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Cervical Intraepithelial Neoplasia 3, Coinfected with HPV-16 and -18

— Case Report —

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Recently, detection of human papillomavirus(HPV)mRNA expression was made possible by in situ hybridization. We described a patient with cervical intraepithelial neoplasia (CIN) 3, showing a distinctive and rare form of co-infection with HPV type 16 and 18. HPV-16 was detected in high grade squamous intraepithelial neoplastic lesion (CIN 3) and HPV-18 was in low grade lesion just adjacent to the HPV-16 infected area.

This case suggests that HPV infection may be one of the most responsible causative agents producing malignant transformation and two distinctive HPV types can also simultaneously infect the squamous epithelium of the uterine cervix.

Key Words: *Human papillomavirus (HPV), in situ hybridization, cervical intraepithelial neoplasia (CIN)*

INTRODUCTION

Invasive cervical cancer is preceded by a progressive spectrum of abnormalities of the cervical epithelium which are considered precancerous lesions, such as CIN 1, 2, and 3 (Richardt, 1973; Hertig, 1979). The evidence linking HPVs with CIN has been derived from clinicopathological investigations and from molecular studies examining the presence and expression of HPV genes in preinvasive cervical tissues (Gissmann et al., 1986; Gupta et al., 1989; Park et al., 1991b). HPV-16 and 18 are usually associated with high grade (2 and 3) CIN lesions (up to 80%) and invasive cancer (up to 90%). HPV-16 is the predominant virus in cervical neoplasia (Lorincz et al., 1987; Fuchs et al., 1988), and HPV-18 is regarded as a more rapidly progressive or aggressive form of cervical cancer than HPV-16 (Barnes et al., 1988; Kurman et al., 1988).

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A case of CIN 3 showing double infection with different HPV types in the serial histologic sections of the same patient is presented.

CASE REPORT

A 34 year old, para 2, gravida 4, woman was admitted to the Dysplasia Clinic of the Department of Obstetrics and Gynecology, Catholic University Medical College Hospital, Seoul, Korea, when an abnormal cytopathologic result was reported by an outpatient clinic. Until her admission, she has been relatively healthy, except for profuse leukorrhea. There was no history of intermittent vaginal bleeding or contact spotting. On speculum examination a red erosive lesion was seen in the portio of the cervix. Cytologic smear was suggestive of severe dysplasia showing atypical koilocytotic cells and nuclear atypical cells with numerous inflammatory cells. After acetic acid was applied, a colposcopic complex appeared, formed by white epithelium (W-II), punctation (P-II) and several glandular orifices in atypical transformation zone. Therefore, cervical conization was performed and histologic section revealed that dysplastic immature parabasal cells were fully replaced in the whole epithelial layer at the squamo-columnar junction (CIN 3), (Fig. 1 & 2). She was well with no evidence of recurrence after 24 months by cytologic and colposcopic follow-up.

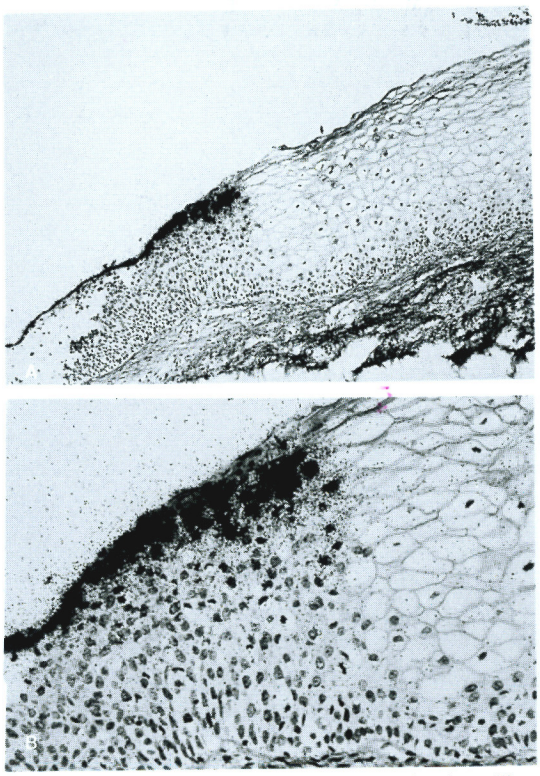

Fig. 1. Section of a cervical cone biopsy showing different grades of cervical intraepithelial neoplasia (CIN) hybridized with a ^{35}S-labelled HPV-16 RNA probe. (Hematoxylin-eosin counterstain)
A) A low-power view (× 100) showing the superficial location of the positive cells.
B) A high-power view (× 250) demonstrates HPV-16 positive cells only in the high grade cervical intraepithelial lesion (CIN 3).

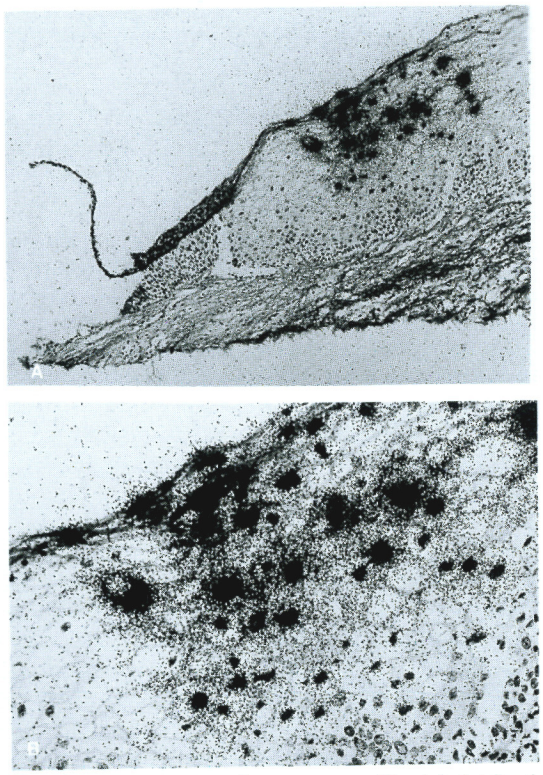

Fig. 2. A serial section of same area as Fig. 1. hybridized with a HPV-18 probe. (Hematoxylin-eosin counterstain)
A) Low magnification showing heavily positive cells in an area of CIN 1 with viral induced changes (× 100).
B) High magnification demonstrates focal high-intensity signals on the koilocytotic cells (× 250).

IN SITU HYBRIDIZATION FINDINGS

To detect the presence of HPV in cervical intraepithelial neoplastic tissue, we employed in situ hybridization with ^{35}S-labeled, single-stranded antisense RNA probes against HPV-6/11, -16 and -18 mRNAs in specific cells in tissue sections. Details of tissue processing and in situ hybridization procedure had been described previously elsewhere (Park et al., 1991a). Viral transcripts of HPV-6 and -11 were not identified in that specimen. The HPV-16 transcripts were definitely identified in high grade dysplastic lesion (CIN 3) just adjacent to low grade CIN lesion (Fig. 1A & B). The HPV-16 signal was not seen in the morphologically normal epithelium or in the stroma. Incontrast, the HPV-18 transcripts were detected only in mild dysplastic lesion (CIN 1) just next to the HPV-16 infected CIN 3 lesion (Fig. 2A & B). The hybridization was strongest toward the surface and became progressively weaker toward the basal layer. The HPV-positive areas displayed focal pattern of hybridization. The hybridization signal was especially intense in well-differentiated, koilocytotic cells of the cervical epithelium (Fig. 2B).

DISCUSSION

The sensitivity of the morphological features of HPV infection of the cervix (koilocytosis and dyskaryosis), as identified by hybridization, has been examined in several recent studies (Sato et al., 1987; Schneider et al., 1987; Park et al., 1989). Histopathologic features of HPV infection were found in 15-68% of HPV-positive lesions and 3% of HPV-negative lesions. These results suggest that the pathognomonic morphological fea-

tures of HPV infection may be detectable in cervical tissues and cells, but the proportion may be lower than we expected.

Among the oncogenic HPV types, HPV-16 is the most common and HPV-16 containing dysplastic lesion is more frequently associated with marked nuclear atypia and an aneuploid karyotype (Crum et al., 1984). In one recent study (Lorincz et al., 1987) comparing the distribution of HPV in CIN versus invasive carcinoma, it was found that HPV-16 accounted for 41% and HPV-18 for 22% of all invasive carcinoma. In contrast, HPV-16 was found in 37% of all grades of CIN whereas HPV-18 was found in only 3%. The detection of high risk HPV infection from the cervical lavages of Korean patients of cervical cancer was identified by Southern blot hybridization and the infection rate of HPV-16 or -18 were 51% (25/49) in the extracted DNAs of cervical neoplasia (Ryu and Song, 1990). In one microinvasive cancer, HPV was identified that hybridized to both HPV-16 and -18. The result of Reid et al. (1987) showed that only six of 416 specimens contained multiple HPV types within the same sample. But the published reports have not described the simultaneous infection of oncogenic HPVs in the same tissue section of CIN with the histologic feature of *in situ* hybridization. In our sample HPV-16 was identified in high grade cervical intraepithelial lesion with HPV-18 mixed infection in just adjacent low grade lesion. It reveals a remarkable specificity of HPV-16 infection in transformed cells. This case also showed the presence of HPV-18 in non-tumorous epithelium. In general, there was no significant difference in the distribution of HPV-16 in CIN as compared with invasive carcinoma. But, of particular interest was the striking deficit of type 18 in intraepithelial neoplasia as compared with invasive carcinoma (Kurman et al., 1988). The deficit in type 18-related CIN compared to invasive carcinoma was thought to be possibly due to the rapid transit time of type 18 associated lesions through the CIN stage. The possibility is considered that is our finding represents the latent status or the helper activity of HPV-18 for the oncogenic ability of HPV-16. HPV genome has also been detected in cervices that are colposcopically, cytologically, and/or histologically normal (Wickenden et al., 1985). The potential biological behavior of HPV infection that occurs in the absence of morphologic lesions is unknown and may not be analogous to the latent state characteristic of the herpes viruses. Although the oncogenic HPV types are capable of immortalizing human keratinocytes in experimental systems (Durst et al., 1987b), the presence of the virus is not a sufficient condition per se to induce invasive neoplastic lesions. Recent studies of invasive cervical cancers have shown that these viral types may be integrated with host DNA (Choo et al., 1987; Cullen et al., 1991) or interact with cellular proto-oncogenes (*myc, ras, raf, erb-A,...*) (Durst et al., 1987a; Riou et al., 1987) and tumor suppressor genes specifically Rb (Dyson et al., 1989) and p53 (Werness et al., 1990). These different lines of evidence have demonstrated that carcinogenesis is a complex, multistep process with several options at different stages of development.

In situ hybridization the detection of DNA or RNA sequences in fixed cells adhered to microscopic slides, permits for histologic assessment of viral nucleic acid localization within tissue. Furthermore, *in situ* hybridization with single-stranded, antisense RNA probes allows detection of viral m RNA expression, i.e., of active viral genome rather than of latent infection (Stoler et al., 1986; Barnes et al., 1988). The distribution of HPV transcripts in our CIN tissue resembles that observed in benign condylomata showing strong hybridization signals specifically in well-differentiated superficial cells of the dysplastic layer. This observation suggests that expression of HPV transcripts is dependent on the stage of maturation of the cell rather than on the stage of the disease.

With the strong relationship that has been established between HPV infection and cervical neoplasia, routine testing for viral typing from every high-risk cervical intraepithelial patient may be advisable. When nonradiolabeled and more sensitive probes become advailable, it should be possible to perform this test in any diagnostic pathology laboratory. The finding of HPV coinfection in apparantly low grade intraepithelial lesion with CIN 3 has implications for the pathogenesis, treatment, and follow-up of CIN. Further, molecular analysis of HPV-associated pathologic tissues may be expected to contribute significantly to the understanding of the natural history of cervical neoplasia.

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