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CORRELATION OF HUMAN PAPILLOMAVIRUS DNA DETECTION IN BIOPSIES OF CERVICAL LESIONS AND THE CORRESPONDING CERVICAL SWABS WITH THE SAME METHOD OF *IN SITU* HYBRIDIZATION

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Summary. – Human papillomavirus types 16 and 18 (HPV 16/18) are considered to play an important role in the pathogenesis of cervical intraepithelial neoplasia (CIN) and invasive cancer of the uterine cervix. The *in situ* hybridization (ISH) is the only method demonstrating a correlation between histopathological findings and the presence of specific HPV DNA, but its sensitivity is limited. To determine whether or not the mode of sample taking contributes to the reported HPV prevalence with this method two observers examined the cervical swabs and tissue sections of 41 CIN lesions independently with the same commercial ISH kits (PathoGene ™/ Enzo Diagnostics Inc., New York). HPV prevalences were almost identical in both sample groups (21/41 HPV positive tissue sections and 23/41 HPV positive swabs). 30/41 samples (73 %) showed identical HPV-ISH results in the biopsy and the corresponding swab. There was only one CIN 1 lesion with different associated HPV types in the corresponding tissue section and cervical swab. By accumulating the results of both sample groups 27 out of 41 CIN lesions had either an HPV positive swab or biopsy or both. The percentage of HPV 16/18 positive samples increased with the severity of the associated lesion.

Key words: human papillomavirus; DNA; cervical lesions; cervicals-wabs; in situ hybridization

Introduction

Strong evidence associates specific HPV with human anogenital cancers, most notably cervical cancer. The approximately 20 HPV types which are found in anogenital lesions can be classified as „low risk", „intermediate risk" and „high risk" according to the risk of progression to malignancy of genital tract lesions which are associated with these viruses. "Low risk" viruses such as HPV 6/11 are usually associated with condylomata accuminata and progress to malignancy

only very rarely, whereas lesions which are associated with „high risk" types such as HPV 16/18 have an increased risk of progression to malignancy (Kataja *et al.*, 1990). HPV DNA 16/18 is hardly ever observed in normal squamous epithelium by means of ISH, but it is detected in 50 to 60 % of CIN 3 lesions and approximately 80 % of invasive squamous cell carcinomas of the uterine cervix (Riou *et al.*, 1990; Schneider *et al.*, 1991).

Numerous experimental data as well as prospective clinical and epidemiological studies based on sensitive methods of HPV-detection such as the polymerase-chain-reaction (PCR) support the concept of an etiological role for „high risk" HPV types in the pathogenesis of cervical cancer (Boshart *et al.*, 1984; Dürst *et al.*, 1983; Howley, 1991; Hurlin *et al.*, 1991; Zur Hausen, 1991). But many observers failed to find an increased risk of anogenital lesions associated with „high risk" HPV types when the ISH or filter ISH (FISH) were used as the method of HPV DNA detection (Franco, 1991). As the ISH of HPV DNA is the only method which can show a morphologic correlation betwen histopathological findings and the presence of HPV DNA, these controversial results raised questions about the role of latent HPV infections in the pathogenesis of cervical cancer, as these infections might escape detection by ISH because of low genome copy numbers (Nuovo *et al.*, 1990; Van den Brule *et al.*, 1990).

Since in some cases of clinical HPV infection the number of infected cells is low, the HPV DNA might be missed in some sections of the lesion and swabs may fail to collect a sufficient number of infected cells from the surface of CIN lesions. In order to determine the importance of the mode of sample taking as regards the detection rate of HPV DNA in CIN lesions, two observers investigated indepently the prevalence of HPV 6/11, 16/18, 31/35/51 in tissue sections and the corresponding cervical swabs by using the same commercial ISH method with biotinylated DNA probes.

Materials and Methods

Patients. Cervical swabs from 41 patients with CIN 1-3 lesions were collected for routine cytological examination and for HPV-ISH the day before conebiopsy was performed. Representative tissue sections of the paraffin-embedded biopsies were used for the control ISH. Age of patients ranged from 23 to 57 years; 8 patients suffered from CIN 1, 14 from CIN 2 and 19 from CIN 3.

Treatment of swabs. For HPV detection swabs were taken with a cytobrush and put into 5 ml PBS. The brush was vigorously vortexed and the suspension was centrifuged for 10 min at 3000 xg. Cell pellets were either processed directly or stored at -22 °C. Similar quantities of each sample were applied to three adhesive pretreated slides and dried at 60 °C for 30 min. Then the slides were incubated with 0.5 ml H_2O_2/NaCl/EDTA solution for 20 min at room temperature and dehydrated in ethanol.

Treatment of biopsies. Three paraffin-embedded sections of each cone biopsy were mounted on adhesive slides, dewaxed in xylol and rehydrated in ethanol. After incubation with 0.1 ml proteinase K (125 µg/ml) for 10 min at 37 °C and subsequent rinsing with PBS, 0.5 ml H_2O_2/NaCl/EDTA was applied to each slide for 10 min at 37 °C. After dehydration in ethanol the sections were ready for hybridization.

ISH with biotin-labelled HPV DNA. One drop of denaturated biotinylated HPV DNA in buffered NaCl/EDTA containing 40 % formamide, 8 % hybridization enhancer and a coverslip were placed on

each sample. One section and one swab sample of each patient were incubated for 10 min at 92 °C and for 30 min at room temperature with „PathoGene" DNA probe assay HPV types 6/11, a second section and swab with HPV types 16/18 and a third section and swab with HPV types 31/35/51 (Enzo Diagnostics Inc., New York).

After discarding the coverslips 0.5 ml of a post-hybridization solution which contains 20 mmol/l phosphate buffer, 260 mmol/l NaCl and 40 % formamide were given to each specimen and incubated for 10 min at room temperature. Subsequently, slides were left in PBS for 15 min at room temperature and incubated with 0.5 ml streptavidin-biotinylated horseradish peroxidase complex for 15 min at 37 °C. Finally, slides were incubated with 2 % 3-amino-9-athylcarbazole and 3 % H_2O_2 in 0.05 mol/l acetate buffer for 10 min at room temperature. Red precipitations within the nuclei of epithelial cells indicated the presence of the specific HPV DNA. The ISH and classification of swab specimens was carried out by one investigator (K. U. P.) whereas the tissue sections were examined by another investigator (E. K.), independently.

Results

The results of ISH for HPV DNA are summarized in Table 1. In 14 cases HPV could not be detected in the tissue sections nor in the corresponding swabs. 10 swabs and the corresponding sections were positive for HPV 16/18 (Fig. 1, 2). Swabs and sections were positive for HPV 31/35/51 in two cases and the corresponding specimens showed positive signs of both HPV 16/18 and HPV 31/35/51 in four cases. Thus in a total of 30 cases (73.17 %) results were identical when ISH for HPV DNA of tissue sections and swabs were compared.

The swabs of 5 patients whose tissue sections were classified as „HPV-negative" were HPV 16/18 positive, whereas vice versa 2 tissue sections of patients with „HPV-negative" swabs stained positive for HPV 16/18. The tissue sections of another patient showed positive signs of HPV 16/18 and HPV

Table 1. Results of ISH for HPV DNA (41 CIN lesions)

		Cases
Biopsy + swab identical	HPV negative	14
	HPV 16/18 positive	10
	HPV 31/35/51 positive	2
	Double infection	4
Swab positive/Biopsy negative	HPV 16/18	5
	HPV 31/35/51	2
Biopsy positive/Swab negative	HPV 16/18	2
	HPV 31/35/51	1
	Double infection	1
Different HPV types		1

Fig. 1

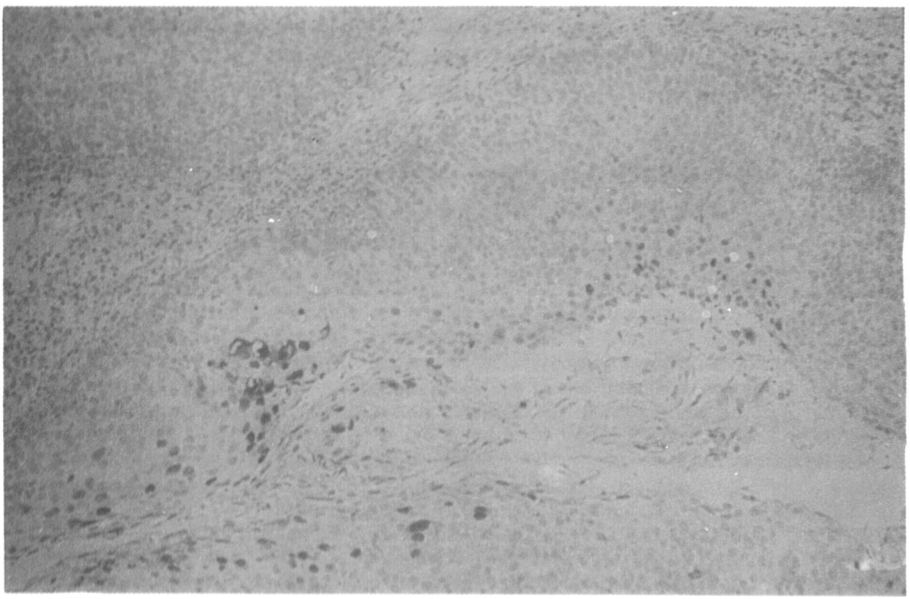

Fig. 2

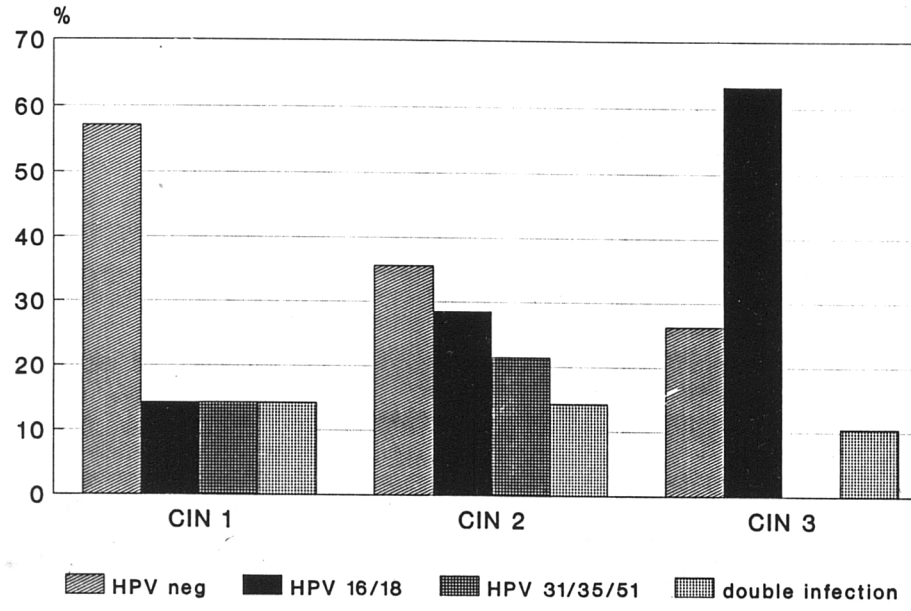

Fig. 3
Accumulated results of ISH for HPV DNA in cervical swabs and biopsies
Total 40 cases, one CIN 1 lesion was excluded because of different HPV types in swab and biopsy.

31/35/51 whereas no HPV DNA could be detected in the corresponding swab specimens.

Two patients had a HPV 31/35/51 positive swab but the biopsy was „HPV-negative", while the cervical swab sample of another woman was „HPV-negative" although HPV 31/35/51 could be detected in the corresponding biopsy.

There was a single case with different HPV types; the swab specimen tested as HPV 16/18 positive and HPV 31/35/51 was associated with the CIN 1 lesion in the tissue sections (Table 1).

27 of the examined 41 women with CIN 1 to CIN 3 lesions had either an HPV positive tissue section or both. Overall HPV 16/18 was the most common type found. Two out of 7 CIN 1 and 5 out of 14 CIN 2 lesions were positive for HPV 16/18 either in the cervical swab or the tissue section or both. Twelve out of 19 CIN 3 lesions stained positive for HPV 16/18 in at least one sample and another

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Fig. 1
Positive ISH for HPV 16/18 DNA in cervical swab

Fig. 2
Positive ISH for HPV 16/18 DNA in cervical tissue section

two CIN 3 lesions were positive both for HPV 16/18 and HPV 31/35/51. We did not find any CIN lesion to be associated with HPV 6/11 among our 41 patients (Fig. 3).

In all 11 cases with different results of cervical swabs and tissue sections in the HPV-ISH we reexamined the samples. In 8 positive samples (3 tissue sections and 5 swabs) we found only a weak staining for HPV DNA (less than 2 % of the squamous epithelial cells). In one case with strong staining for HPV 16/18 and HPV 31/35/51 in the tissue section the corresponding swab was inappropriate because the total number of epithelial cells was too low. There was one CIN 1 lesion with a tissue section positive for HPV 31/35/51 and a cervical swab positive for HPV 16/18.

Discussion

Specific types of HPV are linked to the genesis of neoplasias of the uterine cervix with strong evidence (Howley, 1991; Zur Hausen, 1991). But up to now this concept of a causal role of oncogenic HPV types in the pathogenesis of cervical cancer has not yet resulted in a new strategy regarding how to treat precursor lesions by distinguishing them according to the associated HPV type. Due to the differences in sensitivity between the various methods of HPV DNA hybridization the term „HPV-negative" becomes insignificant, because it summarizes samples which are false-negative due to lack of sensitivity as well as samples with unsequenced HPV types and finally those where papillomavirus is really absent.

The higher HPV 16/18-prevalence in our CIN 3 subgroup compared with the prevalence found in the CIN 1/2 subgroup fits in the concept of HPV 16/18 being a „high risk" factor for progression to malignancy. To the contrary the results „HPV-negative", based on ISH of one sample only was not a „low risk" factor because the false-negative rate was 6/20 for tissue sections and 4/18 for cervical swabs when each sample group was compared with the accumulated results of both groups.

There are some probable reasons for false-negative results of one sample. (1) The number of infected cells might be low and therefore could be missed by one section and/or swab. (2) Although tissue sections were usually chosen from the center of the lesion, an adjacent HPV infection could be missed. Furthermore, most HPV positive cells are found in the superficial layer of the epithelium. Thus previous swabs might result in false-negative tissue sections. (3) As the cell pellet of cervical swabs is divided into three portions the quantity of cells on the three slides of each patient (one for HPV 6/11, one for HPV 16/18 and one for HPV 31/35/51) differs. Therefore the associated HPV type might escape detection if the number of epithelial cells on the corresponding slide is low and infected cells too scarce.

We found an increase in HPV-detection rate when the results of the two methods of sample-taking were summed up. In view of the known relatively low

sensitivity of the ISH a positive staining should demonstrate a permissive HPV infection even if the number of infected cells was low in some cases. We consider as noteworthy that the observed accumulated HPV prevalences of swabs and tissue sections of 41 CIN lesions are comparable with the reported prevalences of PCR based studies (Morrison *et al.*, 1991; Van den Brule *et al.*, 1991).

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