Cyclooxygenase-2 expression in cervical cancer

Ekspresija ciklooksigenaze-2 kod karcinoma grlića materice

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Abstract

Background/Aim. Cyclooxygenase (COX) or prostaglandin H2 synthase is the first enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from arachidonic acid. The aim of the study was to determine the expression level of COX-2 in patients with cervical cancer and compare it with that in the control group with no cervical pathology. **Methods.** The study included 76 patients divided into two groups: the control group – 30 patients without histopathological changes and the group A – 46 patients with cervical cancer, FIGO stage IB-IIA. Histopathological and immunohistochemical analyses were performed in these two groups of patients. **Results.** In the control group, the expression of COX-2 was not confirmed compared to the group A of 26 (56.52%) patients. The expression of COX-2 showed a statistically significant difference in the presence of lymphocytic stromal infiltration (p = 0.0053). The expression of COX-2 was more pronounced in the stromal tissue without lymphocytic infiltration (80% *vs* 20%). **Conclusion.** A higher expression of COX-2 in cervical carcinoma without stromal lymphocytic infiltration suggests a possible paradoxical effect of COX-2 in immunosuppression. Frequent COX- 2 expression in the subgroup with poor prognostic histological parameters in the group A indicates the importance of COX-2 expression in the carcinogenesis of cervical cancer.

Key words:
uterine cervical neoplasms; prostaglandin-endoperoxide synthases; immunohistochemistry; gene expression; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Ciklooksigenaza (COX) ili prostaglandin H2 sintaza (PGHS) prvi je enzim koji katalizuje prva dva koraka biosinteze prostaglandina iz arahidonske kiseline. Cilj rada bio je da se ispita prisustvo i stepen ekspresije COX-2 u tkivu grlića materice ispitanica sa cervikalnim karcinomom i uporedi sa kontrolnom grupom bez cervikalne patologije. **Metode.** Istraživanjem je obuhvaćen patohistološki materijal uzet od 76 bolesnica podeljenih u dve grupe: kontrolna grupa – 30 bolesnica bez patohistoloških promena na grliću materice i grupa A – 46 bolesnica sa verifikovanim karcinomom grlića materice, FIGO stadijum IB-IIA. U obe grupe na patohistološkom materijalu izvršene su histološke i imunohistohemijske analize. **Rezultati.** U kontrolnoj grupi nije potvrđena ekspresija COX-2, a u grupi A jeste kod 26 (56,52%) bolesnica što je statistički značajna razlika u odnosu na kontrolnu grupu. Ispitivanjem ekspresije COX-2 i patohistoloških parametara, uočena je statistički značajna razlika u odnosu na postojanje limfocitne infiltracije (p = 0,0053). Ekspresija COX-2 bila je izraženija u tkivu bez limfocitne stromalne infiltracije (80% *vs* 20%). **Zaključak.** Ekspresija COX-2 bila je izraženija kod karcinoma grlića materice bez limfocitne stromalne infiltracije što navodi na zaključak o mogućem paradoksalnom efektu COX-2 na imunosupresiju u tumorskom tkivu. Nalaz češće ekspresije COX-2 u podgrupi sa lošijim prognostičkim patohistološkim parametrima u grupi A upućuju na značaj aktivnosti ekspresije COX-2 u procesu karcinogeneze karcinoma grlića materice i uticaja na njegovu progresiju.

Ključne reči:
grlić materice, neoplazme; prostaglandin sintetaza; imunohistohemija; geni, ekspresija; osetljivost i specifičnost.

Introduction

According to the global scale, cervix uteri carcinoma remains in the second place among female population of diseased from the malignant neoplasia, with about 400,000 newly diagnosed cases *per* year with annual mortality of about 250,000 women. Approximately 83% of cervical carcinoma is diagnosed in underdeveloped and developing countries, which do not have any adequate screening programs [1]. According to the data obtained from the Cancer Registry of the Central Serbia, cervical carcinoma is the most frequent malignant tumor of the female reproductive organs with the incidence rate of 26.9/100,000. The same data from 2001 show the frequency of cervical carcinoma of 9.4% in

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the total number of the diseased and 5.8% in total mortality from malignant tumors of the female population [2]. According to the Malignant Diseases Registry of the Oncology Institute of Vojvodina, the incidence of 26.6/100,000 was registered in Vojvodina, for the period from 1993 to 2002 [3]. Former research in oncology contributed to the definition of the cyclooxygenase-2 (COX-2) expression significance in tumor cell oncogenesis. In oncogenetic expression, a more significant role is played by COX-2, whose expression is related to various pathophysiological conditions of inflammation or oncogenesis [4]. Furthermore, studies show the significance of COX-2 expression in regulation of apoptosis, disease progression, neoangiogenesis and the therapeutical response [4–7]. Liu et al. [8] presented COX-2 expression growth in more severe cases of esophagus squamous neoplasia. Lim et al. [9] also confirmed the increase in COX-2 expression in adenomatous and metaplastic stomach lesions. Saukonen et al. [10] presented COX-2 expression in 44% of patients with dysplastic changes in stomach. Also, COX-2 expression was confirmed in other neoplastic lesions of the breast, urinary bladder and pancreas [7, 11, 12]. Ferrandina et al. [13] also published the results of COX-2 expression study in precancerous and cancerous changes of vulva. They confirmed a higher level of COX-2 expression in more advanced stages of vulva carcinoma (FIGO stages III/IV), with metastatic lymph nodes and deeper stromal infiltration.

COX-2 expression was a significant factor in the increase of tumor angiogenesis and the reduction of apoptosis, which appeared as a possible, important connection within the development of carcinogenesis and tumor growth [14, 15].

The aim of this paper was to: test the presence and the level of COX-2 expression in cervical tissue of the female patients divided into two groups – the control group and the group A (cervical carcinoma, FIGO stage I-IIA), and to compare the COX-2 expression level in the patients diagnosed with the cervical carcinoma in relation to the level of tumor differentiation, stromal invasion, tumor size, the presence of lymphovascular invasion and the existence of metastases in the lymph nodes.

Methods

The study included histopathological material from 76 patients who underwent surgery with performed hysterectomy with or without adnexectomy due to benign changes in the uterus (myomas) or, with performed radical hysterectomy on the basis of biopsy verified cervical carcinoma, FIGO stage IB-IIA. The trial was conducted at the Clinic for Operative Oncology and the Center for Pathology and Diagnostic Cytology of the Oncology Institute of Vojvodina in Sremska Kamenica.

Based on the definite histopathological findings, the patients were divided into two groups: the control group (without changes at the cervix uteri) and the group A (cervical carcinoma, FIGO IB-IIA).

The control group included histopathological material of 30 patients who underwent total hysterectomy due to benign changes in the uterus and/or ovaries.

Exclusion criteria for this group of patients were the patients with: previous excision or ablation of the cervix uteri: precancerous or cancerous lesions diagnosed in the cervix uteri; verified chronic inflammation of the cervix uteri; verified malignant disease of the genital tract; verified malignant disease of any other localization.

The group A included histopathological material of 46 patients with verified cervical carcinoma FIGO stage IB-IIA, who underwent radical Piver, class III surgery, with lymphadenectomy.

Exclusion criteria for this group were the patients: with verified cervical carcinoma who were previously treated by irradiation therapy or neoadjuvant cytostatic therapy; who previously underwent an excision or ablative type of cervical treatment; with verified malignant disease of the genital tract of other localization; with verified malignant disease of any other localization.

The obtained surgical material was sent to histopathological (HP) examination.

Histopathological examination enabled the definition of the final histopathological diagnosis, determination of the stage of the tumor disease and the analysis of the standard histopathological prognostic parameters: histological type of the tumor; tumor size and the depth of the stromal invasion; grade of histological differentiation; the presence of lymphovascular invasion; the total number of removed lymphatic nodes; the presence of metastases and the number of metastatically changed lymphatic nodes.

Examination included all resection edges of parametrium and vagina, for determination of the presence or absence of the tumor.

Based upon the HE stained preparations, a representative sample from the examined material was selected for immunohistochemical testing.

Immunohistochemical analysis of COX-2

For immunohistochemical analyse, the selected tissue samples from the control group (hysterectomy due to myomatous uterus) and the group A (radical hysterectomy) were used. The samples were fixed in formalin and blocked in paraffin, sliced into sections of 4 micron thickness, and then "glued" to Superfrost (Menzel-Glaser) positively electrified glass slides, previously prepared for immunohistochemical reactions. After deparaffinization, we started blockage of endogenous peroxidase with 3% H_2O_2 for 5 minutes. Immunohistochemical identification of the tested antigens was performed by application of Streptavidin-biotin-peroxidase technique (B-0SA), according to the standard LSAB procedure (Dakocytomation-DAKO). The fragments were incubated for 30 minutes at room temperature with biotinylated anti-mouse antibody, and then incubated with streptovidin-peroxidase complex system, in duration of 30 minutes. As a hromogenous substrate, a 3-amino-9-ethylcarbazole (AEK, DAKO) was applied. After each incubation the samples were rinsed in Tris buffer solution (TBS: 0.05 M, pH 7.6). Contrasting was performed by hematoxylin. The tissue samples, which, during the treatment missed the primary antibody, were used as the negative control for antibody, while the

other phases of the immunohistochemical procedure were applied. The analysis of immunohistochemically processed tumor tissue samples was performed by light-microscopy, by qualitative and semi-qualitative method, expressed as the percentage of positive cells in relation to the total number of cells in the representative fields.

The value of COX-2 expression was analyzed semi-qualitatively, by determination of the percentage of the stained tumor cells [16]: absence of expression – negative findings; mild level of expression - $< 25\%$ of the changed cells were positive; medium level of expression – > 25.1–50% of the changed cells were positive; high level of expression – $\geq 50.1\%$ of the changed cells were positive.

As internal negative control, tumor unchanged epithelial cells of the cervix were used. As positive external tissue control for COX-2 expression, a high-grade transitional cellular urinary bladder carcinoma was used [17].

During statistical analysis of data, descriptive statistics were calculated – frequencies, percentages, mean values, and a standard deviation.

We used graphical presentation of data and the results with the aid of column diagrams and box-whiskers diagrams.

Comparisons were performed by the *t*-test, numerical-feature-variance analysis.

For attributive features, the non-parametric, Pearson's χ^2-test and Fisher's exact test were used.

Statistically significant differences ($p < 0.05$) were marked by the asterisk (*), and highly significant difference ($p < 0.01$) by two asterisks (**).

Statistical data analysis was performed with the aid of the software package STATISTICA 9.0 for which, there is a University license at the Novi Sad University. Data analysis and presentations (tables and graphs) were prepared by the computer technique in programs Microsoft Word, Excel and Power Point.

Results

The study results were obtained by the analysis of histopathological material from 76 patients and their statistical processing. The material was collected at the Gynecological Oncology Department and analyzed at the Pathology and Cytodiagnostics Department of the Oncology Institute of Vojvodina in Sremska Kamenica.

The mean age of the patients in the control group was 46.19 years. The youngest patient was 30 and the oldest one 67 years of age ($\bar{x} \pm SD = 46.19 \pm 8.284$).

The mean age of the patients in the group A was 50.13 years. The youngest patient was 31 and the oldest 66 years of age ($\bar{x} \pm SD - 50.13 \pm 10.417$).

In the control group, histopathological findings showed the normal cervix uteri without any histopathological changes.

In the group A, in all 46 patients, a planocellular type of carcinoma was verified in the final histopathological findings. According to the FIGO classification in 34 (74%) of the patients, the disease was staged as IB1, i.e. stage IB2 in 12 (26%) of the cases.

Beside histological type and the stage of the disease, histopathological examination also analyzed standard prognostic parameters: size of the tumor, depth of stromal invasion, total number of removed lymphatic nodes, the number of metastatically changed lymphatic nodes, degree of histological differentiation, the presence of lymphovascular invasion, involvement of parametrial and vaginal resection edges, involvement of "isthmus" of uterus and the presence of lymphocyte infiltrate. The tumor size was up to 2 cm in 47.83% of the patients. In 69.57% of the patients, the depth of stromal invasion was greater than 10 mm, and more than 10 lymph nodes were removed in 71.74% of the patients. In 17 patients, out of 46, the presence of metastases in the lymph nodes was diagnosed. In 11 patients, two or more lymph nodes were positive. The degree of histological differentiation was distributed in the following manner: G1 (17.39%), G2 (58.69%) and G3 (23.92%). Lymphovascular infiltration was not registered in 60.87% of the patients. "Isthmus" of uterus infiltration was verified in 23.91% of the patients. In 56.52% of the patients, there was lymphocyte stromal infiltration. Parametrial and vaginal infiltration of the cuff was not confirmed in 93.48%, i.e. 91.30% of the patients (Table 1).

After histopathological analysis, the preparations were stained immunohistocemically for testing of the COX-2 expression characteristics. Fisher's exact test and Pearson's χ^2-test were used for testing of the difference between the examined groups.

In the control group, there was no COX-2 expression confirmed, while in the group A, it was verified in 26 (56.52%) patients (Figure 1).

By comparison of COX-2 expression between the examined groups, it was determined that there was a highly statistically significant difference between the control group and the group A ($p = 0.0001$) (Figure 2).

The control group did not show COX-2 expression in the entire examined histopathological material. Further comparison of expression was tested within the group A.

The level of COX-2 expression was divided into: the absence of expression or negative COX-2; mild level of expression – $< 25\%$ of the changed cells were positive; medium level of expression – 25.1–50% of the changed cells were positive; high level expression – $\geq 50.1\%$ of the changed cells were positive.

In the group A, mild, medium and high expression of COX-2 was in 17.39%, 15.22% and 23.91% of cases of the examined histopathological material respectively (Figure 3).

Testing of COX-2 expression and histopathological parameters showed a statistically significant difference in relation to the existence of lymphocyte infiltration ($p = 0.0053$). Positive COX-2 expression was greater in the tissue of the patients without lymphocyte stromal infiltration (16/20, 80%). In the patients with lymphocyte stromal infiltration, positive expression was verified in 10/26 (38.46%) patients. There was no statistically significant difference confirmed regarding other histopathological parameters ($p > 0.05$) (Table 2).

Table 1

**Frequency of histopathological parameters in the group A (with cervical cancer)**

Histopathological variables	Frequency	Percentage
Size of the tumor		
up to 2 cm	22	47.83
over 2 cm	24	52.17
Total	46	100
Depth of stromal invasion		
up to 10 mm	14	30.43
over 10 mm	32	69.57
Total	46	100
Number of lymph nodes		
up to 10	13	28.26
more than 10	33	71.74
Total	46	100
Number of lymph nodes with metastases		
1 positive lymph node	6	35.29
2 or more positive lymph nodes	11	64.71
Total	17	100
Degree of histological differentiation		
G1	8	17.39
G2	27	58.69
G3	11	23.92
Total	46	100
Lymphovascular infiltration		
Yes	18	39/13
No	28	60.87
Total	46	100
Lymphocyte stromal infiltration		
Yes	26	56.52
No	20	43.48
Total	46	100
"Isthmus" infiltration		
Yes	11	23.91
No	35	76.09
Total	46	100
Parametrial infiltration		
Yes	3	6.52
No	43	93.48
Total	46	100
Vaginal cuff infiltration		
Yes	4	8.70
No	42	91.30
Total	46	100
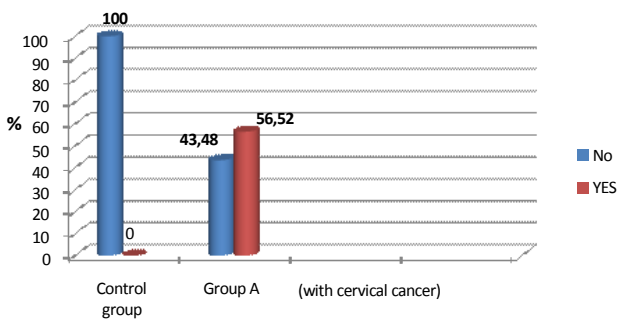
Fig. 1 – Frequency of COX-2 expression *per* groups.
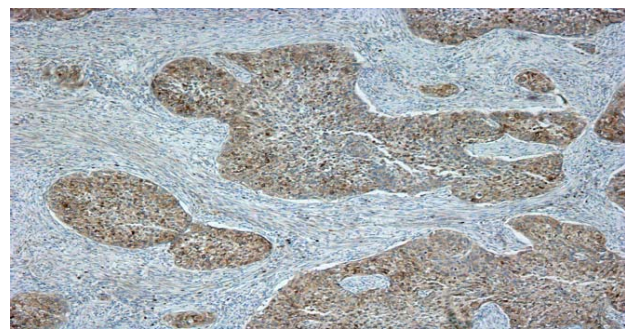
Fig. 2 – Immunohistochemical identification of COX-2 in the group A (with cervical cancer) (LSBA, ×200).
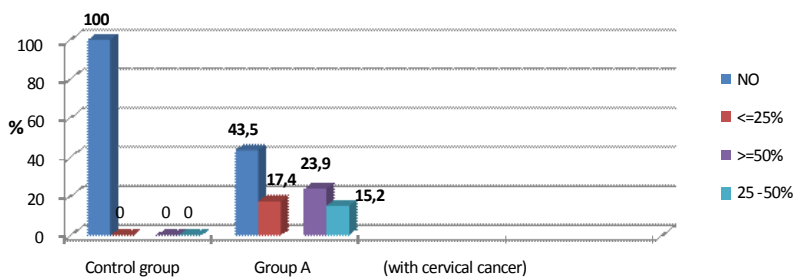
Fig. 3 – Frequency of COX-2 expression level.

Comparison of COX-2 expression of lymphocyte stromal infiltration in the group A (with cervical cancer)

COX-2 expression	Positive, n (%)	Negative, n (%)	Total, n (%)	Fisher exact test p – values
Lymphocyte stromal infiltration				
Yes	10 (38.46)	16 (71.54)	26 (100)	0.0053*
No	16 (80)	4 (20)	20 (100)	
Total	26 (56.52)	20 (43.48)	46 (100)	

In the subgroup of patients with worse histopathological prognostic parameters, positive lymph nodes (17/46), lymphovascular infiltration (18/46), "isthmus" of uterus infiltration (11/46), there was a statistically significant difference in relation to the existence of COX-2 expression (positive COX-2 – 70.59%, 66.66%, 63.54%, and negative COX-2 – 29.41%, 33.34%, 36.36%, respectively; $p < 0.05$) (Figure 4).
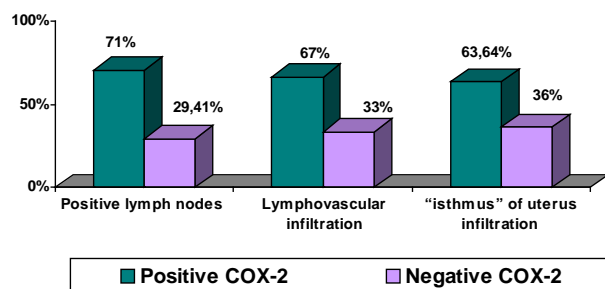

Fig. 4 – Frequency of COX-2 expression in the subgroup of patients with worse histopathological prognostic parameters within the group A (with cervical cancer).

In the other subgroups in the patients with the tumor greater than 2 cm, the level of histological differentiation and the depth of stromal invasion greater than 10 mm, there was no statistically significant difference in relation to COX-2 expression ($p > 0.05$). In a subgroup of patients with parametrial and vaginal infiltration of the cuff, due to a small sample (3, i.e. 4), statistical analysis was not performed.

The existence of a highly statistically significant difference in relation to the presence of lymphocyte stromal infiltration ($p = 0.003$) and the degree of COX-2 expression was determined. In patients with a medium degree of expression, 100% of the tested samples did not have lymphocyte stromal infiltration. Lymphocyte stromal infiltration was not present in 54.55% of the patients with the high COX-2 expression (over 50%) (Figure 5).
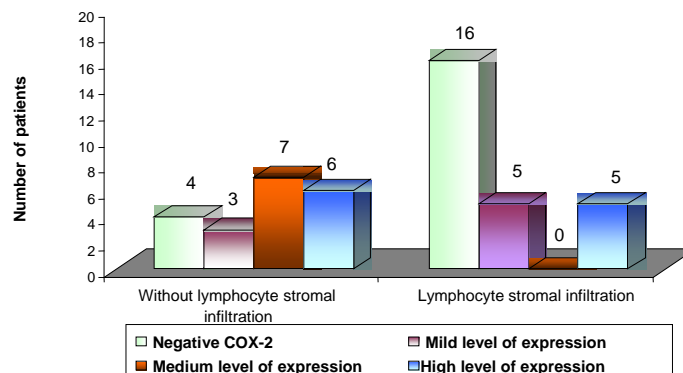
The degree of COX-2 expression in relation to the other histopathological parameters did not show a statistically significant difference ($p > 0.05$).

Discussion

Over 85% of deaths caused by cervical carcinoma are registered in the undeveloped countries. Observing the entire epidemiological picture, at the moment, Serbia is in the fifth place (19.6/100,000) in Europe for cervical carcinoma incidence, and in the third place for mortality (8.6/100,000)[18].

Researching COX-2 expression as a pro-oncogene activator contributed to wider knowledge of oncogenesis, but also imposed some new questions and goals to researchers. COX expression was proved in many premalignant, malignant and metastatic diseases regardless the tumor type and localization[4, 19]. Studies confirmed COX-2 expression in premalignant breast changes, adenomatous colon polyp, leukoplakia of buccal mucosa[20–22]. Expression was also determined in urinary bladder carcinoma, breast, colon, lung, pancreas, stomach, kidneys, skin carcinoma, lymphoma, sarcoma, leukemia, brain tumor[19, 23–30]. Researching COX-2 expression, its role in carcinogenesis and as a prognostic factor, was also presented in studies on malignant diseases of the lower genital systems in women.

Li et al.[31] presented expression of COX-2 in ovarian low malignant potential tumor (borderline) (57.9%) and carcinoma (81.5%) in relation to benign ovarian tumors (38.9%). They also determined a statistically significant difference between COX-2 expression and the clinical stage of ovarian low malignant potential tumor (borderline) (FIGO stage I/II –51.7% and stage III/IV – 90.9%). Denkert et al.[32] analyzed COX-1 and COX-2 expression in 117 ovarian tumor samples and 2 ovarian tissue samples without any changes. COX-2 was only detectable in malignant, changed tissue (42%). In univariate analysis, COX-2 expression was a bad prognostic factor. The mean survival in the patients with

Fig. 5 – Frequency of the degree of COX-2 expression in relation to lymphocyte stromal infiltration.

negative COX-2 was 52 months in comparison to 30 months in COX-2 positive. In this study, the multivariate analysis showed that COX-2 expression is an independent bad prognostic factor (relative risk, RR = 2.74, 95% CI : 1.38–5.47). Chou et al. [33] showed more expressed COX-2 expression in ovarian carcinoma (which was related to endometriosis) than in the isolated carcinoma (27.8% *vs* 5.6%). In endometrial type of ovarian adenocarcinoma, a statistically significantly greater COX-2 expression was confirmed when compared to the isolated ovarian carcinoma (50% *vs* 0%; *p* = 0.023). Nasir et al. [34] tested COX-2 expression in endometrial adenocarcinoma (EAK), atypical complex hyperplasia (AKH) and endometrial hyperplasia (EH). COX-2 expression was proved in 88% of EAK, 80% of AKH and 44% of EH. The mean value of COX-2 immunohistochemical score for EH i.e. EAK was 33 (SD ± 24.11) and 76 (SD ± 54.57), respectively; *p* = 0.022. Ferrandina et al. [35] tested 69 samples of primary endometrial carcinoma. The expression of COX-2 was confirmed in 39.1% of cases. The COX-2 positivity was more pronounced (60.8%) in case of endometrial expansion to the cervix and outside the uterus, in contrast to carcinoma limited to the body of uterus (28.3%; *p* = 0.017). The authors showed the increase of COX-2 expression with the increase of the histological grade level (G1 – 13.6%, G2 – 41.7%, G3 – 60.9%; *p* = 0.0049). A statistically significant difference in expression was determined in relation to the depth of the myometrial invasion (< 50–15.6%, > 50–66.7%). Nofech-Mozes et al. [36] tested the immunohistochemical score of COX-2 expression in a group with inflammatory changes in the vulva (1.6), VIN I and II (1.4), VIN III and carcinoma *in situ* (0.7) and invasive vulvar carcinoma (1.2) with the existence of a statistically significant difference, but not the relation with the level of dysplasia or age.

In our study, we tested COX-2 expression in cervix uteri tissue without pathological changes and with carcinomatous changes. The expression of COX-2 was not confirmed in the control group, in opposition to the group A (56.52%). A statistically significant difference was determined in relation to positive expression between the control group and the group A. The control group did not show COX-2 expression in the entire examined material and thus, it was confirmed as an internal negative control for normal cervix uteri tissue. The specificity of COX-2 protein expression is in its significant role in an inflammatory process, and an important precursor of premalignant and malignant changes in the cervix uteri is a long-term inflammatory process [37]. A significant role in this mechanism has a persistent viral infection with human papillomaviruses of high oncogenic potential. Subbaramaiah et al. [38] presented a complex mechanism of COX-2 expression activation, activated by oncoproteins HPV 16 E6 and E7 at line cells of normal cervix and carcinoma. The activation of COX-2, induced by HPV 16 E6 was done through a complex mechanism by activation of epidermal growth factor receptors (*EGFR*), Ras, mitogen-activated protein kinase (*MAPK*) and activator protein-1 (*AP-1*). The expression of E6 and E7 leads to corepressor inhibition (*NCoR*). A potential mutual activity also coactivates the corepressor with HPV16 E6 and E7 oncoproteins in a complex mechanism of mutual activation and degradation, inducing expression of COX-2. Farley et al. [39] published a paper on the expression of COX-2 in precancerous cervical changes. The study included 62 cervical samples obtained by the LEEP technique (loop electrosurgical excision procedure), which included 18 CIN 1, 19 CIN 2 and 25 CIN 3 changes. The positive expression of COX-2 was marked if there was positivity in more than 50% of cells in the examined sample. In CIN 1 changes, the expression of COX-2 was observed in 50%, CIN 2 in 42% and CIN 3 in 68% of the patients. The average intensity of expression was growing with the level of dysplasia (CIN 1 – 1.6; CIN 2 – 1.8; CIN 3 – 2.1). The authors pointed out COX-2 expression, which might play a role in carcinogenesis of cervical carcinoma. Dai et al. [16] presented 45% of patients with CIN changes, who were positive to COX-2 expression. Similar results were confirmed by Kim et al. [40] with the expression of COX-2 in 24% of the patients with CIN 3 changes in the cervix uteri, in 37.9% in microinvasive cervical carcinoma and in 51.6 % of patients with invasive carcinoma. In their study, Dursun et al. [41] tested the expression of COX-2 in CIN changes of the cervix uteri and planocellular carcinoma and made comparisons with clinicopathological factors. The study included 25 patients with CIN 3 changes and 67 patients with cervical carcinoma. Positive expression of COX-2 was confirmed in 24% of patients in the group with CIN 3 changes, while it was confirmed in 55.2% of patients with carcinoma. A correlation of COX-2 expression and the presence of lymphovascular invasion (LVI) showed a statistically significant difference (positive LVI – 61.9% of the patients with positive COX-2; negative LVI – 33.3%, *p* = 0.02). Furthermore, statistically significant difference was shown in relation to the size of the tumor (up to 4 cm – 39% of patients with positive COX-2 and over 4 cm – 65.9%; *p* = 0.028). A statistically significant difference related to parametrial infiltration, lymph node status or recurrence disease and survival was not confirmed. In multivariate analysis, lymphovascular invasion was the only factor connected to the expression of COX-2, unlike the size of the tumor [41]. In Khunamornpong et al. [42] study COX-2 expression was significantly associated with lymph node metastasis but lacked a significant correlation with tumour stage, size, histologic grade, deep stromal invasion, lymphovascular space invasion (LVSI), and parametrial involvement. COX-2 expression was not associated with lymph node metastasis in the absence of parametrial involvement or LVSI. In the cases with LVSI, COX-2 expression was significantly associated with lymph node metastasis.

Comparison of risk factors and the stage of the disease in relation to COX-2 expression and its level within the group A did not prove a statistically significant difference. Luo et al. [43] tested COX-2 expression in cervical carcinoma and its clinical significance. Seventy-two cervical samples with invasive carcinoma and 16 cervical samples without tumor were examined. Within the group with the invasive carcinoma, COX-2 expression was present in 88.9% of cases and in the group without the tumor in 12.5 %. COX-2 expression was positively related to metastases in lymph nodes

and stromal invasion. A similar study was conducted by Manchana et al. [44] where they tested the prevalence of COX-2 and compared it with the clinicopathological factors. The study included 89 samples of cervical carcinoma, which were obtained after radical hysterectomy. COX-2 expression was confirmed in 49.4% of samples, while the greatest number was related to adenocarcinoma (86.7%) when compared to planocellular type (40.6%). A statistically significant difference was confirmed by comparison of COX-2 expression, the presence of lymph nodes metastases (100% *vs* 46.4%) and the presence of parametrial infiltration (80% *vs* 47.6%). A significant difference was not confirmed in relation to age, size of the tumor, depth of stromal invasion and lymphovascular invasion. The correlation between COX-2 expression and a five-year-long survival was not confirmed (positive COX-2 – 81%, negative COX-2 – 98%). Although the correlation of COX-2 expression, histological type and lymph node status was not confirmed, the authors concluded that COX-2 expression cannot be pronounced as a significant prognostic factor with absolute certainty.

A meta-analysis of Huang et al. [45] indicated that COX-2 overexpression might be an unfavorable prognostic factor and chemoradiation resistance predictive factor for cervical cancer.

An association between COX-2 expression and para-aortic lymph node recurrence has been reported in advanced stage patients treated with radiation therapy [46]. Also, some reports documented a better pathological response to chemotherapy in patients with negative COX-2 protein [47]. Still further studies need to correlate the expression of COX-2 with reccurence cervical cancer and the correlation of survival rate with COX-2 expression.

In the group A, with planocellular histologic type of the tumor and FIGO stage of the disease IB, COX-2 expression was confirmed in 56.5% of cases, similar to previously published results. A correlation of histopathological parameters and COX-2 expression, unlike the presented results of the other authors, did not show a statistically significant difference, but it should be noted that their data also differed.

This difference in the results is related to clinicopathological factors and it is possible that it lies in heterogeneity of the samples (number, stage, histological type), methods of testing of COX-2 expression (immunohistocemically, titer in blood, COX-2mRNA). The results of more frequent positive expression of COX-2 in the subgroup with worse

prognostic histopathological parameters refer to the significance of the activity of these factors in carcinogenesis of cervix uteri carcinoma.

Observing COX-2 expression in our study, a statistically significant difference was determined in relation to lymphocyte stromal infiltration ($p = 0.0053$). In the available published papers, the presence of lymphocyte infiltration was not observed as a clinicopathological factor. Immunosuppression is an important factor in regulation of the activity and aggressiveness of a malignant disease. This represents a controversy in positive expression of COX-2 and its role in prostaglandin stimulation in tumor tissue, which leads to suppressing of immune system cells, thus creating an immunosuppressive area with the reduced immunological defense mechanism against the tumor tissue. Fourteen years ago, Staveley-O'Carroll et al. [48] described the induction anergy of T-lymphocytes in early oncogenesis. T-cell and dendritic cell defect, caused by the production of prostaglandin, which are stimulated by COX-2 expression, can play a significant role in tumor evasion of the immune system [4]. This can be indirectly observed in relation to the presence or the absence of lymphocyte infiltration in and around tumor tissue in relation to COX-2 expression.

Conclusion

There was the outstandingly significant COX-2 expression in the group with cervical carcinoma when compared to the control group. The positive correlation of COX-2 expression in the group with carcinoma shows a possible correlation with carcinogenesis of cervix uteri carcinoma. A statistically significant positive correlation between COX-2 expression and certain individual histopathological parameters in the group A was not proved, which was the basis for discarding the possibility of implementation of COX-2 as a marker for prognostic purposes in patients with neoplastic cervical lesions. COX-2 expression was more pronounced in cervical carcinoma without lymphocyte stromal infiltration, which led to the conclusion of a possible paradoxical effect of COX-2 to immunosuppression in tumor tissue. This conclusion requires some additional research. More frequent COX-2 expression in the subgroup with worse prognostic histopathological parameters in the group A, points to the significance of COX-2 expression activity in the process of cervix uteri carcinoma carcinogenesis and the impact to its progression.

R E F E R E N C E S

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