

IMMUNOHISTOCHEMICAL INVESTIGATIONS OF P16^{INK 4A} EXPRESSION IN CARCINOMAS AND HIGH GRADE CERVICAL LESIONS

M. Karcheva, S. Popovska*, R. Nachev*

Department of Epidemiology of Infectious Diseases
Medical University-Pleven, Bulgaria

*Department of General and Clinical Pathology
University Hospital-Pleven, Bulgaria

SUMMARY:

During the years the role of viruses as a cause of oncological illness was known. One of them is cervical carcinomas, related to Human papilloma viruses (HPV's). There are about 450000 new cases of cervical cancer worldwide each year, and 250000 deaths of this diagnosis. By the diagnosis cervical cancer get there with two procedures: after mass screening or clinical suspicious for ill. By the availability apparent tumor diagnosis is put with biopsy. By the discreet lesions require utilization modern diagnostic methods. One of them is immunohistochemical method. In situ hybridization (ISH) assays for high-risk human papillomavirus (HR-HPV) and immunohistochemical (IHC) assays for surrogate markers such as p16^{INK 4a} can be useful in detecting HR-HPV in cervical dysplasia. The aim of the following study was to find difference the level for expression of the tumor specific gene p16^{INK 4a} in reactive, dysplastic and invasive changes in biopsy specimens from uterine cervix. In the lesions of the uterine cervix, overexpression of p16^{INK 4a} is induced by HPV and is associated with the process of cancerogenesis in the epithelium. Almost 100% of the high grade lesions and the invasive carcinomas demonstrated high level of expression of p16^{INK 4a} while non displastic epithelium is always negative.

Key words: cervical carcinomas, human papilloma viruses, p16^{INK4a} expression

INTRADUCTION:

Viruses have important meaning for human pathology. During the years the role of viruses as a cause of oncological illness was known. One of them is cervical carcinomas, related to Human papilloma viruses (HPV's). WHO Regional Office for Europe reported by cervix cancer morbidity. Data for selected countries are presented on **fig.1**. There are about 450000 new cases of cervical cancer worldwide each year, and 250000 deaths of this diagnosis. Every year new cases of cervical dysplasia, related to HPV's, are assess at 10 million cases with CIN II/III, 30 million cases with CIN I. Success in diagnostics of this disease is due to

the use of Pap-test /citological smear analysis/. However Pap-test gives significant portion of both false-positive and false-negative conclusions. Amendments of the diagnostic procedure are desirable. Aetiological role of papillomaviruses in cervical cancer is established while the role of cellular gene alterations in the course of tumor progression is less clear. Several research groups including us have recently named the protein p16^{INK 4a} as a possible diagnostic marker of cervical cancer. To evaluate whether the specificity of p16^{INK 4a} expression in dysplastic and neoplastic cervical epithelium is sufficient for such application we undertook a broader immunohistochemical registration of this protein with a highly specific p16^{INK 4a} monoclonal antibody.

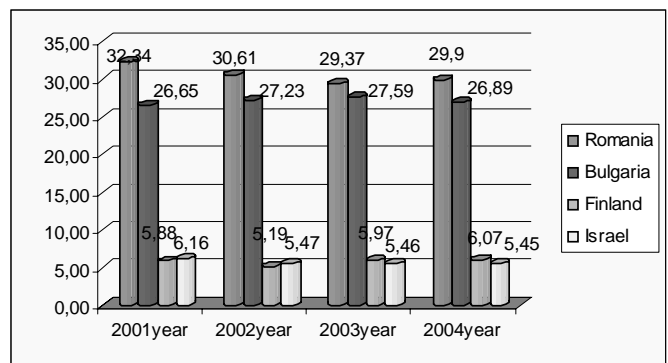


Fig. 1. Morbidity (%000) by cancer of cervix in selected countries since 2001 for 2004 year.

AIM:

The aim of the following study was to find difference the level for expression of the tumor specific gene p16^{INK 4a} in reactive, dysplastic and invasive changes in biopsy specimens from uterine cervix.

MATERIALS AND METHODS:

Fifty six biopsy specimens were investigated in the following study. They were separated in four groups as follows:

- 1st group - reactive non dysplastic changes (n=13);
- 2nd group - different levels of dysplasia to Ca in situ (n=19)
- 3rd group - invasive squamous cells carcinoma (n=16)
- 4th group - endocervical lesions - Microglandular hyperplasia, Ca in situ and invasive endocervical adenocarcinoma (n=8)

Standard 4 µm slides were stained with H&E. An immunohistochemical method with monoclonal antibodies against the tumor-suppressor gene p16^{INK4a} was applied on some specimens, according to the instructions provided by the manufacturer (DAKO). The evaluation of the positive immunohistochemical reactions for p16^{INK4a} was also semiquantitative (0 when there is no positive reaction, 1+ from 15% to 20% staining of the nuclei and cytoplasm, 2+ from 25% to 75% and 3+ >75%). Weak cytoplasm staining (<5%) was considered negative. For statistical analysis of the results was used the method of correlative analyses and χ^2 . Values of $p < 0.05$ were considered as statistically significant.

RESULTS:

The results from the expression of p16^{INK4a} are presented in table 1. In the cases with dysplastic lesions and invasive carcinomas was found strong correlation between the level of expression of p16^{INK4a} and the level of cervical neoplasia ($p < 0.01$).

Tabl. 1. Grade of p16^{INK4a} expression

Histological diagnosis	0	1+ (<25%)	2+ (25-75%)	3+ (>75%)	Îáúî
1. Non-dysplastic lesions					
Unmature squamous metaplasia	5	0	0	0	5
Atypical squamous metaplasia	1	0	0	0	1
Inflammation	5	0	0	0	5
Inflammatory atypia	2	0	0	0	2
2. Dysplastic lesions					
CINI	1	2	0	0	3
CINII	1	2	5	0	8
CINIII	0	0	1	7	8
Invasive squamous carcinomas	0	0	1	15	16
Microglandular hyperplasia	2	0	0	0	1
Endocervical dysplasia	0	0	2	0	1
Endocervical carcinomas	0	0	0	5	5

All 13 cases (100%) of non dysplastic cervical lesions are negative for p16^{INK4a} (fig.2). The most cases of CIN III group (7cases-87,5%) showed strong (+++) cytoplasmic and nuclear expression of p16^{INK4a} in the whole depth of the epithelium (fig. 3, 4). Strong mainly nuclear overexpression was found in all invasive cervical adenocarcinomas. (fig. 5).

DISCUSSION:

In the lesions of the uterine cervix, overexpression of p16^{INK4a} is induced by HPV and is associated with the process of cancerogenesis in the epithelium. These observations show that, the p16^{INK4a} immunostaining can make easier the precise identification of dysplastic cervical epithelial cells on histological specimen. It displays the cellular changes in the infected cell, as a result of the genomic integration of the viral oncogenes. Because of the fact that overexpression is not found in normal, non-transformed cells p16^{INK4a} is reliable marker for identification of dysplastic and malignant epithelium. Almost 100% of the high grade lesions and the invasive carcinomas demonstrated high level of expression of p16^{INK4a} while non dysplastic epithelium is always negative.

CONCLUSION:

This statistically significant different expression of p16^{INK4a}, in different types of lesions in the uterine cervix, contributes for their identification and makes this marker helpful and objective for the diagnosis of the cervical pathology. So far no indications for a difference in the p16^{INK4a} staining pattern of cervical lesions caused by different HR-HPV types have been found. The velocity of neoplastic transformation seems to be different for different HR-HPV types, but once the oncogene products of HR-HPV have caused cell cycle disturbances, as one of the first steps in carcinogenesis, overexpression of p16^{INK4a} should be detectable independent of the individual HR-HPV type.

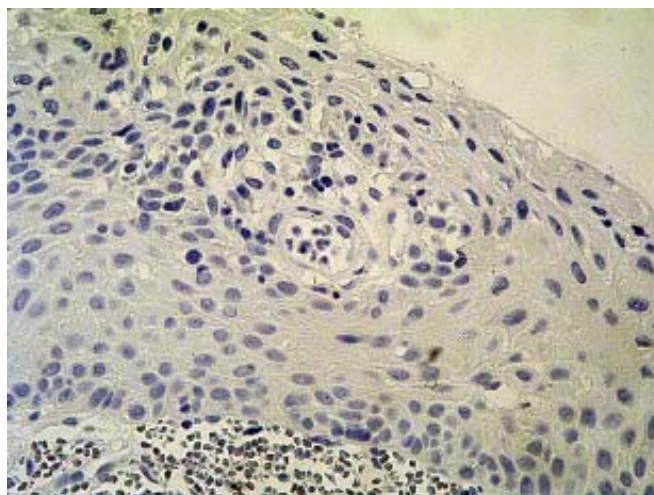


Fig. 2. Non dysplastic lesion negative for p16^{INK4a}

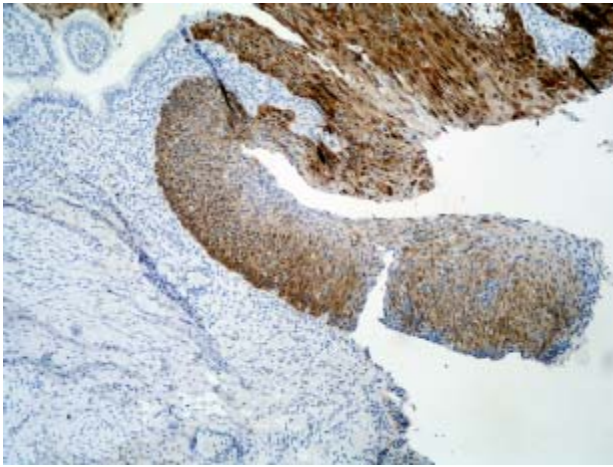


Fig. 3 CIN III - expression of p16^{INK4a}

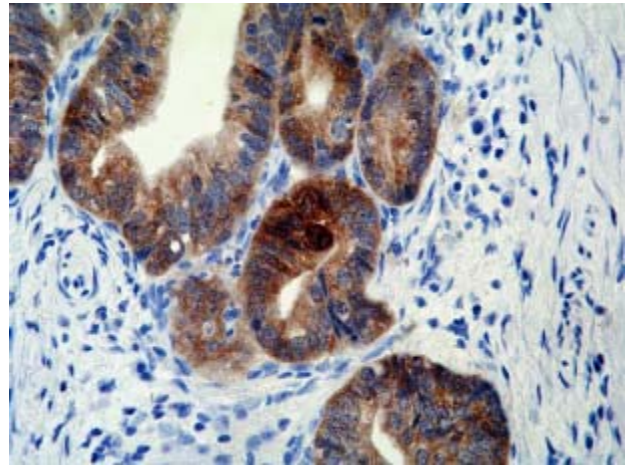


Fig. 4 Adenocarcinoma in situ - expression of p16^{INK4a}

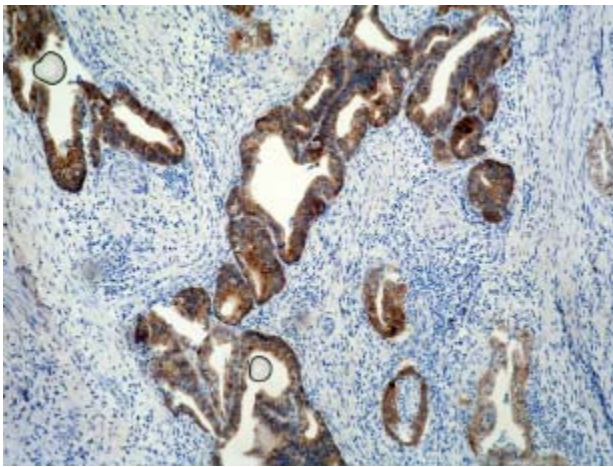


Fig. 5 Invasive cervical adenocarcinoma - expression of p16^{INK4a}

REFERENCES

1. Carozzi F., S. Cecchini, M. Confortini, et al., Role of p16^{INK4a} Expression in Identifying CIN 2 or More Severe Lesions among HPV-positive Patients Referred for Colposcopy after Abnormal Cytology, *Cancer Cytopathology*, 108/2, April 25, 2006, 119-123
2. Giannoudis A, Herrington SC. Human papillomavirus variants and squamous neoplasia of the cervix. *J Pathol* 2001; 193: 295-302.
3. Holladay E., S. Logan, J. Arnold, et al., A Comparison of the Clinical Utility of p16^{INK4a} Immunolocalization With the Presence of HPV by Hybrid Capture 2 for the Detection of Cervical Dysplasia / Neoplasia, *Cancer Cytopathology*, 108/6, December 25, 2006, 451-461
4. Kalvatchev Z., P. Draganov, Human Papilloma Viruses and Malignancy, Clinical application of immunology, Vol 3 /Number 2/ September 2004, 361-365
5. Keating J. T., Ince T., Crum C. P. Surrogate biomarkers of HPV infection in cervical neoplasia screening and diagnosis. *Adv Anat Pathol*, 2001;8:83-92.
6. Klaes R., Friedrich T., Spitkovsky D, et al. Overexpression of p16^{INK4A} as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer*. 2001;92:276-284.
7. Negri G, Egarter-Vigl E, Kasal A et al: p16^{INK4a} Is a Useful Marker for the diagnosis of Adenocarcinoma of the Cervix Uteri and Its Precursor. *Am J Surg Path* 2003;27(2):187-193
8. Sano T, Oyama T, Kashibwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 1998;153:1741-8.
9. Sano T, Oyama T, Kashiwabara K, et al. Overexpression of p16 protein associated with intact retinoblastoma protein expression in cervical cancer and cervical intraepithelial neoplasia. *Pathol Int* 1998;48:580-8.
10. Zur Hausen, H. Papillomavirus infections: a major cause of human cancers. *Biochim Biophys Acta* 1996;1288:F55-78.

Address for correspondence:

Dr. Milena Karcheva
 Department of Epidemiology of Infectious Diseases, University of Medicine
 1 "St. Kliment Ohridsky" Str., 5000 Pleven, Bulgaria
 Phones: +359/64/884 269, 884 144; Fax: +359/64/801 603
 e-mail: milena_karcheva@abv.bg