EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AND HUMAN PAPILLOMAVIRUS (HPV) L1 CAPSID PROTEIN IN CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS

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Abstract: We analyzed the immunohistochemical pattern of epidermal growth factor receptor (EGFR) in cervical squamous intraepithelial lesions (SILs) in correlation with L1 HPV capsid protein, in order to determine the relationship between EGFR expression and the infection status of human papillomavirus (HPV). The study included 40 cases, 24 LSIL (low grade SIL) (CIN1, cervical intraepithelial neoplasia) and 16 HSIL (high grade SIL) (6 cases of CIN2 and 10 cases of CIN3). The immunoexpression of L1 HPV protein was assessed on conventional cervico-vaginal smears and EGFR was immunohistochemically evaluated on the corresponding cervical biopsies. The HPV L1 capsid protein was expressed in 45.83% of LSIL and 25% of HSIL. EGFR was overexpressed in 62,4% of HSIL (58,4% CIN2 and 41,6% CIN3) and 37,6% LSIL. The immunoexpression of L1 HPV has clinical application in the progression assessment of the cervical precancerous lesions without a correlation to the grade of the cervical SIL. EGFR is expressed by all proliferating squamous epithelial cells, thus corresponding with the grade of SIL. The evaluation of EGFR status, correlated with L1 HPV protein expression, can provide useful data of progression risk of cervical squamous intraepithelial lesions.

INTRODUCTION

EGFR (epidermal growth factor receptor) is a potent angiogenic factor present in different tumors, which seems to have, together with c-erbB2 (HER2/neu) and c-myc, an important role in prognostic of advanced cervical cancer. Epidermal growth factor receptor (EGFR) is a member of the ErbB family, the tyrosine kinase receptors with growth promoting effects (Rogers et al, 2005). Human EGFR gene is localized on chromosome 7 and encodes a surface transmembranar glycoprotein which binds EGF (epidermal growth factor), transforming growth factor-α (TGF-α), amphiregulin, and HBEGF (heparin-binding growth factor). HPV-E5 oncogene may be envolved in EGFR activation and this can be done without concomitant increase of receptors’s number (Pim et al, 1992; Gonzales, 2007). HPV-E6 oncogene may determine afterwards the increase of EGFR mARN level and the stabilization of the protein, thus, increasing the signal transduction in the cells. HPV-E5 determines an acceleration of HER2/neu (c-erbB2) gene protein activation. EGFR is expressed in several carcinomas (Janinis et al, 1994; Nakopoulou et al, 1995) and high levels of expression are a common feature of the malignant phenotype in many solid human tumors (Bianco et al, 2007).

HPV L1 capsid protein is expressed in the active phase of the viral infection and is necessary in viral cellular cycle completion. Consequently, viral protein detection, by immunohistochemical reaction is an evidence of active HPV infection in examined tissue (Gu et al, 2007). L1 viral capsid protein is considered a major target of the cellular immune response (Melsheimer et al, 2003). LSIL and moderate SILwithout immunohistochemically detected L1 are correlated, in more than 80% of cases, with dysplasia progression. Moser and co. certify these aspects, evidentiating that minor and moderate lesions without L1 capsid protein expression are significantly more exposed to a progression in comparison to L1 positive cases (Griesser et al, 2004). Most probably, the lack of HPV antigen is determined by a weak protein synthesis, under immunohistochemical test minimum level. As L1 represents the major target of the immune cellular response (Steele et al, 2002), its deficient translation may result in an inefficient epuration of the infected cells, promoting viral DNA integration in host cellular genome and the transformation of immature epithelial cells. The observation that the decrease of the HPV16 capsid positivity in cervical cancer patients serum is an indicator of a poor prognosis sustains the importance of a specific humoral response. Immunohistochemical detection of L1 capsid, on Papanicolau smears, may consequently indicate the defence status locally induced on HPV infection and may offer prognosis information in different squamous intraepithelial lesions.

The purpose of the present study was to analyze the immunohistochemical pattern of epidermal growth factor receptor (EGFR) in cervical squamous intraepithelial lesions (SILs) in correlation with L1 HPV capsid protein, in order to determine the relationship between EGFR expression and the infection status of human papillomavirus (HPV).

MATERIALS AND METHODS

The present study involved 40 women with cytological and histopathological confirmed LSIL (low grade SIL) (CIN1, cervical intraepithelial neoplasia) (n=24) and HSIL (high grade SIL) (6 cases of CIN2 and 10 cases of CIN3) (n=16). The immunoexpression of L1 HPV protein was assessed on conventional cervico-vaginal smears and EGFR was immunohistochemically evaluated on the corresponding cervical biopsies.

The cervico-vaginal smears were fixed and stained with Papanicolaou method. After cytodiagnosis, the cervico-vaginal 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) in a standardized protocol. Epithelial cells with positive nuclear staining were scored as positive, considering one stained nucleus enough for scoring.

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

Proteinase-induced epitope retrieval (PIER) technique was performed using Proteinase K (code S 3020, DAKO, Denmark), for 5 minutes at room temperature. 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, for 30 minutes, at room temperature. 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), under microscope control. The sections were finally counterstained with Lille's modified Mayer's Hematoxylin and mounted.

Quality control performed by external and internal negative and positive controls was necessary to monitor the accuracy of tissue processing, staining procedures and reagents effectiveness. The primary antibody specificity sought to be assesed by their negative controls.

EGFR immunohistochemical expression was quantified according to the EGFR PharmDx scoring guidelines. Thus, the complete or incomplete circumferential membranous staining in $\geq$1% of squamous cells was considered positive. The absence of membrane or cytoplasmic staining was reported as negative. The immunostaining was scored as follows: 1- weak, 2- moderate, and 3 - strong. The percentage of stained cells was assessed as follows: 1–10, 10–50, and >50%.

RESULTS

In our study, the histopathological diagnoses were consistent with the cytodiagnoses, as follows: 24 cases with LSIL (CIN1) and 16 cases with HSIL (6 cases CIN2 and 10 cases CIN3).

HPV infection was morphologically confirmed by the presence of cytopatic HPV effect (koilocytes) in the smears and biopsies.

From all cervical smears, the HPV L1 capsid protein was expressed in 45,83% of LSIL and 25% of HSIL.

The positive reaction was evidentiated by the strong staining of the whole nucleus, surrounded by a cytoplasm with no background. In most cases, positive reaction for HR-HPV L1 was positive in typical koilocytes or in dyskeratocytes, presenting nuclear characteristics for HSIL (CIN 2 or CIN 3). In LSIL cases, the positivity of the nuclei was presented only in typical koilocytes (figures 3,8).

From all cervical biopsies, EGFR was overexpressed in 62,4% of HSIL (58,4% CIN2 and 41,6% CIN3) and 37,6% LSIL.

The EGFR staining pattern was predominantly membranous 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. In HSIL cases, the

staining distribution was as follows: 74% full thickness (figures 5,6,7), 26% basal and intermediate (figure 4). The staining intensity for HSIL cases was strong in 85% (figures 4, 6), moderate in 10% (figures 5,7), and weak in 5% accordingly. The immunostaining was more intense in CIN2 lesions than in CIN3. Regarding LSIL category, the staining distribution was in basal and parabasal cells and in koilocytes. The staining intensity of LSIL cases was strong in 10%, moderate in 68% (figures 1,2), and weak in 22%.

DISCUSSIONS

The sequence of morphologic events in the genesis of invasive cancer of the uterine cervix was thoroughly studied. A progression of intraepithelial lesions from slight to marked and furthermore to invasive cancer has been postulated (Koss and Melamed, 2006; Cain and Howett, 2000). Although a transformation of the initial low grade lesions to high grade lesions may occur, it is a relatively uncommon event. Most high-grade lesions develop independently in adjacent segments of endocervical epithelium (Koss and Melamed, 2006).

Cervical carcinoma arises in women who present a persistent infection with a high risk HPV type and progresses through a multistage process of carcinogenesis (Schoell et al, 1999).

L1 capsid protein is expressed in the active phase of HPV infection and is necessary in viral cellular cycle completion. Viral protein detection, by immunohistochemistry is an evidence of active HPV infection in cells and tissues (Gu et al, 2007). LSIL and moderate SIL (CIN2) without immunohistochemical detected L1 are correlated, in more than 80% of cases, with dysplasia progression (Fiedler et al, 2006). Mild and moderate lesions without L1 capsid protein expression are significantly more exposed to a progression in comparison to L1 positive cases (Griesser et al, 2004). Most probably, the lack of HPV antigen is determined by a weak protein synthesis, below the minimum level of the immunohistochemical test.

From all cervical smears, our data revealed that the HPV L1 capsid protein was expressed in 45,83% of LSIL and 25% of HSIL. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. As we previously mentioned, because of the low rate of HR-HPV L1 positivity found in LSIL cases in our study, we can admit that HPV is not helpful in grading cervical SIL, which is in accordance with the literature data (Yildiz et al, 2007).

In our study, patients’ mean age was 28 years. This fact is consistent with the literature data, where it has been demonstrated that the prevalence of HPV infection varies with age and geographical region, reaching highest rates below 35 years of age (Cox, 1999).

The presence of L1 immunopositive nuclei of squamous epithelial cells could be correlated with the clinical course. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. Mild and moderate dysplastic cervical lesions without immunohistochemical positive reaction of HPV L1 capsid protein are more likely to progress as compared to positive cases (Griesser et al, 2004). Researches consider that lack of detectable HPV antigen in the Pap smears is due to low protein synthesis in squamous epithelial cells below the limit of the immunocytochemical test. The loss of L1 capsid protein immunoexpression can be the result of the integration of the viral DNA into the human genome. Although most of cervical carcinomas show integration of viral DNA, it is detectable only in a small proportion of LSIL and HSIL (Klaes et al, 1999).

The development of HPV capsid antigen L1 depends upon transcriptional factors, which only can be expressed during maturation process from basal epithelial cell to superficial epithelial cell (Gu et a, 2007). In HSIL, the normal structure as well as maturation of the epithelium are disturbed, thus the dysplastic basal squamous cells represent the predominant cell type with reduced L1 capsid protein expression.

In our study, from all cervical biopsies, EGFR was overexpressed in 62,4% of HSIL (58,4% CIN2 and 41,6% CIN3) and 37,6% LSIL. The EGFR staining pattern was predominantly membranous 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, which is in accordance with the majority of reports on EGFR expression (Magkou et al, 2008).

EGFR staining was observed in basal and parabasal cells, in koilocytes and in dysplastic squamous cells of the intraepithelial lesions. This is in accordance with previous studies, which affirm that EGFR is expressed by all proliferating squamous epithelial cells and as such correlates with the grade of SIL (Chapman et al, 1992).

Regarding HSIL lesions, the immunostaining was more intense in CIN2 lesions than in CIN3. These findings are in concordance with the literature data, which assert that the elevated expression of EGFR may be linked to the neoplastic state of the cervical squamous epithelia (Wistuba et al, 1994).

Findings from previous studies confirm that EGFR staining is mainly membranous and observed in basal and parabasal cells, in normal squamous epithelium and atypically proliferating keratinocytes in CIN and nonkeratinizing cells of cervical carcinoma (Mittal et al, 1990) . These are in accordance with our study, indicating a possible role of EGFR in neoplastic proliferation and differentiation of the cervical epithelium.

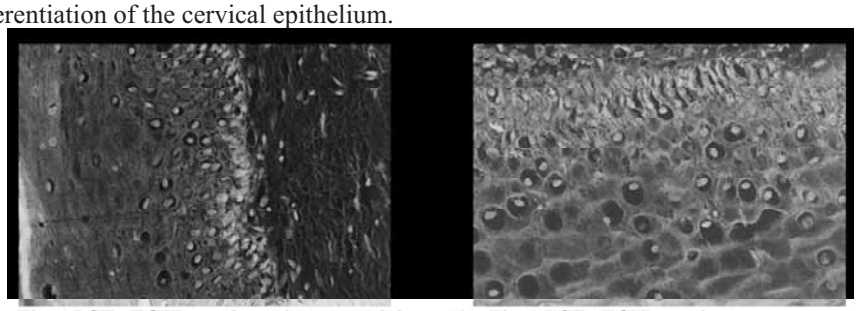

Fig. 1 LSIL, EGFR, moderate immunostaining x 10

Fig. 2 LSIL, EGFR, moderate immunostaining x 20

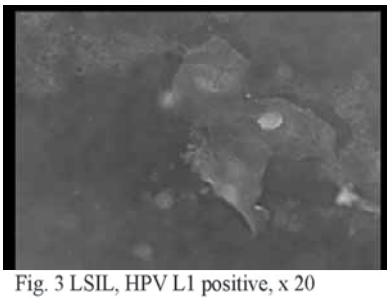

Fig. 3 LSIL, HPV L1 positive, x 20

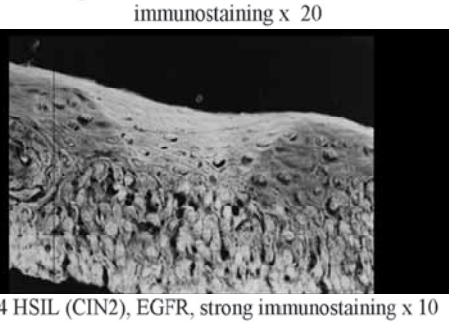

Fig. 4 HSIL (CIN2), EGFR, strong immunostaining x 10

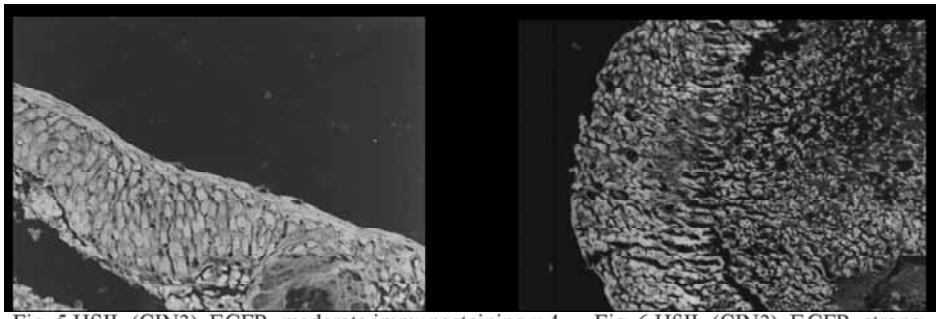

Fig. 5 HSIL (CIN3), EGFR, moderate immunostaining x 4

Fig. 6 HSIL (CIN3), EGFR, strong immunostaining x 10

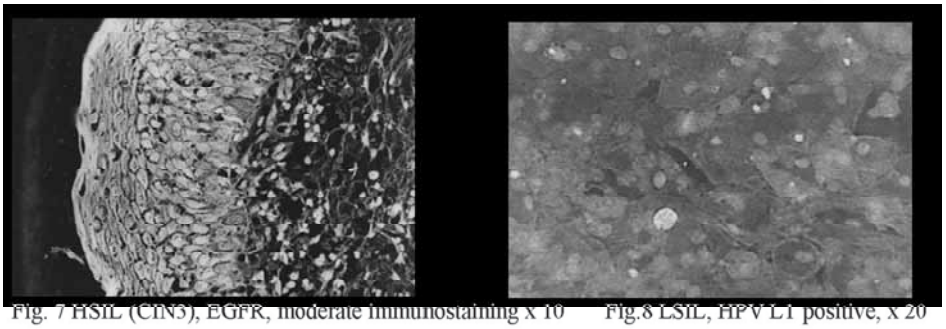

Fig. 7 HSIL (CIN3), EGFR, moderate immunostaining x 10

Fig.8 LSIL, HPV L1 positive, x 20

CONCLUSIONS

The immunoexpression of L1 HPV has clinical application in the progression assessment of the cervical precancerous lesions without a correlation to the grade of the cervical SIL. EGFR is expressed by all proliferating squamous epithelial cells, thus corresponding with the grade of SIL. The evaluation of EGFR status, correlated with L1 HPV protein expression, can provide useful data of progression risk of cervical squamous intraepithelial lesions.

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