# Expression of gelatinase (MMP-2 and MMP-9) and cyclooxygenase-2 (COX-2) in endometrial carcinoma

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**Summary**

*Objective:* Matrix metalloproteinases (MMPs) are key players in the degradation of extracellular matrix and basement membranes, and are thus important in tumor invasion. Gelatinases (MMP-2 and MMP-9) in particular are prognostic factors in many solid tumors. In this study the immunohistochemical expression of both COX-2 and matrix metalloproteinases has been shown for the first time in endometrium carcinoma.

*Methods:* Forty-two endometrial carcinoma tissues were immunostained for MMP2 antibody (1:100, Rabbit polyclonal), MMP9 antibody (1:100, Rabbit polyclonal) and CoX2 antibody (1:100, Epitope specific rabbit antibody).

Results: 90.5% of the cases were positive for MMP-2 and MMP-9, and 83.3% of the cases were positive for COX-2. A statistically significant association was found between COX-2 overexpression and FIGO stage ($p = 0.001$). A positive correlation was also found with histological grade ($p = 0.006$), myometrial invasion ($p = 0.033$), vascular invasion ($p = 0.017$), and lymphatic invasion ($p = 0.007$). A positive correlation was found between MMP-2 overexpression and vascular and lymphatic invasion ($p = 0.030$ and $p = 0.003$, respectively). MMP-9 overexpression was also found to be correlated with vascular and lymphatic invasion ($p = 0.001$ and $p = 0.012$, respectively). Furthermore, there was a statistically significant correlation between MMP-2 and MMP-9 overexpression ($p = 0.0001$).

*Conclusion:* The results showed that COX-2, MMP-2 and MMP-9 were expressed in a high percentage of primary endometrial carcinomas and their expressions may be associated closely with parameters of tumor aggressiveness.

*Key words:* Endometrial carcinoma; MMP-2; MMP-9; COX-2.

## Introduction

Endometrial carcinoma is currently the most frequent pelvic malignancy in women in the United States and Europe [1]. It is the third most common cause of gynecologic cancer deaths following ovarian and cervical cancer [2].

Currently, the International Federation of Gynecology and Obstetrics (FIGO) stage, depth of myometrial invasion, and grade of differantiation are used to predict the clinical outcome and to plan treatment modalities for patients with endometrial carcinoma [3]. Most endometrial carcinomas are diagnosed in early stages and are associated with a favorable outcome. However, the prognosis of advanced disease is poor [1-3]. The outcome is dependent on the formation of lymph node metastases and distant metastases, which are partially influenced by the degree of tumor differentiation [1-3].

Angiogenesis is essential for the development, growth and advancement of solid tumors [4]. Angiogenic factors from tumors induce and activate matrix metalloproteinase (MMP) plasminogen activator, collagenase and other enzymes in endothelial cells [5].

Matrix metalloproteinases (MMPs) are members of the family of zinc(Zn)-dependent endopeptidases that degrade the extracellular matrix. The MMP family currently consists of 25 enzymes, which are classified according to their structures into eight distinct classes: five secreted and three membrane-type MMPs (MT-MMPs) [6]. These enzymes are produced by many types of cells in response to inflammation or tumor progression. Among the variety of proteinases potentially implicated in tumor progression, the MMPs have attracted considerable interest because of their ability to collectively degrade essentially all protein constituents of connective tissues. This process is mediated by proteolytic enzymes that degrade the extracellular matrix and basement membranes, facilitating tumor invasion and metastasis [7-9].

Increased expression of certain MMPs in advanced tumors leads to degradation of the extracellular matrix (ECM). MMP-2 (gelatinase A), a 72-kDa protein, and MMP-9 (gelatinase B), a 92-kDa protein, are able to degrade basement membranes and several specified extracellular matrix macromolecules such as type I, IV, and V collagen, elastin and laminin [8]. These proteases have been linked to the malignant potential of tumors by enhancing invasion and metastases [9].

Recently much attention has been focused on cyclooxygenase (COX), which is a rate-limiting enzyme in the biosynthesis of prostaglandins from arachidonic acid [10]. Two isoforms of COX are known, COX-1 and COX-2, of eicosanoids, including prostaglandins $D_2$, $E_2$, $I_2$, $F_{2\alpha}$ and tromboxane A [10].

COX-2 is an inducible immediate-early gene, which is up-regulated by various stimuli, including mitogens, cytokines, growth factors and tumor promoters [11]. Thus, COX-2 plays a key role in early stages of carcinogenesis by promoting the proliferation of tumoral cells

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and their resistance to apoptosis, as well as angiogenesis, tumor cell invasion and setting up of the metastatic process. It has been reported that COX-2 works on angiogenesis associated with tumor growth advancement of various tumors such as advanced ovarian serous carcinomas [12], breast cancers [13], gastric cancers [14], renal cell carcinomas [15], head and neck squamous cell carcinomas [16] and colon cancers [17]. Several studies have shown that COX-2 exspression is aberrantly increased in various human epithelial cancers [12-17]. These findings suggest that the cellular up-regulation of COX2 may be a common mechanism in epithelial carcinogenesis.

## Materials and Methods

*Patients and samples*

Tissue samples from 42 patients with endometrial carcinoma that were diagnosed between 1998 and 2005 at the Department of Pathology, Suleyman Demirel University School of Medicine, were included in the study. The age, tumor type, myometrial and vascular invasion, lymph node metastasis, peritoneal cytology, FIGO grade and stage were evaluated by reviewing the medical charts and pathological records. Glass slides were reviewed for histological classification according to the FIGO criteria [4]. Three independent observers did the histopathological evaluations twice.

*Immunohistochemistry*

Immunohistochemical analysis for MMP-2, MMP-9 and COX-2 were performed on formalin-fixed, paraffin-embedded archival tissue using the streptavidin-biotin-peroxidase technique. For all cases, 4 μm histologic sections were deparaffinized in xylene and dehydrated in a descending dilution of ethanol. For the antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by 20 min of incubation with 0.3% hydrogen peroxidase. Slides were tested with MMP-2 antibody (1:100, Rabbit polyclonal, LabVision), MMP-9 antibody (1:100, Rabbit polyclonal Lab Vision) and COX-2 antibody (1:100 Epitope specific rabbit antibody, Lab Vision). Sections were tested with the streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP, Lab Vision, USA), and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, and mounted with mounting medium. The positive controls for MMP-2 and MMP-9 were placental tissue. Tissue of colon cancer from humans served as the positive control in the COX-2 immunostaining.

Three independent observers blinded for clinical data analyzed the staining for MMP-2, MMP-9 and COX-2. The total area of the sample was analyzed, and the percentage of positive immunostaining of cancer cells was estimated visually. The number of positive immunostained cancer cells was regarded as the most important element, while the intensity of staining was also taken into consideration. The cut-off value for positivity was set at 20% of cancer cells stained. The staining was considered intensive when more than 50% of cancer cells showed a positive reaction. The immunostaining was thus recorded in three categores; negative (-), positive (+), and intensively positive (++) staining.

*Statistical analysis*

For statistical evaluation, the SPSS software version 12 was used. We used Spearman's correlation test to analyze correlations among expressions of MMP-2, MMP-9 and COX-2 and several clinicopathological parameters. A p value $< 0.05$ was considered as significant.

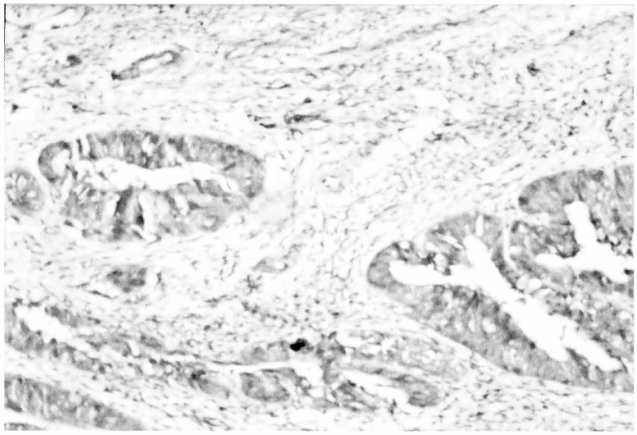
## Results

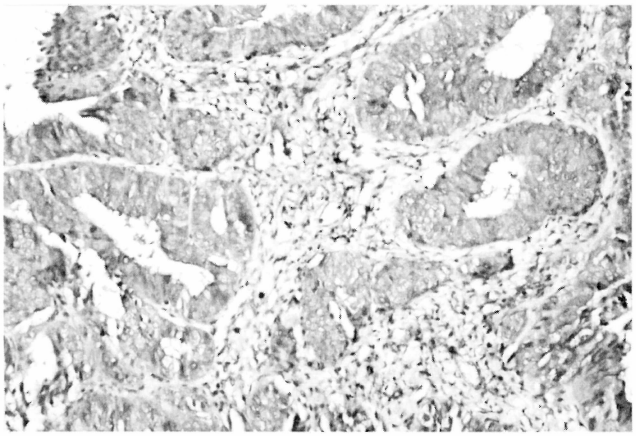
Our group consisted of 42 patients. Ages of the patients ranged from 37 to 80 years (median 57 years). There were four patients under 46 years of age (9.5%) and 38 patients over 46 years of age (90.5%). All endometrial carcinomas were endometrioid carcinoma. Among 42 endometrial carcinomas studied, there were 25 patients (59.5%) with histological grade 1, ten patients (23.8%) with histological grade 2, and seven patients (16.7%) with histological grade 3 according to FIGO. There was invasion in less than half of the myometrium in 20 patients (47.6%). Twenty-two (52.4%) patients had invasion of more than half of the myometrium. Twenty-three patients (54.8%) were in Stage 1, five (11.9%) were in Stage 2, and 14 (33.3%) were in Stage 3. Eleven cases (26.2%) had lymph node metastases and 14 cases (33.3%) had vascular invasion. The clinicopathologic characteristics of the endometrial cancer patients are shown in Table 1.

Histologically, MMP-2, MMP-9 and COX-2 expressions showed a cytoplasmic pattern. MMP-2 expression was detected in 38 (90.5%) out of 42 cases. MMP-2 showed positive staining in all histological grades. MMP-2 was expressed not only in tumoral tissue but also in the endothelial cells of intratumoral and peritumoral microvessels and with fibroblasts and macrophages located in the stroma within or around the tumor aggregates (Figure 1). MMP-9 expression was detected in 38 (90.5%) out of 42 cases. MMP-9 was also expressed by

Table 1. — *Clinicopathological characteristics of the endometrial cancer population.*

| All cases | No. = 42 |
|---|---|
| Age (years) | |
| < 45 | 4 (9.5%) |
| > 46 | 38 (90.5%) |
| FIGO grade | |
| I | 25 (59.5%) |
| II | 10 (23.8%) |
| III | 7 (16.7%) |
| FIGO stage | |
| I | 23 (54.8%) |
| II | 5 (11.9%) |
| III | 14 (33.3%) |
| Myometrial invasion | |
| < 1/2 | 20 (47.6%) |
| > 1/2 | 22 (52.4%) |
| Lymph node status | |
| Negative | 31 (73.8%) |
| Positive | 11 (26.2%) |
| Vascular invasion | |
| Negative | 28 (66.7%) |
| Positive | 14 (33.3%) |





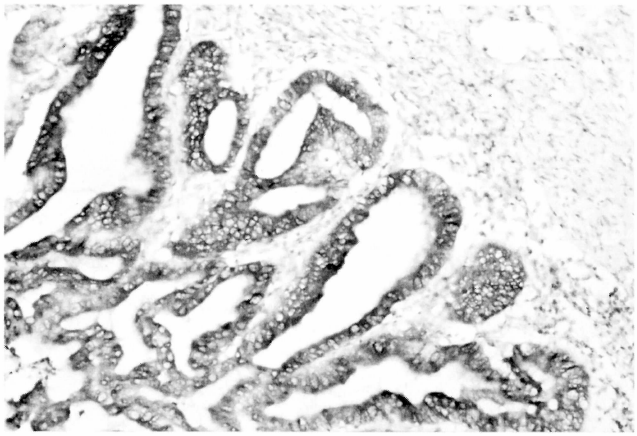


Figure 1. — Immunohistochemical staining for MMP-2 in FIGO grade 1 endometrial adenocarcinoma. Also some stromal cells, inflammatory cells and microvessels showing immunoreactivity can be seen (DAB x 100).

Figure 2. — MMP-9 immunolocalized to abnormal uterine glands in FIGO grade 1 adenocarcinoma. Stroma staining is also evident particularly in areas adjacent to tumor tissue (DAB x 100).

Figure 3. — Immunohistochemical staining for COX-2 in endometrial cancer in FIGO grade 1 endometrial adenocarcinoma. COX-2 positive tumor showing intense cytoplasmic immunreaction in tumor cells and immunoreaction in inflammatory cells (DAB x 100).

stromal cells, including endothelial cells, fibroblasts and macrophages (Figure 2). MMP-9 showed positive staining not only in tumor tissue but also in stromal cells and inflammatory cells around the tumor.

COX-2 expression was detected in 35 (83.3%) out of 42 cases. Although COX-2 showed a diffuse cytoplasmic staining pattern in tumor cells (Figure 3), perinuclear and nuclear expression was also detected in some tumor cells.

Table 2. — *COX-2, MMP-2 and MMP-9 expressions and clinicopathological characteristics.*

| | COX-2 expression | | | | MMP-2 expression | | | | MMP-9 expression | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (–) | (+) | (++) | r value<br>p value | (–) | (+) | (++) | r value<br>p value | (–) | (+) | (++) | r value<br>p value |
| FIGO Grade | | | | r = 0.418** | | | | r = 0.013$^{NS}$ | | | | r = 0.124$^{NS}$ |
| I | 7 | 5 | 13 | p = 0.006 | 2 | 5 | 18 | p = 0.934 | 3 | 3 | 19 | p = 0.566 |
| II | 0 | 2 | 8 | | 1 | 3 | 6 | | 1 | 3 | 6 | |
| III | 0 | 0 | 7 | | 1 | 0 | 6 | | 0 | 0 | 7 | |
| FIGO Stage | | | | r = 0.555** | | | | r = 0.129$^{NS}$ | | | | r = 0.236$^{NS}$ |
| I | 7 | 6 | 10 | p = 0.000 | 3 | 4 | 16 | p = 0.417 | 3 | 2 | 3 | p = 0.487 |
| II | 0 | 1 | 4 | | 0 | 3 | 2 | | 0 | 2 | 3 | |
| III | 0 | 0 | 14 | | 1 | 1 | 12 | | 1 | 1 | 12 | |
| Myometrial invasion | | | | r = 0.330* | | | | r = 0.060$^{NS}$ | | | | r = 0.159$^{NS}$ |
| < 1/2 | 5 | 5 | 10 | p = 0.033 | 3 | 3 | 14 | p = 0.060 | 3 | 1 | 16 | p = 0.765 |
| > 1/2 | 2 | 2 | 18 | | 1 | 5 | 16 | | 1 | 5 | 16 | |
| Lymph node status | | | | r = 0.413** | | | | r = 0.450** | | | | r = 0.385* |
| Negative | 7 | 7 | 17 | p = 0.007 | 4 | 5 | 22 | p = 0.003 | 4 | 15 | 12 | p = 0.012 |
| Positive | 0 | 0 | 11 | | 0 | 0 | 11 | | 0 | 2 | 9 | |
| Vascular invasion | | | | r = 0.367* | | | | r = 0.335* | | | | r = 0.593* |
| Negative | 6 | 7 | 15 | p = 0.017 | 3 | 4 | 21 | p = 0.030 | 4 | 16 | 8 | p = 0.000 |
| Positive | 1 | 0 | 13 | | 1 | 1 | 12 | | 0 | 1 | 13 | |
| Age (years) | | | | 0.593 | | | | 0.677 | | | | 0.788 |
| < 45 | 2 | 0 | 2 | | 0 | 1 | 3 | | 4 | 0 | 0 | |
| > 46 | 5 | 7 | 26 | | 4 | 7 | 27 | | 0 | 6 | 18 | |

r = Spearman's correlation test, correlation coefficient; * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

COX-2 expression was detected in stromal inflammatory cells, fibroblasts and capillary endothelial cells.

There was a statistically significant correlation detected between the COX-2 expression and FIGO grade ($r = 0.418$, $p = 0.006$), FIGO stage ($r = 0.555$, $p = 0.000$), myometrial invasion depth ($r = 0.330$, $p = 0.033$), vascular invasion ($r = 0.0.367$, $p = 0.017$), and lymph node metastases ($r = 0.413$, $p = 0.007$). A statistically significant relationship was detected between COX-2 expression and MMP-9 expression ($r = 0.372$, $p = 0.015$). There was a statistically significant relationship between MMP-2 expression and vascular invasion and lymph node metastases ($r = 0.335$, $p = 0.03$ and $r = 0.450$, $p = 0.003$, respectively). A statistically significant relationship was present between MMP-9 expression and vascular invasion and lymph node metastasis ($r = 0.593$, $p = 0.000$ and $r = 0.385$, $p = 0.012$, respectively). There were no statistically significant relationships between MMP-2 and MMP-9 expressions and FIGO grade, FIGO stage, and myometrial invasion depth ($p > 0.05$).

In addition, there was a statistically significant relationship between MMP-2 expression and MMP-9 expression ($r = 0.644$, $p = 0.0001$). COX-2, MMP-2 and MMP-9 expressions and clinicopathological characteristics are shown in Table 2.

## Discussion

Invasion and metastases of cancer cells are known to be initiated by an interaction between cancer cells and host cells. The extracellular matrix (ECM) plays a major role in the recognition and migration of cancer cells [8]. The mechanisms involved in cellular invasion and migration include cell-cell or cell-ECM attachment by proteolytic modification of ECM, and then migration through the modified matrix by a cancer cell. Among various cellular mediating components, MMPs and integrins have been implicated in this process [9].

Recent studies have shown that the role of MMPs during tumor progression is not restricted to only metastasis and invasion. They also contribute to steps like tumor growth, angiogenesis and migration [18-22]. It is known that increased MMP-2 expression is correlated with the stage and progression of many human tumors [11-17].

In the present study, it was shown that COX-2 MMP-2, and MMP-9 are expressed in primary endometrial carcinomas and their expressions may be associated closely with tumor agressiveness.

Aglund *et al.* detected a significant relationship between MMP-9 expression and histological grade in their immunohistochemical study of 88 patients with endometrial carcinoma [23]. In our study, there was no statistically significant relationship between histological grade and MMP-9 expression. Aglund *et al.* did not find a significant correlation between myometrial invasion depth and MMP-2 and MMP-9 expression [23]. In the current study, there was no statistically significant relationship between myometrial invasion depth and MMP-2 and MMP-9 expressions which is in agreement with their results.

Di Nezza *et al.* detected a relationship between MMP-9 and vascular and lymphatic invasion [24]. Our findings were in line with these results. On the other hand, Lopata *et al.* have confirmed elevated levels of latent and active forms of MMP-2 and MMP-9 in uterine lavage samples from endometrial cancer patients [25]. They found no statistically significant association between MMP score and histological grade, vascular invasion and depth of myometrial invasion.

Epidemiologic and basic science conducted thus far would suggest that prostaglandins play a significant role in cancer biology. Many studies have reported recently that COX-2 is highly expressed in endometrial adenocarcinoma [26-34]. Although the mechanism of COX-2 upregulation is unknown, recent studies suggest that it may result from deregulation of key steps in the epidermal growth factor receptor signaling pathways, including the ras oncogene whose expression has been detected in endometrial tumors [27, 29].

Ferrandina *et al.* showed a higher percentage of COX-2 positivity in endometrial carcinomas invading greater than 50% of myometrial thickness compared with tumor confined to the inner half of the myometrium [32]. In our study, the relationship between COX-2 expression and myometrial invasion depth was in accordance with their results.

In 63 cases with endometrial cancer, Fujiwaki *et al.* found that immunohistochemically evident COX-2 expression in endometrial cancer cells correlated significantly with intratumoral microvessel count [27]. Their findings suggest that COX-2, which is produced by endometrial cancer cells, may be at least partially responsible for the important process of angiogenesis in the development of human endometrial cancer. In our study, there was a significant correlation between lymphatic and vascular invasion depth and COX-2 expression.

In 110 cases with primary endometrial carcinoma, Lambropoulou *et al.* detected a positive correlation between COX-2 expression and histological grade, FIGO stage and myometrial invasion, as in our study [26]. Fujiwaki *et al.* did not find a correlation between COX-2 expression and histological grade and myometrial invasion [27]. However, we detected a significant correlation between COX-2 expression and histological grade, FIGO stage and myometrial invasion.

## Conclusions

The present data show that elevated COX-2 expresssion is associated with histological grade, FIGO stage, myometrial invasion, lymph node metastases and vascular invasion while MMP-9 and MMP-2 expressions are associated with only lymph node metastases and vascular invasion. A combined use of MMP-9 together with MMP-2 and COX-2 showed that MMP-9 expression was positively correlated both with COX-2 and MMP-2 expressions.

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