The triage of squamous cell abnormalities of cervical cytology by human papilloma virus screening

A. A. Ozsaran[1], *Assoc. Prof.*; **Y. Dikmen**[1], *Assoc. Prof.*; **F. Akercan**[1], *Assist. Prof.*; **O. Zekioglu**[2], *Specialist*; **M. C. Terek**[1], *Specialist*; **L. Mgoyi**[1], *Resident*; **I. Altuglu**[3], *Specialist*

[1]*Department of Obstetrics and Gynecology;* [2]*Department of Pathology,*
[3]*Department of Microbiology, Ege University Faculty of Medicine, Izmir (Turkey)*

Summary

Objective: The aim of the study was to determine the presence of human papilloma virus (HPV) infection in cervical swabs by the use of the Digene® Hybrid Capture assay in a cohort of patients with squamous cell abnormalities found in cervical cytologic screening.

Materials and methods: Thirty-four (0.3%) of 1,100 patients who came for their routine cervical cytologic screening and diagnosed as having squamous cell abnormalities were enrolled in the study. Colposcopy-directed biopsy was obtained from all study patients. HPV DNA was sought in cervical swab specimens placed in Digene® transport medium by the use of the Digene® Hybrid Capture assay. The findings of cervical cytology, colposcopy-directed biopsy and HPV screening were compared.

Results: In a total of 34 women who were diagnosed as having squamous cell abnormalities in their routine cervical cytologic screening, 15 women had atypical squamous cell lesions of undetermined significancy (ASCUS), 16 women had low-grade cervical intraepithelial lesions (LGSIL), and three women had high-grade cervical intraepithelial lesions (HGSIL). Five (15%) of these women tested positive for HPV screening in cervical swabs where four women had infection with high-risk and one woman had infection with low-risk subtypes. None of the patients with koilocytotic changes of the squamous cells in the class of LGSIL histopathologically tested positive for HPV screening. In addition, one patient diagnosed as having invasive cervical carcinoma histopathologically tested negative for HPV screening. Atypical vascularization was seen colposcopically in this 37-year-old woman who had ASCUS cytologically.

Conclusion: HPV screening seems to have value in the triage of patients with ASCUS with no clear advantage to colposcopy-directed biopsy. The routine performance of HPV screening for the triage of patients with squamous cell abnormalities has no advantage over colposcopy-directed biopsy.

Key words: HPV; Squamous cell abnormality; Colposcopy; Cervical cytology; ASCUS; LGSIL; HGSIL.

Introduction

Cervical cytologic screening has made it possible to detect precancerous and cancerous lesions before they can progress to invasive cervical cancer. Women at increased risk for cancer include those who have had more than one sexual partner, who were sexually active beginning at a young age, who have a history of genital warts and who smoke. Any woman who has been sexually active is at risk for cervical cancer.

The role of human papilloma virus (HPV) infection in cervical cancer has been constantly established by many studies [1, 2]. The International Biological Study on Cervical Cancer reported detection of HPV in 99.7% of cervical cancers, suggesting that a HPV-negative high-grade cervical intraepithelial lesion (HGSIL) has little malignant potential [3, 4]. HPV infections are among the most frequent of the sexually transmitted diseases [5]. Although HPV infections resolve spontaneously, some of them will persist with a small percentage progressing to high-grade preinvasive lesions [6, 7].

The aim of the study was to determine the presence of HPV infection in cervical swabs by the use of the Digene® Hybrid Capture assay in a cohort of patients with squamous cell abnormalities during cervical cytologic screening.

Materials and Methods

Patients. During the period between January 2001 and December 2002, 34 (0.3%) of 1,100 patients who came for their routine cervical cytologic screening and diagnosed as having squamous cell abnormalities were enrolled in the study. Informed consents of the patients were obtained.

Cervical cytology. The patients were advised not to douche for 48 hours before undergoing cervical cytologic screening, not to use vaginal creams for one week before the procedure and to abstain from coitus for 48 hours in advance. The cervical smear specimens were collected from each woman via administration of a dry speculum. Cervical smears were not performed during menstruation or vaginal bleeding. The samples were taken from the endocervix with a cytobrush and from the exocervix with an Ayre spatula. Cervical smears were fixated and then stained by Harris' hematoxylin and EA 36 polychrome stain. Cytologic evaluation was performed by an experienced pathologist without any knowledge of the patient's clinical condition. Abnormal results were categorised according to the Bethesda classification [8].

HPV DNA detection. HPV DNA detection was performed by a signal amplified solution hybridization antibody capture che-

Revised manuscript accepted for publication March 13, 2003

miluminescent assay (Digene hybrid capture system, Digene, London, UK) for the qualitative detection of HPV types 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52 and 56 analysis of HPV DNA low- and high-risk groups in cervical specimens: HPV types 6/11/42/43/44 and 16/18/31/33/35/45/51/52/56. Cervical specimens were transported by Digene Specimen Transport Medium (Digene, London, UK). The Digene Hybrid Capture System is a signal amplified solution hybridization antibody capture assay that utilizes chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids are captured onto the surface of a tube coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as relative light units on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen.

Colposcopy. In the dorsal lithotomy position, vaginal seretions and excess cellular debris were cleansed by saline-moistened cotton swabs after insertion of a speculum. Cervical topography was examined with the colposcope. Then, 5% acetic acid was applied to the cervix and allowed to remain for at least 30 seconds. Punch biopsy was taken from the abnormal or suspicious areas by a Kevorkian punch biopsy tool. Finally, endocervical curettage was performed.

Results

In a total of 34 women who were diagnosed as having squamous cell abnormalities in their routine cervical cytologic screening, 15 women had atypical squamous cells of undetermined significancy (ASCUS), 16 had a low-grade cervical intraepithelial lesion (LGSIL), and three had HGSIL.

Among 15 women who were diagnosed of having ASCUS in their cervical smear, three were found to have chronic cervicitis, nine were found to have LGSIL, two were found to have HGSIL and one woman was found to have invasive carcinoma of the cervix after histologic examination of colposcopy-directed cervical biopsy specimens. In a total of 16 women who were found to have LGSIL on the cytologic examination of the cervical smears, histopathologic evaluation of colposcopy-directed biopsy specimens revealed the same pathologic findings in 15 women, and chronic cervicitis in one woman. Among three patients diagosed of having HGSIL cytologically, histologic examination revealed LGSIL in two women and HGSIL in one women. The distribution of patients with positive human papilloma virus screening test results compared to pathologic diagnosis is demonstrated in Table 1.

Five (15%) of 34 women with abnormal squamous cell cytology tested positive for HPV DNA hybridization in the cervical specimens where four women had infection with high-risk and one woman had infection with low-risk subtypes. The distribution of patients with a positive HPV DNA hybridization test is summarized in Table 3. Among four women who were diagnosed as having infection with high-risk HPV, two women had ASCUS cytologically, one had LGSIL and one HGSIL histopathologically.

Table 1. — *Distribution of patients with positive human papilloma virus screening test results compared to pathologic diagnosis.*

Cervical smear		Cervical biopsy		No. of patients with positive HPV
Cytopathology	*No. of patients*	*Histopathology*	*No. of patients*	–
ASCUS	15	Chronic cervicitis	3	–
		ASCUS	–	–
		LGSIL	9	2
		HGSIL	2	1
		Carcinoma	1	–
LGSIL	16	Chronic cervicitis	1	–
		ASCUS	–	–
		LGSIL	15	1
		HGSIL	–	–
		Carcinoma	–	–
HGSIL	3	Chronic cervicitis	–	–
		ASCUS	–	–
		LGSIL	2	–
		HGSIL	1	1
		Carcinoma	–	
Total			34	5

ASCUS: Atypical squamous cells of undetermined significancy; **LGSIL:** Low grade cervical intraepithelial lesion; **HGSIL:** High grade cervical intraepithelial lesion; **HPV:** Human papilloma virus.

One women in the high-risk HPV subgroup exhibited mosaic pattern colposcopically, while LGSIL was observed both cytologically and histopathologically. The other woman in the high-risk HPV subgroup had HGSIL both cytologically and histopathologically while white epithelium and punctation were shown on colposcopic evaluation. One woman who was found to be infected with low-risk HPV had ASCUS and LGSIL on her cytologic and histopathologic examinations, respectively. Punctation was observed on colposcopic evaluation of this woman (Table 2).

Discussion

Cervical cancer appears to be etiologically related to infection of the cervix with sexually transmitted oncogenic strains of HPV. HPV subtype 16 is the most common

Table 2. — *Characteristics of patients with positive human papilloma virus screening test.*

No.	Age	Cervical cytology	Colposcopic findings	Histopathology	HPV subtype
1	50	ASCUS	Acetowhite epithelium	LGSIL	High-risk
2	49	ASCUS	Acetowhite epithelium	HGSIL	High-risk
3	53	ASCUS	Punctation	LGSIL	Low-risk
4	31	LGSIL	Mosaic pattern	LGSIL	High-risk
5	42	HGSIL	Acetowhite epithelium and punctation	HGSIL	High-risk

ASCUS: Atypical squamous cells of undetermined significancy; **LGSIL:** Low grade cervical intraepithelial lesion; **HGSIL:** High grade cervical intraepithelial lesion; **HPV:** Human papilloma virus.

HPV type in invasive cancer and in cervical intraepithelial neoplasia-2 (CIN-2) and –3 (CIN-3) and is found in 47% of women in both categories [9]. HPV subtype 16 is also the most common HPV subtype in women with normal cytology and it has been found in 16% of women with low-grade cervical lesions and up to 14% of women with normal cytology. HPV subtype 18 has been found in 23% of women with invasive cervical cancers [10].

A proportion of patients with mildly abnormal smears will present with high-grade disease [11]. Identifying this subgroup to target appropriate management is an important clinical issue. Rebello *et al.* [12] tested 333 patients with persistent borderline or mildly dyskaryotic cervical smears with Digene Hybrid Capture assay to test the presence of high-risk types of HPV by using cervical brush specimens who were treated by large loop excision of the transformation zone. Subjects aged under 30 years (n=166) were more likely than older subjects (n = 167) to test positive for HPV (79% versus 45%, $p = 0.001$) and to have CIN-2 or CIN-3 (high-grade cervical intraepithelial lesions) (43% versus 27%, $p < 0.01$). The clearest role for HPV testing at the moment is said to be in the management of women with borderline or mildly dyskaryotic smears. In particular, those aged above 30 years who test positive for high-risk types could be referred immediately for colposcopy, while those younger than 30 years who test negative could receive less intensive surveillance [13].

Woodman *et al.* [14] studied the natural history of the incidence of cervical HPV infection and its relation to the development of cervical intraepithelial neoplasia. In 1,075 women who were cytologically normal and HPV negative at recruitment, the cumulative risk at three years of any HPV infection was 44%; HPV type 16 was the most common type. In the same study the risk of HGSIL was greatest in women who tested positive for HPV type 16 and this risk was a maximum of 6-12 months after the first detection of HPV type 16. Five women who progressed to high-grade CIN consistently tested negative for HPV.

In the present study, none of the patients with only koilocytotic changes of squamous cells in the classification of LGSIL histologically, tested positive for HPV screening. Eight of 26 women who had LGSIL had koilocytotic changes. Furthermore, one patient diagnosed with invasive cervical carcinoma histologically tested negative for HPV screening. Atypical vascularization was seen colposcopically in this women who was enrolled in the study with cytologic results of ASCUS.

Crabtree *et al.* [15] by use of the HPV Hybrid Capture II reported that significant lesions may be discovered in patients in the "high-risk HPV-positive ASCUS" category who had no previous abnormal cervical cytology history. They suggested a more aggressive clinical approach in the management of new onset ASCUS positive cases for high-risk HPV subtypes. Of the 50 patients presented in their study, histologic follow-up demonstrated evidence of squamous carcinoma in one patient (2%), HGSIL in eight patients (16%), and LGSIL in 19 patients (38%). The other 22 (44%) showed chronic cervicitis, reactive changes, or no pathologic changes.

Altuglu *et al.* [16] studied the prevalence of HPV infection in a group of patients with mucopurulent endocervicitis. HPV DNA was sought in cervical swab specimens placed in Digene transport medium by use of the Digene Hybrid Capture assay. HPV infection was detected in 5.4% (8/148) of the patients with mucopurulent endocervicitis. HPV DNA was detected in eight patients: five had infections with low-risk subtypes, one with high/intermediate risk subtypes and one with the combination of high- and low-risk subtypes.

Kulasingam *et al.* [17] stated that HPV DNA testing of women having cervical cytology showing atypical squamous cells of undetermined significance has clinical usefulness. They found that testing for HPV had higher sensitivity but lower specificity than thin-layer cervical cytology screening. They concluded that, in some settings, particularly where screening intervals are long or haphazard, screening for HPV DNA may be a reasonable alternative to cytology-based screening of reproductive-age women. However, the usefulness of HPV DNA testing alone in primary screening remains to be determined.

In conclusion, HPV screening seems to have value in the triage of patients with ASCUS with no clear advantage to colposcopy-directed biopsy. The routine performance of HPV screening for the triage of patients with squamous cell abnormalities has no advantage over colposcopy-directed biopsy.

References

[1] Anon. Human papillomaviruses, vol 64. Lyon. International Agency for Research on Cancer, 1995.

[2] Ozsaran A.A., Atest T., Dikmen Y., Zeytinoğlu A., Terek C., Erhan Y. *et al.*: "Evaluation of the risk of cervical intraepithelial neoplasia and human papilloma virus infection in renal transplant patients receiving immunosuppressive therapy". *Eur. J. Gynaecol. Oncol.,* 1999, *20*, 127.

[3] Walboomers J.M., Jacobs M.V., Manos M.M.: "Human papillomavirus is a necessary cause of invasive cancer worldwide". *J. Pathol.*, 1999, *189,* 12.

[4] Herrington C.S.: "Do HPV-negative carcinomas exist?". *J. Pathol.*, 1999, *189,* 1.

[5] Schiffman M.H., Brinton L.: "The epideniology of cervical carcinogenesis". *Cancer,* 1995, *76,* 1888.

[6] Tate J.E., Resnick M., Sheets E.E., Crum C.P.: "Absence of papillomavirus DNA in normal tissue adjacent to most cervical intraepithelial neoplasms". *Obstet. Gynecol.,* 1996, *88*, 257.

[7] Remmink A.J., Walboomers J.M., Helmerhorst T.J.M., Voorhost F.J., Rozendaal L., Risse E.K. *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.

[8] National Cancer Institute Workshop.: "The 1988 Bethesda system for reporting cervical/vaginal cytological diagnoses". *JAMA,* 1989, *262,* 931.

[9] Bauer H.M., Ting Y., Greer C.E., Chambers J.C., Tashiro C.J., Chimera J., Reingold A., Manos M.M.: "Genital human papilloma virus infection in female university students as determined by a PCR-based method". *JAMA,* 1991, *265*, 472.

[10] Lorincz A.T., Reid R., Jenson A.B., Greenberg M.D., Lancester W., Kurman R.J.: "Human papilloma virus infection of the cervix. Relative risk associations of 15 common anogenital types". *Obstet. Gynecol.,* 1992, *79*, 328.

[11] Kinney W.K., Manos M.M., Hurley L.B., Ransley J.E.: "Where's the high-grade cervical neoplasia? The importance of the minimally abnormal Papanicolaou diagnoses". *Obstet. Gynecol.,* 1998, *91*, 973.

[12] Rebello G., Hallam N., Smart G., Farquharson D., McCafferty J.: "Human papillomavirus testing and the management of women with mildly abnormal cervical smears: an observational study". *Br. Med. J.,* 2001, *322,* 893.
[13] Cuzick J., Sasieni P., Davies P., Adams J., Normand C., Frater A.: "A systematic review of the role of human papillomavirus testing within a cervical screening programme". *Health Technol. Assess.*, 1999, *3*, 14.
[14] Woodman C.B.J., Collins S., Winter H., Bailey A., Ellis J., Prior P.: "Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study". *Lancet,* 2001, *357,* 1831.
[15] Crabtree D., Unkraut A., Cozens D., Smith T., Lucas C., Pennington D. *et al.*: "Role for HPV testing in ASCUS: A cytologic-histologic correlation". *Diagn. Cytopathol.,* 2002, *27*, 382.
[16] Altuglu I., Terek M.C., Ozacar T., Ozsaran A.A., Bilgiç A.: "The prevalence of human papilloma virus DNA in women with mucopurulent endocervicitis". *Eur. J. Gynaecol. Oncol.,* 2002, *23,* 166.
[17] Kulasingam S.L., Hughes J.P., Kiviat N.B., Mao C., Weiss N.S., Kuypers J.M., Koutsky L.A.: "Evaluation of human papiloma virus testing in primary screning for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral". *JAMA,* 2002, *288,* 1749.

Address reprint requests to:
F. AKERCAN, M.D.
Department of Obstetrics and Gynecology
Ege University Faculty of Medicine
Bornova, Izmir 35100 (Turkey)