxic) = 34.7% (n.v. 26.8+6.0) T4/T8 ratio = 1.06 (n.v. = 1.75+0.5), OKT11 = 79.8+ and DR = 11.6%.

DISCUSSION

These data reveal that the patients considered " positive " for HPV infection by cytologic and/or colposcopic examination showed, on average, a greater tendency to promiscuity and precocity in sexual intercourse in comparison with patients considered " negative " for HPV infection. The patients considered positive also showed a lower tendency to use sanitary practices before and/or after coitus and to use the condom as contraceptive method . In addition, compared to the " negative » patients, the " positive " ones revealed a greater tendency to some viral infections, particulary Herpes labialis and warts, and general diseases, such as psoriasis and hypersensibility type I in which the immunological system seems to play an important role.

All the epidemiologic variations considered, showed no significant changes in the statistics as far as the incidence between the " positive " patients and the "negative" ones to the colposcopic and cytologic examination was concerned.

Our study seemed to have once more stressed the great importance played by the " male factor " in the cervical and/or vaginal HPV relapsing and/or persisting infections. In fact, male partners might represent understimated sources of the viral infection and might also explain the causes of relapses, and therapeutic failures in the treatment of the cervical and/or vaginal HPV infections.

Therefore, the right therapeutic approach to women with relapsing and/or persisting genital infections should not disregard the examination of the male partner. The association between colposcopic and cytologic examinations was needed in order to improve the diagnostic reliability (table 5): 8 (11%) of patients with no cytologic alteration showed by means of the colposcopic examinations, alterations referrable to a probable viral infection and, on the other hand, 3 (4%) of the patients whose colposcopic examinations were considered negative, presented cytologic alterations that were probably due to the HPV infection.

Dealing with the 18 (26%) patients considered negative by the cytologic and colposcopic examinations and according to the fact that the positive patients reveal a greater predisposition than the negative patients to some viral infections, particularly Herpes labialis and warts, and to general diseases, such as psoriasis and hypersensibility type I, we further underline the importance of the interaction between the immunological system of the host and the viral antigenic heterogeneity.

From these data, it is possible to reveal that many of male partners of women with cervical and/or vaginal HPV infection relapsing and/or persisting after medical and/or surgical therapies, show alterations referable to the viral infection. Therefore, HPV infection is often present in both members of a couple and, so far, both the male partner and the female patients can play an important role in sustaining the infection within the couple itself.

Therefore, for the therapy to be successful, diagnoses must consider both

partners. The fact that there is a great incidence of HPV infection and HPV associated lesions and the risk of transmitting a possibly oncogenic virus in the genital organs, emphasizes the importance of both partners being diagnosed in order to ensure a correct diagnostic and therapeutic course and follow-up in recurring HPV genital infections in the couple [22, 23, 24, 25].

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