The prevalence of human papilloma virus DNA in women with mucopurulent endocervicitis

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Summary

Objective: The aim of the study was to determine the prevalence of human papillomavirus (HPV) infection in a group of patients with mucopurulent endocervicitis.

Materials and methods: One hundred and forty-eight patients who came for their routine medical screening and were diagnosed with mucopurulent endocervicitis were enrolled in the study. HPV DNA was sought in cervical swab specimens placed in digene transport medium by use of the Digene Hybrid Capture assay.

Results: HPV infection was detected in 5.4% (8/148) of the patients with mucopurulent endocervicitis. The mean age of the patients was 36.4 ± 8.2 (18-54) years. Approximately 40% (59/148) of the patients used intrauterine devices currently or in the past, while 16.2% (24/148) used combined oral contraceptives as the contraceptive method. 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. The mean age of the HPV infected patients was significantly lower than the HPV negative patients (28.2 ± 6.3 versus 36.9 ± 8.1 years, p = 0.003). Risk factors for HPV infection did not differ between the infected and uninfected groups.

Conclusion: HPV infection should be sought in patients with clinical evidence of mucopurulent endocervicitis even without risk factors for cervical neoplasia.

Key words: HPV; Mucopurulent endocervicitis.

Introduction

Laboratory-based research has firmly established a role for human papillomavirus (HPV) infection in cervical cancer. The epidemiological evidence that supports this finding is largely based on case-control studies, which have consistently revealed a strong association between cervical neoplasia and the detection of HPV

DNA in samples of exfoliated cervical cells were taken at, or subsequent to, diagnosis of disease [1, 2]. The International Biological Study on Cervical Cancer reported detection of HPV in 99.7% of cervical cancers, suggesting that the HPV-negative high-grade cervical intraepithelial lesion has little malignant potential [3, 4]. HPV infections are among the most frequent of the sexually transmitted diseases [5]. The presence of HPV is best identified by DNA detection even in the subset of women who manifest cytological abnormalities detected by a Pap smear. Most HPV infections resolve, though some will persist with a small percentage progressing to high-grade pre-invasive lesions [6, 7].

The mucosal HPV types are commonly grouped in 'high-risk' and 'low-risk' categories on the basis of known epidemiologic associations. High-risk types are those similar to the types frequently found in anogenital malignancies; low-risk types are those similar to the types found in condylomata [8]. HPV 16 is the virus most predominantly associated with cervical cancers in worldwide studies and accounts for about 50% of the cancers. HPV 18 is associated with 10 to 20% of the cervical cancers and is not distributed uniformly in different geographic areas [9].

The strong association between HPV infection and cervical cancer brought the need for accurate diagnostic methods for HPV. In the diagnosis of HPV, clinical manifestations and cytologic screening with the Pap test continue to play a major role. Other conventional methods for the diagnosis of HPV infection are as an adjunct to direct visualization colposcopy and serologic assays that detect seroreactivity to the E6 and E7 proteins of oncogenic HPV types. HPVs can be specifically identified by nucleic acid-based assays. Tests in common use are polymerase chain reaction based assays, hybridization of unamplified tissue DNA with viral probes by hybrid capture, Southern blot or dot blot hybridization and in situ hybridization of tissue sections to localize the viral genome to specific cells [9, 10].

The aim of the study was to determine the prevalance of HPV infection in cervical swab specimens by use of the Digene Hybrid Capture assay in a cohort of patients with mucopurulent endocervicitis. Discrimination between high risk and low risk types was also determined.

Materials and Methods

Patients: During the period between January 2001 and June 2001, 148 patients who came for their routine medical screening and were diagnosed with mucopurulent endocervicitis were enrolled in the study. Mucopurulent endocervicitis was diagnosed clinically as described previously [11]. Patients were

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evaluated regarding the age, number of sexual partners, cigarette smoking, presence and duration of intrauterine device and the use of combined hormonal oral contraceptives.

HPV DNA Detection: Cervical samples were collected with a commercial swab specimen collection kit (Digene Swab specimen collection kit, Beltsville, MD, USA). Samples were stored at -20°C until tested. The commercially available Hybrid capture assay (Digene Hybrid Capture System, Beltsville, MD, USA) was used for the detection of HPV DNA. The same assay was used to test for high and low risk HPV types in all specimens by using probe A (low-risk HPV types 6, 11, 42, 43 and 44) and probe B (high/intermediate HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56). 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 with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatese 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.

Statistical analysis: The groups were compared with the Mann Whitney U nonparametric test. The ordinal values were compared with the chi-square test; p values lower than 0.05 were accepted as significant.

Results

HPV infection was detected in 5.4% (8/148) of the patients with mucopurulent endocervicitis. The mean age of the patients was 36.4 ± 8.2 (18-54) years. Demographic variables are summarized in Table 1. Approximately 40% (59/148) of the patients used intrauterine devices currently or in the past, while 16.2% (24/148) used combined oral contraceptives as the contraceptive method. 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.

The comparison of demographic variables is shown in Table 2. The mean age of the HPV infected patients was significantly lower than the HPV negative patients (28.2 ± 6.3 versus 36.9 ± 8.1 years, p = 0.003). Risk factors for HPV infection did not differ between the infected and uninfected groups (Table 3).

Table 1. — *Demographic variables of the patients with mucopuruelent cervicitis*

	Minimum	Maximum	Mean	Standard deviation
Age	18	54	36.4	8.2
Gravida	0	5	1.01	1.2
Parity	0	7	1.9	1.1
IUD use (months)	2	240	51.4	41.9
OCP use (years)	1	16	5.1	4.8

IUD: Intrauterine device, OCP: Oral contraceptive pill

Table 2. — *Comparison of variables between human papilloma virus infected and uninfected groups*

	HPV uninfected group (n=140)	HPV infected group (n=8)	P value
Age	36.9±8.1	28.2±6.3	0.003
Gravida	1.01±1.2	0.8±0.8	0.95
Parity	2.01±1.1	1.2±0.7	0.03
IUD use (months)	53.7±41.8	9.0±7.9	0.01
OCP use (years)	5.3±4.8	2±0	0.55

IUD: Intrauterine device, OCP: Oral contraceptive pill,
HPV: Human papilloma virus

Table 3. — *Comparison of risk factors for human papillomava virus infection*

	HPV positive (n)	HPV negative (n)	P value
Presence of IUD (current or past)	3	56	0.8
Multiple sexual partners	0	8	0.4
Low socioeconomical status	1	30	0.5
Cigarette smoking	4	45	0.2
OCP use (current or past)	1	23	0.7
Multiparity	4	97	0.2

HPV: Human papilloma virus, IUD: Intrauterine device,
OCP: Oral contraceptive pill

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 HPV type in invasive cancer and in cervical intraepithelial neoplasia II/III and is found in 47% of women in both categories [12]. HPV subtype 16 is also the most common HPV subtype in women with normal cytology and it could be found in 16% of women with low-grade cervical lesions and up to 14% of women with normal cytology. HPV subtype 18 is found in 23% of women with invasive cancer [13].

Woodman *et al.* [14] studied the natural history of incident 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 cummulative 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 high-grade cervical intraepithelial neoplasia was greatest in women who tested positive for HPV type 16 and this risk was maximum 6-12 months after first detection of HPV type 16. Five women who progressed to high-grade cervical intraepithelial neoplasia consistently tested negative for HPV.

Although clinical examination and cytologic screening remain the mainstays of HPV diagnosis, DNA testing has expanded the options available for the detection and study of HPV disease. Because HPV DNA asays can reveal the type of HPV involved, these assays may be valuable in providing prognostic information for patients with cervical intraepithelial neoplasia (CIN). HPV DNA

tests may also be useful in confirming HPV infection in patients with equivocal Pap smears and following viral response to treatment. Because of these reasons in recent years tests that detect the presence of HPV DNA have provided key epidemiologic and pathogenic information concerning HPV infection [15].

In a study by Erensoy *et al.* [16] 59 paraffin-embedded biopsy specimens with different diagnoses were tested for the presence of HPV DNA with an in situ hybridization assay. No HPV was detected in patients diagnosed with squamous hyperplasia of the vulva and koilocytosis. In patients with condyloma acuminatum/koilocytotic atypia, 63.6% were positive for HPV DNA. In the last group, diagnosed as CIN or epidermoid cancer, 21.9% were found to be positive [16]. In the present study, HPV infection was detected in 5.4% (8/148) of the patients with mucopurulent endocervicitis.

Co-factors such as cigarette smoking, use of oral contraceptives and serologic evidence of current or past infection with Chlamydia trachomatis have been inconsistently linked with development of CIN or invasive cervical cancer [17]. The association of the presence of HPV DNA with behavioral and reproductive factors has been examined and the prevalance of HPV infection has been found to decline with increasing age. Minimal associations were found between cervical infections with HPV and certain behavioral determinants for cervical neoplasia, such as a high number of sexual partners, younger age at first intercourse and cigarette smoking. Other correlates of cervical cancer, such as infection with herpes simplex virus type 2 and duration of oral contraceptive use were not associated with HPV infection [10]. In the present study, the mean age of the HPV infected patients was significantly lower than the HPV negative patients. There was no association between HPV infection and presence of intrauterine device, oral contraceptive pill use, low socio-economic status, cigarette smoking and multiparity. It is hard to make a comment on the association between HPV infection and having multiple sexual partners because of the limited number of patients in this group.

It has been demonstrated over the past 20 years that cervical cancer is related strongly to an infectious carcinogen, HPV. The presence of HPV is best identified by DNA detection. Hybrid capture DNA assay is a nonradioactive, relatively rapid, liquid hybridization assay and is designed to detect 14 HPV types divided into high-risk and low-risk groups. In conclusion, HPV infection should be sought in patients with clinical evidence of mucopurulent endocervicitis even without risk factors for cervical neoplasia.

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