An observational study of women with positive HPV-DNA tests and normal cytology and colposcopy

E. Paraskevaidis[1], E. J. Davidson[3], V. Malamou-Mitsi[2], P. M.-Hirsch[3], L. Pappa[2], G. Koliopoulos[1], E. Lolis[1], K. Zikopoulos[1], M. Paschopoulos[1], V. Doussias[1], N. Agnantis[2]

[1]Departments of Gynecology and [2]Cytopathology, Ioannina Medical School, Ioannina (Greece)
[3]Academic Unit of Obstetrics and [3]Gynaecology, St. Mary's Hospital, Manchester (UK)

Summary

Purpose: High risk human papillomaviruses (HPV) are implicated in the aetiology of malignant cervical disease. The usefulness of HPV DNA tests in identifying women at risk of cervical cancer as an adjunct to cervical cytology is under evaluation.

Patients and Methods: This is a retrospective analysis of 47 women positive for high risk HPV but with negative cytology and negative colposcopy at the start of the study. Women were observed for three years or more (in 96% cases) using six-monthly combined HPV DNA tests, cytological and colposcopic evaluation.

Results: At the end of follow-up, 29/47 (62%) women were still positive for high risk HPV, 45/47 (96%) women had normal cytology and 47/47 (100%) women continued to have normal colposcopy.

Conclusions: Normal colposcopy has an excellent negative predictive value for HPV positive women with normal cytology. These women can be safely screened cytologically on a three-yearly basis.

Key words: HPV DNA test; Cervical cytology; Colposcopy.

Introduction

The detection of high risk human papillomaviruses (HPV) in most cervical tumours has established a role for these viruses in the pathogenesis of malignant cervical disease [1]. Persistent infection with oncogenic HPV types is correlated with progressive cervical disease and has been shown to be predictive for the later development of cervical intraepithelial neoplasia grade 3 (CIN 3) [2, 3]. This evidence has led to the suggestion that the positive predictive power of cervical cytology might be improved by the simultaneous use of HPV detection methods on cervical cytological specimens and a number of clinical trials have been initiated in an attempt to answer this question [4].

It is clear, however, that while genital HPV infections are extremely common, most are transient and disappear without ever causing disease [5]. It is anticipated that the use of an HPV DNA test as a primary screening tool would correctly identify women at risk of premalignant disease of the cervix at the cost of also identifying a significant proportion of women who are not [6, 7]. The appropriate management of women with positive HPV DNA tests and negative cervical cytology is uncertain, as is the issue of whether or not to inform women of their HPV status. It is reasonable to assume that such women will be extremely concerned to discover that they carry a potentially carcinogenic virus in their lower genital tract, particularly as we are currently unable to treat it. The aim of this study was to present a retrospective analysis of a group of women with positive HPV DNA tests and normal cervical cytology following several years of careful surveillance.

Patients and Methods

The study population consisted of women with positive HPV DNA tests in combination with negative cytology and colposcopy. HPV DNA detection was performed on cervical cytological specimens by washing the spatula and cytobrush in normal saline to release residual cervical cells for HPV DNA testing after spreading the smear for cytological analysis. A standard PCR technique was used as described previously [8]. Positive HPV DNA tests were taken to be those where smears were found to be positive for high risk HPV types only [16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58]. Normal cervical cytology was defined as those smears where none of the following abnormal features were detected: any degree of SIL, ASCUS or even HPV-type features (including koilocytosis, dyskeratosis, hyperchromatic nuclei, bi-or multinucleation and cleared cytoplasm) [9, 10]. Normal colposcopy was defined as cases where a) colposcopy was satisfactory (the entire transformation zone visible) and b) there was neither evidence of any degree of SIL nor evidence of HPV-type changes (soft, sharply defined acetowhite epithelium or rings of acetowhite around gland openings).

The study comprised two subgroups of women. Group A consisted of women who had participated in a previous study [11] where the efficacy of extended cytological criteria was compared with conventional cytological analysis together with HPV DNA test results in identifying women with SIL. Those women who were found to have positive HPV DNA tests and normal cytology were included in this analysis. Group B consisted of a subset of women who had been recruited into a long term obser-

Revised manuscript accepted for publication Janaury 14, 2002

vational study of women with cytological and colposcopic features of HPV infection +/- CIN 1 [8]. Those women who regressed during follow-up to normal cytology and colposcopy as described above but who were still positive for HPV DNA by PCR were included in the present analysis.

Women were evaluated every six months by HPV DNA testing, cervical cytology and colposcopy. The treatment policy in our department is to 'see and treat'. Women with evidence of high grade disease at cytology and colposcopy are treated by large loop excision of the transformation zone (LLETZ) immediately, while those with low grade phenotype are offered continued surveillance or treatment if they wish. The same policy was scheduled for the women in this study if they went on to develop progressive cervical disease.

Results

In Group A there were 24 women who were eligible for inclusion in the study. In total, 45 women were found to have a positive HPV DNA test in the presence of negative cytology. According to their study design these women were referred for colposcopic evaluation which classified 13 of them as having features of SIL. Eleven of these women subsequently consented to LLETZ and were found to have HPV-type features only (6/11), CIN 1 (4/11) or CIN 2/3 (1/11) by histology. Another eight women in the original group of 45 had a colposcopic impression of HPV-type features only and therefore did not undergo LLETZ, while the remaining 24 had a completely normal colposcopy. These are the women who constitute Group A in the present study.

In Group B there were 23 women with a positive HPV DNA test, negative cytology and negative colposcopy that were included in the study. These women were part of a long-term observational study of a cohort of 330 women with cytological and colposcopic features of HPV +/- CIN 1. During follow-up, 23 women regressed completely to negative cytology and colposcopy but continued to have evidence of high risk HPV infection by PCR.

For both groups of women, entry into the present study was defined as the moment when both cytology and colposcopy were normal in the presence of a positive HPV DNA test. The mean age of entry into the study was 36.2 years (SD 7.3, range 19-53) for Group A and 32.7 years (SD 9.4, range 21-49) for Group B. The mean duration of follow-up for women in Group A was 3.9 years (SD 1.1, range 2.9-4.8 years) and for women in Group B 6.7 years (SD 3.2, range 2.3-10.2 years). Twenty-one of 23 women (91%) in Group B had at least three years' of follow-up. During this follow-up period, women in Group A had an average of eight combined cytological and colposcopic evaluations (SD 2, range 6-10) while women in Group B had an average of 14 visits (SD 6, range 5-21) for combined assessment by cytology and colposcopy.

The HPV status, cytological and colposcopic phenotype of the 47 women at the end of follow-up is shown in Table 1. Twenty-nine women (61.7%) were still positive for high risk HPV DNA by PCR at the end of the study. Eighteen women were HPV negative at the end of follow-up, but eight of these (8/18, 44%) had presented with intermittently positive and negative HPV tests between visits. Forty-five women (95.7%) continued to have normal cytology and this included five women who had shown some evidence of low grade SIL during follow-up, but in whom these abnormalities had regressed back to a normal cytological phenotype by the end of the study. The other two women had cytological features suggestive of HPV infection at the end of the study. All women (100%) had consistently normal colposcopy throughout the study and at the end of follow-up, including those who presented with low grade cytology during the surveillance period.

Table 1. — *HPV status, cytological and colposcopic phenotype of the 47 women at entrance and exit from the study*

	Entry into the study (n)	Exit from the study (n)
Positive HPV DNA test	47	29[1]
Negative cytology	47	45[2]
Negative colposcopy	47	47[3]

[1]Eight women had fluctuating HPV status during the study (i.e., became intermittently positive and negative between visits) but were HPV negative at the exit.
[2]Two women had cytology suggestive of HPV infection at the end of the study. A further 5 women had features of low grade SIL by cytological analysis during follow-up which subsequently regressed during the study period.
[3]All women in both groups remained colposcopically normal throughout the whole of the follow-up period.

Discussion

This is a relatively small study, but all women were under close, careful surveillance with six-monthly HPV DNA testing, cytology and colposcopy. While the two groups of women originated from separate clinical trials, they all shared the same features at entry into the present study: negative cytology and negative colposcopy by expanded, stringent criteria and a positive high risk HPV DNA test. Although we have shown that a low grade cytological and colposcopic phenotype together with a positive HPV DNA test may mask underlying high grade disease in a small proportion of patients [8], the women in the present study did not have even mild evidence of HPV infection by cytology and/or colposcopy.

It is not possible to speculate about the spontaneous evolution of the subgroup of 29 women who were still positive for high risk HPV DNA at the end of follow-up. They may represent a group of women at a relatively high risk of cervical carcinogenesis [5] compared to the general population and they therefore continue to be observed within the research protocol on a six-monthly basis by cytology and colposcopy.

The women in this study were positive at entry for high risk HPV DNA with normal cytology using expanded cytological criteria and normal colposcopy. The majority were followed-up for at least three years (45/47, 96%)

and during this time none of them developed colposcopic abnormalities. This is even true for those women who presented with low grade cytology during follow-up. On the basis of these results, it appears that women with positive high risk HPV DNA tests but negative cytology and negative colposcopy can be safely screened cytologically on a three-yearly basis like the rest of the normal population. As the natural history of HPV infection of the uterine cervix is still unclear, it is likely that women with positive HPV DNA tests may nonetheless be referred for colposcopic evaluation even when they have normal cytology to exclude underlying SIL. In this way, women will receive colposcopic reassurance of the tiny possibility that they have high grade SIL despite normal cytology defined by expanded criteria. However, the financial impact of excess rates of unnecessary colposcopy as well as the shortage of facilities may render such an approach impractical. In addition, and apart from cost effectiveness, one should bear in mind the relatively low reliability of abnormal colposcopy which will in turn inevitably result in high rates of over-management [12].

In conclusion, normal colposcopy had an excellent negative predictive value in this particular group of women which lasted for at least three years, therefore covering the time interval for repeat cytological tests in countries where cervical screening is performed every three years. In other parts of the world, where cervical screening is more frequent or opportunistic, women with positive HPV DNA tests and initial normal cytology will have the opportunity for a repeat smear earlier and can be reassured without the need for referral to colposcopy. However, if colposcopy is available and advocated, both physicians and women alike should be aware of the poor positive predictive value of an abnormal colposcopic finding and the unnecessary treatment rates that will inevitably follow such an approach.

References

[1] Walboomers J. M., Jacobs M. V., Manos M. M. *et al.*: "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide". *J. Pathol.*, 1999, *189 (1)*, 12.

[2] Remmink A. J., Walboomers J. M. M., Helmerhorst T. M. *et al.*: "The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months". *Int. J. Cancer.*, 1995, *61*, 306.

[3] Ho G. Y., Buck R. D., Klein S. *et al.*: "Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia". *J. Natl. Cancer Inst.*, 1995, *87*, 1365.

[4] Kuhn L., Denny L., Pollack A., Lorincz A., Richart R. M., Wright T. C.: "Human papillomavirus DNA testing for cervical cancer screening in low resource settings". *J. Natl. Cancer Inst.*, 2000, *92*, 818.

[5] Nobbenhuis M. A., Walboomers J. M., Helmerhorst T.J. *et al.*: "Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study". *Lancet.*, 1999, *354*, 20.

[6] Clavel C., Masure M., Levert M. *et al.*: "Human papillomavirus detection by the hybrid capture II assay: a reliable test to select women with normal cervical smears at risk for developing cervical lesions". *Diagn. Mol. Pathol.*, 2000, *9*, 145.

[7]Cuzick J., Sasieni P., Davies P. *et al.*: "A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: summary and conclusions". *Br. J. Cancer*, 2000, *83*, 561.

[8] Paraskevaidis E., Kalantaridou S. N., Georgiou I. *et al.*: "Spontaneous evolution of human papillomavirus infection in the uterine cervix". *Anticancer Res.*, 1999, *19*, 3473.

[9]Schneider A., Meinhardt G., de Villiers E. M., Gissman L.: "Sensitivity of the cytologic diagnosis of cervical condyloma in comparison with HPV-DNA hybridisation studies". *Diagn. Cytopathol.*, 1987, *3*, 250.

[10] Solomon R. J., Kurman D.: "The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses". New York: Springer-Verlag, 1994.

[11] Paraskevaidis E., Malamou-Mitsi V., Koliopoulos G. *et al.*: "Expanded cytological referral criteria for colposcopy in cervical screening: comparison with human papillomavirus testing". *Gynecol. Oncol.*, 2001, *82*, 355.

[12] Paraskevaidis E., Koliopoulos G., Malamou-Mitsi V., Pappa L., Agnantis N. J., Lolis D. E.: "Large loop excision of the transformation zone for treating cervical intraepithelial neoplasia: a 12-year experience". *Anticancer Res.*, 2001, *21 (4B)*, 3097.

Address reprint requests to:
V. D. MALAMOU-MITSI, M.D.
Department of Pathology,
Ioannina Medical School,
45110, Ioannina (Greece)