Srp Arh Celok Lek. 2015 Jan-Feb;143(1-2):42-49
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1502042G
UDC: 618.146-074:577.1

Significance of Immunohistochemical Expression of $p16^{INK4a}$ in the Differentiation of Inflammatory and Preneoplastic Cervical Lesions

Živorad Gajanin[1], Rade Vilendečić[2], Vesna Ećim Zlojutro[2], Radoslav Gajanin[2], Pavle Budakov[3]

[1]Healthcare Center, Kotor Varoš, Republic of Srpska, Bosnia and Herzegovina;
[2]Clinical Center Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[3]Department of Pathology, Clinical Center of Vojvodina, Novi Sad, Serbia

SUMMARY

Introduction Most studies point at the main role of humanpapilloma virus (HPV) in the development of dysplasia and cervical cancer. Due to the low specificity and sensitivity of morphological diagnostic methods it is necessary to find an adequate marker which would be introduced in the screening program for cervical cancer. Most research suggests that $p16^{INK4a}$ is a specific and sensitive marker.

Objective The aim of this research was to determine the presence of $p16^{INK4a}$ expression in inflammatory and preneoplastic lesions of the cervix.

Methods The study was performed on 73 samples of cervical biopsy. In 34 patients a preneoplastic change (dysplasia) in the stratified squamous cervix epithelium was found, and in 39 a non-specific inflammatory process was disclosed. In all samples, immunohistochemical analysis using antibodies to $p16^{INK4a}$ was performed.

Results The expression of $p16^{INK4a}$ was verified in 67.65% of cases in dysplastic cervical lesions and 38.5% of the inflammatory lesions. A statistically significant difference was determined in the presence and grade of expression between dysplastic and inflammatory lesions of the cervix (χ^2=24.16; p<0.001). The expression was more frequent and had a higher grade in dysplastic lesions with high grade and showed a statistically significant difference compared to the expression in low-grade dysplasia (χ^2=21.48; p<0.001).

Conclusion The analysis of the presence of $p16^{INK4a}$ can differentiate non-neoplastic from preneoplastic changes in the cervix. It is recommended to use immunocytochemical and immunohistochemical analysis using $p16^{INK4a}$ in interpreting borderline lesions of the cervix.

Keywords: cervical intraepithelial neoplasia; cervicitis; immunohistochemistry; $p16^{INK4a}$; differentiation

INTRODUCTION

Cervical cancer has remained at the top of oncological conditions in the twentieth century. The mortality rate of this disease in the developed countries declined from the first place in 1920 to the eighth place in 1990. Despite these data, the incidence has been minimally changed in those countries where screening programs have not been developed. With cytological techniques it is possible to diagnose changes in the cervix in the premalignant stages of the disease [1].

Histological diagnosis of cervicitis is common enough that is identified with normal results. The cervix is in direct contact with the vagina and is exposed to infectious agents such as viruses, bacteria, fungi and parasites. Cervical infection can rarely be present in the absence of infection in the vagina. The cervix is a reservoir of many infectious pathogens such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Herpes simplex virus (HSV), humanpapilloma virus (HPV), and Mycoplasma spp. Most women, holders of specified microorganisms, have no clinical symptoms, and therefore it is of great importance to establish their presence in women with an increased risk [1, 2].

Preneoplastic changes localized in the lower genital tract are frequently multicentric (often embedded within the epithelium which embryonically originates from anogenital epithelium). About 10% of women with cervical intraepithelial neoplasia (CIN) has a synchronous preinvasive lesions localized on the vulva, vagina or anus [1, 3]. CIN is sometimes marked as a dysplasia and involves disruption of growth and maturation of the epithelium of the cervix. There are three grades of CIN. Mild dysplasia (CIN I) is defined as a disorder localized in the bottom (basal) third of the epithelium, CIN II is localized in two-thirds of the thickness of the basal epithelium.

Severe dysplasia or CIN III involves changes localized in more than two thirds of the thickness of the epithelium, while carcinoma in situ (CIS) is a disorder which occupies the entire thickness of the epithelium (Figure 1). Histological grading is still based on this three-grade grading scale [2]. The prevalence of CIN is different and depends on socio-economic status, geographic factorsand exposure to risk factors and ranges from 1.05% to 13.7%. CIN is most commonly diagnosed in women aged 20 years, CIS in women aged 25-35 years, and cervical cancer is diagnosed after the age of 40 [1, 4, 5].

Correspondence to:
Radoslav GAJANIN
Clinical Center Banja Luka
12 beba, 78000 Banja Luka
Bosnia and Herzegovina
radoslavgajanin@gmail.com

Different markers were analyzed as potential candidates which could perform triage of borderline cytological and histological findings (Ki 67, CDc6, Mcm5, MN) [6]. One of the most intensively studied markers is $p16^{INK4}$ cell protein (p16), which belongs to the CDKI group and shows a high expression in all high grade dysplasias and cervical carcinoma [7, 8]. The analysis of expression of regulatory proteins of the cell revealed that it was highly associated with viral E7 oncoprotein activity at the molecular grade. Continuous inactivation of RB protein by the viral oncoprotein E7 is required for the malignant phenotype expression of cells infected with HPV. Detection of the expression of p16 can be used as highly specific and sensitive "surrogate" marker of RB tumor suppressor protein in HPV infected cells [7, 8].

Most studies point at the main role at HPV in the development of dysplasia and cervical cancer. The analyses confirmed the presence of HPV in more than 80% of CIN lesions and in 99.7% cases of invasive cervical cancer [3, 9, 10].

Two viral oncogenes E6 and E7 are expressed in HPV-infected cells. Products of viral oncogenes are involved in the mechanism of malignant cell genesis. Oncogenic activity of the products of viral genesisis is based on the interplay of cell-specific proteins. Viral E6 protein causes a premature degradation of the p53 tumor suppressor protein. Similarly, E7 binds RB protein and leads to the release of the E2F transcription factor. Functionally, E7 leads to inactivation of the RB protein. Inactivation of the RB protein (due to E7 or mutations, gene deletions) leads to an increased phosphorylation and expression of cyclin-dependent kinases – CDK (CDK4 and CDK6). Nature of $p16^{INK4}$ expression represents a negative feedback control of the loss of RB protein. Thus, the loss of RB protein function leads to increaseof $p16^{INK4a}$. The inactivation of RB protein by E7 viral protein leads to increased expression of $p16^{INK4a}$, which can represent a substantial sensitive and specific biomarker for active expression of HPV oncogenes [6, 11].

Significance of analysing the expression of $p16^{INK4a}$ in samples of cervical tissue was shown in many studies, and it is widely accepted [7, 12]. In cytology, the examination of the expression of $p16^{INK4a}$ was investigated in order to assess the benefit to triage of patients, whose screenings on a routine cytology were interpreted as a boundary case. In most studies, the sensitivity is similar to the HPV testing, but the rate is significantly higher at the side of the specificity of expression of $p16^{INK4a}$ in the cytological material, in particular in the triage of borderline cytological findings [13, 14, 15].

Although numerous studies indicate the significant potential of $p16^{INK4a}$ testing and triage of abnormal Pap findings, they also show significant limitations. Limitations are present mainly due to non-standardized and inadequate immune reaction that occurs due to the use of different, non-standardized antibodies. Also, there are no standardized protocols for the interpretation of a small number of biopsy samples that follow the interpretation of $p16^{INK4a}$.

OBJECTIVE

Aim of the research is to determine the presence of $p16^{INK4a}$ expression in inflammatory and preneoplastic (dysplastic) lesions of the cervix and to determine whether the presence and grade of expression of $p16^{INK4a}$ depends on the grade of dysplasia in the epithelium of the cervix.

METHODS

Our study included biopsy materials of 73 patients, who were operated at the Clinical Center of Banja Luka, Department of Obstetrics and Gynecology. The study was conducted from January until December 2012. Analysis of bioptic tissue samples was performed at the Department of Pathology, Clinical Center of Banja Luka. The material obtained by biopsy was fixed in 10% formalin. After routine processing, paraffin tissue blocks were made, which were cut in a microtome in serial sections and stained with hematoxylin-eosin (HE) staining method. The analysis was one on a binocular microscope Leica (objective 10×, 25×, 35×, 40×; eyepieces 10×) with the width of the visual field of 1.4 mm.

In all subjects the nature of changes in the mucosa of the vaginal portion (mucosa without changes, inflammation, dysplasia in stratified squamous epithelium) was determined. The classification was made according to the recommendations of the World Health Organization (WHO) [16].

Changes are divided in two groups: Group I (34 bioptic samples), in which the histological analysis revealed the diagnosis and the grade of dysplasia in the epithelium of the cervix; and Group II (39 bioptic samples), in which the diagnosis is determined by histological analysis of inflammatory lesions in cervical epithelium.

In all samples (Groups I and II) immunohistochemical analysis was performed using antibodies against $p16^{INK4a}$ (Dako, Denmark). For immunohistochemical analysis we used anti-human $p16^{INK4a}$ monoclonal antibody (clone E6; mtm laboratories AG, Heidelberg, Germany). Tissue samples were cut in a microtome (Leica 2000), to a thickness of 2-4 microns and after that they were deparaffinized. Antigen unmasking was performed in citrate buffer, 0.007 M for 40 minutes at 97 degrees Centigrade, pH of 6.0. For unblocking we used 1.5% hydrogen peroxide for 15 minutes. Slides were stained with primary monoclonal antibody (1:1000 overnight at 4 degrees Centigrade), and the visualization was done by the avidin biotin kit (Ultra Vision Detection System).

Evaluation of the presence of expression was performed as follows: the grade of expression of $p16^{INK4a}$ was assessed semiquantitatively according to the percentage of epithelial cells which showed expression. A positive reaction involved an intense nuclear and cytoplasmic immunoreaction (coloration).

Level of expression was evaluated in the following manner: score 0 – <1% of positive epithelial cells; score 1 –

1-5% of positive epithelial cells; score 2 – 5-25% of positive epithelial cells; score3 – >25% of positive epithelial cells.

Statistical analysis was performed using SPSS, version 17.0. A descriptive statistical analysis (average values) was used in order to describe the total sample and individual groups of the sample. For the analysis of the existence of differences between the groups the following tests were used: Chi square, Wilcoxon signed-rank test and Mann-Whitney test.

RESULTS

The first group consisted of materials of 34 patients who were diagnosed with preneoplastic lesions in cervical samples. In the second group there were samples of 39 patients morphologically diagnosed as non-specific inflammation in cervical samples. The youngest patient was 23 and the oldest had 86 years. The average age of all patients was 49 years. The average age of patients in the first group was 37 years, and in the second group 61 years.

Patients in the second group were older and had a diagnosis of nonspecific inflammation. Mainly, they were operated due to prolapsed uterus, which is most frequently clinically diagnosed in postmenopausal women, thus explaining the age structure of this group of patients. The average age of women in group I was 37 years and they had the diagnosis of dysplasia in the squamous epithelium of the vaginal portion. The average age of our patients fits the data available in the literature.

In all patients in group I (n=34) surgical material in which the analysis had been carried out was a conical clip of vaginal portion of the cervix. In all 39 patients of group II, surgical material in which the analysis was carried out was uterus. Samples analyzed in patients of group II originated from vaginal portions in which non-specific inflam-

Table 1. Level of expression of p16^{INK4a} in samples in groups I and II

Number (%)		Level of expression of p16^{INK4a}				
		0	1	2	3	Total
Group I	CIN I	4 (57.1%)	3 (42.9%)	0	0	7 (100.0%)
	CIN II	6 (54.5%)	2 (18.2%)	2 (18.2%)	1 (9.1%)	11 (100.0%)
	CIN III	1 (6.3%)	0	0	15 (93.8%)	16 (100.0%)
	Total	11 (32.4%)	5 (14.7%)	2 (5.9%)	16 (47.0%)	34 (100.0%)
Group II		24 (61.5%)	13 (33.3%)	2 (5.2%)	0	39 (100.0%)

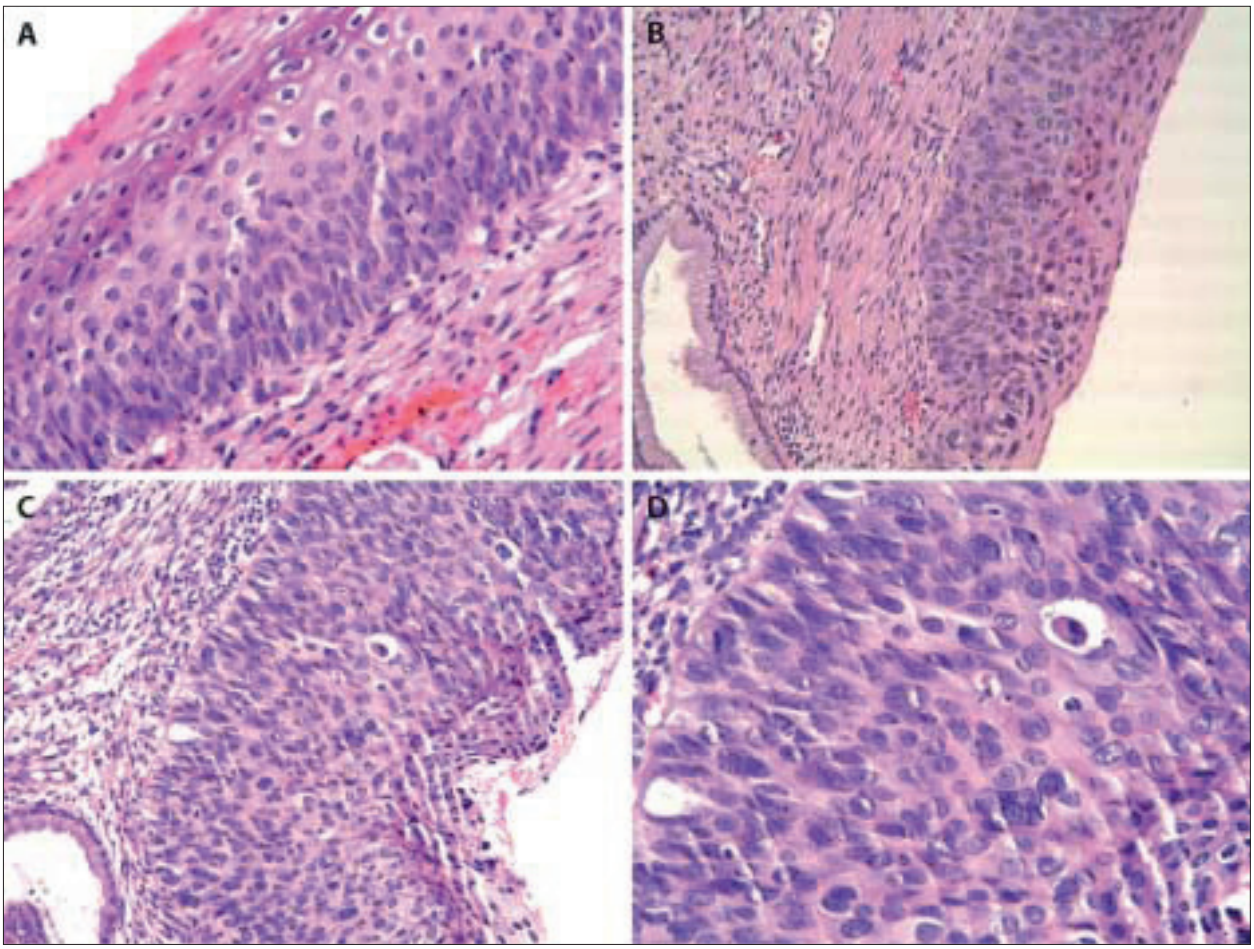

Figure 1. Histologic appearance of dysplasia in the squamous epithelium. A. Dysplasia in the squamous epithelium of low grade cervix, low grade – CIN I; B. Dysplasia of squamous epithelium, moderate grade – CIN II; C-D. Dysplasia of squamous epithelium, severe grade – CIN III (HE, ×200).

doi: 10.2298/SARH1502042G

mation and erosion (defect in stratified squamous epithelium) were verified and were incurred as the result of a mechanical irritation of the mucous of the vaginal portion. In the vicinity of the defect in all samples there were regenerative changes in epithelium, glands and stroma.

Among patients in group I, 17 (50%) cases were diagnosed as dysplasia of squamous epithelium, severe grade (CIN III), 10 (29.41%) cases had a squamous epithelial dysplasia, moderate grade (CIN II) and 7 (20.59%) of patients had a dysplasia of squamous epithelium, low grade (CIN I) (Table 1, Figure 1). In all 39 (100%) cases of patients in group II, histological diagnosis of chronic, nonspecific inflammation of the cervix was established.

The expression of $p16^{INK4a}$ in materials of group I was present in 23 (67.6%) cases. Expression had a range from focal, low expression which was estimated semiquantitatively as score 1 in 5 (14.7%) cases, to high expression which was estimated as score 3 in 16 (47%) of cases. Expression was not present in 11 (32.4%) of the cases (Table 1). The analysis showed that high expression of $p16^{INK4a}$ was present in the materials in which dysplasia of squamous epithelium of the cervix in severe grade or CIN III was diagnosed. The absence or low expression were associated with dysplasia of low or moderate grade (CIN I CIN II) (Table 1).

In patients in group I, a statistically significant difference in the grade of expression of $p16^{INK4a}$ between patients with different grades of dysplasia in the squamous epithelium was found, χ^2 (2, N=34) = 21.48, p<0.001. Patients in group I, who were diagnosed with low grade dysplasia (CIN I), had a lower grade of expression of $p16^{INK4a}$ from patients who were diagnosed with severe grade of dysplasia (CIN III) (Table 1, Figure 2). We analyzed patients who had been diagnosed with dysplasia of low and moderategrade (CIN I + CIN II), compared to those patients who were diagnosed with severe grade of dysplasia (CIN III). There was a statistically significant difference in the grade of expression of $p16^{INK4a}$ between patients with different grades of dysplasia, χ^2 (3, N=34) = 30.36, p<0.01. Patients in group II, with dysplasia of low and moderate grade (CIN I and CIN II) had a lower grade of expression of $p16^{INK4a}$ than patients with severe grade of dysplasia (CIN III) (Table 1).

The expression of $p16^{INK4a}$ in the materials in group II was present in 15 (38.46%) cases. The expression had mostly low grade – 13 (33.3%) cases, while moderate grade was present in only 2 (5.2%) cases. $p16^{INK4a}$ expression was not verified in 24 (61.5%) cases (Table 1).

Between group I (34 patients) and group II (39 patients) there was a statistically significant difference in the grade

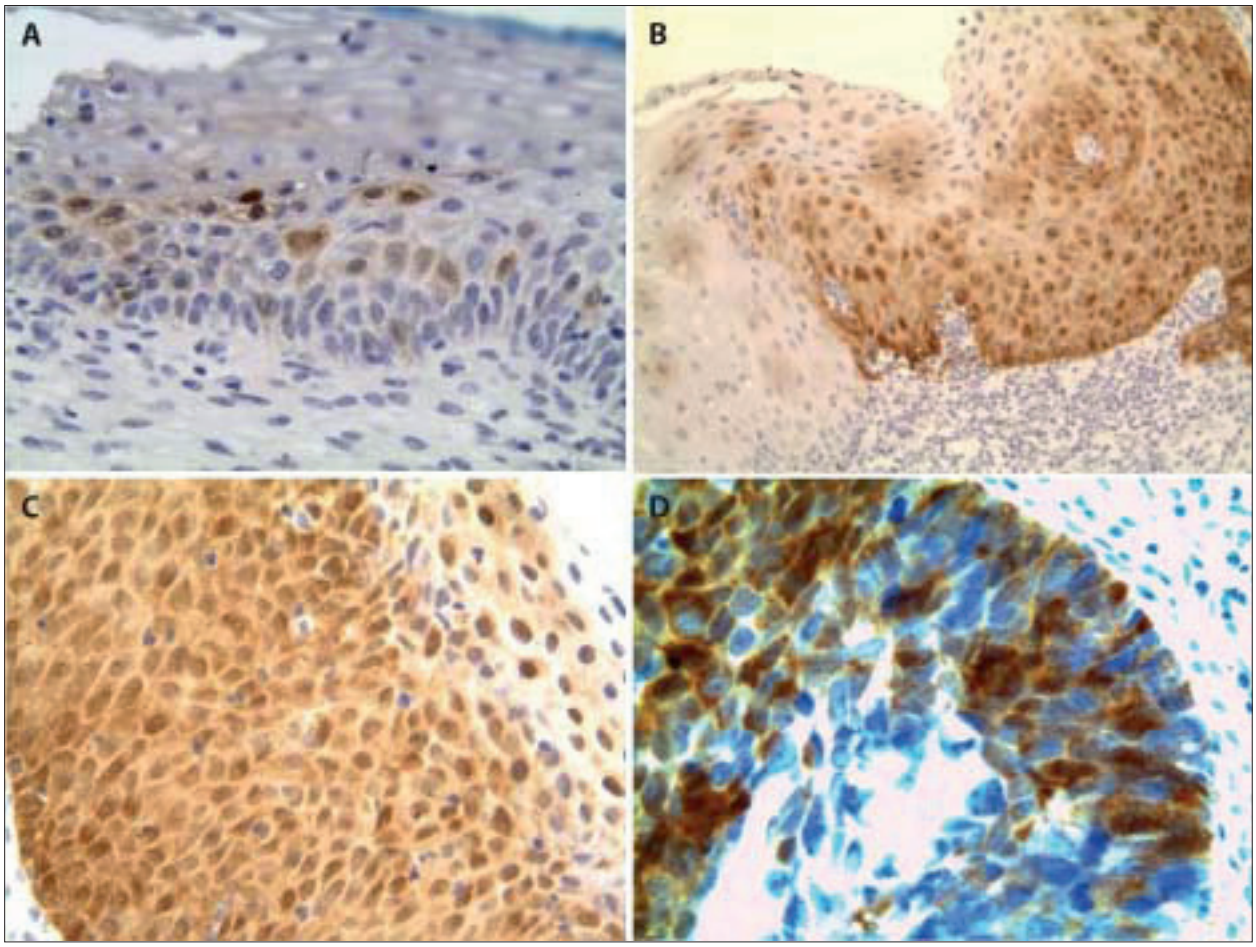

Figure 2. Expression of $p16^{INK4a}$ in the dysplastic epithelium of the cervix. A. Dysplasia in the squamous epithelium, low grade (CIN I); a low grade of expression of $p16^{INK4a}$ in dysplastic epithelium of the cervix (anti-p16, ×200). B-C. Dysplasia of severe grade (CIN III), a high grade of expression of $p16^{INK4a}$ in dysplastic cervical epithelium, and absence of expression in normal epithelium (anti-p16, ×200). D. Dysplasia of severe grade (CIN III), a high grade of expression of $p16^{INK4a}$ a in dysplastic epithelium of the cervix (anti-p16, ×400).

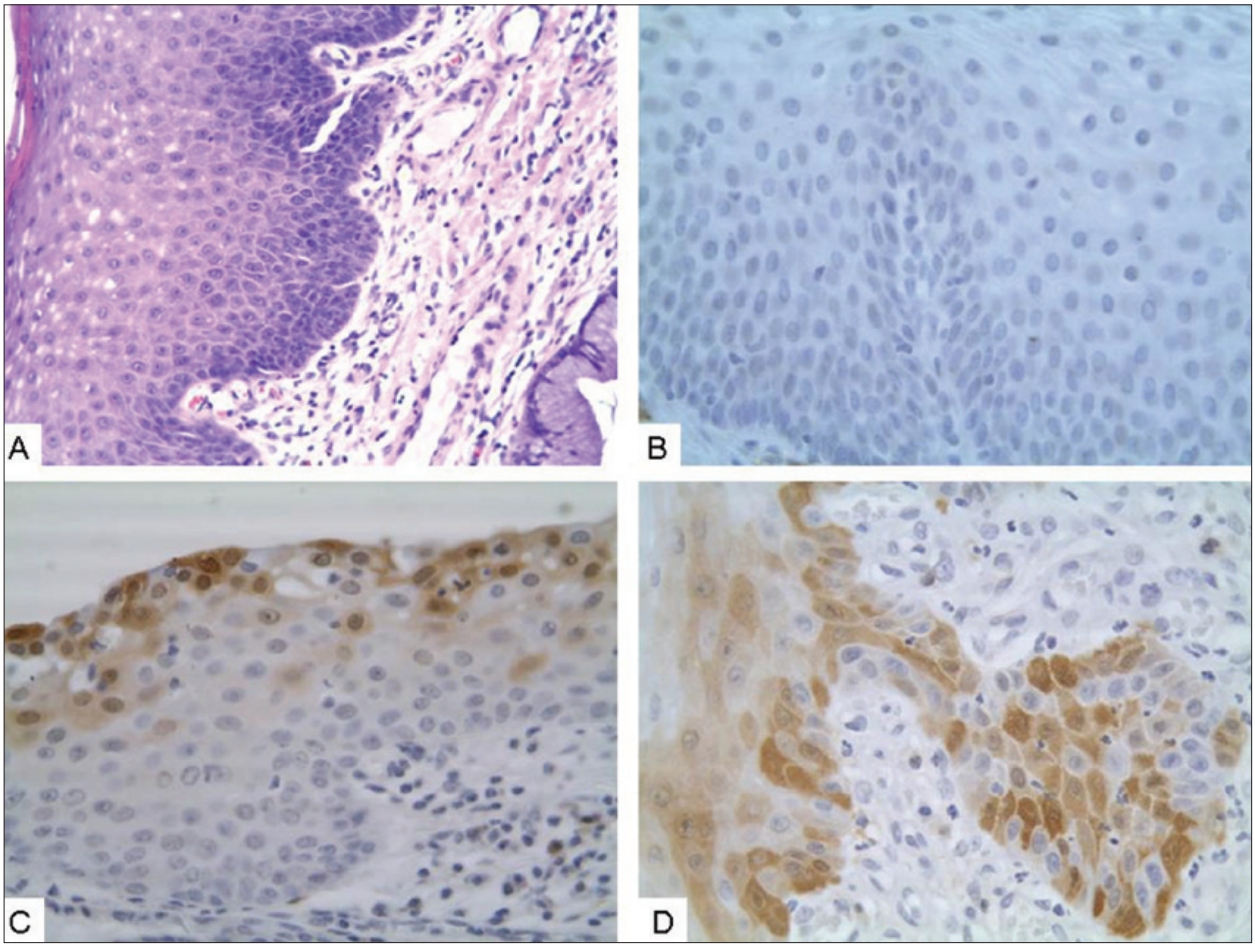

Figure 3. Non-specific chronic cervicitis. A. Chronic non-specific inflammation of the cervical mucosa (HE, ×200); B. Epithelium of the cervix, without p16^{INK4a} expression – score 0 (anti p16, ×200); C. Epithelium of the cervix, low grade of expression of p16^{INK4a} – score 1 (anti p16, ×200); D. Epithelium of the cervix, moderate grade of expression of p16^{INK4a} – score 2 (anti p16, ×200).

of expression of p16^{INK4a}, χ^2 (3, N=73) = 24.16, p<0.001. Patients with non-specific inflammation had a lower grade of expression of p16^{INK4a} compared to patients with preneoplastic lesion (Table 1). The relationship of presence and grade of expression between group II (cervicitis) and materials of group I with different grades of dysplasia was analyzed. The difference was not present in the expression of p16^{INK4a} between group II and low grade (CIN I) and moderate (CIN II) grade, χ^2 (1, N=46) = 0.37, p=0.54; χ^2 (2, N=49) = 2.62, p=0.27, respectively. The difference was present in the expression of p16^{INK4a} between group II and severe grade of dysplasia (CIN III), χ^2 (1, N=56) = 42.98, p<0.001. Patients with non-specific inflammation had a lower grade of expression of p16^{INK4a} than patients with preneoplastic lesion with grade of dysplasia CIN III (Table 1, Figure 3).

DISCUSSION

Persistent HPV infections have long been confirmed as the underlying cause and risk factor for the occurence of invasive cervical cancer. Organized prevention programs in developing countries, aimed at detecting changes that precede the formation of invasive cancer, have led to a significant decrease of morbidity and mortality from cervical cancer. The main diagnostic method is cytological technique ("Pap smear"). This method of diagnosis is successful because the evolution of changes from normal epithelium to invasive carcinoma is long and takes several years [8, 17, 18].

Over the past two decades there has been a considerable progress in understanding of HPV infection and advances in molecular technology (diagnostics). New methods (techniques) were developed, which were used in screening alone or together with cytologic techniques. One of those methods is HPV DNA screening tests that have been used successfully for triage and are more reliable than cytological techniques [19].

The use of liquid-based cytology together with immunocytochemical analysis of presence of P16^{INK4a} expression is very effective for the detection of precancerous and cancerous lesions of the cervix [20, 21, 22]. The sensitivity was in the range from 0.59 to 0.96 and the specificity between 0.41 and 0.96 for the detection of dysplastic lesions. A double immunostaining was introduced, where Ki-67 and P16^{INK4a} were used, which should greatly simplify and standardize assessment [23, 24].

The expression of p16^{INK4a} in our study in patients with CIN lesions was present in 67.6% of cases. It should be also

noted that in CIN I lesions expression in most cases was not present (57.1%), and in cases where the expression was verified it had a low grade, i.e. expression in less than 5% of the epithelial cells (42.9%). Similar was the expression of $p16^{INK4a}$ in CIN II lesions: absent in 54.5% of cases, low grade in 18.2% of cases, moderate grade of expression (5-25% of the cells showed expression) was detected in 18.2% of the cases, while severe grade (more than 25% of positive epithelial cells) was verified only in one case (9.1%). $p16^{INK4a}$ expression in CIN III lesions was verified in 94% of cases. Only in one case the expression was not observed (6% of cases) (Table 1).

Statistical testing found that there was a statistically significant difference in the expression of $p16^{INK4a}$ in relation to the grade of dysplasia. Statistically significant difference in the expression of $p16^{INK4a}$ was present between severe grade dysplasias (CIN III) and dysplasias of low and moderate grade (CIN I and CIN II). The expression is more often verified in dysplasias of severe grade (Table 1).

In lesions in which an inflammatory or reactive change were diagnosed, the expression was absent in most of the cases (61.5%) or had a focal, low intensity (33.3%). Moderate grade of expression in inflammatory changes was verified only in 2 cases (5.2%). This and other studies suggest that the use of immunohistochemical techniques, using antibody for $p16^{INK4a}$, can safely differentiate reactive (inflammatory) changes from preneoplastic and neoplastic lesions of the cervix.

A statistically significant difference in our study was found in expression and grade of the expression of $p16^{INK4a}$ between patients with the diagnosis of inflammatory process (cervicitis) compared to patients who were diagnosed with dysplasia (Table 1). It is noteworthy that the statistically significant difference was not found in the expression of $p16^{INK4a}$ between patients who had inflammation and those with low and moderate grade of dysplasia, respectively CIN I and CIN II (Table 1). Our results of expression of $p16^{INK4a}$ in CIN III lesions are consistent with the results of Sano et al. [25].

Sano and associates have conducted the research on expression of RB protein and $p16^{INK4a}$ in reactive, CIN lesions and cancers. They demonstrated the presence of the expression of $p16^{INK4a}$ in all cancers of the cervix, and the grade of expression was high in 97% of cases. $p16^{INK4a}$ expression in CIN lesions ranged from 53% in CIN I lesions to 100% in CIN III lesions [25].

Results similar to ours were showed by Agoff et al. [26]. They proved that the grade of expression of $p16^{INK4a}$ and Ki-67 correlated highly with the grade of dysplasia in the cervical epithelium, and also with HR-HPV infection.

Klaes et al. [27] examined the association of expression of $p16^{INK4a}$, high-risk HPV infection and the presence of dysplasia. They found that in 139 samples with CIN lesions and 60 samples of the cervical cancer the expression of $p16^{INK4a}$ is generally intensive and diffuse, in contrast to the expression in the surrounding normal stroma and epithelium. Most of the samples showed a cytoplasmic expression. The same authors found that most inflammatory lesions on the cervix (75%) showed no expression of $p16^{INK4a}$. If expression is present in inflammatory lesions, it is generally focal in individual cells. They proved the association of $p16^{INK4a}$ expression and high-risk HPV infection [27].

Due to the large differences in the interpretation of cytological and histological lesions in the mucous membrane of the cervix and the low sensitivity of Pap tests, we are still striving to find an objective biomarker to aid in the diagnosis of cervical neoplasia.

Klaes et al. [28] have made an analysis of the interpretation of biopsy specimens of HE treated samples and the combination of HE staining and immunohistochemical staining using anti-$p16^{INK4a}$. On cervical samples the following diagnoses were determined: normal findings (no dysplasia), cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) and carcinoma of the cervix. The matching in the diagnosis of invasive carcinoma was high (94%), while the matching in the interpretation of CIN lesions ranged from 35% (CIN I) to 72% (CIN III) in materials that are processed only by HE staining. In materials processed with HE staining and immunohistochemistry using the anti-$p16^{INK4a}$, congruence among pathologists in all categories was above 90% [28]. They concluded that the use of $p16^{INK4a}$ reduced the difference in the interpretation of lesions on the cervix among pathologists [28]. Similar results were reached by other authors [29, 30, 31].

In recent years, immunohistochemical and immunocytochemical analysis is of great importance in the interpretation of localized lesions on the cervix and other anatomical regions. The results should be interpreted only in conjunction with the results of other analyses, particularly in accordance with morphological changes. The fact that immunohistochemical analysis of $p16^{INK4a}$ expresion can be used to differentiate lesions with high risk of progression is of great importance for routine diagnostics. It is likely that in the coming years, immunohistochemical and molecular techniques will have an increasingly important role in the diagnosis of lesions localized on the cervix [3].

Our research may lead to a more reliable differentiation of borderline case lesions in the cervical epithelium, in cytological and histological material. Using immunocytochemical and/or immunohistochemical analysis using antibodies to $p16^{INK4a}$ can help us to differentiate lesions cytologically interpreted as ASC-US or ASC-H, and in histology, which are interpreted as uncertain preneoplastic lesions (CIN I, CIN II). Also, we can prevent erroneous diagnosis of reactive changes and their classification as preneoplastic lesions in histological materials and prevent unnecessary treatment (usually surgical). Certainly, and vice versa, this will help prevent the classification of dysplastic lesions in non-dysplastic lesions, and therefore provide timely and appropriate treatment of patients.

CONCLUSION

The expression of $p16^{INK4a}$ in inflammatory lesions of the cervix is rarely present, focal and low intensity. The expression of $p16^{INK4a}$ in preneoplastic lesions of the uterine cervix is often present. Presence and grade of $p16^{INK4a}$ ex-

pression depends on the grade of dysplasia. The expression of $p16^{INK4a}$ in severe grade dysplasias is present, diffuse, and has a high intensity, while in low grade dysplasias it is uncommon, focal, and has a low intensity.

The definitive conclusion about the nature and evolution of lesions of the cervix requires a correlation among observed morphological changes, presence and degree of expression of $p16^{INK4a}$. $p16^{INK4a}$ is a reliable marker that can help in differentiation of borderline lesions of the cervix. By using a reliable biomarker, the diagnostic accuracy in interpreting changes in the cervix is increased and the best treatment of patients is ensured.

ACKNOWLEDGMENT

This paper is a part of the master thesis presented at the Medical Faculty in Banja Luka on February 2013 entitled „Expresion of Inflammatory, Preneoplastic and Neoplastic Cervical Changes – Clinical Significance".

REFERENCES

1. Berek JS. Berek &Novak's Gynecology. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
2. DiSaia PJ, Creasman WT. Clinical Gynecologic Oncology. 7th ed. Philadelphia: Mosby Elsevier; 2007.
3. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA, Sani S, et al. Potential diagnostic value of P16 expression in premalignant and malignant cervicallesions. J Res Med Sci. 2012; 17(5): 428-33.
4. Wei Q, Fu B, Liu J, Xu J, Zhao T. Combined detection of $p16^{INK4a}$ and IMP3 increase the concordance rate between cervical cytologic and histologic diagnosis. Int J Clin Exp Pathol. 2013; 6(8):1549-57.
5. Solomon D, Nayar R. The Bethesda System for Reporting Cervical Cytology. 2nd ed. New York: Springer; 2004.
6. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U. Overexpresion of $p16^{INK4a}$ as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer. 2001; 92:276-84.
7. Liao GD, Sellors JW, Sun HK, Zhang X, Bao YP, Jeronimo J, et al. $p16^{INK4a}$ immunohistochemical staining and predictive value for progression of cervical intraepithelial neoplasia grade 1: a prospective study in China. Int J Cancer. 2014; 134(7):1715-24.
8. Tota JE, Ramana-Kumar AV, El-Khatib Z, Franco EL. The road ahead for cervical cancer prevention and control. Curr Oncol. 2014; 21(2):255-64.
9. Matsukura T, Sugase M. Pitfalls in the epidemiologic classification of human papillomavirus types associated with cervical cancer using polymerase chain reaction: driver and passenger. Int J Gynecol Cancer. 2008; 18(5):1042-50.
10. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin. 2012; 62(3): 147-72.
11. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Saunders Elsevier; 2010.
12. Tsoumpou I, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P, et al. $p16^{INK4a}$ immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treatment Rev. 2009; 35:210-20.
13. Wentzensen N, Bergeron C, Cas F, Vinokurova S, von Knebel Doeberitz M. Triage of women with ASCUS and LSIL cytology: use of qualitative assessment of $p16^{INK4a}$ positive cells to identify patients with high-grade cervical intraepithelial neoplasia. Cancer. 2007; 111:58-66.
14. Indarti J, Fernando D. Comparison of p16INK4a immunocytochemistry with the HPV polymerase chain reaction in predicting high grade cervical squamous intraepithelial lesions. Asian Pac J Cancer Prev. 2013; 14(9):4989-92.
15. Jović M, Nenadić D, Magić Z, Zoltarevski L, Djurdjević-Vukomanović B, Tatomirović Ž, et al. Reliability of the CINtecTM p16INK4a immunocytochemical test in screening cervical precancerous lesions. Vojnosanit Pregl. 2008; 65(3):211-19.
16. Tavassoli FA, Devilee P. Pathology and genetics of tumors of the breast and female genital organs. Lyon: IARCPress; 2003.
17. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types ssociated with cervical cancer. N Engl J Med. 2003; 348(6):518-27.
18. Ferenczy A, Franco E. Cervical-cancer screening beyond the year 2000. Lancet Oncol. 2001; 2(1):27-32.
19. Sahasrabuddhe VV, Luhn P, Wentzensen N. Human papillomavirus and cervical cancer: biomarkers for improved prevention efforts. Future Microbiol. 2011; 6(9):1083-98.
20. Balasubramanian A, Hughes J, Mao C, Ridder R, Herkert M, Kiviat NB, et al. Evaluation of an ELISA for p16INK4a as a screening test for cervical cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18(11):3008-17.
21. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol. Biomarkers Prev. 2008; 17(10):2536-45.
22. Tsoumpou I, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P, et al. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009; 35(3):210-20.
23. Petry KU, Schmidt D, Scherbring S, Luyten A, Reinecke-Lüthge A, Bergeron C, et al. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. Gynecol Oncol. 2011; 121(3):505-9.
24. Schmidt D, Bergeron C, Denton KJ, Ridder R; European CINtec Cytology Study Group. p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL Papanicolaou cytology: results from the European equivocal or lowly abnormal Papanicolaou cytology study. Cancer Cytopathol. 2011; 119(3):158-66.
25. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajlama T. Immunohistochemical overexpresion of p16 protein associated with intact retinoblastoma protein expresion in cervical cancer and cervical intraepithelial neoplasia. Pathol Int. 1998; 48(8):580-5.
26. Agoff SN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA. $p16^{INK4a}$ expresion correlates with degree of cervical neoplasia: a comparasion with Ki-67 expresion and detection of high HPV types. Mod Pathol. 2003; 16(7):665-73.
27. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, et al. Overexpresion of $p16^{INK4a}$ as a specific marker for dysplastic and neoplsatic epithelial cells of the cervix uteri. Int J Cancer. 2001; 92(2):276-84.
28. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D, et al. $p16^{INK4a}$ Immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002; 26(11):1389-99.
29. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA, Sani S, et al. Potential diagnostic value of P16 expression in premalignant and malignant cervicallesions. J Res Med Sci. 2012; 17(5):428-33.
30. Grenko RT, Abendroth CS, Frauenhoffer EE. Variande in the interpretation of cervical biopsy specimens obtaines for atypical squamous cells of undeterminated significance. AM J Clin Pathol. 2000; 114(5):735-40.
31. de Vet HC, Knipschild PG, Schouten HJ, Koudstaal J, Kwee WS, Willebrand D, et al. Sources of interobserver variation in histopathological grading of cervical dysplasia. J Clin Epidemiol. 1992; 45(7):785-90.

Значај имунохистохемијске експресије *p16^{INK4a}* у разликовању инфламацијских и пренеопластичних лезија грлића материце

Живорад Гајанин[1], Раде Вилендечић[2], Весна Ећим Злојутро[2], Радослав Гајанин[2], Павле Будаков[3]

[1]Дом здравља, Котор Варош, Република Српска, Босна и Херцеговина;
[2]Клинички центар Бања Лука, Бања Лука, Република Српска, Босна и Херцеговина;
[3]Универзитет у Београд, Медицински факултет, Нови Сад, Србија

КРАТАК САДРЖАЈ

Увод Већина истраживања указује на главну улогу хуманог папилома вируса (ХПВ) у настанку дисплазије и карцинома грлића материце. Због ниске специфичности и сензитивности морфолошких дијагностичких метода, потребно је пронаћи одговарајући маркер који би био уведен у скрининг-програм цервикалног карцинома. Већина истраживања потврђује да је *p16^{INK4a}* специфичан и сензитиван маркер.

Циљ рада Циљ истраживања је био да се утврди експресија *p16^{INK4a}* у инфламацијским и пренеопластичним лезијама грлића материце.

Методе рада Истраживање је урађено на 73 биоптичка узорка грлића материце. Код 34 испитанице утврђена је пренеопластична промена (дисплазија) у плочасто-слојевитом епителу грлића материце, а код 39 утврђен је неспецифични запаљењски процес. На свим узорцима је урађена имунохистохемијска анализа употребом антитела на *p16^{INK4a}*.

Резултати Експресија *p16^{INK4a}* је потврђена у 67,65% случајева у диспластичним лезијама грлића материце и 38,5% случајева у инфламацијским лезијама. Статистички високо значајна разлика у погледу постојања и степена експресије забележена је између диспластичних и инфламацијских лезија грлића материце (χ^2=24,16; p<0,001). Експресија је била чешћа и већег степена у диспластичним лезијама тешког степена и показала је статистички значајну разлику у односу на експресију у дисплазијама ниског степена (χ^2=21,48; p<0,001).

Закључак Анализом заступљености *p16^{INK4a}* може се диференцирати ненеопластична промена на грлићу материце од пренеопластичних. Препоручује се примена имуноцитохемијске и имунохистохемијске анализе употребом *p16^{INK4a}* у тумачењу граничних лезија на грлићу.

Кључне речи: цервикална интраепителна неоплазија; цервицитис; имунохистохемија; *p16^{INK4a}*; диференцијација

Примљен • Received: 14/01/2014 **Прихваћен • Accepted:** 03/06/2014