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Immunohistochemical assessment of p16, COX-2 and EGFR in HPV-positive cervical squamous intraepithelial lesions

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Abstract

The protein capsid L1 of the human papilloma virus (HPV) – a key factor in the cervical carcinogenesis – is considered, together with p16, EGFR and COX-2, a characteristic marker for the evaluation of the malignancy progression and prognostic, in terms of tumoral aggressiveness. The purpose of the present study was to make a comparative assessment between the immunohistochemical pattern of p16, EGFR and COX-2 and immunochemical expression of L1 HPV capsid protein, in low grade and high-grade cervical squamous intraepithelial lesions, in order to determine the relationship of these tumoral markers with the infection status of HPV, and their practical applicability in patients diagnosis and follow-up. The study group included 50 women with cytological and histopathological confirmed LSIL (low grade SIL) and HSIL (high-grade SIL). The immunoexpression of L1 HPV protein was assessed on conventional cervico-vaginal smears and EGFR, COX-2 and p16 were immunohistochemically evaluated on the corresponding cervical biopsies. From all cervical smears, the HPV L1 capsid protein was expressed in 52% of LSIL and 23% of HSIL. From all cervical biopsies, p16 was positive in 64% of LSIL, 82% of CIN2 and 100% of CIN3, EGFR was overexpressed in 67% of HSIL (56% CIN2 and 43% CIN3) and 32% LSIL. For COX-2, the Allred score was higher in HSIL when compared to LSIL. Our data revealed 33 cases belonging to both LSIL and HSIL categories with the same Allred score. Immunochemical detection of L1 capsid protein, on cervico-vaginal smears, indicates an immune status induced by the HPV infection and may offer prognosis information, mainly in LSIL lesions. The assessment of p16, EGFR, and COX-2 allows to an integrative approach for the progression of squamous intraepithelial lesion, associated or not with the HPV infection.

Keywords: HPV L1 protein capsid, p16, EGFR, COX-2, LSIL, HSIL.

Introduction

In the cervical carcinogenesis, the human papilloma virus (HPV) is essential, the presence of high-risk (including intermediate-risk) HPVs being documented in nearly all invasive cancers and in up to 90% of precancerous lesions [1–3]. Many observations have indicated the importance of immune response in HPV infection. HPV L1 capsid protein is expressed in the active phase of the viral infection and is necessary in viral cellular cycle completion. Thus, the immunochemical expression of the capsid protein is an evidence of active HPV infection in examined tissue [4]. L1 viral capsid protein is considered a major target of the cellular immune response [5]. LSIL and moderate SIL without immunochemically detected L1 are correlated, in more than 80% of cases, with dysplasia progression [6]. Most probably, the lack of HPV antigen is determined by a weak protein synthesis, under the minimum level of the immunochemical test. The immunochemical detection of L1 capsid, on Papanicolau smears, may consequently indicate the defense status locally induced on HPV infection and may offer prognosis information in different squamous intraepithelial lesions.

The tumor suppressor protein p16 is a cyclin-dependent kinase inhibitor that regulates transition from the G1 to the S phase of the cell cycle [7, 8]. The high immunoexpression of the p16 was previously reported to be characteristic for dysplastic and neoplastic epithelium of the cervix [9–11]. The viral oncoprotein E7 accounts for the major transforming and immortalizing activity in high-risk types of HPV. E7 contains also a binding site for retinoblastoma gene (Rb) [12]. Rb is involved in regulation of cell proliferation, suffering various phosphorilation degrees during cell cycle. PRb inhibits also the transcription of an inhibitory gene of cyclin-dependent kinase p16(INK4A), with a role in cell cycle proliferation. Through blocking of pRb function is produce overexpression of p16(INK 4A) in cells. Recent studies have proposed that p16 is a useful marker for HR (high-risk) HPV-type related cervical neoplasia and for predicting SIL progression [13, 14].

The Epidermal Growth Factor Receptor (EGFR) is a member of the ErbB family, the tyrosine kinase receptors with growth promoting effects [15], including a recognized angiogenic potential. Human EGFR gene is localized on chromosome 7 and codifies a surface transmembranar glycoprotein, which binds Epidermal Growth Factor (EGF), Transforming Growth Factor alpha, amphiregulin, and Heparin-binding Growth Factor. In the EGFR activation, HPV-E5 oncogene may be involved and this can occur without concomitant growth of the receptors number [16, 17]. HPV-E6 oncogene may establish afterwards the increase of EGFR mARN level and the stabilization of the protein, and therefore will increase the signal transduction in the cells. HPV-E5 oncogene establishes an acceleration of Her-2/neu (c-erbB2) gene protein activation. EGFR seems to have, together with c-erbB2 and c-myc, an important role in the prognostic of advanced cervical cancer. However, its involvement in early stages or in the development of preneoplastic lesions is still unclear and controversial, being mentioned years before, abandoned and resumed again.

The cyclooxygenase 2 (COX-2) regulates the prostaglandins synthesis, and the metabolic residues produced by the COX-2 action against the arachidonic acid are involved in the carcinogenesis through several mechanisms, such as the inhibition of apoptosis and immune surveillance, and the increase of the neoangiogenesis [18]. Consequently, COX-2 has role in the onset and progression of malignancies, including the cervical carcinoma, and is considered as a marker of tumor aggressiveness [18, 19]. Unfortunately, only a small number of studies reported in the publication mainstream focus on the relationship between the COX-2 expression and the HPV detection, in pre-invasive cervical lesions [18, 20, 21].

The purpose of the present study was to make a comparative assessment between the immunohistochemical pattern of p16, EGFR and COX-2 and immunochemical expression of L1 HPV capsid protein, in low grade and high-grade cervical squamous intraepithelial lesions, in order to determine the relationship of these tumoral markers with the infection status of HPV, and their practical applicability in patients diagnosis and follow-up.

Materials and Methods

The study group included 50 women with cytological and histopathological confirmed LSIL (low grade SIL) (CIN1, cervical intraepithelial neoplasia) (n=32) and HSIL (high-grade SIL) (eight cases of CIN2 and 10 cases of CIN3) (n=18). The immunoexpression of L1 HPV protein was assessed on conventional cervicovaginal smears and EGFR, COX-2 and p16 were immunohistochemically evaluated on the corresponding cervical biopsies.

The cervico-vaginal smears were fixed and stained with Papanicolaou method. After the routine cytodiagnosis, the cervicovaginal smears were used to detect HPV L1 capsid protein by immunocytochemistry, using the monoclonal antibodies (Cytoactiv HPV L1 High Risk Set REF SCA0850, Cytoimmun Diagnostics GmbH). Epithelial cells with positive nuclear staining were scored as positive, considering one stained nucleus enough for scoring.

The tissue sections obtained from the cervical biopsies were investigated by routine histopathological exam and by immunohistochemistry, using p16, EGFR, and COX-2 antibodies. The collected tissues were fixed for 24 hours in buffered formalin and processed for paraffin embedding. The serial sections of 4–5 μm were dewaxed and stained with Hematoxylin–Eosin, or furthermore prepared for immunohistochemistry.

For EGFR, PIER (proteinase-induced epitope retrieval) technique was performed using Proteinase K (code S3020, DAKO, Denmark), for 5 minutes at room temperature. For COX-2 and p16-D25 antibodies, HIER (heat-induced epitope retrieval) technique was performed using Target Retrieval Solution pH 9 (code S2367, DAKO, Denmark) and respectively, Target Retrieval Solution pH 6 (code S1700, DAKO, Denmark). After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with the primary antibodies, anti-EGFR mouse monoclonal antibody (clone E30, code M7239, DAKO, Denmark), dilution range 1:50, anti-COX-2 mouse monoclonal antibody (clone CX-294, code M3617, DAKO, Denmark), dilution range 1:80 and anti-p16 mouse monoclonal antibody (clone D25, code sc-81613, Santa Cruz, USA), dilution range 1:100. The immune reaction was amplified using the appropriate secondary antibody and the Streptavidin–Biotin– Peroxidase HRP complex (code K5001, DAKO, Denmark). Sections were then developed using 3,3'-diaminobenzidine tetrahydrochloride chromogen (DAB, code K5001, DAKO, Denmark) and counterstained with Lille's Hematoxylin.

The presence of the p16 protein was scored based upon the estimated proportion of immunopositive cells, as follows: 0 – none, 1 – <25% immunopositive cells, 2 – between 25–75% immunopositive cells, 3 – >75% immunopositive cells. The intensity of the immunohistochemical reaction, either weak, moderate or strong, was not considered for scoring.

The EGFR immunohistochemical expression was quantified according to a proposed score system [22], as follows: 0 score: no staining observed, or membrane staining in <10% neoplastic cells, negative; 1+ score: weak complete and/or incomplete membrane staining in >10% neoplastic cells, positive; 2+ score: moderate complete and/or incomplete membrane staining in >10% neoplastic cells, positive; 3+ score: strong complete and/or incomplete membrane staining in >10% neoplastic cells, positive.

The COX-2 immunohistochemical expression was quantified in accordance to Allred score [23], and compared across histological categories. Allred score was established using a 0–8 scale based upon the sum of a proportion score (percent of stained cells) and intensity score (weak, intermediate, and strong) (Table 1). The possible values of Allred score are: 0 – Allred 0*; 1 – Allred 2, 3, 4; 2 – Allred 5, 6; 3 – Allred 7, 8 (*Allred score 1 is not possible).

Table 1 – *The Allred score*

Proportion score (PS)		Intensity score (IS)	
Value	*Significance*	*Value*	*Significance*
0	None	*0*	None
1	<1%	*1*	Weak
2	1–10%	*2*	Intermediate
3	10–33%	*3*	Strong
4	33–66%		
5	>66%		

Results

In all 50 studied cervical biopsies, the first cytological diagnosis was consistent with the histopathologic diagnosis (32 cases with LSIL (CIN1), 18 cases with HSIL (eight cases of CIN2 and 10 cases of CIN3/CIS)). The HPV infection was morphologically confirmed by the presence of cytopatic HPV effect in the intermediate and superficial squamous cells (koilocytes) from the smears and biopsies.

Immunochemistry in cervical smears

From all cervical smears, the HPV L1 capsid protein was expressed in 52% of LSIL and 23% of HSIL. The expression of L1-capsid protein was significantly reduced for HPV-positive HSIL. In HPV-positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. The strong staining of the nucleus surrounded by a cytoplasm, with no background, confirmed the positive reaction. The reaction for HR–HPV L1 was positive in typical koilocytes or in dyskeratocytes, presenting nuclear morphological characteristics for HSIL (CIN 2 or CIN 3). In LSIL cases, the nuclei were positive only in typical koilocytes (Figure 1).

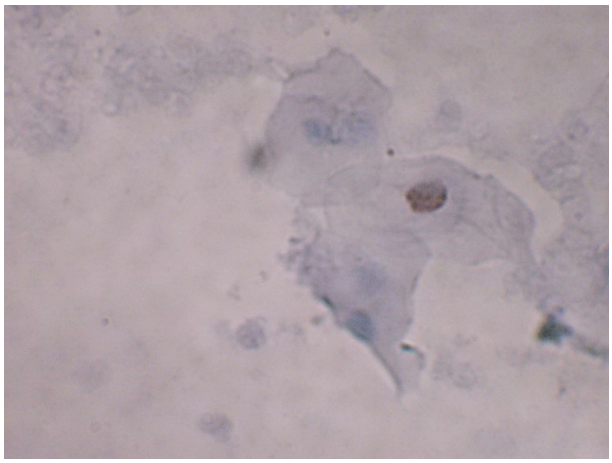

Figure 1 – ***Conventional smear, LSIL, superficial squamous cell with HPV cytopatic effect, with positive nuclear staining (anti-HR HPV L1, ob. ×40).***

Immunohistochemistry in cervical biopsies

From all cervical biopsies, p16 was positive in 64% of LSIL (two cases with score 1, six cases with score 2 and 12 cases with score 3) (Table 2), 82% of CIN2 and 100% of CIN3 (two cases with score 1, eight cases with score 2 and six cases with score 3) (Table 3).

Table 2 – *The semi-quantitative assessment for p16, EGFR and COX-2 in LSIL cases*

Case No.	LSIL				
	P16	*EGFR*	*COX Allred score*		
	Score [%]	*Score [%]*	*PS*	*IS*	*Total*
1.	1	0	2	2	4
2.	0	0	2	1	3
3.	3	0	3	3	6
4.	0	0	2	2	4
5.	3	0	3	3	6
6.	3	2	4	3	7
7.	2	1	2	3	5
8.	2	0	3	3	6
9.	3	2	4	3	7
10.	2	0	3	2	5
11.	0	1	2	2	4
12.	1	0	2	3	5
13.	2	0	4	2	6
14.	3	2	3	4	7
15.	0	0	3	2	5
16.	0	0	2	1	3
17.	3	2	4	3	7
18.	2	0	3	2	5
19.	3	0	4	2	6
20.	3	3	4	3	7
21.	0	0	2	2	4
22.	0	0	2	1	3
23.	2	0	3	2	5
24.	0	1	3	2	5
25.	3	2	4	3	7
26.	0	0	2	1	3
27.	0	0	3	2	5
28.	3	2	4	2	6
29.	0	0	2	2	4
30.	0	0	2	1	3
31.	3	0	3	3	6
32.	3	0	3	2	5

Table 3 – *The semi-quantitative assessment for p16, EGFR and COX-2 in HSIL cases*

Case No.	HSIL				
	P16	*EGFR*	*COX Allred score*		
	Score [%]	*Score [%]*	*PS*	*IS*	*Total*
1.	0	0	3	3	6
2.	3	3	4	3	7
3.	3	3	5	3	8
4.	3	3	5	3	8
5.	1	2	4	3	7
6.	0	0	3	2	5
7.	2	1	4	2	6
8.	3	3	5	3	8
9.	2	3	5	3	8
10.	2	0	5	2	7
11.	3	3	5	3	8
12.	2	3	5	2	7
13.	2	0	4	3	7
14.	2	0	4	3	7
15.	2	3	5	3	8
16.	1	0	3	3	6

Case No.	HSIL				
	P16	*EGFR*	*COX Allred score*		
	Score [%]	*Score [%]*	*PS*	*IS*	*Total*
17.	2	3	5	2	7
18.	3	3	5	3	8

The p16 staining pattern was predominantly nuclear with occasional cytoplasmic positivity. Most cases presented heterogeneity of staining, with positive cells admixed with negative cells. P16 presented a positive immune reaction in the precancerous lesions, with or without L1 HPV-positivity.

The proportion of biopsies with intense immunoexpression of p16 increased with the severity of cytological abnormality. In LSIL cases, the staining distribution was basal in 72% of cases (Figure 2) and dispersed in 28%. The staining intensity of LSIL cases was strong in 26% of cases, moderate in 16% of cases, and weak in 58% of cases. Regarding HSIL category, the staining distribution was as follows: 62% – full thickness (Figure 3), 38% – 1/3 and 2/3 of the epithelium thickness. The staining intensity for HSIL cases was strong in 75% of cases, moderate in 12% of cases and weak in 13% of cases.

From all biopsied cases, EGFR was overexpressed in 32% of LSIL (with score values: 1 – three cases, 2 – six cases, 3 – one case) (Table 2) and 67% of HSIL (56% CIN2 and 43% CIN3) (with score values: 1 – one case, 2 – one case, 3 – 10 cases) (Table 3). The EGFR staining pattern was predominantly membranar with occasional cytoplasmic positivity. Most cases presented heterogeneity of staining, with positive cells admixed with negative cells. The proportion of biopsies with intense immunoexpression of EGFR increased with the severity of cytological abnormality. EGFR staining was observed in basal and parabasal cells, in koilocytes and in dysplastic squamous cells of the intraepithelial lesions. Regarding LSIL category, the staining distribution was identified in basal and parabasal cells and in koilocytes (Figure 4), and was considered strong in 10% cases, moderate in 64% cases, and weak in 26% cases. In HSIL cases, the staining distribution was as follows: 72% full thickness, 28% in basal and intermediate layers, and the staining intensity was assessed as strong in 87% cases, moderate in 7% cases, and weak in 6% cases, being more intense in CIN2 lesions (Figure 5) than in CIN3.

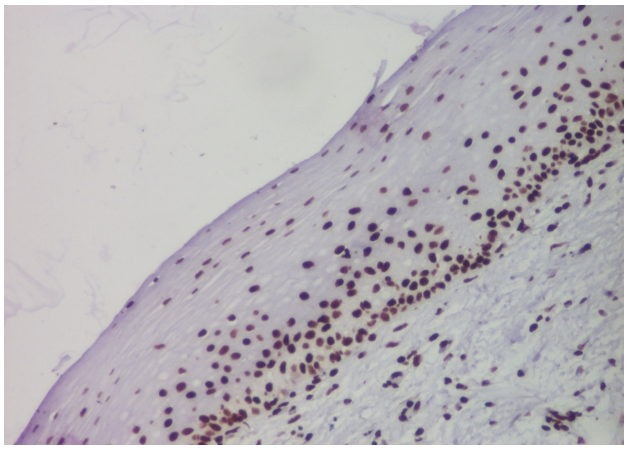

Figure 2 – ***Stratified squamous exocervical epithelium displaying LSIL, strong nuclear staining in the basal third of the epithelium (anti-p16, ob. ×10).***

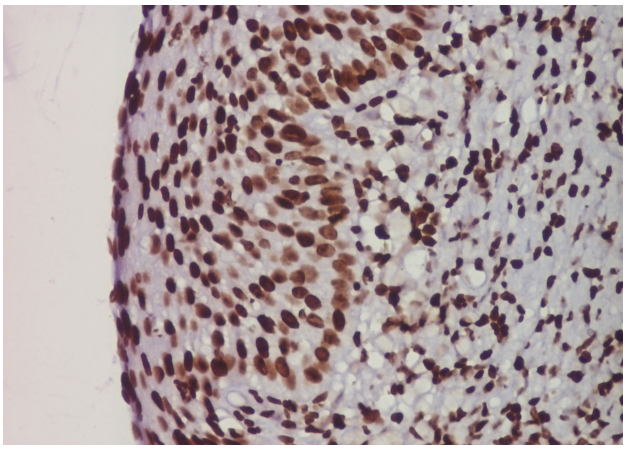

Figure 3 – ***Stratified squamous exocervical epithelium displaying HSIL, corresponding to CIN3 lesion, revealed strong nuclear staining in the entire thickness of the epithelium (anti-p16, ob. ×20).***

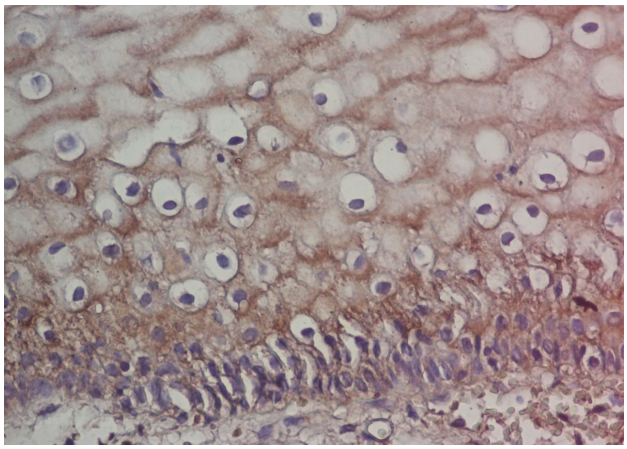

Figure 4 – ***Stratified squamous exocervical epithelium displaying LSIL, revealed a strong membranar staining in koilocytes and dysplastic basal squamous cells (anti-EGFR, ob. ×20).***

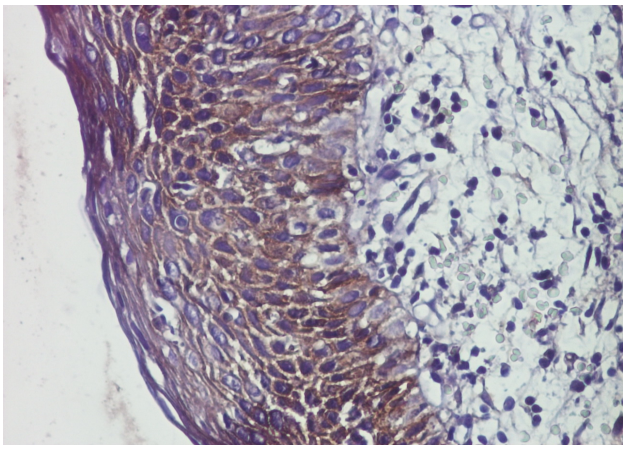

Figure 5 – ***Stratified squamous exocervical epithelium displaying HSIL, corresponding to CIN2 lesion, revealed a strong membranar and diffuse cytoplasmic staining, excepting the superficial layer (anti-EGFR, ob. ×20).***

COX-2 expression showed finely granular cytoplasmic staining with occasional membrane staining, especially in koilocytes. The stromal inflammatory cells, in cases with associated chronic cervicitis, were also intense positive for COX-2.

The results of the semiquantitative exam, based on the Allred score applied to the immunohistochemical reactions, were summarized in Tables 2 and 3. The general score was higher in HSIL when compared to LSIL. Our data revealed 33 cases belonging to both LSIL and HSIL categories with the same Allred score, as follows: nine LSIL cases and one HSIL case with value 5, seven LSIL cases and three HSIL cases with value 6, six LSIL cases and seven HSIL cases with value 7. Regarding the intensity of cytoplasmic COX-2 immunostaining, a weaker expression was observed in specimens with LSIL (Figure 6) and a stronger one in those diagnosed with HSIL (Figure 7). The highest score was noted in HSIL corresponding to CIS lesions. No correlation between the intensity of the COX-2 immunostaining and the presence of the koilocytes within the squamous dysplastic epithelium was found.

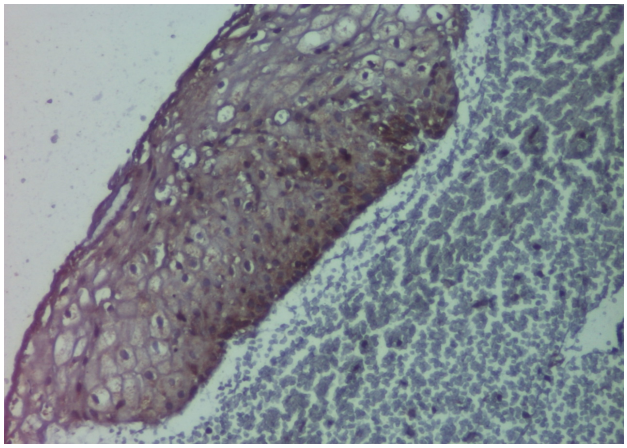

Figure 6 – ***LSIL, showed a strong, heterogeneous cytoplasmic staining in the basal third of the stratified squamous exocervical epithelium (anti-Cox2, ob. ×10).***

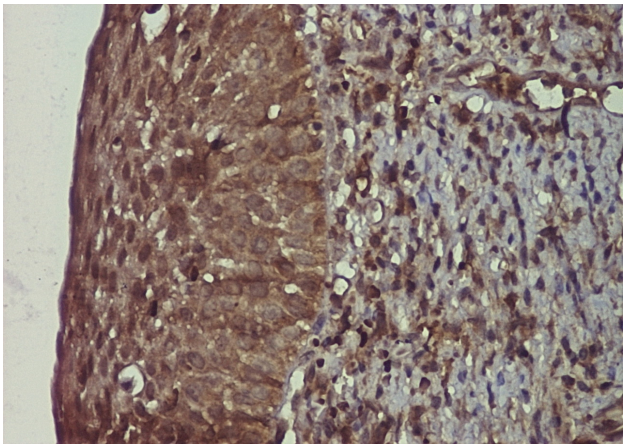

Figure 7 – ***HSIL, corresponding to CIN2 lesion, showed a strong, heterogeneous cytoplasmic staining of the stratified squamous exocervical epithelium; the stromal lymphocytes display also moderate cytoplasmic staining (anti-Cox2, ob. ×20).***

Discussion

The HPV infection of the squamous epithelium occurs in the basal layer of the cervical epithelium, this being the only part of the epithelium capable of mitotic activity necessary to induce epithelial transformation. There are still many questions about the mechanism of HPV infection, regarding the presence of receptors on the target cells, the infection of the target epithelium by mature virions or sequences of viral DNA, or the pathway of viruses' travel across the cytoplasm to reach the nucleus [24]. It is considered that the carcinogenic role of the HPV can only manifest under certain conditions that favor its persistence, one of them being the host immunodeficiency [24]. A high frequency of viral infection and precancerous lesions was observed in immunosupressed women, particularly women infected with human immunodeficiency virus (HIV) and women with AIDS [25, 26]. According to the literature, there are no specific associations of HPV types with the precursor lesions of cervical cancer [24]. All HPV types (low-risk, intermediate, and high-risk) can occur in precancerous lesions, but their severity and behavior cannot be correlated with viral type [24].

Because L1 capsid protein is expressed in the active phase of HPV infection, the viral protein immunodetection is an evidence of the active HPV infection in the examined tissue [4]. It is well known that L1 viral capsid protein is considered a major target of the cellular immune response [5]. The squamous intraepithelial lesions, without L1 HPV immunodetection are correlated, in more than 80% of cases, with dysplasia progression [6]. Immunochemical detection of L1 capsid protein, on conventional Papanicolau smears, may be a local defense status induced by HPV infection and may offer prognosis information, mainly in LSIL lesions.

Our current data, based also on our previous studies [27], sustain that L1 HPV positivity represent a favorable expression of the immune status and, consequently, ensure a relative protection against the progression of the lesions toward the malignant framework. In our group, HPV L1 capsid protein was expressed in 52% of LSIL and 23% of HSIL, diagnosed on the cervicovaginal smears. Expression of L1 capsid protein was significantly reduced for HPV-positive HSIL and less reduced in HPV-positive LSIL. Because of the low rate of HR-HPV L1 positivity found in LSIL cases in our study, we can suggest that HPV is not helpful in grading cervical SIL, which is in accordance with the literature data [28].

The tumor-suppressor protein p16 regulates transition from the G1 to the S-phase of the cell cycle [7, 8]. The viral oncoprotein E7 accounts for the major transforming and immortalizing activity in high-risk types of HPV. E7 contains also a binding site for retinoblastoma gene (Rb) [12], which is involved in regulation of cell proliferation, suffering various phosphorilation degrees during cell cycle. PRb inhibits also the transcription of an inhibitory gene of cyclin-dependent kinase p16(INK4A), with a role in cell cycle proliferation. The overexpression of p16(INK4A) in cells is the consequence of pRb blocked function.

Recent researches highlight the role of p16, as an

extremely sensitive marker for cervical epithelial dysplasia and high-risk HPV-type related neoplasia [9–11, 29, 30], and for predicting SIL progression [13, 14]. Strong and full thickness staining of p16 in the cervical epithelium is highly supportive of HSIL, while weak and basal/rare staining favors LSIL [8].

In our study, from all cervical biopsies, p16 was positive in 64% of LSIL, 82% of CIN2 and 100% of CIN3. The proportion of biopsies with intense immunoexpression of p16 increased with the severity of cytological abnormality, which is consistent with our previous study [27] and with the data reported in the literature [31–34]. For the early diagnosis, p16 can contribute as an adjuvant tool in the follow-up of cervical intraepithelial lesions when the cytology sample is collected in the standard way [35]. Moreover, the combination between L1 HPV capsid protein and p16 is considered to be more useful, having a higher accuracy than L1 or p16 alone [36]. There are also opinions for the relevance of p16 expression in cervical squamous and glandular epithelium, as a marker of dysplasia or malignancy irrespective of the HPV infection status [37]. Our results reveal an overexpression of p16 in the precancerous lesions, with or without L1 HPV positivity, as follows: p16-positive with HPV L1-positive in 28% of LSIL and 6% of HSIL; p16 positive with HPV L1-negative in 36% of LSIL and 17% of HSIL.

These data can be interpreted in the context of the HPV immune behavior, translated by the avoidance of the host immune response, through late expression of antigenic protein responsible for antibodies production. HPV induces local immune dysfunction characterized by the decrease of the intraepithelial antigen presenting cells and of the cytotoxic T-lymphocytes. It is possible that in the case of a low secretion of the protein L1 below the limit of its immunodetection, there is no antibodies production and no lymphocytes activation. Thus, will increase the chance for infection to persist and for the intraepithelial squamous lesion to progress, statement in concordance with our results presented above, in which the lack of immunoexpression of the HPV capsid protein L1 was more correlated with the overexpression of p16. Therefore, our results support the idea that the squamous intraepithelial lesions from the patients with an altered or disturbed immune status are more likely to progress toward a high-grade dysplasia or carcinoma, than those from the patients with an active HPV infection, in which the cellular immune response is manifested through its particular mechanisms.

At the cervical level, EGFR is present in normal status and malignant conditions with varying degrees of expression. In normal cervical mucosa, EGFR is expressed in the cytoplasm and the membrane of the cells within the basal layer, and as cells differentiate, there is a shift toward the cytoplasm [38]. It is admitted that the high expression of EGFR, in collaboration with viral oncoproteins (E6 and E7) and the activation and overexpression of mTOR pathway (mammalian target of rapamycin inhibitor), plays a key role in both high-grade squamous intraepithelial lesions and invasive squamous cell carcinoma [39]. HPV infection may change the biology of EGFR expression by preventing EGFR degradation [40]. Recently, controversial data are published regarding the association of EGFR overexpression with the poor prognosis of the squamous precancerous lesions [41, 42]. The exact biological mechanisms, which promote cell growth and the relationship between viral proteins, gene amplification, decreased levels of phosphatase, and coexpression of EGF, TGF-α, amphiregulin are not completely understood and still represent subjects of investigation [43].

In our study, from all cervical biopsies, EGFR was overexpressed in 67% of HSIL (56% CIN2 and 43% CIN3) and 32% LSIL. The staining pattern was predominantly membranar, with occasional cytoplasmic positivity. Most cases presented heterogeneity of staining, with positive cells admixed with negative cells. The number of biopsies with intense immunoexpression of EGFR increased with the severity of cytological abnormality.

Regarding high-grade squamous lesions, the immunostaining was more intense in CIN2 lesions than in CIN3. According to our findings, the expression of EGFR can be associated with HPV infection, as EGFR expression increases with increasing grade of the intraepithelial squamous lesion, but not with HPV type.

It is already admitted that the inflammatory COX–prostaglandins axis is elevated in ovarian, endometrial and cervical cancers [44–48]. This pro-inflammatory pathway can be induced by a variety of stimuli (cytokines, growth factors and tumor-promoting chemical carcinogens) [49, 50]. The overexpression of COX-2 could impair host immune responses, as suggested by the ability of COX-2 inhibitors to revert tumor-induced immunosuppression [51].

Opposite opinions are published, some papers showing that the expression of COX-2 increases with the severity of the grade of cervical dysplasia [52, 53], other considering no correlations with the disease severity [20]. As we mentioned above, there are few information on the COX-2 expression induced by the HPV infection, in pre-invasive cervical lesions and cervical cancers [18, 21, 54].

In the present study, we investigated also the immunoexpression of COX-2 in cervical precancerous lesions of low grade and high-grade. Our data show that COX-2 levels are increased with the progression of the squamous intraepithelial lesions, with different degrees of overexpression from LSIL (CIN1) to HSIL (CIN2, CIN3/CIS). Furthermore, the stromal inflammatory cells of associated chronic cervicitis were also intense positive for COX-2. Consequently, we consider our results as supplementary evidences in order to sustain that COX-2 induction begins in the premalignant phase of cervical carcinogenesis and is correlated with inflammation [55]. Additionally, in the context of the confirmed HPV infection, we must stress the COX-2 expression of the cases presenting p16 positivity.

Nevertheless, our general results suggest that COX-2 and EGFR are closely related to each other and this interaction play an important role in the tumor development and progression of the squamous pre-

cancerous lesions induced by HPV infection. Certainly, there are other several factors that regulate EGFR expression and activity, thus COX-2 alone cannot determine the absolute expression level of EGFR in neoplastic cells [56]. Other studies support our result [57, 58], despite convergent data that suggest the possibility of a specifically down-regulated EGFR expression through the COX-2 overexpression [19].

Conclusions

Immunochemical detection of L1 capsid protein, on cervicovaginal smears, indicates an immune status induced by the HPV infection and may offer prognosis information, mainly in LSIL lesions. The assessment of p16, EGFR, and COX-2 allows to an integrative approach for the progression of squamous intraepithelial lesion, associated or not with the HPV infection.

Acknowledgments

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