A Descriptive study of Expression of p16^{ink4a} in Different Types of Lesions in Uterine Cervix

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Abstract—*Human papilloma virus (HPV) is the main reason for cervical carcinoma. The viral E7 oncogene induces increasing expression of the cyclin dependent kinase inhibitor p16 INK4a in dysplastic cells. This can be used to identify dysplastic cells in histological slides. The aim of this study was to determine the presence of p16INK4a expression and to evaluate the diagnostic value of p16 immunohistochemical (IHC) investigation in different types of lesions in the uterine cervix. The study was performed on 112 samples of cervical biopsy. All samples were selected from the records of Pathology services in University Hospital-Pleven, Bulgaria. The samples were collected in four separate groups: reactive non dysplastic changes (n=26); different degrees of intraepithelial dysplasia (n=38); invasive squamous cell carcinoma (n=32); endocervical lesions with glandular origin – microglandular hyperplasia, Adenocarcinoma in situ and invasive endocervical adenocarcinoma (n=16). In all samples immunohistochemical analysis using antibodies to p16INK4a was performed. Results. In the cases with dysplastic lesions and invasive carcinomas was found strong correlation between the level of expression of p16INK4a and the level of cervical neoplasia (p<0.01). All 26 cases (100%) of non-dysplastic cervical lesions are negative for p16INK4a. The most cases of CIN III group (14cases-87.5%) showed strong cytoplasmic and nuclear expression of p16INK4a in the whole depth of the epithelium. Strong mainly nuclear overexpression was found in all invasive cervical adenocarcinomas. Conclusions. P16INK4a overexpression is associated to high-grade precancerous lesions and cervical carcinomas. Immunohistochemical evaluation can be useful biomarker in identifying HR-HPV- infected low-grade lesions.*

Key words: Cervical dysplasia, Cervical cancer, HPV,p16INK4a

I. INTRODUCTION

Incidence and prevalence of cervical cancer in Bulgaria is higher than the European average. For 2012 data show the incidence for Bulgaria is 28.5 per 100 000 women, compared with the European average of 13.4 per 100 000 women. Mortality from cervical cancer in Bulgaria is higher than the European average – 8.8 and 4.9 per 100 000 women, respectively. The causal role of human HPV infection in cervical cancer has been documented.[1]

The p16 is a cyclin-dependent kinase-4 inhibitor that is expressed in a limited range of normal tissues and tumors. In recent years, immunohistochemistry with p16 antibodies has been used as a diagnostic aid in various scenarios in gynecologic pathology. Positivity with p16 in the cervix can be regarded as a surrogate marker of the presence of high-risk human papillomavirus (HPV).[2, 3]

Causal relationship between genital human papillomavirus (HPV) infection and cervical dysplasia/carcinoma is well established. The viral E7 oncogene induces increasing expression of the cyclin dependent kinase inhibitor p16^{INK4a} in dysplastic cells. This can be used to identify dysplastic cells in histological slides. [3]

Many authors[4-10] had conducted researches in this direction and had variable findings so this study was conducted with the aim to determine the presence of $p16^{INK4a}$ expression and to evaluate the diagnostic value of p16 immunohistochemical (IHC) investigation in different types of lesions in the uterine cervix.

II. METHODOLOGY

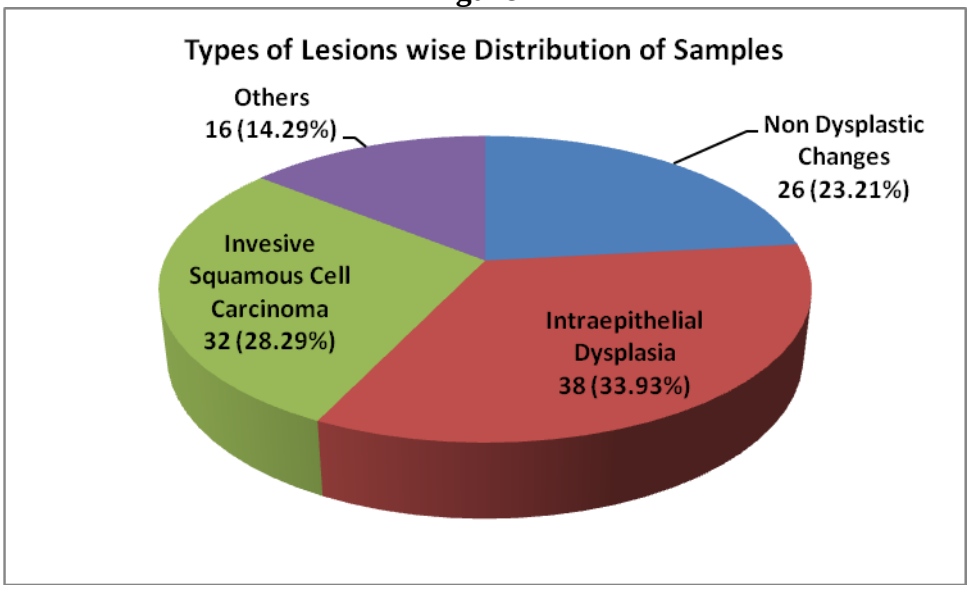
This study was performed on 112 samples of cervical biopsy. All samples were selected from the records of Pathology services in University Hospital-Pleven, Bulgaria. The samples were collected in four separate groups: reactive non dysplastic changes (n=26); different degrees of intraepithelial dysplasia (n=38); invasive squamous cells carcinoma (n=32); endocervical lesions with glandular origin – microglandular hyperplasia, adenocarcinoma in situ and invasive cervical adenocarcinoma (n=16).

In all samples an immunohistochemical method with monoclonal antibodies against the tumor-suppressor gene $p16^{INK\ 4a}$ was applied, according to the instructions provided by the manufacturer (DAKO). The evaluation of the positive immunohistochemical reactions for $p16^{INK4a}$ was also semi quantitative (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.

III. RESULTS

Out of all 112 samples of cervical biopsy, 26 (%) were reactive non dysplastic changes, 38 (%) were different degrees of intraepithelial dysplasia , 32 (%)invasive squamous cells carcinoma and 16 (%) were in other group including endocervical lesions with glandular origin – microglandular hyperplasia, adenocarcinoma in situ and invasive cervical adenocarcinoma. (Figure 1)

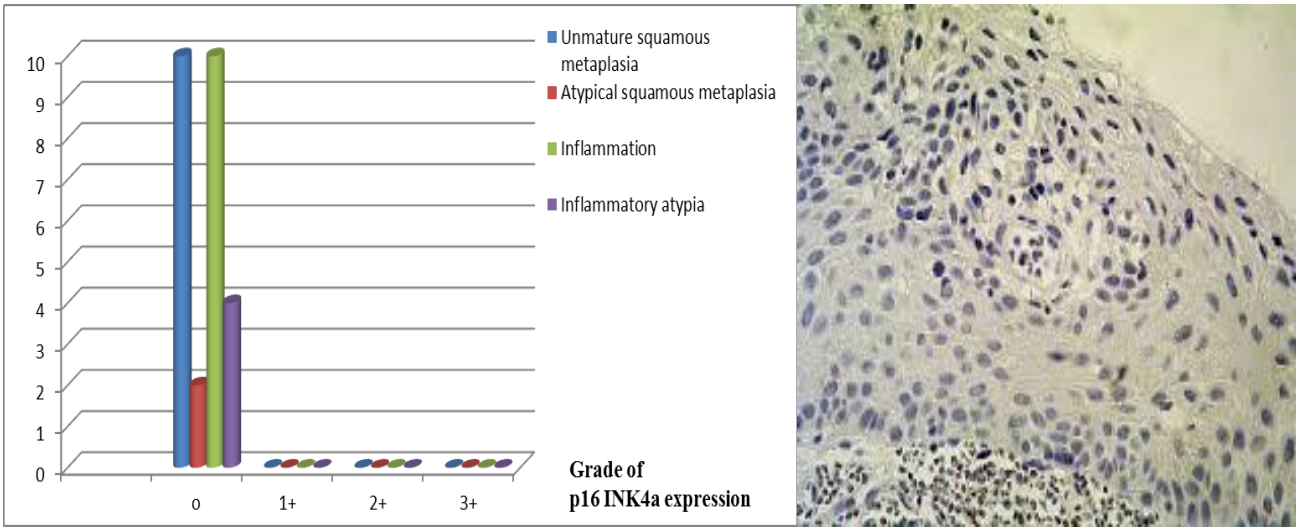
Figure 1



All 26 cases of non-dysplastic cervical lesions are negative for $p16^{INK4a}$ (Fig 2a) i.e. there is 100% $p16^{INK4a}$ negativity in non-dysplastic cervical lesions. Microscopic finding of these samples were presented in Fig 2b.

Figure: 2a **Figure: 2b (Microscopy)**

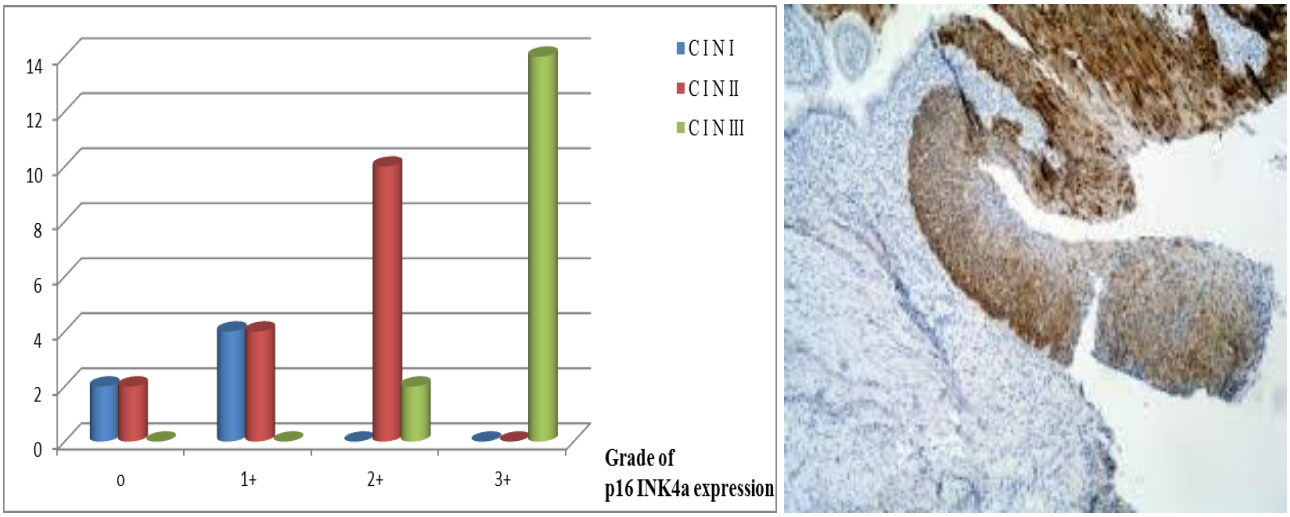
Grade of $p16^{INK4a}$ expression in non-dysplastic lesion Non-dysplastic lesion negative for $p16^{INK4a}$



Most cases of CIN III group (14 cases - 87.5%) showed strong cytoplasmic and nuclear expression of $p16^{INK4a}$ in the whole depth of the epithelium (Fig 3a). Microscopic finding is presented in Fig 3b.

Figure: 3a **Figure: 3b**

Grade of $p16^{INK4a}$ expression in CIN **CIN III - expression of $p16^{INK4a}$**



Thirty cases (93.75%) of invasive squamous cell carcinoma showed strong expression of $p16^{INK4a}$ (Fig 4a). Microscopic finding is presented in Fig 4b.

Figure: 4a

Grade of p16^{INK4a} expression in invasive SCC

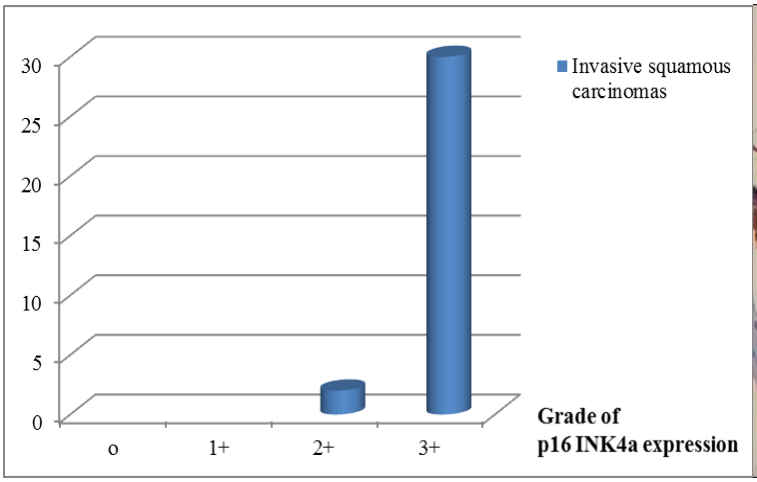
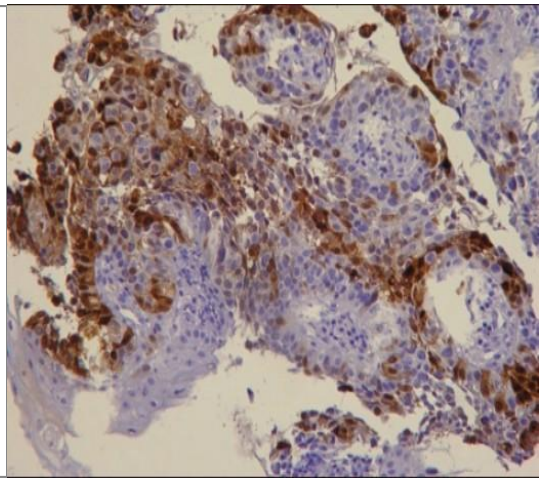


Figure: 4b

SCC - expression of p16INK4a expression



Strong mainly nuclear overexpression was found in all invasive cervical adenocarcinomas. Ten cases (62.5%) with adenocarcinoma in situ showed Grade (2+) p16^{INK4a} expression (Fig 5a). Microscopic finding is presented in Fig 5b and 5c.

Figure: 5a

Grade of p16^{INK4a} expression in Endocervical lesions with glandular origin

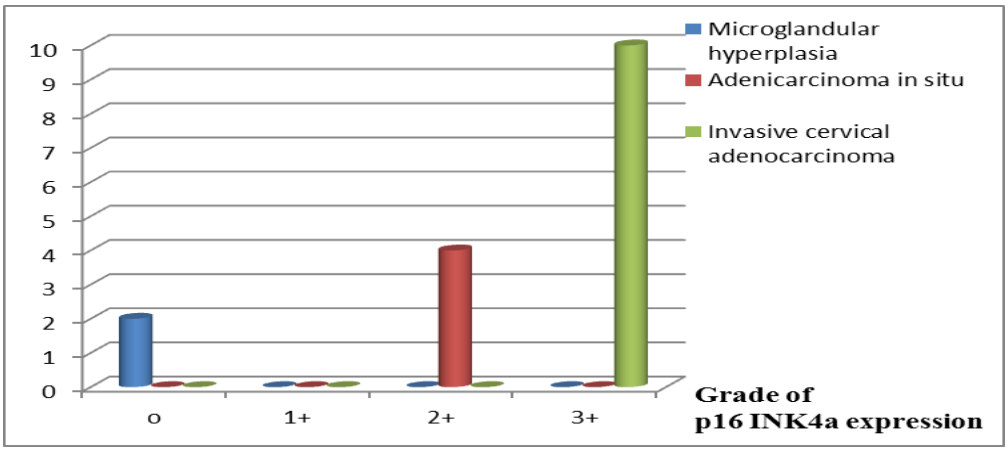


Figure: 5b

Adenocarcinoma in situ - expression of p16^{INK4a}

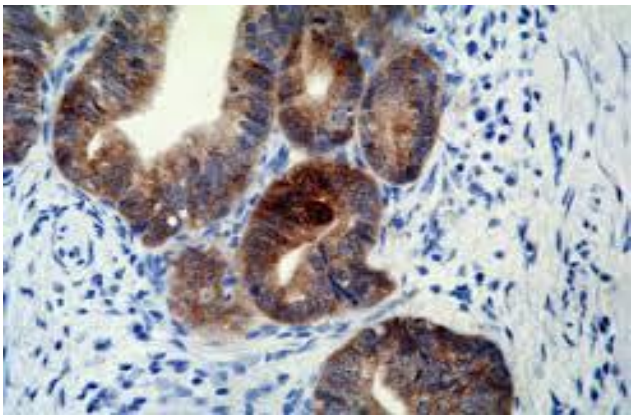
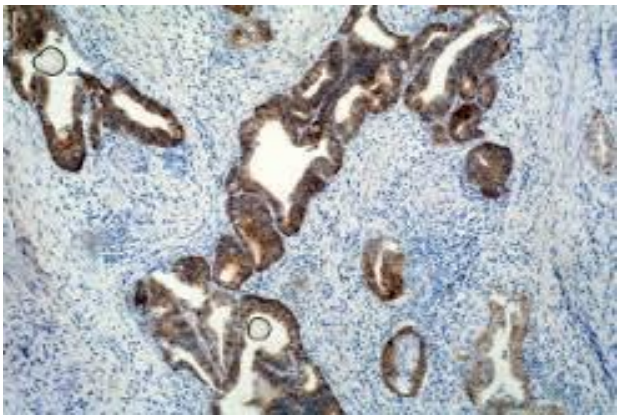


Figure: 5c

Invasive cervical adenocarcinoma - p16^{INK4a}



IV. DISCUSSION

This study highlight the need to identify specific biomarkers with a value in detecting the presence of cervical dysplasia or cancer. The p16 over expression was evaluated in 112 cervical biopsies including histological negative cases, low - and high-grade lesions, as well as squamous cervical carcinomas and adenocarcinomas. 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 ($p<0.01$). In our opinion p16-positive CIN I and CIN II are the low-grade lesions which need particular clinical attention.

Nieth etall[10] reported that 36% AGC-containing cases were actually squamous abnormalities and p16 Immunocytochemical stain was reactive in 61% of smears. Out of these p16 Immunocytochemical stain was reactive weakly/sporadically in 6% and strongly positively in 55%. There was 1 case of high grade squamous intraepithelial lesion showing negative immunostaining for p16

One of the priorities of cancer control is associated with early diagnosis of malignant diseases. The timely detection of the disease is related not only to a more favorable outcome and consequently greater survival and quality of life of patients, but is associated with significant social and economic benefits for society.

Like observations made by this study, M. benevolo etall[3] also reported that p16 overexpression is associated to high-grade precancerous lesions and cervical carcinomas, and further demonstrated that immunohistochemical evaluation of p16 may be a useful biomarker in identifying HR-HPV-infected low-grade lesions.

V. CONCLUSION

$P16^{INK4a}$ overexpression is associated to high-grade precancerous lesions and cervical carcinomas. Immunohistochemical evaluation can be useful biomarker in identifying HR-HPV- infected low-grade lesions.

CONFLICT OF INTEREST

None declared till now.

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