Original article

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EVALUATION OF P16^{INK4A} PROTEIN AS A BIOMARKER FOR CERVICAL INTRAEPITHELIAL NEOPLASIA AND SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

Biljana Đorđević and Nikola Živković

The association of human papilloma virus (HPV) infection and cervical intraepithelial neoplasia (CIN) is well known. Interaction of HPV proteins with cellular regulatory proteins leads to up regulation of p16INK4A. The aim of this study was to evaluate p16^{INK4A} protein as a biomarker for CIN lesions and squamous cell carcinoma on biopsy specimens of patients who underwent biopsy of the uterine cervix due to abnormal cytological finding.

The authors analyzed biopsies from 50 patients with CIN and invasive squamous cell carcinoma of the uterine cervix. Expression of p16^{INK4A} in CIN and invasive squamous cell carcinoma was immunohistochemically analyzed by using monoclonal anti-p16^{INK4A} antibody.

A total of 50 patients with CIN and invasive squamous cell carcinoma of the uterine cervix (mean age 40.2±11.5 years, range 20-74 years) were analyzed. CIN I lesions were found in 27 (54%), CIN II/CIN III lesions in 9 (18%), and invasive squamous cell carcinoma in 14 (28%) patients. Differences in the expression of p16^{INK4A} between CIN I, CIN II/CIN III and squamous cell carcinoma were statistically significant ($p<0.0001$). Expression of p16INK4A showed low sensitivity (7%), specificity (8%), positive predictive value (8%), and negative predictive value (7%) for CIN I. Sensitivity, specificity, positive predictive value, and negative predictive value of p16^{INK4A} were 78%, 61%, 30%, and 93% for CIN II/CIN III, and 100%, 75%, 61%, and 100% for squamous cell carcinoma, respectively.

Results of this study suggest that p16^{INK4A} protein may be a sensitive biomarker for CIN II/CIN III lesions and invasive squamous cell carcinoma of the uterine cervix. Acta Medica M*edianae 2011;50(2):29-33.*

Key words: *cervical intraepithelial neoplasia, squamous cell carcinoma, p16^{INK4A}*

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Introduction

The incidence and mortality rate for cervical cancer has dramatically declined since the introduction of cytological screening by using the Papanicolaou (Pap) test (1). The Bethesda System for interpretation of Pap smear findings has established more uniform diagnostic criteria and reduced interpretational errors in cervical cytology (2).

The last revision of the Bethesda System differentiates four categories of cervical squamous cells abnormalities, including atypical squamous cells (ASC), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (2). In histopathology, the cervical intraepithelial neoplasia (CIN) terminology divides precursors of the squamous cell carcinoma into three groups: CIN I, CIN II, and CIN III. Actually, LSIL corresponds to CIN I lesion, and HSIL corresponds to both CIN II and CIN III lesions (3).

Human papilloma virus (HPV) is the most important etiologic agent in the pathogenesis of CIN lesions and cervical carcinoma. It has recently been shown that persistent HPV infection is necessary for morphological changes in the squamous cells of the uterine cervix (4). Integration of HPV DNA into the genome of cervical epithelial cells may lead to an abnormal expression of some cellular proteins such as p16^{INK4A}. It is considered that p16^{INK4A} is a surrogate marker for HPV types of high oncogenic risk and the overexpression of p16^{INK4A} is established in CIN lesions and invasive cervical carcinoma (5-10).

The aim of this study was to evaluate p16^{INK4A} protein as a biomarker for CIN lesions and squamous cell carcinoma on biopsy specimens of patients who underwent biopsy of the uterine cervix due to abnormal cytological finding.

Patients and methods

A retrospective study was conducted in 50 patients with cytological diagnoses of ASC-US, LSIL, HSIL and invasive squamous cell carcinoma, who underwent colposcopy and punch biopsy of the uterine cervix at the Clinic of Gynecology and Obstetrics in Niš, between January 2008 and December 2010.

Cytological findings of the patients were classified according to the 2001 Bethesda System (3).

Histopathological analysis of routinely processed cervical specimens stained with standard hematoxylin-eosin method and immunohistochemical analysis of p16^{INK4A} were done at the Institute of Pathology, Faculty of Medicine, University of Niš.

For p16^{INK4A} detection, a mouse monoclonal anti-p16^{INK4A} antibody (clone 6H12, IgG2b / Newcastle) at a dilution of 1:40 and a standard avidin-biotin immunoperoxidase complexes detection system according to the manufacturer's protocol (Dako LSAB2R system-HRP) were used.

Immunohistochemical reaction for p16^{INK4A} protein was present in the nuclei and/or cytoplasm of cells. The evaluation of p16^{INK4A} protein expression was performed only in the nuclei of cells. Immunohistochemical findings were classified as p16^{INK4A} negative (if ≤10% of the nuclei were stained) or p16^{INK4A} positive (if >10% of the nuclei were stained).

The statistical analysis was performed by using Excel 2000 and Statcal. The results were presented as average, standard deviation, interval of variation (minimum-maximum) and index of structure (%). Chi-square test was used for the evaluation of significance of determined differences for attributive features. Differences were considered significant at $p < 0,05$.

The values for sensitivity, specificity, positive predictive value and negative predictive value of immunohistochemical findings in relation to the histopathological findings were determined as follows: sensitivity = true positive / (true positive + false negative), specificity = true negative / (true negative + false positive), positive predictive value = true positive / (true positive + false positive) and negative predictive value = true negative results / (true negative + false negative).

Results

A total of 50 patients aged from 20 to 74 years were examined. The mean age of patients was 40,2±11,5 years.

Table 1 shows the distribution of cytological and histopathological findings. Cytological finding were classified as ASC-US in 5 (10%) patients. LSIL (CIN I) lesions were found in 20 (40%) patients after cytological examination and 27 (54%) patients after histopathological examination. In 18 (36%) patients, HSIL lesions were cytologically verified and in 9 (18%) patients HSIL (CIN II/CIN III) lesions were histopathologically verified. Squamous cell carcinoma was found in 7 (14%) patients after cytological examination and 14 (28%) patients after histopathological examination.

Immunohistochemical analysis revealed a positive nuclear expression of p16^{INK4A} in 7,41% of CIN I (LSIL) lesions, 77,78% of CIN II/CIN III (HSIL) lesions and 100% of squamous cell carcinomas (Figures 1-3, Table 2). These differences were statistically significant ($\chi 2$ test, $p<0,0001$).

Table 1. Cytological and histopathological finding

Finding	N (%)
Cytological finding	
ASC-US	5 (10)
LSIL	20 (40)
HSIL	18 (36)
Carcinoma	7 (14)
Histopathological finding	
CIN I (LSIL)	27 (54)
CIN II/CIN III (HSIL)	9 (18)
Carcinoma	14 (28)

ASC-US – *atypical squamous cells of undetermined significance;*
LSIL - *low grade squamous intraepithelial lesion*;
HSIL - *high grade squamous intraepithelial lesion*;
CIN – *cervical intraepithelial neoplasia*

Table 2. Histopathological finding and 16^{INK4A} expression

Histo-pathological finding	p16 ^{INK4A} expression			χ^2 test
	N	Positive N (%)	Negative N (%)	(*p*)
CIN I (LSIL)	27	2 (7,41)	25 (92,59)	<0.0001
CIN II/CIN III (HSIL)	9	7 (77,78)	2 (22,22)	
Carcinoma	14	14 (100)	0 (0)	
Total	50	23 (46)	27 (54)	

LSIL - *low grade squamous intraepithelial lesion*;
HSIL - *high grade squamous intraepithelial lesion*;
CIN – *cervical intraepithelial neoplasia*

*Table 3***.** Sensivity, specificity, positive predictive value, and negative predictive value of p16^{INK4A} depending on the histopathological diagnosis

	Histopathological diagnosis		
	LSIL (CIN I) N=27	HSIL (CIN II/CIN III) N=9	Carcinoma N=14
Sensivity	7%	78%	100%
Specificity	8%	61%	75%
Positive predictive value	8%	30%	61%
Negative predictive value	7%	93%	100%

LSIL - *low grade squamous intraepithelial lesion*;
HSIL - *high grade squamous intraepithelial lesion*;
CIN – *cervical intraepithelial neoplasia*

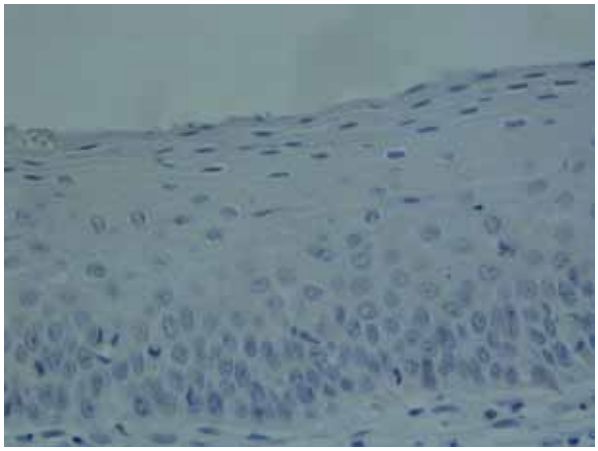

Figure 1. Negative expression of p16^{INK4A} in CIN I lesion (LSAB, x400)

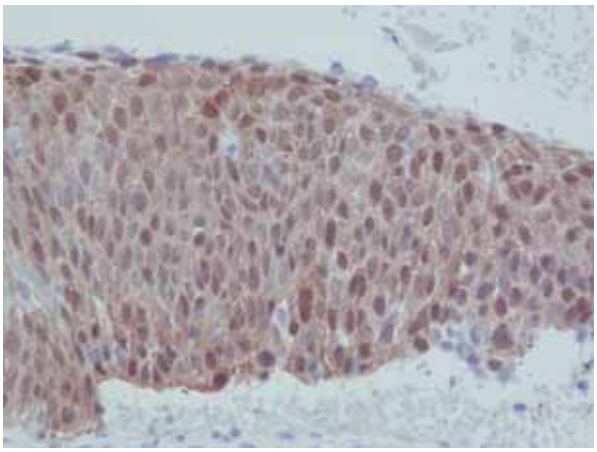

Figure 2. Positive expression of p16^{INK4A} in CIN III lesion (LSAB, x400)

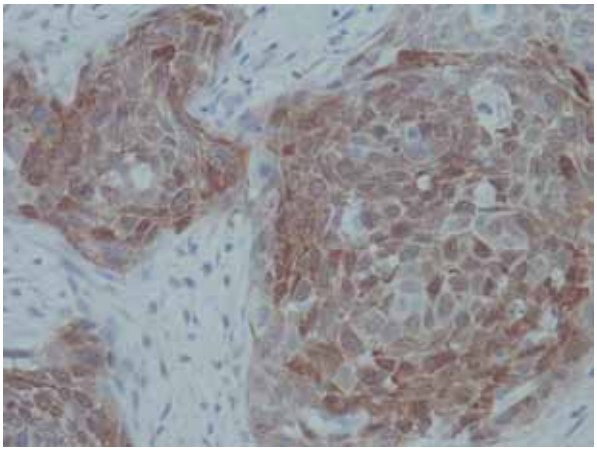

Figure 3. Positive expression of p16I^{NK4A} in squamous cell carcinoma (LSAB, x400)

Table 3 shows values for sensitivity, specificity, positive predictive value and negative predictive value of p16^{INK4A}, depending on the histopathological diagnosis. Expression of p16^{INK4A} showed low sensitivity (7%), specificity (8%), positive predictive value (8%) and negative predictive value (7%) for CIN I (LSIL) lesions. In contrast, the sensitivity, specificity, positive predictive value and negative predictive value of p16^{INK4A} were 78%, 61%, 30% and 93% for CIN II/CIN III (HSIL) lesions and 100%, 75%, 61% and 100% for invasive squamous cell carcinoma.

Discussion

The occurrence of invasive squamous cell carcinoma of the uterine cervix is preceded by precancerous lesions, which are termed cervical intraepithelial neoplasia (CIN). According to the severity, CIN lesions are divided into CIN I, CIN II and CIN III. It is now believed that CIN II and CIN III lesions occur as a result of a persistent infection with HPV types of high oncogenic risk, but also in presence of bacterial vaginosis (11). In persistent HPV infections, HPV DNA was integrated into the host DNA (4). Integration of viral DNA into the genome of cervical epithelial cells leads to abnormal expression of several cellular proteins. One of them is p16^{INK4A} protein that regulates cell cycle.

There are different data regarding the expression of p16^{INK4A} in CIN lesions and invasive cervical carcinoma (12-29). According to the data, p16^{INK4A} is expressed in 0-100% of LSIL (CIN I) lesions, 45-100% of HSIL (CIN II/CIN III) lesions and 93-100% of invasive cervical carcinoma. In this study, p16^{INK4A} expression was found in 77,78% of HSIL (CIN II/CIN III) lesions and 100% of invasive squamous cell carcinoma of the uterine cervix. Also, expression of p16^{INK4A} was found in 7,41% of LSIL (CIN I) lesions. Similar results were registered by Haidopoulos et al. (27), who found expression of p16 ^{INK4A} in 6,07%of LSIL lesions.

In this study, the sensitivity of p16^{INK4A} expression in detecting of CIN II/CIN III lesions and invasive squamous cell carcinoma of the uterine cervix was high (75% for CIN II/CIN III lesions and 100% for invasive squamous cell carcinoma). In contrast, the sensitivity of p16^{INK4A} expression in detecting of CIN I lesions was low (7%). The specificity of p16^{INK4A} expression for CIN I lesions, CIN II/CIN III lesions and invasive squamous cell carcinoma was 8%, 61% and 75%, respectively. The positive predictive value of p16^{INK4A} expression was 8% for CIN I lesions, 30% for CIN II/CIN III lesions and 61% for invasive squamous cell carcinoma. The negative predictive value of p16^{INK4A} expression for CIN I lesions, CIN II/CIN III lesions and invasive squamous cell carcinoma of uterine cervix was 7%, 93% and 100%, respectively.

Conclusion

Based on the results of this study, it was concluded that p16^{INK4A} protein may be a sensitive biomarker for CIN II/CIN III lesions and invasive squamous cell carcinoma of the uterine cervix.

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EVALUACIJA P16^{INK4A} PROTEINA KAO BIOMARKERA ZA CERVIKALNU INTRAEPITELNU NEOPLAZIJU I SKVAMOCELULARNI KARCINOM GRLIĆA MATERICE

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Udruženost infekcije humanim papiloma virusima (HPV) i cervikalne intraepitelne neoplazije (CIN) dobro je poznata. Interakcija HPV proteina sa ćelijskim regulatornim proteinima dovodi do ushodne regulacije 16^{INK4A}. Cilj rada bila je evaluacija p16INK4 proteina kao biomarkera za CIN lezije i skvamocelularni karcinom na biopsijskim uzorcima bolesnica koje su bile podvrgnute biopsiji grlića materice zbog nenormalnog citološkog nalaza.

Autori su analizirali biopsijske uzorke 50 bolesnica sa CIN lezijama i invazivnim skvamocelularnim karcinomom grlića materice. Ekspresija p16^{INK4A} u CIN lezijama i invazivnom skvamocelularnom karcinomu grlića materice analizirana je imunohisto-hemijski upotrebom monoklonskog anti- p16^{INK4A} antitela.

Analizirano je ukupno 50 bolesnica sa CIN lezijama i invazivnim skvamocelularnim karcinomom grlića materice prosečnog starosnog doba 40.2±11.5 godina (opseg 20-74 godine). CIN I lezije nađene su kod 27 (54%), CIN II/CIN III lezije kod 9 (18%) i invazivni skvamocelularni karcinom kod 14 (28%) bolesnica. Razlike u ekspresiji p16^{INK4A} između CIN I lezija, CIN II/CIN III lezija i invazivnog skvamocelularnog karcinoma bile su statistički značajne ($p<0.0001$). Ekspresija p16INK4A pokazivala je nisku senzitivnost (7%), specifičnost (8%), pozitivnu prediktivnu vrednost (8%) i negativnu prediktivnu vrednost (7%) za CIN I. Senzitivnost, specifičnost, pozitivna prediktivna vrednost i negativna prediktivna vrednost p16^{INK4A} iznosile su 78%, 61%, 30% i 93% za CIN II/CIN III i 100%, 75%, 61% i 100% za skvamocelularni karcinom.

Rezultati ove studije ukazuju da p16^{INK4A} protein može biti senzitivni biomarker za CIN II/CIN III lezije i invazivni skamocelularni karcinom grlića materice. *Acta Medica Medianae 2011;50(2):29-33.*

Ključne reči: *cerviklna intraepitelna neoplazija, skvamocelularni karcinom, p16^{INK4A}*