Prevalence of human papilloma virus infection of the uterine cervix in women with abnormal cervical cytology

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Summary

Purpose of investigation: To determine the prevalence of human papillomavirus (HPV) using polymerase chain reaction (PCR) in women with abnormal cytology results.

Methods: A prospective study of 215 women with abnormal cytology results referred consecutively to the cervical pathology clinic was carried out. A second cervical cytology using the Bethesda System was performed on all the patients to confirm the initial diagnosis, as well as to test for the presence of HPV by PCR and a colposcopy and punch biopsy in cases presenting with an abnormal pattern on colposcopy. The sensivitiy, specificity, and positive and negative predictive value (PPV and NPV) were calculated using 2 x 2 tables.

Results: The women aged 35 years or younger presented a higher percentage of HPV infection (85.6%) than the women over 35 years of age (54%). The highest percentage of women with a positive result for HPV was found in those with a cytological high-grade squamous intraepithelial lesion (HSIL) (85.5%), as compared with 47.4% of the women with a cytological low-grade squamous intraepithelial lesion (LSIL). HPV infection has a high negative predictive value (93.2% of cases) and a high sensitivity (93.5%) for the detection of HSIL by biopsy, although the specificity and positive predictive value were low, 51.5% and 52.1%, respectively.

Conclusion: Patients with cytological HSIL have a high prevalence of HPV infection.

Key words: Prevalence; Human papillomavirus infection; Abnormal cytology.

Introduction

In recent years several studies have appeared in the literature [1-4] relating human papillomavirus infection (HPV) with cancer of the cervix and its precursor lesions. This has generated great interest in the scientific community for two reasons: first, because in spite of advances in treatment and early diagnosis, 437,000 new cases of cervical cancer are detected worldwide every year, this being the malignancy with the second highest incidence and mortality in women after breast cancer [5, 6]. In large-scale studies of cancer of the cervix [1] in which tumor biopsies were analyzed for the presence of HPV using DNA amplification techniques (PCR) and in situ hybridization, HPV was detected in 92.7% of cases, and some authors have questioned whether malignant tumors of the cervix negative for HPV even exist [7]. This is why an attempt has been made to use HPV testing to improve the sensitivity of cervical cytology for the detection of cervical cancer, especially in cases in which the cytology results may be equivocal (ASCUS) or in order to decide how to manage cases of low-grade lesions. Nevertheless, there is still no commonly accepted protocol for the use of HPV testing to facilitate the work of clinicians and improve rates of early detection of high-grade lesions, or to determine which low-grade lesions should be treated in order to prevent them from developing into high-grade lesions.

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Material and Methods

In this prospective study, the study group consisted of 215 women referred consecutively to the cervical pathology clinic for abnormal cytology results. A second cervical cytology was performed on all of them, and a final cytological diagnosis was made using the Bethesda System. All were tested using PCR for the presence of human papillomavirus. Of the 215 women, 198 were examined by colposcopy, and punch biopsy specimens were obtained from patients presenting with abnormal colposcopy.

Colposcopy was performed following application of 2% acetic acid. The classification used was that proposed by the International Federation of Cervical Pathology and Colposcopy (IFCPC) in Rome in 1990 [8].

Biopsy was performed by colposcopic-directed punch biopsies on the 143 women who presented with abnormal colposcopy results. The biopsy specimens were then fixed in formalin, analyzed by the pathologist, and classified as follows: negative, low-grade lesions, high-grade lesions, changes suggestive of HPV infection, and carcinoma.

Detection of human papillomavirus samples was obtained using a swab applied to the surface of the cervix. The contents were then dissolved in 0.5 ml of saline solution (pH 7.2) and the type of HPV was identified by PCR using the "Line Probe assay" (LIPA) method (Innogenetics Laboratories®), which permits identification of up to 25 different HPV genotypes from a single sample.

Loop excision of the transformation zone (LETZ) was performed on women with persistent low-grade lesions or in cases of discordance between the initial cytology and the cytological results obtained in our hospital.

Conization using a more extensive loop excision of the transformation zone in the form of a cone, which also included part of the endocervical canal, was performed on all women with a cytological diagnosis of a high-grade squamous intraepithelial lesion (HSIL) at our hospital, or those whose biopsy results yielded a diagnosis of HSIL independently of the result of cervical cytology.

Statistical method the sensitivity, specificity, positive and negative predictive value (PPV and NPV) of colposcopy and of HPV PCR testing for the detection of HSIL in cervical biopsy specimens were calculated using 2 x 2 tables.

Results

The average age of the women in the study was 37 years. In ten cases, cytological abnormalities were associated with the presence of vulvar condylomata. The characteristics of the study group are summarized in Table 1. The largest percentage (46%) consisted of women with a diagnosis of HSIL (99 cases) (Table 2). Table 2 also shows the prevalence of HPV infection in the different cytological diagnoses. Concordance between the cytology results obtained in our hospital and the diagnosis made in the hospital from which the patient was referred was quite low, coinciding in only 55 women (25.5%). Human papillomavirus was detected in 130 women (61.3%), in 48 of whom (22.6%) more than one HPV genotype was isolated, and genotypes with a high risk of cancer were predominant (85.8%). As Table 3 shows, 22 types of HPV were detected, although the predominant type was HPV 16.

Colposcopy results were abnormal in 143 women, and the three predominant colposcopic images were mosaic (56 cases), followed by leukoplasic (35 cases) and punctation (24 cases).

As shown in Table 4, 143 colposcopically directed cervical biopsies were performed, in 42 of which the results were normal, and the predominant pathological diagnosis was HSIL (44 women). Concordance between the biopsy results and cytology results obtained in our hospital was 42.3% (91 women), and if we take into account the results of both cytologies (the one performed in our hospital and the one for which the patient was referred) the concordance rises to 67.9% (146 women). Concordance between the histological results for the 80 women who underwent both punch biopsy and either conization or LETZ was 50% (40 cases).

A total of 113 women were treated, 66 by conization and 47 with LETZ. The results of the histological study of both procedures are summarized in Table 5.

Table 1. — *Characteristics of the study population.*

Variable	Mean	Range
Age (years)	37.8	19-69
Number of children	1.3	0-8
Number of abortions	0.4	0-9
Age of initiation of sexual relations (years)	18	13-28
Number of partners	3.2	1-15
Follow-up in months	7.8	1-41

Table 2. — *Cytology diagnosis for which the patient was referred and prevalence of HPV infection.*

Cytological diagnosis	Number	Percentage %	HPV infection	Percentage of HPV infection %
High-grade SIL	99	46	82	82.8
Low-grade SIL	59	27.4	31	52.5
HPV infection	24	11.1	8	33.3
ASCUS	20	9.3	10	50
AGUS	8	3.7	1	12.5
Squamous carcinoma	5	2.3	5	100

Table 3. — *Different genotypes of HPV isolated.*

HPV genotype	Number	Percentage
6*	5	2.3
11*	6	2.8
16	51	24
18	8	3.7
31	16	7.5
33	12	5.6
35	5	2.3
39	5	2.3
42*	2	0.9
43*	3	1.4
44*	2	0.9
45	3	1.4
51	20	9.4
52	14	6.6
53	12	5.6
54*	4	1.8
56	7	3.3
58	4	1.8
66	12	5.6
68	5	2.3
70*	4	1.8
74	2	0.9
X	3	1.4

*Low cancerigenic risk; X: unknown HPV genotype.

Table 4. — *Results of colposcopically directed punch biopsy.*

Histologic result of punch biopsy	Number of cases n = 143	Number of HPV-negative cases
High-grade SIL	44	3
Low-grade SIL	36	8
HPV infection	18	4
Carcinoma	3	0
Negative	42	18

Table 5. — *Results of the histological analysis of conization and LETZ.*

Histologic result of conization	Number of cases n = 66	Number of HPV-negative cases
High-grade SIL	46	2
Low-grade SIL	11	3
Negative	4	1
Microinvasive carcinoma	3	0
Invasive carcinoma	2	0
Histologic result of LETZ	**Number of cases n = 47**	**Number of HPV-negative cases**
High-grade SIL	18	2
Low-grade SIL	24	3
HPV infection	2	0
Negative	3	1

Twelve hysterectomies were performed, three for microinvasive cancer, five for positive cone margins (in three of these cases there was a lesion in the uterus), two for relapse of HSIL (in one case there was a lesion in the uterus) and two Wertheim's operations in cases of invasive cancers.

It is important to note that in no case of cervical cancer did the patient test negative for HPV, and only five of 77 women (6.4%) with a positive biopsy result for HSIL tested negative for HPV. Of the 61 women with a low-grade SIL biopsy, 13 (21.3%) tested negative for HPV, and of the 19 women with histologic signs of HPV infection, four (21%) tested negative.

Discussion

The low concordance between the cytology performed in our hospital and the hospital from which the patients were referred is striking, although it is similar to that observed in other large series [9]. The percentage of women testing positive for HPV was higher than that observed in other series: 61.3% as compared with 28.8%-53.6% [9-11], although this may be influenced by a variety of factors such as the technique used to test for HPV and the percentage of women with high-grade lesions (higher in our study). The highest percentage of women testing positive for HPV – 82.8% – was found among women with a cytological diagnosis of HSIL, as compared with 52.5% of women with LSIL (Table 2). In a similar study [12], the percentage of HPV infection was 76.1% for women with a cytological diagnosis of LSIL. Women aged 35 years or younger presented a higher percentage of HPV infection, 85.6% as compared with women over the age of 35 (54%). In the literature, HPV genital infection affects between 3.7% and 47.9% of study populations [13, 14], but it should be kept in mind that our population was one selected for cervical pathology. We observed that the frequency of HPV infection is greater in young women, a finding also reported by other authors [15].

In only nine women with HPV infection was a single low-risk HPV genotype detected, in four women with a cytological diagnosis of HSIL, four with LSIL, and in one case of ASCUS. Colposcopy was performed on 198 women: in 30 women it was not satisfactory, in 25 cases it was normal and in 143 it was abnormal. In those women with unsatisfactory or normal colposcopy results and a cytological diagnosis of HSIL, conization was performed. If the lesion was a low-grade type, the women were followed by colposcopy and cytologies scheduled every three to six months, and only if the lesion persisted for two years or longer or in women who opted for treatment was LETZ performed on an outpatient basis. In the remaining cases, cytological and colposcopic follow-up was scheduled every six months. The sensitivity of colposcopy for the detection of a high-grade lesion or a carcinoma in this study (94%) was similar to that observed by other authors (96%), and its specificity (18.1%) falls within the range of 10% to 48% reported in the literature [8].

The percentage of women with a biopsy result positive for HSIL who presented with high-risk HPV infection was 92.2%, similar to the percentage reported in other large studies (93.3%) [16]. In our study, therefore, HPV infection had a high negative predictive value of 93.2% for the detection of HSIL in a biopsy, and a high sensitivity of 93.5%, although the specificity and positive predictive value were low, 51.5% and 52.1%, respectively.

In only two cases, in one of which the cervical biopsy result showed carcinoma and the other high-grade SIL, the HPV infection was of a low-risk type (types 6 and 11, respectively).

It should be noted that in 18 of the 42 cases (42.8%) with a cytological or punch biopsy diagnosis of LSIL in which LETZ was performed, the final diagnosis based on the loop specimen was HSIL; that is, an occult high-grade lesion was detected. In only two cases out of the 18, the result of HPV testing was negative, and in all but one case HPV genotypes with a high risk of carcinogenic transformation were identified.

We may conclude that the prevalence of HPV infection is higher in patients with cytological HSIL, and that the presence of a HSIL is rare in a cervical biopsy specimen from women who test negative for HPV test.

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