Journal of Interdisciplinary Histopathology
www.scopmed.org
DOI: 10.5455/jihp.20150923115314

Original Research

Immunohistochemical expression of cyclooxygenase-2 in uterine serous carcinoma tissue

Joseph Menczer[1], Letizia Schreiber[2], Esther Berger[1], Tally Levy[2]

[1]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, E. Wolfson Medical Center, Holon, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel,
[2]Department of Pathology, E. Wolfson Medical Center, Holon, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel

Address for correspondence:
J. Menczer, M.D.
Gynecologic Oncology Unit
Dept. of Obstetrics and Gynecology
E. Wolfson Medical Center, Holon, Israel
E-mail: joseph12@internet-zahav.net

Received: August 27, 2015
Accepted: September 23, 2015
Published: October 7, 2015

ABSTRACT

Objective: The aim of the present study was to assess cyclooxygenase-2 (COX-2) expression in uterine serous carcinoma (USC) tissue, to correlate it with clinicopathological prognostic factors in order to assess whether selective COX-2 inhibitors may be effective in the treatment in this tumor. **Materials and Methods:** COX-2 expression assessment by immunohistochemistry was performed on deparaffinized sections of paraffin-embedded tissue blocks of consecutive available USC uterine specimens of patients diagnosed from 2000 to 2014. Staining of more than 10% of the cells was considered positive. Staining intensity was graded on a 0-3 scale. A scoring index was calculated by multiplying the intensity grade by the percentage of stained cells and considered low when it was equal to 1 or less and high when it was more than 1. Clinicopathological data were retrospectively abstracted from the records of the study group patients. **Results:** The study comprised uterine specimens of 31 USC patients. Positive immunohistochemical staining was observed in 25 (80.6%), USC specimens and a high score in 6 (19.4%) of them. No association between immunohistochemical staining parameters and clinicopathological prognostic factors was observed. **Conclusion:** Although our findings should be verified in larger series, it seems that in view of the lack of association between immunohistochemical COX-2 staining parameters in USC tissue and clinicopathological prognostic factors; this aggressive tumor is not a candidate for the use of selective COX-2 inhibitors.

KEY WORDS: Cyclooxygenase-2 expression, clinicopathological prognostic factors, uterine carcinosarcoma

INTRODUCTION

Cyclooxygenase-2 (COX-2) is involved in various steps in the process of malignant tumorigenesis and progression of endometrial carcinoma. It is associated with angiogenesis, increased proliferation and reduced apoptosis [1]. Expression of COX-2 in endometrial cancer has been assessed in the multiple investigations [2-23]. All studies deal with endometrioid endometrial carcinoma or the hystotype of endometrial cancer is not specified in them. Only few studies mentioned COX-2 expression in non-endometrioid carcinoma [11,17,24,25]. None of these studies dealt specifically with uterine serous carcinoma (USC).

USC represents only 10% of endometrial cancer cases. It is an aggressive tumor with a poor prognosis that accounts for a disproportionate number of uterine cancer-related deaths. Even in apparent clinical early stage disease, USC is found to have unfavorable pathological prognostic factors such as lymphovascular space invasion (LVSI), lymph node involvement, and microscopic intraperitoneal spread [26,27].

The aim of the present study was to assess COX-2 expression in USC tissue, to correlate it with clinico-pathological prognostic factors in order to assess whether selective COX-2 inhibitors may be effective in the treatment in this tumor.

MATERIALS AND METHODS

Paraffin-embedded tissue blocks of available consecutive USC uterine specimens of patients diagnosed from 2000 to 2014 were examined after Institutional Review Board approval.

Formalin-fixed hematoxylin–eosin-stained 6 μm slides from the tissue of the same cases were newly performed and reviewed by an expert pathologist (LS) to verify the diagnosis.

The records of the study group patients were retrospectively abstracted, and their clinico-pathological data were recorded.

Immunohistochemistry was performed on deparaffinized 4 μm sections of paraffin - embedded tissue blocks, on a Ventana BenchMark Ultra staining system (Roche Diagnostics, Ventana product, Tucson AZ. the USA). The detection of COX-2 was done using rabbit monoclonal antibody (Diagnostic BioSystem CA. the USA) diluted to 1:50, and a biotin free, multimer technology based on ultra-view universal DAB detection system.

The immunohistochemical cell membrane staining in all specimens was evaluated with microscopy by counting 10 high power fields (×400) with a minimum of 1000 cells counted per slide. Staining of more than 10% of cells was considered positive.

The pathologist assessed two parameters in each section:

a. The proportion of the positively stained cells of the total number of tumor cells counted in the fields examined, ranging from 0% to 100%.
b. The intensity of immunohistochemical staining that was graded subjectively on a 0-3 scale, in which 0 reflected no detectable staining and 3 represented very intense staining.

The scoring index was then calculated by multiplying the intensity grade of the stained cells by the percentage of the positively stained cells. The score was considered low when the index was equal to 1 or less and high when it was more than 1.

Sections of colon carcinoma tissue known to contain COX-2 served as positive controls. The pathologist was blinded to clinical data.

Distribution of categorical variables such as depth of myometrial invasion, (LVSI), lymph node involvement and stage were compared to staining status and score using Fischer's exact test. Survival was calculated by the Kaplan–Meier analysis and differences in survival by the log-rank method. A $P < 0.05$ was considered significant.

RESULTS

The study comprised uterine specimens of 31 USC patients. The mean age of the patients at diagnosis was 74 (range 57-84). Additional selected characteristics of the study group patients are presented in Table 1. Post-menopausal bleeding was the presenting symptom in the great majority (93.6%) of the patients. Only 29.0% of the patients were diagnosed in Stage 1. Most patients were treated by adjuvant chemotherapy ± irradiation (70.4%). Positive immunohistochemical staining was observed in 25 (80.6%), USC specimens and a high score in 6 (19.4%) of them.

Intense immunohistochemical staining in a specimen of serous endometrial carcinoma is shown in Figure 1.

Immunohistochemical COX-2 expression status and score according to histopathological prognostic factors and stage are presented in Tables 2 and 3, respectively. No association between positive staining and high score and clinicopathological prognostic factors was observed. The percentage of USC specimens with LVSI and a high score was higher than those with a low score (50% vs. 10.5%), but the difference did not reach significance ($P = 0.07$). There was no statistically significant correlation between staining parameters and stage or survival after a median follow-up of 18.5 months (range 3-100 months).

Table 1: Selected characteristic of the study group patients

Characteristics	*n* (%)
Total	31 (100.0)
Presenting symptom	
Post-menopausal bleeding	29 (93.6)
Other	2 (6.4)
CA125 prior to treatment	
Elevated	9 (29.0)
Within normal range (≤35 U/mL)	9 (29.0)
Unknown	13 (42.0)
Stage	
I	9 (29.0)
II-IV	22 (70.1)
Treatment	
Surgery+chemotherapy±irradiation	23 (74.2)
Neoadjuvant chemotherapy+surgery+chemothrapy	2 (6.4)
Surgery only	6 (19.4)
Immunohistochemical staining	
Positive	25 (80.6)
Negative	6 (19.4)
Immunohistochemical score; *n*=25 (100%)*	
High	6 (24.0)
Low	19 (76.0)

*6 with negative staining not included

Table 2: COX-2 expression in uterine serous carcinoma tissue according to immunohistochemical staining status

Staining	*n* (%)		*P*
	Positive	Negative	
Total	25 (100.0)	6 (100.0)	
Myometrial invasion (%)			NS
≤50	12 (48.0)	3 (50.0)	
>50	13 (52.0)	3 (50.0)	
LVSI			NS
Present	5 (20.0)	2 (33.3)	
Absent	20 (80.0)	4 (66.7)	
Lymph nodes*			NS
Involved	6 (33.3)	3 (50.0)	
Not involved	12 (66.7)	3 (50.0)	
Stage			NS
I	7 (28.0)	2 (33.3)	
II-IV	18 (72.0)	4 (66.7)	
Median survival (months)	28.1	29.8	NS

*In 7 with positive staining, lymph nodes were not assessed,
NS: Non-significant, COX-2: Cyclooxygenase-2

DISCUSSION

We found that COX-2 positive immunohistochemical staining was present in 80.6%, and a high score in 19.4% of 31 USC specimens examined by us. However, there was no association between COX-2 expression parameters and clinico-pathological prognostic factors.

A PubMed search located only 4 previous studies that mentioned COX-2 expression in non-endometrioid endometrial carcinoma. One study [17] found that the expression of COX-2 was not significantly different between 276 endometrioid and 60 non-endometrioid tumors. Another study [27] that comprised 152 endometrial cancers included 5 cases of non-endometrioid carcinoma, and positive COX-2 expression was found in one of them. An additional study [11] of 110 endometrial carcinoma

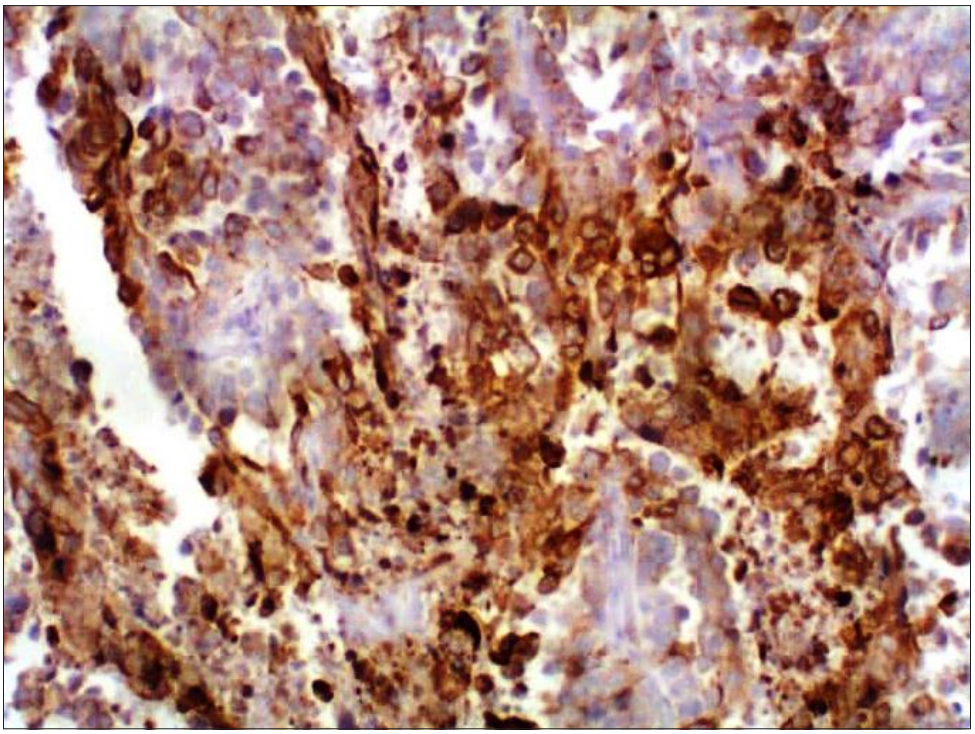

Figure 1: Intense immunohistochemical cyclooxygenase-2 staining in a specimen of uterine serous carcinoma

Table 3: COX-2 immunohistochemical expression in uterine serous carcinoma tissue according to the score of immunohystochemical staining

Score	*n* (%)		*P*
	High	Low	
Total	6 (100.0)	19 (100.0)	
Myometrial invasion (%)			NS
≤50	3 (50.0)	9 (47.4)	
>50	3 (50.0)	10 (52.6)	
LVSI			0.07
Present	3 (50.0)	2 (10.5)	
Absent	3 (50.0)	17 (89.5)	
Lymph nodes*			NS
Involved	3 (50.0)	2 (16.7)	
Not involved	3 (50.0)	10 (83.3)	
Stage			NS
I	2 (33.3)	5 (26.3)	
II-IV	4 (66.7)	14 (73.7)	
Median survival (months)	Not reached	27.7	NS

*Total number with known data=18 (100.0%), NS: Non-significant, COX-2: Cyclooxygenase-2

patients included 15 non-endometrial carcinomas and 6 of them had positive COX-2 staining. The exact histotype of the non-endometrioid tumors was not provided in any of these studies. Only one study [26] of 69 endometrial carcinomas specified that it included 6 cases of serous carcinoma and 3 of them were COX-2 positive.

COX-2 expression in endometrial endometrioid carcinoma was found to be associated with unfavorable prognostic factors in several studies [11,13,20,24]. However, in several other investigations it was found not to be of prognostic value [10,17,18]. Similarly, we found that in USC as well there is no association between COX-2 expression and unfavorable clinicopathological factors. That this is due to the small number of cases in each category cannot be ruled out.

The authors of several studies suggested that selective COX-2 inhibitors may be effective in the treatment of COX-2 positive endometrial cancers [10,14,15,21,22,28]. We could not locate any studies that dealt with the use of selective COX-2 inhibitors in USC.

Although our findings should be verified in larger series, it seems that in view of the lack of association between immunohistochemical COX-2 staining parameters in USC tissue and clinicopathological prognostic factors; this aggressive tumor is not a candidate for the use of selective COX-2 inhibitors.

REFERENCES

1. Ohno Y, Ohno S, Suzuki N, Kamei T, Inagawa H, Soma G, *et al*. Role of cyclooxygenase-2 in immunomodulation and prognosis of endometrial carcinoma. Int J Cancer 2005;114:696-701.
2. Tong BJ, Tan J, Tajeda L, Das SK, Chapman JA, DuBois RN, *et al*. Heightened expression of cyclooxygenase-2 and peroxisome proliferator-activated receptor-delta in human endometrial adenocarcinoma. Neoplasia 2000;2:483-90.
3. Jabbour HN, Milne SA, Williams AR, Anderson RA, Boddy SC. Expression of COX-2 and PGE synthase and synthesis of PGE(2) in endometrial adenocarcinoma: A possible autocrine/paracrine regulation of neoplastic cell function via EP2/EP4 receptors. Br J Cancer 2001;85:1023-31.
4. Cao QJ, Einstein MH, Anderson PS, Runowicz CD, Balan R, Jones JG. Expression of COX-2, Ki-67, cyclin D1, and P21 in endometrial endometrioid carcinomas. Int J Gynecol Pathol 2002;21:147-54.
5. Fujiwaki R, Iida K, Kanasaki H, Ozaki T, Hata K, Miyazaki K. Cyclooxygenase-2 expression in endometrial cancer: Correlation with

microvessel count and expression of vascular endothelial growth factor and thymidine phosphorylase. Hum Pathol 2002;33:213-9.
6. Uotila PJ, Erkkola RU, Klemi PJ. The expression of cyclooxygenase-1 and -2 in proliferative endometrium and endometrial adenocarcinoma. Ann Med 2002;34:428-33.
7. Landen CN Jr, Mathur SP, Richardson MS, Creasman WT. Expression of cyclooxygenase-2 in cervical, endometrial, and ovarian malignancies. Am J Obstet Gynecol 2003;188:1174-6.
8. Kilic G, Gurates B, Garon J, Kang H, Arun B, Lampley CE, *et al.* Expression of cyclooxygenase-2 in endometrial adenocarcinoma. Eur J Gynaecol Oncol 2005;26:271-4.
9. Toyoki H, Fujimoto J, Sato E, Sakaguchi H, Tamaya T. Clinical implications of expression of cyclooxygenase-2 related to angiogenesis in uterine endometrial cancers. Ann Oncol 2005;16:51-5.
10. Hasegawa K, Ohashi Y, Ishikawa K, Yasue A, Kato R, Achiwa Y, *et al.* Expression of cyclooxygenase-2 in uterine endometrial cancer and anti-tumor effects of a selective COX-2 inhibitor. Int J Oncol 2005;26:1419-28.
11. Lambropoulou M, Alexiadis G, Limberis V, Nikolettos N, Tripsianis G. Clinicopathologic and prognostic significance of cyclooxygenase-2 expression in endometrial carcinoma. Histol Histopathol 2005;20:753-9.
12. Li W, Xu RJ, Zhang HH, Jiang LH. Overexpression of cyclooxygenase-2 correlates with tumor angiogenesis in endometrial carcinoma. Int J Gynecol Cancer 2006;16:1673-8.
13. Ohno S, Ohno Y, Suzuki N, Soma G, Inoue M. Cyclooxygenase-2 expression correlates with apoptosis and angiogenesis in endometrial cancer tissue. Anticancer Res 2007;27:3765-70.
14. Genç S, Attar E, Gürdöl F, Kendigelen S, Bilir A, Serdaroglu H. The effect of COX-2 inhibitor, nimesulide, on angiogenetic factors in primary endometrial carcinoma cell culture. Clin Exp Med 2007;7:6-10.
15. Nasir A, Boulware D, Kaiser HE, Lancaster JM, Coppola D, Smith PV, *et al.* Cyclooxygenase-2 (COX-2) expression in human endometrial carcinoma and precursor lesions and its possible use in cancer chemoprevention and therapy. *In Vivo* 2007;21:35-43.
16. Erkanli S, Bolat F, Kayaselcuk F, Demirhan B, Kuscu E. COX-2 and survivin are overexpressed and positively correlated in endometrial carcinoma. Gynecol Oncol 2007;104:320-5.
17. Fowler JM, Ramirez N, Cohn DE, Kelbick N, Pavelka J, Ben-Shachar I, *et al.* Correlation of cyclooxygenase-2 (COX-2) and aromatase expression in human endometrial cancer: Tissue microarray analysis. Am J Obstet Gynecol 2005;192:1262-71.
18. Jongen VH, Briët JM, de Jong RA, Joppe E, ten Hoor KA, Boezen HM, *et al.* Aromatase, cyclooxygenase 2, HER-2/neu, and p53 as prognostic factors in endometrioid endometrial cancer. Int J Gynecol Cancer 2009;19:670-6.
19. Keser SH, Gül AE, Barisik NO, Cakir C, Sensu S, Kandemir NO, *et al.* The relationship of COX-2 expression with estrogen receptor, progesterone receptor and prognostic parameters in endometrial carcinomas. J Obstet Gynaecol Res 2010;36:560-6.
20. Lambropoulou M, Papadopoulos N, Tripsianis G, Alexiadis G, Pagonopoulou O, Kiziridou A, *et al.* Co-expression of survivin, c-erbB2, and cyclooxygenase-2 (COX-2): Prognostic value and survival of endometrial cancer patients. J Cancer Res Clin Oncol 2010;136:427-35.
21. Hasegawa K, Torii Y, Ishii R, Oe S, Kato R, Udagawa Y. Effects of a selective COX-2 inhibitor in patients with uterine endometrial cancers. Arch Gynecol Obstet 2011;284:1515-21.
22. Hasegawa K, Ishikawa K, Kawai S, Torii Y, Kawamura K, Kato R, *et al.* Overcoming paclitaxel resistance in uterine endometrial cancer using a COX-2 inhibitor. Oncol Rep 2013;30:2937-44.
23. Knapp P, Chabowski A, Blachnio-Zabielska A, Walentowicz-Sadlecka M, Grabiec M, Knapp PA. Expression of estrogen receptors (a, ß), cyclooxygenase-2 and aromatase in normal endometrium and endometrioid cancer of uterus. Adv Med Sci 2013;58:96-103.
24. Ferrandina G, Legge F, Ranelletti FO, Zannoni GF, Maggiano N, Evangelisti A, *et al.* Cyclooxygenase-2 expression in endometrial carcinoma: Correlation with clinicopathologic parameters and clinical outcome. Cancer 2002;95:801-7.
25. Jeon YT, Kang S, Kang DH, Yoo KY, Park IA, Bang YJ, *et al.* Cyclooxygenase-2 and p53 expressions in endometrial cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1538-42.
26. del Carmen MG, Birrer M, Schorge JO. Uterine papillary serous cancer: A review of the literature. Gynecol Oncol 2012;127:651-61.
27. Fader AN, Santin AD, Gehrig PA. Early stage uterine serous carcinoma: Management updates and genomic advances. Gynecol Oncol 2013;129:244-50.
28. Hasegawa K, Kawamura K, Kato R, Komiyama S, Kaneko C, Udagawa Y. The effects of the selective cyclooxygenase-2 inhibitor on endometrial cytological findings in uterine endometrial cancer patients. Acta Cytol 2012;56:394-400.

Source of Support: Nil, Conflict of Interest: None declared.