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Immunohistochemical evaluation of COX-2 expression in HPV-positive cervical squamous intraepithelial lesions

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Abstract

Cyclooxygenase 2 (COX-2) regulates the prostaglandins production and it seems to have a role in the onset and progression of different malignant tumors, being overexpressed in numerous human malignancies and premalignant conditions. Some cellular elements from chronic inflammatory processes, together with stromal cells may be involved in neoplastic transformation of proliferative stem cells and in the process of tumor invasion. Cervical carcinoma, as a commonly pattern of different tumors, can express COX-2 in association with glutathione-S-transferase isoenzymes and can be considered as possible molecular targets in antitumoral therapy. The purpose of this study was to evaluate the expression of cyclooxygenase (COX)-2 in cervical squamous intraepithelial lesions of low-grade (LSIL) and high-grade (HSIL), with morphologic evidence of HPV infection. Immunostains with COX-2 antibodies were performed on formalin-fixed and paraffin-embedded tissue sections from 20 cervical biopsies: 10 with LSIL histopathologic diagnosis and 10 with HSIL histopathologic diagnosis. All LSIL biopsies and four HSIL cases (equivalent to CIN2) presented also intermediate squamous cells, with pathognomonic morphology of HPV infection (koilocytes). The Allred immunohistochemical score for the intensity of staining and the percent of cells stained was assigned. The slides were scored by three independent pathologists and compared across histological categories. Regarding the intensity of cytoplasmic COX-2 immunostaining, a weaker expression was observed in specimens with LSIL and a stronger one in those diagnosed with HSIL, the highest score being noted in HSIL corresponding to CIN3 lesions. The increase of COX-2 expression in cervical cancer precursors certifies that COX-2 may have a role in the development and progression of cervical squamous intraepithelial lesions.

Keywords: COX-2, immunohistochemistry, HPV, LSIL, HSIL.

Introduction

Cyclooxygenases (COXs) represent important enzymes in the conversion of arachidonic acid to prostaglandins (PGs). The two isoforms, COX-1 and COX-2 are different according to their pattern of expression, COX-1 being expressed in most of the tissues, and COX-2 being usually absent [1]. It is assumed that COX-1 has a minor impact in the carcinogenesis and COX-2 has a certified role in the cancer development. In the development of cervical squamous intraepithelial lesions and cervical cancer, the human papillomavirus (HPV) is essential but not exclusive, as other cofactors are required (e.g. inflammatory cytokines) [2].

Cyclooxygenase 2 (COX-2) regulates the prostaglandins production and it seems to have a role in the onset and progression of different malignant tumors, being overexpressed in numerous human malignancies and premalignant conditions. The metabolic residues produced by the COX-2 action against arachidonic acid are involved in carcinogenesis mechanisms. It was established that the expression of isoform COX-2 is induced by numerous stimuli (mitotic and inflammatory) [3]. Some cellular elements from chronic inflammatory processes, together with stromal cells may be involved in neoplastic transformation of proliferative stem cells and in the process of tumor invasion.

A great number of tumors, including cervical carcinoma can co-express COX-2 and glutathione-S-transferase isoenzymes and can be considered as possible molecular targets in antitumoral therapy. COX-2 is overexpressed both in transformed cells and in malignant tissues [4–7]. Although all these recognized data, the literature has only few reports [8] regarding the COX-2 profile in relationship with HPV infection in the squamous intraepithelial cervical lesions.

The purpose of this study was to evaluate the expression of COX-2 in cervical squamous intraepithelial lesions of low-grade (LSIL), corresponding to CIN1 (cervical intraepithelial neoplasia type 1) and high-grade (HSIL), which includes CIN2 and CIN3 lesions, associated with a morphologic evidence of HPV infection.

Materials and Methods

The tissue sections were obtained from 20 cervical biopsies. The cases were investigated by routine histopathological exam and by immunohistochemistry, using COX-2 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. Heat-induced epitope retrieval technique was performed using Target Retrieval Solution pH 9 (code S2367, DAKO, Denmark). After blocking the endogenous peroxidase and non-specific binding, the sections were incubated with the primary antibodies, anti-COX-2 mouse monoclonal antibody (clone CX-294, code M3617, DAKO, Denmark), dilution range 1:80. 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 Mayer's Hematoxylin.

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 assessed by their negative controls.

COX-2 immunohistochemical expression was quantified in accordance to Allred score [9], by three independent pathologists 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 – ***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 20 studied cervical biopsies, the histopathologic diagnosis revealed 10 cases with LSIL and 10 cases with HSIL (six cases of CIN3 and four cases of CIN2). All LSIL biopsies and four HSIL cases (equivalent to CIN2) presented also intermediate squamous cells, with pathognomonic morphology of HPV infection (koilocytes). COX-2 expression showed finely granular cytoplasmic staining with occasional cytoplasmic membrane staining, especially in koilocytes. The staining pattern was frequently homogeneous. The results of the semiquantitative exam, based on the Allred score applied to the immunohistochemical reactions, were summarized in Table 2.

Table 2 – ***Allred scores quantified in the investigated cases***

Case no.	LSIL			Case no.	HSIL		
	PS	*IS*	*Total score*		*PS*	*IS*	*Total score*
1.	2	2	4	1.	3	3	6
2.	2	1	3	2.	4	3	7
3.	3	3	6	3.	5	3	8
4.	2	2	4	4.	5	3	8
5.	3	3	6	5.	4	3	7
6.	4	3	7	6.	3	2	5
7.	2	3	5	7.	4	2	6
8.	3	3	6	8.	5	3	8
9.	4	3	7	9.	5	3	8
10.	3	2	5	10.	5	2	7

Although the general score was higher in HSIL when compared to LSIL, we observed 13 cases belonging to both categories with the same Allred score (two LSIL cases and one HSIL case with value 5, three LSIL cases and two HSIL cases with value 6, two LSIL cases and three HSIL cases with value 7). Regarding the intensity of cytoplasmic COX-2 immunostaining, a weaker expression was observed in specimens with LSIL (Figures 1 and 2) and a stronger one in those diagnosed with HSIL (Figures 3 and 4).

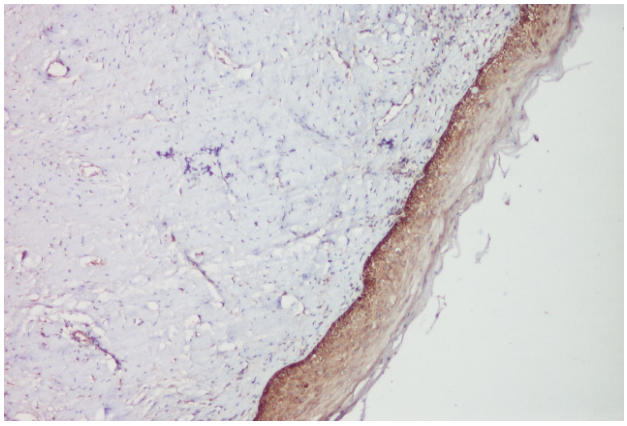

Figure 1 – ***Stratified squamous exocervical epithelium displaying LSIL, revealed a weak cytoplasmic staining, slightly stronger in the basal layer (anti-COX-2, ×50).***

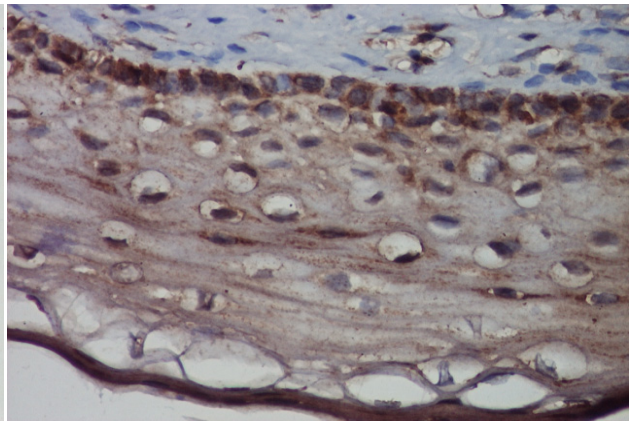

Figure 2 – ***Detailed image showing LSIL exhibiting evident koilocytic atypia, weak cytoplasmic staining in the whole epithelium (anti-COX-2, ×200).***

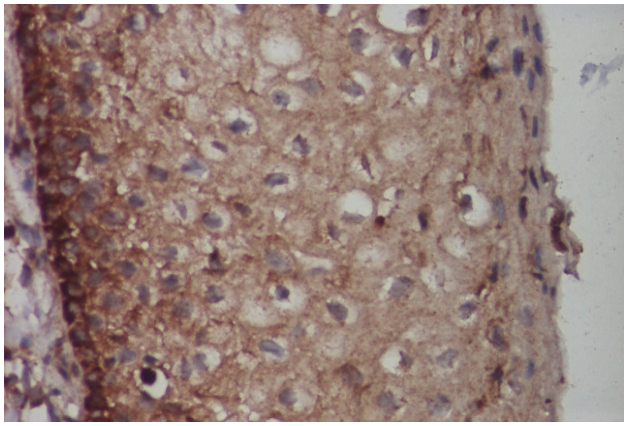

Figure 3 – ***Stratified squamous exocervical epithelium displaying HSIL, revealed a moderate cytoplasmic staining (anti-COX-2, ×20).***

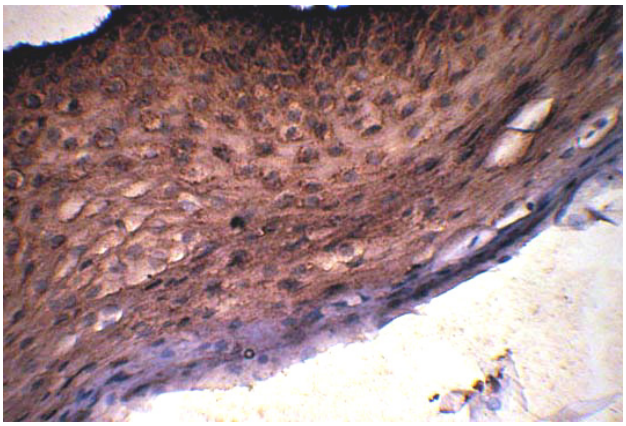

Figure 4 – ***Stratified squamous exocervical epithelium displaying HSIL, revealed a strong cytoplasmic staining, excepting the superficial layer (anti-COX-2, ×200).***

The highest score was noted in HSIL corresponding to CIN3 lesions (Figure 5).

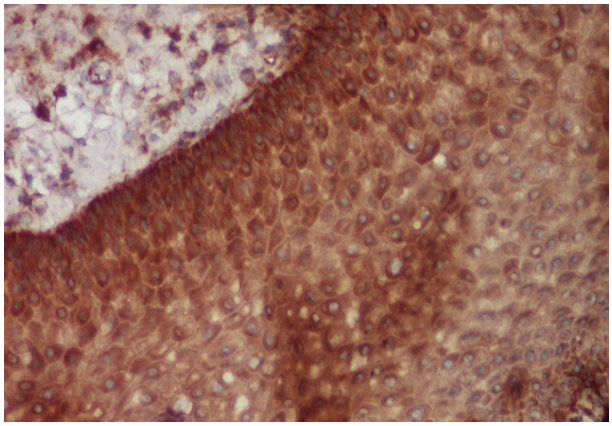

Figure 5 – ***HSIL, corresponding to CIN3 lesion, showed a strong, heterogeneous cytoplasmic staining in the entire thickness of the stratified squamous exocervical epithelium (anti-COX2, ×200).***

The stromal inflammatory cells, in cases with associated chronic cervicitis, were also intense positive for COX-2.

There was no association between the intensity of the COX-2 immunostaining and the presence of the koilocytes within the squamous dysplastic epithelium.

Discussion

Over the years, many attempts have been made to establish a logical sequence of morphologic events in the genesis of invasive cancer of the uterine cervix. A progression of intraepithelial lesions from slight to marked and furthermore to invasive cancer has been postulated [10, 11]. 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 [10].

Cervical carcinoma arises in women who present a persistent infection with a high risk HPV and progresses through a multistage process of carcinogenesis [12]. For example, CIN3, a precursor lesion detected in screening programs, can progress to invasive cancer, the premalignant phase of cervical carcinogenesis lasting for 5–10 years. COX exists in two distinct forms: one is constitutively expressed (COX-1), and the other is inducible (COX-2). COXs catalyze the formation of prostaglandins from arachidonic acid. Both isoforms have a structural similitude of 60% but perform different biological functions. COX-1 regulates the renal blood flow, the gastric mucosa protection and the control of platelet aggregation. COX-2 is not, generally, detected in most of the tissues, but can be easily induced by cytokines, endotoxins, mitogenic factors, and hormones [13–16]. Recent studies suggest that COX-2 is important in carcinogenesis. COX-2 is overexpressed in transformed cells and in malignant tissues [17–21].

In the present study, we investigated the immunoexpression of COX-2 in cervical squamous intraepithelial lesions of low-grade and high-grade. Our data show that COX-2 levels are increased as the squamous lesion progresses. Previously, COX-2 was found to be up-regulated in normal cervix and uterus during certain stages of the estrous cycle and in pregnancy [22, 23]. In the uterine cervix carcinoma, the immunoexpression of COX-2 is preferentially localized at the stromal-invasive tumor interface [5]. Our data revealed that COX-2 is overexpressed both in LSIL (CIN1) and HSIL (CIN2, CIN3), aspect which is in concordance with other data already reported in the literature [24].

It was previously admitted that serum levels of progesterone and estradiol appear to be correlated with increased COX-2 expression in squamous intraepithelial lesions, emphasizing that the evaluation of expression of tumor markers must take into account the SIL grade [25]. These data support our findings.

The stromal inflammatory cells, in cases with associated chronic cervicitis, were also intense positive for COX-2, aspect that was previously described in literature [26].

In our study, there was no association between the intensity of the COX-2 immunostaining and the presence of the koilocytes within the squamous dysplastic epithelium. The present study does not intend to establish a correlation between HPV types and COX-2 expression. A possible association between certain types of HPV and COX-2 detection may represent a future direction of our research.

Concerning the HPV types, it is accepted that HPV16

and HPV18 are often present in HSIL and cervical carcinoma. It was demonstrated that HPV16 oncogenes activate MAPKs [27] and the AP-1 family of transcription factors [28]. Besides these effects, HPV16 oncoprotein E6 influences the degradation of p53 [29], a result that could also enhance COX-2 levels [30]. These data are consistent with our results, showing an increased expression of COX-2 in high-grade lesions, which more probably are linked to high risk HPV types. In our opinion, HPV typing is needed to determine the possible biological mechanism that links HPV infection to COX-2 expression. In other cells, the overexpression of COX-2 has been certified to inhibit apoptosis [31], suppress immune function [32], promote angiogenesis [33], and enhance the invasiveness capacities of malignant cells [34]. Therefore, the higher expression of COX-2 noted in HSIL suggests that COX-2 plays a role in tumor progression rather than tumor initiation.

Conclusions

The present study suggests that COX-2 induction is an early event in cervical carcinogenesis and can be correlated with inflammation. The increase of COX-2 expression in cervical cancer precursors and the correlation of the immunohistochemical score to the severity of SIL certify that COX-2 may have a role in the development and progression of cervical squamous intraepithelial lesions. HPV typing is needed to determine the possible biological mechanism initiated by HPV infection, resulting in COX-2 expression.

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