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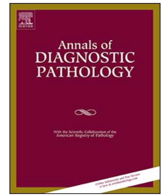

Different amplification patterns of 3q26 and 5p15 regions in cervical intraepithelial neoplasia and cervical cancer

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ABSTRACT

Purpose: The aim of this study was to evaluate and correlate the amplification of chromosomal regions 3q26 and 5p15 in different cytological and histological subgroups of patients and to compare the sensitivity and specificity of amplification tests with cytology, colposcopy and HPV status.

Methods: The work was conducted at the Department of Obstetrics and Gynaecology in cooperation with the Institute of Pathological Anatomy, JFM CU in Martin and UNM during years 2013–2016. Prospective longitudinal study included 131 patients. We focused on the FISH diagnosis of the amplification of regions encoding the components of telomerase enzyme (3q26, 5p15) in cytology specimens. We manually evaluated 100 atypical cells per slide and analysed the amplification patterns. Correlations between cytological, histological, HPV DNA results and amplification patterns of chromosomal regions 3q26 and 5p15 were analysed by chi-squared test and non-parametric Man - Whitney *U* test.

Results: The results showed that the amplification of chromosomal regions increases with the degree of dysplasia toward the invasive disease ($p < 0.001$). Whereas the increase in the amplification of 3q26 is noticeable already at CIN 2 + lesions ($p < 0.01$), 5p15 amplification is shifted up toward CIN 3/CIS ($p < 0.001$) and cervical cancer. Amplification of selected regions correlated with each other and also with hrHPV-positive status ($p < 0.01$).

Conclusion: The analysis of the amplification of 3q26 and 5p15 regions may serve in the future for the differential diagnosis of cervical lesions and to determine their malignant potential. High specificity of these tests can improve the excellent sensitivity of HPV DNA test.

1. Introduction

Cervical cancer is one of the most common malignancies in the female population. The worldwide incidence in 2012 reached 528,000 new cases and that mortality exceeded the 266,000 [1]. Currently, standard screening for cervical precanceroses includes cytological examination of cervical smears in combination with HPV DNA diagnostics. E6, E7 mRNA, p16 protein, methylation markers, glycomics, proteomics and the telomerase activity are the new diagnostic tools for cervical dysplasia and cervical cancer.

In our study we focus on amplification patterns of chromosomal regions 3q26 and 5p15 tightly connected with the telomerase activity. 3q26 chromosomal region contains TERC gene that codes the RNA template of telomerase enzyme. The second gene localised in this region is PIK3CA encoding the catalytic subunit of phosphatidylinositol-3-kinase which represents the intracellular messenger. On the other hand, region 5p15 contains TERT gene responsible for the synthesis of catalytic subunit of telomerase enzyme. Increased telomerase activity is an early process in cervical carcinogenesis and its activity and expression could be a valuable marker for the diagnosis and prognosis of patients with cervical neoplasia [2].

2. Material and methods

The study was carried out between years 2013–2016 at the Department of Gynaecology and Obstetrics and at the Institute of Pathology, Jessenius Faculty of Medicine in Martin, Comenius University, Slovakia. The prospective study involved 131 patients. Twenty patients formed the control group with negative cytology (NILM) and negative high-risk human papillomavirus (hrHPV) finding.

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Patients younger than eighteen years, pregnant women and patients who underwent the operative treatment for cervical lesions in the past were excluded from the study. The study was approved by the ethics committee (27 January 2012) and before each examination the informed consent was signed.

The cytological samples for HPV and fluorescent in situ hybridization (FISH) were obtained during colposcopic examination. The brush was immersed in a liquid fixation medium (Liqui-Prep vial, LiquiPrep™, LGM International Inc.). We continued with advanced colposcopy with 3% acetic acid solution and. Schiller test. In case of insufficient finding we used endocervical speculum (Kevorkian - 3 and 7 mm) for cervical channel visualisation. Colposcopic findings were evaluated according to the latest international nomenclature approved in Rio de Janeiro in 2011. Based on the biopsy results the patients were further managed either conservatively or surgically. The more severe diagnosis (from the biopsy or conisation) was categorized as the definite one. Cytological specimens were classified according to the Bethesda system (2001) whereas HPV status was analysed by the method HC2 (Digene Hybrid Capture 2 High-Risk HPV DNA Test, Gaithersburg, MD).

2.1. FISH hybridization

LiquiPrep™ specimens were processed according to the producer's manual, centrifuged at 1000*g* for 10 min, mixed with a LiquiPrep™ cellular base with a ratio of 1:3, pipetted manually on SuperFrost slides (Menzel-Glaser, Braunschweig, Germany) and dried at room temperature.

Slides were pre-treated and incubated for 2 min at 37 °C in 2× saline-sodium citrate (SSC) buffer (pH 7.0) and 1 min with the pepsin solution (concentration of 0.5 mg/mL) afterwards. After incubation the slides were washed twice in 1× phosphate-buffered saline at room temperature each for 3 min (pH 7.4), dehydrated in 70, 85 and 95% ethanol at room temperature for 1 min each and air-dried. The FISH probe FHACT™ (Cancer Genetics Inc., Rutherford, NJ, USA) was vortexed briefly in a microcentrifuge. Subsequently, 10 μL of the probe was applied on a slide and covered with a cover glass (24 × 24 mm). The edges of the cover slide were sealed thoroughly with rubber cement. Afterwards the slides were co-denatured for 3 min at 80 °C on a temperature-controlled hot plate protected from direct light, incubated for 48 h in a humidified environment at 37 °C and protected against the light. After the hybridization process the cover glass was removed and the slide was washed for 2 min in 2× SSC/0.1% Igepal at room temperature, 2 min at 72 °C in 0.4× SSC/0.3% Igepal, 1 min at room temperature in 2× SSC/0.1% Igepal, dehydrated in 70, 85 and 95% ethanol at room temperature and air dried. The slide was briefly rinsed in distilled water and air dried. In the end 10 μL of diamidino-2-phenylindole solution (DAPI, 0.1 μg/mL) was applied to the hybridized area and covered with a cover glass (24 × 24 mm).

We used an Olympus BX61 fluorescent microscope for the slide evaluation and picture acquisition. The slides were screened with a 100× objective. The FISH probe FHACT™ (The FISH-based HPV-Associated Cancer Test – Cancer Genetics Inc., Rutherford, NJ, USA) is designed to determine copy number changes of the 3q26, 5p15, 20q13 and Cen7 regions. We evaluated 100 most atypical cells on each slide and analysed the amplification patterns of 3q26 and 5p15 regions.

Statistical analysis was performed by PASW Statistics 18 (IBM ®) software. Nonparametric Man-Whitney *U* test was used for the correlation between cytological and histological results, HPV DNA status and amplification patterns of 3q26 and 5p15 amplification ROC (Receiver Characteristics Operators) curves were used to determine the cut-off values for the determination of sensitivity, specificity, positive and negative predictive values of amplification tests.

3. Results

The average age of patients included in our study was 39.4 years.

Table 1
Summary of definite histological, cytological and hrHPV results.

		WNL	CIN1	CIN2	CIN3/CIS	SCCA/AC
hrHPV negat	ASCUS	6	4	–	–	–
	LSIL	2	3	–	–	–
	ASC-H	5	0	–	–	–
	HSIL	0	1	–	–	–
	SCCA/AC	0	0	–	–	1
hrHPV pozit	ASCUS	2	1	5	2	–
	LSIL	11	4	11	6	–
	ASC-H	1	1	0	3	2
	HSIL	0	1	8	14	4
	SCCA/AC	0	0	0	0	13

The definite cytological, histological and hrHPV findings are summarised in Table 1.

(WNL – within normal limit, CIN –cervical intraepithelial neoplasia, CIS – carcinoma in situ, SCCA – squamocellular carcinoma, AC – adenocarcinoma, NILM – negative for intraepithelial lesion or malignancy, ASCUS – atypical squamous cells of undetermined significance, LSIL – low-grade squamous intraepithelial lesion, ASC-H – atypical squamous cells, cannot exclude high-grade, HSIL – high-grade squamous intraepithelial lesion, hrHPV – high-risk human papillomavirus)

3.1. FISH analysis

By the ROC curves, we determined the most appropriate cut-off values for amplification tests of chromosomal region 3q26 and 5p15 in detecting CIN 2+ lesions. The results showed that the amplification increases with the degree of dysplasia ($p < 0.001$). While the increase in the 3q26 amplification is evident even at CIN 2+ lesions ($p < 0.01$), 5p15 amplification is shifted to CIN3+ lesions ($p < 0.001$). The absolute number of positive cases for 3q26 (cut off > 11 cells with > 2 signals per case) and 5p15 (cut-off > 3 cells with > 2 signals per case) is shown in Tables 2 and 3.

(WNL – within normal limit, CIN –cervical intraepithelial neoplasia, CIS – carcinoma in situ, SCCA – squamocellular carcinoma, AC – adenocarcinoma, NILM – negative for intraepithelial lesion or malignancy, ASCUS – atypical squamous cells of undetermined significance, LSIL – low-grade squamous intraepithelial lesion, ASC-H – atypical squamous cells, cannot exclude high-grade, HSIL – high-grade squamous intraepithelial lesion)

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Significant differences were observed between the negative histology and light dysplasia for 3q26 amplification. We did not detected statistically significant difference between ASCUS and LSIL cytology group for the amplification pattern of both genes ($p = 0.910$ (3q26),

Table 2
Number of positive cases of 3q26 amplification in cytological and histological categories (cut-off for 3q26 amplification: > 11 cells with > 2 signals).

	WNL	CIN1	CIN2	CIN3	CA	Total
NILM						1/20
ASCUS	0/8	1/5	2/5	1/2	–	4/20
LSIL	1/13	1/7	5/11	6/6	–	13/37
ASC-H	0/6	0/1	–	3/3	2/2	5/12
HSIL	–	2/2	4/8	11/14	4/4	21/28
CA	–	–	–	–	14/14	14/14
Total	1/27	4/15	11/24	21/25	20/20	

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