Colposcopy, cytology and HPV-DNA testing in HIV-positive and HIV-negative women

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Summary

In this study we examined the incidence of colposcopic-colpocytologic findings and analyzed Human Papilloma Virus (HPV)-DNA testing by Polymerase Chain Reaction (PCR) in 104 Human Immunodeficiency Virus (HIV) serous positive women (Group 1) and 218 HIV-negative women (control Groups 2 and 3). The aim of the study was to evaluate the most appropriate and efficacious diagnostic methods for screening programs for cervical cancer in HIV-positive women. For Group 1 we also considered the value of CD4+ T-lymphocytes and morphologic and molecular follow-up from 3 to 6 months. The results showed that the abnormal transformation zone (ANTZ) was present in 66.3% of the cases in Group 1 compared with 31.4% in control-Group 2 ($p<0.001$), and with 58.93% of the cases in control-Group 3 ($p=0.257$); intraepithelial squamous lesions (SIL) were found in 50% vs 5.66% ($p<0.001$) and vs 56.25% of the cases ($p=0.433$), respectively.

In 28.85% of the HIV-positive patients the first cytological screening exam was not evaluable due to inflammation but in 56.67% of the cases colposcopy revealed ANTZ. The subsequent colpocytological checkup after therapy showed 10 cases (30%) of low risk squamous intraepithelial lesions (LSIL) and two cases (6.6%) of high risk squamous intraepithelial lesions (HSIL). HPV-DNA testing by PCR was positive in 53.8% of the cases in Group 1, in 6.6% in control-Group 2 and in 42% in control-Group 3. In HIV-positive patients multiple HPV genotypes were simultaneously present in 21.43% of the cases and high risk genotypes were present in 70% of the cases of HSIL. In Group 1, 36.61% of the cases had lesions of the lower genital tract. The value of CD4+ T-lymphocytes was <200 cells/ml in 30% of the cases of HSIL.

Our data, like those of other Authors, confirm a high incidence of HSIL, abnormal colposcopic findings, and HPV infections in HIV-positive women with respect to control-Group 2, while there was not much difference between Group 1 and control-Group 3.

Such frequency again suggests that an integrated morphological diagnostic approach with colposcopy-colpocytology in the screening of immunosuppressed subjects would be worthwhile.

Key words: Human Immunodeficiency Virus (HIV); Human Papilloma Virus (HPV); Colposcopy; Cytology; Polymerase Chain Reaction (PCR).

Introduction

Numerous epidemiological studies have correlated infectious pathology and neoplasia in the anogenital tract to the local and general immunological state of subjects [1-5]. In the last decade HIV infection has assumed epidemic proportions in many countries according to the reports of the World Health Organization (WHO). In Italy those at highest risk are drug users. Now the new infected cases are, above all, heterosexuals and it is expected that in the future women will outnumber men [6, 7]. The first study that showed an association between HIV infection and cervical epithelial neoplasia (CIN) was in 1987 [8]. Subsequent reports demonstrated an elevated incidence of intraepithelial neoplasia and cellular alterations by HPV infections in HIV-positive women. However, cases of invasive cervical carcinoma are rarely diagnosed [9-11]. At the moment there is more data favoring a correlation between HIV and CIN than between AIDS and invasive carcinoma [12].

For the morphological diagnosis of lesions of the lower genital tract generally only colpocytology is used. However, a discrete number of false negatives and false positives have been found. Thus the routine use of colposcopy together with colpocytology in HIV-positive women has been suggested [13-15]. The most recent molecular diagnostic procedures for HPV-DNA testing have confirmed an elevated incidence of genital HPV, and particularly the simultaneous presence of various genotypes [16-19].

In this study we examined the incidence of the morphological colposcopic-colpocytologic findings and analyzed HPV-DNA testing by PCR in HIV-positive and HIV-negative women to evaluate the most appropriate diagnostic methods and the efficacy of the screening programs for cervical cancer in HIV-positive women. In this latter group variations in time of the morphological and molecular findings were also considered in order to obtain a better understanding of the natural history of cervico-vaginal neoplasias in immunosuppressed women and to establish a more adequate cervical cancer secondary prevention.

Materials and Methods

The study included 322 women of whom 104 were HIV-positive in various stages. It was carried out from January 1997 to January 1999 at the Department of Gynaecology and Obstetrics at the University of Padua. All the women underwent Pap tests, colposcopy, HPV-DNA testing by PCR, gynacological exams and counseling during the same visit. The cytological samples for the Pap tests and for HPV-DNA testing, the colposcopy and the gynacological exam were performed by the same Gynacologist.

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The patients were divided into three groups: HIV-positive women (Group 1) were compared with control-groups of HIV-negative women (Groups 2 and 3), separately.

Group 1 consisted of 104 HIV-positive women, from 24 to 76 years old (median 35 years) who came for gynacological screening. All the patients with HSIL and those with LSIL with undetermined dysplasia and with reactive and reparative changes that had abnormal colposcopy findings underwent target biopsy. The cases with LSIL were followed-up at six months. The value of CD4+ T-lymphocytes and viral load at the gynacological checkup were correlated with the cyto-morphological findings.

Group 2 – Controls – included 106 HIV-negative women from 21 to 70 years of age (median 32 years) who came for routine screening with previous negative colpocytological findings.

Group 3 – Controls – was comprised of 112 HIV-negative women from 19 to 74 years old (median 31 years) with previous morphological diagnoses of alterations related to HPV infections associated or not with CIN. The patients were submitted to target biopsy according to the colposcopic and colpocytological indications.

In addition to the abnormal colposcopic findings in the HIV-positive and HIV-negative women we also evaluated the simultaneous presence of pathological areas in the vagina, vulva, perineum and the mapping of the lesions, particularly the perimetrial extension of ANTZ.

The sensitivity, the specificity, and the positive and negative predictive values of the cytologic smears were compared between HIV-seropositives and control-Group 3.

Before undergoing colposcopy all the women examined were submitted to two cytologic samples, one for a Pap test and one for HPV-DNA-PCR testing. The Pap smears were taken with an Ayre spatula and a cytobrush in the standard way for all patients. The sample for molecular biology was collected in a sterile tube containing a phosphate-buffered solution. The colposcopic exams were done according to the usual method using 5% acetic acid and a solution of Lugol. The Bethesda II Classification [20] was used for the colpocytologic diagnosis and the Italian Colposcopic Classification [21] for colposcopy.

The HIV-positive women underwent morphological-molecular follow-up from three to six months, always with the same gynacologist. Patients with high grade lesions underwent excisional surgical therapy with adequate follow-up.

HPV-DNA testing was carried out by PCR. Cellular DNA obtained from the cervico-vaginal cell suspensions was amplified in 50 µl reaction mixture using the consensus primers MY09/MY11. To control the quality of DNA to be amplified, PC04 and GH20 primers (Genset), specific for a 268 bp sequence of beta-globin gene, were used in a separate reaction. Ten µl of each PCR amplified product (length range: 449-455 bp) were digested with 4U of one or more of the restriction enzymes (Res) Rsa I, Hae III, Pst I, Bam HI and Sau IIIA (Boeringher, Mannheim. Germany), in a final volume of 20 µl at 37 °C overnight; digestion products were then run on an 8% polyacrylamide (acryl/bis-acryl 19:1) gel with ethidium bromide and photographed under UV light.

Results

The colposcopic findings for the three groups of patients are reported in Tables 1 and 2 and in Figures 1 and 2. Using the chi-square test a significant statistical difference in incidence was found both in ANTZ and SIL ($p<0.001$) in the screening of the HIV-positive (Group 1) versus HIV-negative women (Group 2). Instead there was no significant difference between Group 1 and control-Group 3 ($p=0.257$ and $p=0.433$, respectively). Table 3 shows the correlations between the cytomorphologic and colposcopic findings in HIV-positive patients. It can be seen that colposcopy was always abnormal (ANTZ) in all women with high grade lesions and in 17 (56.67%) of the cases where the first cytological screening was not evaluable due to inflammation (Table 3). In these 17 cases the colpocytology after therapy revealed 10 cases (30%)

Table 1. — *Colposcopic findings in HIV-positive and HIV-negative women.*

Colposcopy	HIV-positive		HIV-negative			
	Group 1		Group 2		Group 3	
	N	%	N	%	N	%
NTZ	**34**	**32.7**	**73**	**68.86**	**46**	**41.07**
ANTZ	**70**	**67.3**	**33**	**31.14**	**66**	**58.93**
G0	16	15.4	25	23.6	6	5.36
G1	44	42.3	8	7.54	44	39.29
G2	10	9.6	–	–	16	14.28
Total	104	100	106	100	112	100
HPV findings	53	51	11	10.4	28	25

NTZ = normal transformation zone
ANTZ = abnormal transformation zone

Table 2. — *Cytomorphology in HIV-positive and HIV-negative women.*

Cytomorphology	HIV-positive		HIV-negative			
	Group 1		Group 2		Group 3	
	N	%	N	%	N	%
Unsatisfactory due to inflammation	30	28.85	9	8.5	13	11.61
Within normal limits	1	0.96	69	65.1	11	9.82
Reactive and reparative changes	11	10.57	22	20.74	14	12.5
Undetermined dysplasia	9	8.66	–	–	11	9.82
SIL	**52**	**50**	**6**	**5.66**	**63**	**56.25**
LSIL	42	40.4	6	5.66	46	41.07
HSIL	10	9.6	–	–	17	15.18
Microinvasive carcinoma	1	0.96	–	–	–	–
Total	104	100	106	100	112	100

Table 3. — *Correlation between cytomorphology and colposcopic findings in HIV-positive women.*

Cytomorphology	Colposcopy					
	NTZ		ANTZ		Total	
	N	%	N	%	N	%
Unsatisfactory due to inflammation	13	43.33	17	56.67	30	29
Within normal limits	1	100	–	–	1	0.96
Reactive and reparative changes	6	54.54	5	45.46	11	10.57
Undetermined dysplasia	1	11.11	8	88.89	9	8.66
LSIL	13	30.95	29	69.05	42	40.4
HSIL	–	–	10	100	10	9.6
Microinvasive carcinoma	–	–	1	100	1	0.96
Total	34	32.7	70	67.3	104	100

Table 4. — *HPV prevalence by cytomorphology in HIV-positive and HIV-negative women.*

Cytomorphology	HIV-positive			HIV-negative					
	Group 1			Group 2			Group 3		
	N	HPV+		N	HPV+		N	HPV+	
		N	%		N	%		N	%
Unsatisfactory due to inflammation	30	12	40	9	1	11.1	13	2	15.4
Within normal limits	1	–	–	69	2	2.9	11	1	9.1
Reactive and reparative changes	11	2	18.2	22	3	13.63	14	5	35.7
Undetermined dysplasia	9	8	88.9	–	–	–	11	6	54.5
LSIL	42	24	57.1	6	1	16.7	46	19	41.3
HSIL	10	9	90	–	–	–	17	14	82.3
Microinvasive carcinoma	1	1	100	–	–	–	–	–	–
Total	104	56	53.8	106	7	6.6	112	47	41.9

Table 5. — *Distribution of HPV genotypes in HIV-positive and HIV-negative women.*

HPV types	HIV-positive		HIV-negative			
	Group 1		Group 2		Group 3	
	N	%	N	%	N	%
Low risk (6, 11, 42, 53, 54, 61, 62, 73)	13	23.2	3	42.86	14	29.8
High risk (16, 18, 31 33, 45, 52, 56, 58, 66)	22	39.3	3	42.86	20	42.55
Novel types (CP141, CP8304, MM8 PAP155, X)	9	16.07	1	14.28	11	23.4
Mixed infection	12	21.43	–	–	2	4.25
Total	56	100	7	100	47	100

Table 6. — *HPV genotype prevalence in HIV-positive women.*

Cytomorphology	N	HPV							
		Low risk		High risk		Mixed		Total HPV positive	
		N	%	N	%	N	%	N	%
Unsatisfactory due to inflammation	30	7	23.33	4	13.33	1	3.3	12	40
Within normal limits	1	–	–	–	–	–	–	–	–
Reactive and reparative changes	11	1	9	1	9	–	–	2	18.18
Undetermined dysplasia	9	1	11.1	4	44.4	3	33.3	8	88.8
LSIL	42	6	14.28	12	28.57	6	14.28	24	57.1
HSIL	10	–	–	7	70	2	20	9	90
Microinvasive carcinoma	1	–	–	1	100	–	–	1	100
Total	104	15	14.42	29	27.88	12	11.53	5.6	53.8

Table 7. — *Lower genital tract associated colposcopic lesions and >2/3 exocervix ANTZ extension in HIV-positive and HIV-negative women.*

Low genital tract associated COLPOSCOPIC LESIONS	HIV-positive		HIV-negative			
	Group 1 N=104		Group 2 N=106		Group 3 N=112	
	N	%	N	%	N	%
Lower genital tract associated lesions						
vagina	9	8.6	5	4.7	8	7.14
vulva-perineum	11	10.57	–	–	7	6.25
vagina and vulva	16	36.61	–	–	–	
Total	36	36.61	5	4.7	20	17.85
>2/3 ANTZ extension on exocervix	37	35.57	1	0.94	18	16.07

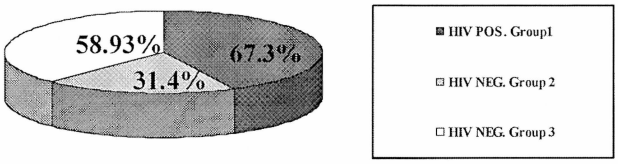
Table 8. — *Correlation between cytomorphology and histology in HIV-positive women.*

Histology	Cytomorphology											
	Reactive and reparative changes		LSIL		Undetermined dysplasia		HSIL		Microinv. carcinoma		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
Minor alterations	3	40	5	17.24	–	–	–	–	–	–	8	15.1
CIN I	1	20	22	75.86	5	62.5	–	–	–	–	28	52.8
CIN II	1	20	2	6.89	3	37.5	3	30	–	–	9	17
CIN III	–	–	–	–	–	–	7	70	–	–	7	13.2
Microinvasive carcinoma	–	–	–	–	–	–	–	–	1	100	1	1.9
Total	5	100	29	100	8	100	10	100	1	100	53	100

Table 9. — *Cytomorphology, CD4+ T-lymphocytes and viral load >50,000 copies/ml in HIV-positive women.*

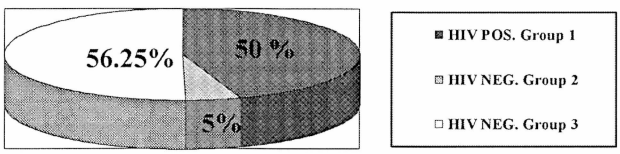
Cytomorphology	CD4+ cells/ml				Virus >50,000 copies	
	CD4+<400		CD4+<200			
	N	%	N	%	N	%
Unsatisfactory due to inflammation	5	16.66	1	3.33	5	16.66
Within normal limits N=1	–	–	–	–	–	–
Reactive and reparative changes N=11	2	18.18	–	–	1	9.09
Undetermined dysplasia N=9	2	22.22	–	–	–	–
LSIL N=42	12	28.57	3	7.14	3	7.14
HSIL N=10	7	70	3	30	4	40
Microinvasive carcinoma N=1	–	–	1	100	1	100

of LSIL and two cases (6.6%) of HSIL. As shown in tables 4, 5, 6 and figure 3 the HPV-DNA prevalence testing rate by PCR was 53.8% in Group 1, 6.6% in control Group 2 and 41.9% in control Group 3. In particular HSIL and undetermined dysplasia were associated with HPV infection, respectively, in 90% and 88.9% of the cases in Group 1. In Table 5 the distribution of the genotypes of HPV isolated in HIV-positive and HIV-negative patients is reported. In the high risk group, HPV 16, the single most frequently detected HPV type, was associated with HSIL in four out of seven cases in Group 1. The novel, yet unclassified types, CP141, CP8304, MM8 (PAP 155) were detected in nine cases (16.07%). In HIV-negative women - control Group 2 - two cases



HIV pos. vs HIV neg. Group 2 p<0.001
HIV pos. vs HIV neg. Group 3 p=0.257

Figure 1. — Abnormal transformation zone prevalence in HIV positive and HIV negative women.



HIV pos. vs HIV neg. Group 2 p<0.001
HIV pos. vs HIV neg. Group 3 p=0.433

Figure 2. — SIL prevalence in HIV positive and HIV negative women.

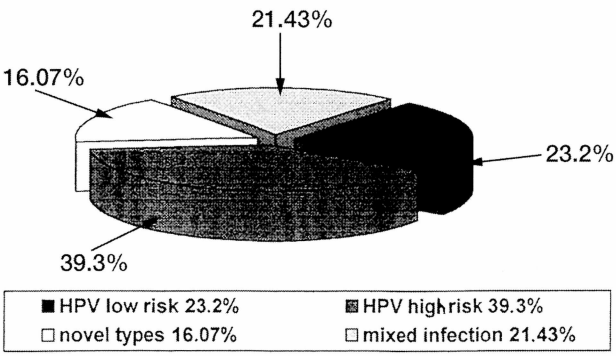


Figure 3. — Distribution of HPV genotypes in HIV-positive women.

revealed HPV-genotype 18 when the colpocytology was within normal limits and colposcopy revealed ANTZ GO and in one case HPV genotype 52 was observed with a diagnosis of reactive and reparative changes and of ANTZ GO.

In Group 1 in 70% of HSIL cases high-risk genotypes were present and in 20% multiple genotypes (Table 6).

In Table 7 the incidence of lower genital tract colposcopic lesions in HIV-positive and HIV-negative women is reported. As for the comparison of the mapping of abnormal colposcopic findings in the groups considered, ANTZ etended into two-thirds of the exocervices in 37 (35.57%) of the patients in Group 1, in one (0.94%) in control Group 2 ($p<0.001$) and in 18 cases (16.07%) in control Group 3 ($p<0.01$), respectively (Table 7). In Group 1, 53 target biopsies were performed. The histological exam confirmed the diagnoses in all cases of HSIL (Table 8). Instead, LSIL showed cytological overestimation in 14.28% and an underestimation in 4.75%. In the cases with reactive and reparative changes that underwent target biopsies based on colposcopic indications, CIN I was diagnosed in 20% of the cases and CIN II in 20% (Table 8). In Group 3, 88 target biopsies were performed which diagnosed 18 cases (20.45%) of minor alterations, 48 (50.54%) of CIN I, 12 (13.63%) of CIN II, and 10 (11.36%) of CIN III. There was cytological underestimation of LSIL in two cases (4.34%), an overestimation in six (13.04%) and diagnostic confirmation in 38 cases (82.6%). All 17 cases of HSIL were also confirmed in this group. Among seropositives, the sensitivity, the specificity and the positive and negative value of cytologic smears for CIN were 91% (48/53), 50% (3/8), 94% (48/53) and 86% (3/8), respectively. The corresponding figures in the controls (Group 3) were 97% (68/70), 67% (12/18), 92% (68/74) and 86% (12/14), respectively, and did not differ significantly from those of seropositives.

In HIV-positive women the value of CD4+ T-lymphocytes was less than 200 cells/ml in 30% of the cases of HSIL and micro-invasive carcinoma. The viral load was greater than 50,000 copies/ml in four cases (40%) of HSIL (Table 9).

Morphological-molecular follow-up in the HIV-positive women included 93 patients who were checked from 3 to 6 months; nine who had excisional surgery for high grade lesions were followed-up at 3 months, and two dropped out. Of the 93 patients, progression in the cytologic lesions was observed in 12 cases (12.9%) and a variation of the viral genotypes was found in 18 (19.35%). Morphological-molecular follow-up at 3 months in the nine women who had undergone surgical therapy revealed 2 cases of persistent lesions (22.22%), 2 cases of LSIL (22.22%) and 5 negative cases (55.56%). As to the presence of HPV, a variation in viral genotype was observed in one case (11.11%), one case showed negativity (11.11%) and persistence of the same genotype was seen in 7 cases (77.78%).

Discussion

Analysis of our data, like that of other Authors [1, 3, 5, 8, 9, 13], confirmed a high incidence of abnormal colposcopic findings, HSIL and HPV infections in the screening of HIV-positive women (Group 1) with respect to control-Group 2, while there was no significant difference between Group 1 and control-Group 3. Thus, the role of immunosuppression in the interaction between HIV and HPV at a cellular level should be considered. The present knowledge of the biological evidence of HPV infection indicates that the various types of morphological lesions represent a visible epiphenomenon of complex levels of interaction between the virus and host cells. Our case series revealed that in HIV-positive and HIV-negative women colpocytology identified all high grade lesions and that there was not a statistically significant difference in sensitivity, specificity and predictive value in Group 1 and control Group 3. However, considering the high number of non-evaluable cytologic exams at the first checkup which subsequently revealed 36.6% of cases of SIL of various grades, the target biopsies which revealed CIN – where colposcopy was determinant – contributed to a better diagnostic accuracy in Group 1. Thus, colposcopy is particularly important for immunosuppressed subjects who often do not return for check-ups and in whom the progression of the lesion can be more rapid. As for the HPV genotype research in the 3 groups we found a higher prevalence of infections among

the HIV-positive women (Group 1) for all the cytomorphological categories considered, particularly high risk genotypes in 70% of the cases of HSIL. The occurrence of mixed infection was also much more common in Group 1. Among the HIV-infected patients CD4+ T-lymphocyte count was <400 cells/ml in high risk HPV and in HSIL, again confirming the role of immunosuppression as a risk factor. In the follow-up of the HIV-positive women various viral genotypes were observed in 19.35% of cases and in 12.9% of cases cytologic progression of the lesion, expressing the interrelation among changes in the immune system, associated risk factors and therapy with multi-antiretroviral drugs. These data stress the importance of the most accurate diagnostics possible in a short time with an adequate period of follow-up. Therefore, we think it is crucial to integrate colpocytology screening with colposcopy, a non-invasive method used to explore the entire anogenital tract, which is often the site of concomitant lesions.

The advantages of routine colposcopy include faster diagnoses and mapping of dysplastic lesions, some of which progress rapidly. The colposcopic exam together with clinical investigation is, in fact, the first morphological expression of cervico-vaginal physiopathology. In its complex and multi-various framework and in its important "details", it suggests a first and precocious morphologic diagnosis subsequently defined by cytohistology and eventually completed by molecular research, thus obtaining a more accurate evaluation of risk. Therapy would therefore be more precocious and personalized. Thus, the cost/benefit balance of the morphological diagnostics in the screening of immunosuppressed subjects should also take into account these aspects.

The compliance in HIV-positive women (Group 1) was satisfactory in that only two patients dropped out. It seems that having the same Gynacologist perform the exams at the same time also contributed to these results.

In conclusion using an integrated diagnostic approach – in particular colposcopic-colpocytology together with adequate counseling and psychological support – seems a valid means in the screening of cervical cancer in HIV-positive women. As is well known, this population is at high risk of onset and progression of dysplastic pathology in the lower genital tract and has notable psychological and interrelational problems.

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