Localized distribution of human papillomavirus genotypes in the uterine cervix

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Summary

Introduction: The localization and distribution of single or multiple HPV genotypes in the uterine cervix has not been studied thus far. The present study was undertaken to determine whether single or multiple HPV genotypes detected in cervical smears originate from a single (dysplastic) area, or from different areas (dysplastic or normal) of the uterine cervix.

Methods: Of eight patients with moderate or severe dysplasia, 31 colposcopically guided biopsies of different dysplastic lesions of the uterine cervix, as well as of normal epithelium were investigated. A highly sensitive, broad spectrum, short fragment polymerase chain reaction (SPF-10 PCR) HPV detection method in combination with a line probe assay (LiPA) for simultaneous genotyping was used.

Results: In the uterine cervix of four of the eight patients, multiple HPV genotypes were detected. These multiple HPV genotypes were detected in different biopsies as well as within a single biopsy. In three patients, all with carcinoma in situ or microinvasive carcinoma, only a single HPV genotype, HPV 16, was found all over the cervix including in the normal epithelium.

Conclusion: Different HPV genotypes can be detected in different dysplastic lesions as well as within single lesions, especially in patients with severe dysplasia. The severity of the lesion may possibly have a relation with the distribution of the HPV genotypes. The low number of patients and biopsies does not allow definite conclusions. However, the impact of these findings on the outcome of screening and vaccination programs remains to be elucidated.

Key words: HPV, Localization, Distribution, Uterine Cervix.

Introduction

Cancer of the uterine cervix ranks number two worldwide in cancers of women, accounting for 6% of all malignancies. Epidemiological and molecular studies over the past two decades have convincingly demonstrated that certain types of human papillomaviruses (HPV) are etiologically related to the development of most cases of cervical cancer. More than 95 genotypes of HPV have been identified so far, of which 35 types were found to infect the genital mucosa [1-3]. Several HPV genotypes, including types 16, 18, 31, 33, and 45 have been implicated in cervical carcinogenesis and are considered high-risk [1-3]. High-risk HPV genotypes are able to disturb cell cycle regulators. Theoretically, these disturbances allow repetitive clonal events to take place which may ultimately, result in cervical carcinoma via the development of premalignant stages [4-6].

During the premalignant stages, different areas, each with a different degree of dysplasia can be identified in the uterine cervix with colposcopy [7]. Cervical smears, ideally sampled from all these areas, contain in the majority a single genotype of HPV. Recently, more sensitive HPV detection methods have been shown to detect multiple HPV genotypes in up to 40% of cervical smears [6, 8-11]. The localization of single or multiple HPV genotypes in the uterine cervix and the distribution of HPV throughout the cervix have not been studied thus far. Studying the localized distribution of HPV may provide new insights in HPV infection and transmission and its complex relation with cervical cancer.

The present study was undertaken to determine whether single or multiple HPV genotypes detected in cervical smears originate from a single (dysplastic) area, or from different areas, either dysplastic or normal, of the uterine cervix.

Materials and Methods

Patients referred to the colposcopy clinic of the University Medical Center Nijmegen, with a cervical smear indicating moderate or severe dysplasia, were asked to participate in the study. A total of eight patients were included. One to three weeks prior to colposcopy, a liquid-based cervical smear was taken from all patients, using a Cervex brush (Rovers; Oss, The Netherlands). These smears were fixed with unifix® and processed into AgarCyto cell blocks, allowing for multiple analyses including HPV testing, as described previously [12]. The cervical smears indicated moderate dysplasia in one patient, severe dysplasia in four, and carcinoma in situ in three patients. The mean age of the patients was 37 years (32-46). None of the patients had previously been treated for dysplasia of the uterine cervix.

The cervix was assessed and mapped during colposcopy using acetic acid and lugol. After local anesthesia, three to five

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biopsies were taken from each patient using a small electrosurgical loop. All cervical areas with a colposcopically suspected different degree of dysplasia were biopsied, including an area of normal epithelium. Subsequently, the transformation zone was removed by large loop electrosurgical excision in order to complete treatment of all cervical abnormalities. The localization of each biopsy was marked in the patient's record.

All biopsies were embedded in parafin and processed separately to avoid cross-contamination with HPV. Histopathological examination was done on each of the biopsies. Single adjacent sections were tested for the presence of HPV. The results of the HPV test were not known during histopathological examination. For HPV detection a highly sensitive broad-spectrum short polymerase chain reaction fragment (SPF10 HPV-PCR) was used as previously described [8,9]. HPV typing was subsequently performed by reverse hybridization in a line probe assay (LiPA). This LiPA technique identifies 25 different low- and high-risk HPV genotypes simultaneously [8,9].

Results

HPV was detected in the cervical smears of seven patients. In two of these patients, double infections with respectively, HPV genotypes 16/31 and 16/52 were detected. All HPV genotypes detected in the cervical smear of each individual patient were also found in the biopsies of that patient. Additional HPV genotypes were detected in the biopsies of patients 1, 3, 4, and 6. The cervix of patients 1, 3, 5, and 6 contained multiple HPV genotypes. Up to four HPV genotypes could be detected within a single biopsy (patient 3). Table 1 shows the HPV genotypes detected in the smears, the histological grading of each biopsy and the HPV genotypes detected in the different biopsies.

Table 1. — *HPV genotypes detected in the cervical smears and in each biopsy, in relation to histologic grading, are shown for each patient.*

	HPV in smear	Biopsy	Histologic grading	HPV in biopsy
Pt. 1	X	1	Metaplasia	Neg
		2	Metaplasia	X
		3	Severe dysplasia	58
		4	Moderate dysplasia	33/58
Pt. 2	16	1	Carcinoma in situ	16
		2	Moderate dysplasia	16
		3	Normal sq. epithelium	Neg
		4	Normal endocx tissue	16
Pt. 3	33	1	Severe dysplasia	33
		2	Severe dysplasia	31/33/52/66
		3	Inflammation	33/66
		4	Endocx. atypia	66
Pt. 4	Negative	1	Moderate dysplasia	51
		2	Mild dysplasia	Neg
		3	Normal sq. epithelium	Neg
Pt. 5	16/31	1	Severe dysplasia	16/31
		2	Normal sq. epithelium	Neg
		3	Metaplasia	16
Pt. 6	16/52	1	Severe dysplasia	16/18
		2	Severe dysplasia and Endocx. atypia	16/18/52
		3	Severe dysplasia	16/18
		4	Mild dysplasia	16
Pt. 7	16	1	Carcinoma in situ	16
		2	Mild dysplasia	16
		3	Normal sq. epithelium	16
		4	Micro invasive ca.	16
Pt. 8	16	1	Severe dysplasia	16
		2	Severe dysplasia	16
		3	Normal sq. epithelium	16
		4	Micro invasive ca.	16
		5	Micro invasive ca.	16

Normal sq. epithelium = Normal squamous epithelium, Normal endocx. tissue = normal endocervical tissue, X = HPV type not specified.

Figure 1 shows a graphic representation of the localization of the biopsies related to the histological grading and the types of HPV detected in the cervix of each patient. HPV was detected in all biopsies showing dysplasia, except for a mild dysplastic lesion in patient 4. HPV was detected in two of the three biopsies with metaplasia, and in two of the five biopsies with normal squamous epithelium. Only in patients 7 and 8, both with a microinvasive carcinoma, was HPV detected in normal squamous epithelium.

In all patients with severe dysplasia as the most severe lesion (patients 1, 3, 5, and 6), more than one genotype of HPV was detected in the cervix, and multiple HPV genotypes were present within a single biopsy of each of these patients. In the other four patients a single HPV genotype was detected. Two of these patients had invasive carcinoma, and one patient had carcinoma in situ. In the patients with invasive carcinoma, HPV 16 was detected throughout the cervix, including normal squamous epithelium. Even the severe dysplastic areas in these patients only contained this single genotype of HPV.

Discussion

This study shows that multiple HPV genotypes can be detected within the uterine cervix in different dysplastic areas as well as within a single dysplastic area. The finding of different dysplastic areas in the uterine cervix, identified by colposcopy, has been described previously and it was suggested that these areas were not related to each other, nor were part of a developing process of cervical cancer [7]. Our results confirm those of another study which detected different HPV genotypes in different biopsies of the uterine cervix [13]. However in contrast with our results, only one biopsy with severe dysplasia in that study contained two HPV genotypes [13]. We detected multiple HPV genotypes in seven of 18 biopsies obtained from patients with severe dysplasia as the highest grade of abnormality, and within a single biopsy up to four different HPV genotypes were detected. It is possible that the detection of different HPV genotypes in different cervical lesions might be attributed to coincident infections causing separate lesions as has been suggested before [13]. In cases with the same HPV genotype detected in different lesions, morphologic progression 'in situ' was more likely [13]. The morphologic progression theory supposes that repetitive clonal events occur early in the development of dysplasia under the influence of a single HPV genotype [14]. Although it has to be emphasized that the number of patients and biopsies included in our study is very small, the multiple HPV genotypes

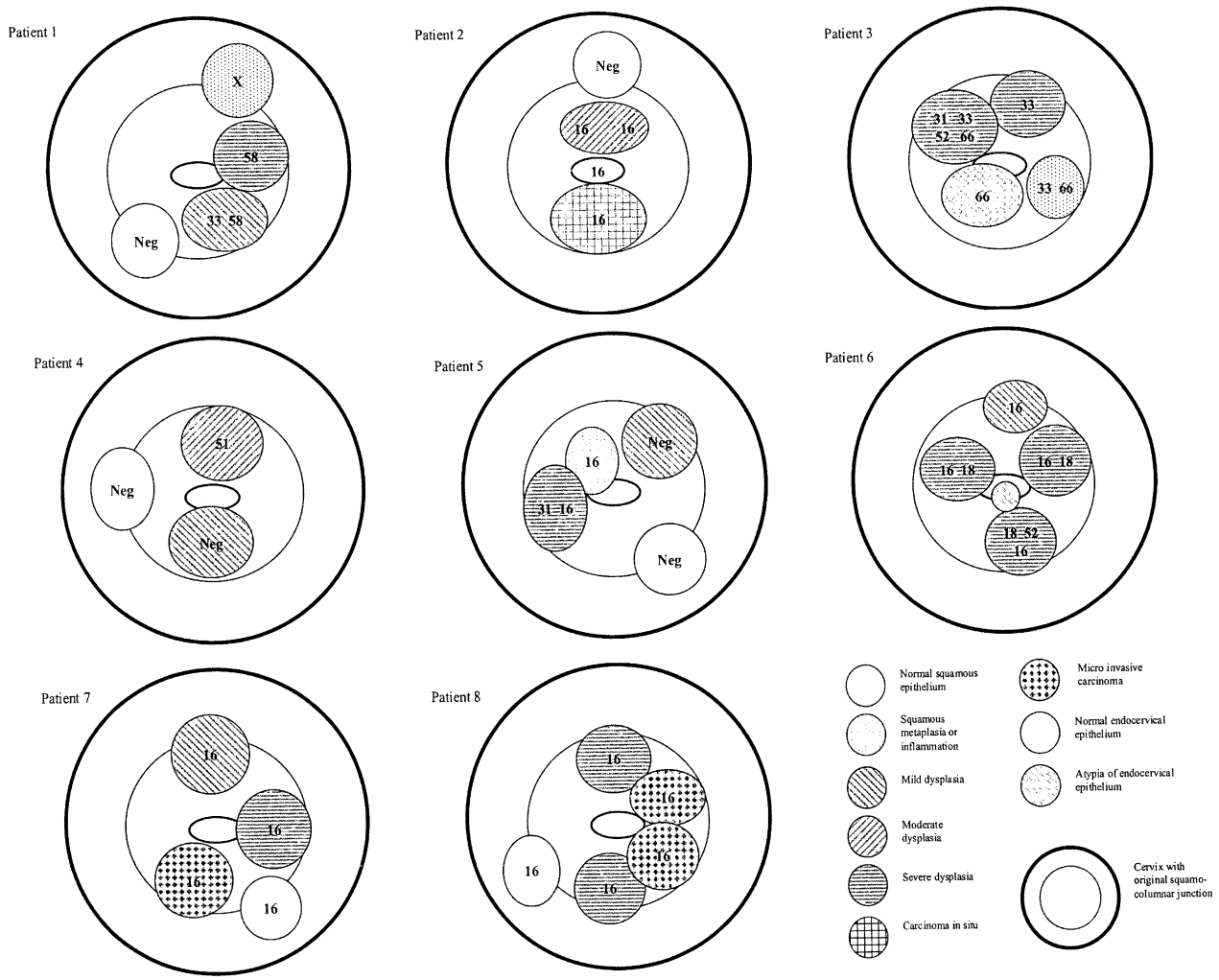

Figure 1. — *Graphical representation of the localization of the biopsy, histologic grading, and HPV genotypes detected within the biopsy.*

present in our patients are in contrast with the basics of the morphologic progression theory. On the other hand, it is quite possible that a clonal process attributed to a specific single HPV genotype is in progress, while other HPV genotypes detected are merely superfluous or transient, either newly acquired, or a reactivation of latent HPV infections. The presented data suggest that the detection and distribution of single or multiple HPV genotypes, may possibly have a relation with the stage of the disease. In patients with severe dysplasia as the most severe lesion, multiple HPV genotypes were detected within a single biopsy as well as in different biopsies, while in the patients with carcinoma in situ or invasive carcinoma, a single HPV genotype was detected in all biopsies including areas with different degrees of dysplasia and normal epithelium. More studies are required to solve these issues.

We found a lower detection rate of multiple HPV genotypes in cervical smears than in the corresponding biopsies. The assumption that HPV genotypes detected in cervical smears are a true reflection of all types of HPV present in the cervix may therefore not always be true. This observed underestimation in cervical smears may be due to a sampling error, but may also indicate that only the most prominent HPV genotype(s) are detected in a cervical smear. The replication of these genotypes may be more pronounced, or they may be localized in more superficial layers of the epithelium. This facilitates detection in cervical smears and masks the presence of other, possibly latent genotypes of HPV in the deeper epithelial layers. Indeed, in the literature more evidence to support the presence of latent HPV is described. The prevalence of HPV during pregnancy increases with gestational age to 40% compared with 5-20% prevalence in the non-pregnant population [15-17]. This increase could not be explained by other risk factors [16]. Patients above 55 years and patients with immune suppression also show a higher prevalence of HPV than in a regular population, and there is a strong correlation between the prevalence of HPV and the severity of the immune-suppression [11,18,19].

More sensitive HPV detection methods are able to detect a higher prevalence of genital HPV infection in the population and do more often detect multiple HPV

genotypes [6,8,9]. The highly sensitive SPF-10 LiPA-PCR HPV detection method used in this study has been validated before [8]. With this detection method, HPV genotypes were correctly identified in 97.2% of 238 samples and showed to be more sensitive than other PCR primers (MY 09/11, GP5(+)/6(+)) especially when multiple HPV genotypes were present [8].

As shown in this study, multiple HPV genotypes can be found within a single biopsy of a dysplastic lesion of the uterine cervix, indicating that the relation between HPV and cervical cancer is very complex. The relation between multiple HPV infections and cervical cancer as well as factors influencing latency, recurrence and clearance of HPV need further study.

The presence of multiple HPV genotypes may have a direct impact on the outcome of screening and vaccination programs and further analysis is required.

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