

HSV₂ AND CERVICAL INTRAEPITHELIAL NEOPLASIA: CYTOLOGICAL, HISTOLOGICAL AND SEROLOGICAL FEATURES

P. PULIGHEDDU - R. NIEDDU - F. MEDDA - C. MARCELLO - U. LECCA
Clinica Ostetrica e Ginecologica - Università degli Studi, Cagliari (Italy)

Summary: An epidemiological investigation on the incidence of HSV₂ infection in 1216 women with acute or recurrent vulvovaginitis was performed. HSV₂ research was carried out by means of clinical, cytological, colposcopic, histologic examinations and vaginal pad cultures and antibody titration. Of the patients examined, 12.6% presented signs of genital herpes; among the diagnostic techniques used, the most reliable proved to be the serological method. Incidence of HSV₂ was higher among women in the under privileged social classes, those who had sexual relations with different partners and those who were multiparous. A higher percentage of dysplastic and/or neoplastic lesions of the uterine cervix among HSV₂ positive ($P < 0.025$) with respect to controls was also found. A higher incidence of neoplastic pathologies among women who are positive for HSV₂ was therefore confirmed.

INTRODUCTION

In recent years particular attention has been dedicated to the problem of Herpes Simplex infection of the female genital tract (HSV₂)^(1, 2, 3, 4). Up to the beginning of the 1960s this affection was limited in its diffusion, but today it is progressively increasing all over the world. The etiologic agent of HSV₂ is an epidermotropic virus with double-filament DNA, surrounded by a protein sheath having an icosahedral symmetry, in turn protected by a lipoglyco-proteinic lining, which is essential to virus infections. The virion has a diameter of 100 nanometers and contains 162 capsomeres. The complete viral particle reaches a size of 150 nm.

The primary infection, usually transmitted by sexual intercourse, manifests itself clinically with the appearance of typical vesicles in the shape of bunches on the external genitals, the vagina and the uterine cervix. These vesicles break in about 24-48 hours, leaving small ulcerated formations. The symptoms are characterized by a burning sensation, an overall feeling

of indisposition, and disappear in two or three weeks.

The disease can, however, remain latent (virus migration to the pelvic ganglia) and reappear periodically following aspecific stimuli.

Many different criteria can be followed for the diagnosis: clinical evidence; cytological smear (characters); colposcopic and histologic pictures; culture isolation; antibody titration. The infection presents cytomorphological lesions characterized by giant, multinucleate cells with an opaque nucleus (frosted glass cells), sometimes with intranuclear eosinophile inclusions^(5, 6, 7).

The most important histological alterations are found at the level of the squamocolumnar junction, with hyperplasia of the basal cells and the presence of single, scattered giant cells^(5, 8).

Cultural isolation is possible in about 4.5% of cases having clear symptomatology cases⁽⁹⁾.

With sero-immunological methods it is possible to detect the presence of anti-HSV₂ antibodies in the host organism, thus revealing it even asymptomatic^(5, 10).

In the last few years a large number of epidemiological studies^(11, 12, 13, 14) have prospected the existence of a possible connec-

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tion between HSV₂ infection and the development of uterine cervix carcinoma in the uterine cervix. It has been demonstrated that the HSV₂ virus can produce tumours in laboratory animals^(16, 17), and the presence of antigens of the HSV₂ has been demonstrated in cells from neoplastic cervix tissue, as a confirmation of the genetic transfer HSV₂ in tumour cells^(14, 18). Epidemiological data⁽¹⁷⁾ show a greater frequency of dysplastic and/or neoplastic cervical lesions in women with herpes infection and a higher incidence of anti-HSV₂ antibodies in women with cervical dysplasia v/s controls⁽¹⁹⁾. Studies in perspective have demonstrated that women with herpetic infections develop cervical anaplasia more frequently than do controls.

All epidemiological data^(21, 22, 23) also confirm a greater frequency of cervical carcinoma in women with early sexual intercourse, with different partners and who belong to underprivileged social classes: these are situations in which the diffusion of HSV₂ is very high.

These data have led to the hypothesis of a sexually transmitted viral pathology⁽²⁾.

The herpes infection has, however, a much higher incidence than does cervical carcinoma. Therefore the cancer appearance can be determined by various conditions; such as transitory weakness in cell mediated immunity and still unknown cocarcinogen factors.

Many authors have isolated HSV₂ in asymptomatic male subjects; isolation has been carried out in the prostate, deferents and urethra. Therefore, the partner is often the asymptomatic carrier of the HSV₂^(22, 23). The concept of "male carrier" has been developed⁽²¹⁾, with repeated HSV₂ infection or partners with herpes infections and cervical dysplasia: it was still considered as virus natural reservoir.

In order to evaluate the incidence of HSV₂ infection and its possible association with dysplastic/neoplastic alterations of

the uterine cervix, we carried out an epidemiological study on our population. The research was carried out using several different diagnostic methods so as to keep false negatives to a minimum.

MATERIAL AND METHODS

Between 1983 and 1985, at the Cytology Service of the Obstetrics and Gynecologic Clinic of the University of Cagliari (Italy), 1216 women between the ages of 15 and 74 (average age 41.2 years) were examined.

All women underwent the following examinations and tests:

- gathering of anamnesic data, above all those regarding recurrent genital virus infections and sexual activity;
- gynecological examination;
- colposcycological examination according to Papanicolau;
- colposcopic examination;
- target biopsy and histological examination in cases with atypical colposcopic images;
- search for herpes viruses on vaginal pads inoculated in cultures of HEP₂ and VERO cells obtained respectively from a laryngeal carcinoma and a kidney carcinoma of a particular species of African monkey, followed by typifying the viral stock found by means of neutralization with specific antiserum (anti-HSV₁ and anti-HSV₂);
- blood sampling in order to titrate the anti-HSV₁ and anti-HSV₂ serum antibodies, which was always carried out with the neutralization method.

We considered positive those sera which presented neutralization at dilutions above 1/1000 so as to exclude cross-reactivity between the two viral types present at lower dilutions.

Data obtained were statistically analysed by means of Fischer's χ^2 test.

RESULTS

Of the 1,216 women examined, 154 (12.6%) presented signs of a genital herpes infection (table 1). In 24 (15.5%)

Table 1. - *Incidence of herpes infection.*

Total No. cases	HSV ₂ positivity No. %	
1.216	154	12.6

of these cases we succeeded in isolating the virus by culture in vaginal pads, while positivity for HSV₂ was significantly higher with cyto-colpo-histologic ascertainment (27.2%), anamnestic and clinical examinations (52%), reaching highest values (74%) with anti-HSV₂ antibody titration (table 2).

Table 2. - Type of positivity referred to entire sample and to the 154 HSV₂ positive cases.

	No.	%	%
Clinical anamnestic	80	6.5	52
Cytological colposcopic histological	42	3.4	27.2
Cultural	24	2	15.5
Serological (1/1000)	114	9.3	74

The results of the colpo-cytological examination over the entire sample and in 154 cases of positivity for HSV is given in table 3: in subjects positive for herpes genitalis a higher percentage was found for classes III, IV and V ($P < 0.0001$).

Table 3. - Comparison of colpo-cytological results on total subjects examined to cases positive for HSV₂.

	Entire sample		Cases positive for HSV ₂	
	No.	%	No.	%
Class I - II	980	80.6	104	67.5
Class III	194	16	42	27.2 *
Class IV - V	42	3.4	8	5.2 *
Total	1216			

(* = $P < 0.001$)

With the colposcopic examination, 928 cases of typical and 288 cases of atypical images were showed (table 4). On the latter a systematic histological ascertainment was carried out following a target biopsy; 168 cases of cervicitis

Table 4. - Colposcopic pictures.

	No.	%
Atypical images	288	23.7
Typical images	928	76.3
Total	1216	

(58.3%), 68 of CIN I (23.6%), 22 of CIN II (7.6%), 20 of CIN III (7%) and 10 invasive carcinomata (3.5%) (table 5) were found.

Table 5. - Histological results on 288 biopsies.

	No.	%
Cervicitis	168	58.3
CIN I	68	23.6
CIN II	22	7.6
CIN III	20	7.0
Invasive K.	10	3.5
Total	288	

Of the 288 biopsies carried out, 78 were also HSV₂ positive; 34 cases presented cervicitis (43.5%), 24 CIN I (30.8%), 8 CIN II (10.8%) ($P < 0.025$), 10 CIN III (12.8%) ($P < 0.025$) and 2 invasive carcinomata (2.6%) (table 6).

Table 6. - Comparison of histological results for dysplasia and neoplasia on total biopsies (A) to those relating to cases positive for HSV₂ (B).

	A		B	
	No.	%	No.	%
Cervicitis	168	58.3	34	43.5
CIN I	68	23.6	24	30.8
CIN II	22	7.6	8	10.8
CIN III	20	7.0	10	12.8 *
Invasive K.	10	3.5	2	2.6
Total	288		78	

(* = $P < 0.025$)

Analysis of subjects' socio-economic status (table 7) showed up the low frequency of the herpes infection in the higher classes (7.8%) and its prevalence (46.8%) in the lower socio-economic classes ($P < 0.0001$).

Table 7. - Comparison of socio-economic condition of total cases examined to those found to be HSV₂ positive.

	Total cases No.	%	HSV ₂ positives No.	%
High	48	3.9	12	7.8
Middle	928	76.3	70	45.4
Low	240	19.7	72	46.8 *
Total	1216		154	

(* = $P < 0.0001$)

As regard childbirth (table 8), women who had borne more than one child and who were HSV₂ positive represented ($P < 0.025$) with respect to the other categories examined.

Table 8. - Parity distribution over total cases examined and HSV₂ positives.

	Total cases No.	%	HSV ₂ positives No.	%
Nulliparous	230	18.6	40	26
Primiparous	200	16.4	16	10.3
Secundiparous	346	28.4	36	23.3
Multiparous	440	36.1	62	40.2 *
Total	1216		154	

(* = $P < 0.025$)

84.7% of the entire sample and 76.6% of the women with a herpes inflammation reported having relations with only one partner; among the women who had more than one partner there was a significant difference for those who were HSV₂ positive ($P < 0.025$) (table 9).

Table 9. - Comparison of number of sexual partners of total cases examined to HSV₂ positives.

	Total cases No.	%	HSV ₂ positives No.	%
0	24	1.9	6	3.9
1	1030	84.7	118	76.6
2	98	8	16	10.3
>2	64	5.2	14	9 *
Total	1216		154	

(* = $P < 0.0025$)

DISCUSSION

In Literature there are some quite discordant data on the incidence of herpetic infection in the female genital tract. This infection is undergoing a progressive increase on a world-wide basis and is directly connected with the liberalization of customs and sexual promiscuity⁽²⁷⁾.

American authors report rather high incidences; according to Gardner and Kaufman⁽²⁴⁾, 22% of the patients in the public hospitals in Houston present serological evidence of a previous HSV₂ infection. These authors place HSV₂ among the most important sexually transmitted diseases in the last decade, with about 10.000.000 cases per year in the United States alone. Analogous epidemiological evaluations in Europe (15.26) put the number of cases at a much lower level, with an incidence varying from Kleger's 0.6% to Coleman's 8.6%. In our study, HSV₂ positivity was found to be 12.6%. This rather high incidence may find an explanation in the fact that the women investigated were chosen on the basis of symptomatology involving acute or recurring vaginal inflammation. Furthermore, by using different diagnostic methods on the same subject, the possibility of positive cases escaping diagnosis was greatly reduced. Among the methods adopted, the most effective and reliable proved to be the serological examination, which allowed the finding of an antibody

titre above 1/1000 in 74% of positive cases; the vaginal pad culture was less reliable, and this confirms the transitory presence of the virus in the vagina.

Herpetic disease had the highest incidence among women of the underprivileged socio-economic classes, those who had borne more than one child and those who had had different sexual partners. Our results fit in with those of other studies^(25, 26), thus confirming the importance the herpes genitalis phenomenon has reached even in our population.

The significance of our data is even more evident if one considers that the investigation was carried out on women coming from a wide variety of environments, having in common only the symptomatology of vulvo-vaginal inflammations, both acute and recurrent. On the one hand, this confirms the existence of the high risk of contracting the herpes disease for certain categories (in particular) and on the other hand the importance of having included HSV₂ inflammation among the most important venereal diseases of the last decade.

Comparison of the cyto-colpo-histological results for the entire sample examined and for the cases positive for the presence of HSV₂ inflammation appears to confirm what has been observed by other Authors in similar works^(28, 29, 30) concerning the existence of a possible causal connection between Herpes and dysplastic and/or neoplastic states of the uterine cervix^(28, 29, 30).

In our report, 28.5% of the women found positive for HSV₂ showed a more or less high degree of dysplasia as against 9.8% of the entire group considered, following histological examination of target biopsies.

Naib and Aurelian^(31, 32) had already underlined the association between herpetic viruses and cervical cancer and they had hypothesized a cytopathological action of the virus on the nucleus, thus

pushing cell replication in the direction of dysplasia^(33, 34).

This is certainly not sufficient to allow us to state that there is a direct cause and effect relationship between herpes and tumours: the infection may simply precede the tumour.

The significant difference we found between women with cervical dysplasia and herpetic infection with respect to those with dysplasia but who were HSV₂ negative tends to confirm the concept of an important etiological correlation^(31, 32, 33, 34) between virus and cancer.

We propose to follow up women with HSV₂ infection to see how many of them develop cervical anaplasia in the years to come. Data now available, however, allow us to catalogue HSV₂ as an important carcinogen factor which, in association with other still unknown agents is capable of dysplastic cell transformation. We therefore now have the real possibility of selecting a category of women facing a high risk of developing cervical cancer and therefore also the possibility of taking all necessary diagnostic and therapeutic steps in order to prevent the cancer development or at least to detect them at the earliest stage.

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