Immunohistochemical expression of MMP-2, MMP-9 and COX-2 in Stage IA malignant polyps of the endometrium

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Summary

Objective: To study whether endometrioid type malignant endometrial polyps (MEP) are different from endometrium cancer not associated with polyps (ECNAP) in means of immunohistochemical expressions of MMP-2, MMP-9 and COX-2. *Methods:* Archived tissue samples of eight MEP, eight ECNAP and 16 benign endometrial polyps were selected and immunohistochemically analyzed for MMP-2, MMP-9 and COX-2 expression. *Results:* MMP-2 and MMP-9 were overexpressed in ECNAP compared to MEP and benign endometrial polyps ($p < 0.05$). MMP-2 and MMP-9 expressions were not different in the malignant part of MEP, benign part of MEP and benign endometrial polyps. COX-2 expression was found to be higher in benign lesions, although this was not statistically significant. *Conclusion:* Similar immunohistochemical expression of MMP-2, MMP-9 and COX-2 within a polyp and with benign polyps may indicate an immunohistochemically indolent characteristic of MEP.

Key words: Endometrial polyp; Endometrium cancer; Cyclooxygenase 2; Matrix metalloproteinase 2.

Introduction

Endometrial cancer is the most common gynecologic cancer in developed countries [1]. It usually affects postmenopausal women and manifests as uterine bleeding early in the course of disease. Therefore, it is usually identified in FIGO Stage I [2]. Tumor type, histologic grade, myometrial invasion, lymphovascular space involvement and lymph node metastasis are important prognostic factors [3, 4]. The extracellular matrix and basal membrane are the main barriers to tumor progession.

Matrix metalloproteinases (MMP) are a group of zinc-dependent endopeptidases which play a pivotal role in the degration of extracellular matrix [4]. The extracellular matrix is composed of type I, type II and type III collagen. The basement membrane is especially composed of type IV collagen. These are degraded particularly by gelatinases (MMP-2 and MMP-9) in tumor invasion [4, 5]. The frequency of MMP-2 and MMP-9 expression is reported to be increased with advancing histologic grade and with increasing depth of myometrial invasion [6-8]. Endometrial cancer invading the basal membrane and myometrium by gelatinases penetrates to lymphovascular spaces and disseminates. The role of gelatinases in this sequence has been well-established in recent reports [6-8].

Cyclooxygenases (COX) synthesize prostoglandins from arachidonic acid. COX-1 is constitutively expressed in most of the tissues [9]. However, COX-2 transcription can be induced by cytokines, growth factors, phorbol esters and mitogens. COX-2 is found to be upregulated in malignant cells [10, 11] . COX-2 is related to angiogenesis, tumor growth and progression [12]. COX-2 is reported to be overexpressed in endometrium cancer [13] and it is proposed that COX-2 inhibitors may be of benefit in the treatment of endometrial cancer [14].

Malignant endometrial polyps are rarely encountered [15]. Seventy-eight percent of endometrial polyps are benign, and 13% are with endometrial hyperplasia without atypia. Polyps with endometrial hyperplasia with atypia and carcinomatous polyps comprise 1% and 2% of endometrial polyps, respectively. [16]. The low prevalence of malignant endometrial polyps undervalues its entity and makes it difficult to study. It is not known whether malignant endometrial polyps are different from malignancies not arising in a polyp [17]. Malignant polyps may have access to the lymphovascular system even though they are low-stage [17]. Characteristics of malignancies arising in polyps may be different from malignancies not assoiated with polyps. In the present study, immunohistochemical expressions of gelatinases and COX-2 were studied in benign endometrial polyps, early-stage malignant endometrial polyps and early-stage endometrial cancer. This may give clues regarding interaction of malignant endometrial polyp tumors (MEP) and endometrial cancer not associated with polyps (ECNAP) with extracellular matrix.

Material and Methods

Specimens of eight MEP, eight ECNAP and 16 benign endometrial polyps were included in the study. All the malignant specimens were grade 1, endometrioid type endometrium cancer in FIGO Stage IA. We defined a malignant polyp according to Coeman et al.'s strict criteria [18]. Coeman *et al.* reported that the pedicle and surrounding endometrium must be benign and the carcinoma must be confined to the polyp surface to recognize malignancy originating in a polyp [18].

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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, a 4 μm histological section was deparaffinized in xylene and dehydrated in descending dilution of ethanol. For 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, USA), MMP-9 antibody (1:100, rabbit polyclonal, Lab Vision, Fremont, CA, USA) and COX-2 antibody (1:100 Epitope specific rabbit antibody, Lab Vision, Fremont, CA, USA). Sections were tested with the streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP, Lab Vision, Fremont, CA, 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 control for MMP-2 and MMP-9 was placental tissue. Tissue of colon cancer from humans served as the positive control in the COX-2 immunostaining.

Two independent observers blinded for clinical data analyzed the staining for MMP-2, MMP-9 and COX-2. Scoring was done on a point scale, the IRS [19]. Staining intensity (weak, 1 point; moderate, 2 points; strong, 3 points) and percentage groups of positive tumor cells (< 10%, 1 point; 11%-50%, 2 points; 51%-80%, 3 points; > 80%, 4 points) were multiplied to achieve a score between 1 and 12. IRS for MMP-2, MMP-9 and COX-2 were calculated in benign polyps, ECNAP and MEP. Two IRS encounting for benign and malignant sections of MEP were recorded for each MEP. Results were analyzed by the One-way ANOVA test and chi-square test. The level of statistical significance was chosen to be $p < 0.05$. Statistical analysis was performed using the SPSS 13.0 software program (SPSS, Chicago, IL, USA).

Results

Cox-2 expression

None of the examined specimens had a maximum IRS of 12. Mean of COX-2 IRS was 3.2 ± 2.0 points, 2.8 ± 1.9 points, 3.5 ± 2.5 points and 4.3 ± 1.4 points in ECNAP, malignant part of MEP, benign part of MEP and benign endometrial polyp, respectively ($p > 0.05$) (Figure 1).

Percentage of stained cells was similar in all groups. Intensity of staining in benign lesions was higher than malignant lesions ($p < 0.05$); COX-2 was stained strongly in benign endometrial polyps compared to ECNAP and the malignant part of MEP ($p < 0.05$), while intensity of COX-2 staining in benign endometrial polyps and the benign part of MEP was similar. There was no difference in intensity of COX-2 staining within the benign and malignant parts of the each MEP (Table 1).

MMP-2 expression

None of the examined specimens had a maximum IRS of 12. Mean of MMP-2 IRS was 3.7 ± 0.7 points, 2.8 ± 1.8 points, 2.1 ± 1.8 points, 1.5 ± 1.3 points in ECNAP, malignant part of MEP, benign part of MEP and benign endometrial polyp, respectively ($p < 0.05$). IRS of MMP-2 in endometrium cancer is higher than benign polyps ($p < 0.05$) and malignant polyps ($p > 0.05$) (Figure 2). Percentage of stained cells and intensity of stained cells were significantly different in ECNAP and the malignant section of MEP than benign lesions (Table 1).

MMP-9 Expression

None of the examined specimens had a maximum IRS of 12. Mean of MMP-9 IRS was 4.2 ± 1.2 points, 2.7 ± 1.9 points, 2.2 ± 2.1 points, 1.9 ± 1.5 points in ECNAP, the malignant part of MEP, the benign part of MEP and benign endometrial polyps, respectively ($p < 0.05$). IRS was found to be higher than benign endometrial polyps ($p < 0.05$) and MEP ($p > 0.05$) (Figure 3). Percentage of stained cells and intensity of stained cells were significantly different in ECNAP and malignant sections of MEP than benign lesions (Table 1).

Table 1. — *Intensity of immunohistohemical expression of COX-2, MMP-2 and MMP-9.*

Intensity & percentage stained cells	ECNAP	Malignant part of MEP	Benign part of MEP	Benign endometrial polyp	p
Cox-2	–	12.5%	12.5%	–	< 0.05
No staining	50%	37.5%	25%	–	
Weak	50%	37.5%	25%	50%	
Moderate	–	12.5%	37.5%	50%	
Strong					
No staining	–	12.5%	12.5%	–	
< 10%	25%	12.5%	25%	25%	NS
11-50%	50%	75%	62.5%	75%	
51-80%	25%	–		–	
> 80%	–	–		–	
MMP-2	–	–	–	12.5%	< 0.05
No staining	12.5%	37.5%	75%	56.3%	
Weak	87.5%	50%	12.5%	31.3%	
Moderate	–	12.5%	12.5%	–	
Strong					
No staining	–	–	–	12.5%	< 0.05
< 10%	–	37.5%	62.5%	68.8%	
11-50%	100%	62.5%	37.5%	18.8%	
51-80%	–		–	–	
> 80%	–		–	–	
MMP-9	–	–	12.5%	12.5%	< 0.05
No staining	12.5%	50%	50%	43.8%	
Weak	62.5%	50%	25%	37.5%	
Moderate	25%	–	12.5%	6.3%	
Strong					
No staining	–	–	12.5%	12.5%	< 0.05
< 10%	–	50%	50%	50%	
11-50%	100%	37.5%	37.5%	37.5%	
51-80%	–	12.5%	–	–	
> 80%	–	–	–	–	

Discussion

In the present study, immunohistochemical expression of important markers which have prognostic value and are involved in the pathogenesis of endometrial cancer were compared in ECNAP, MEP and benign endometrial polyps.

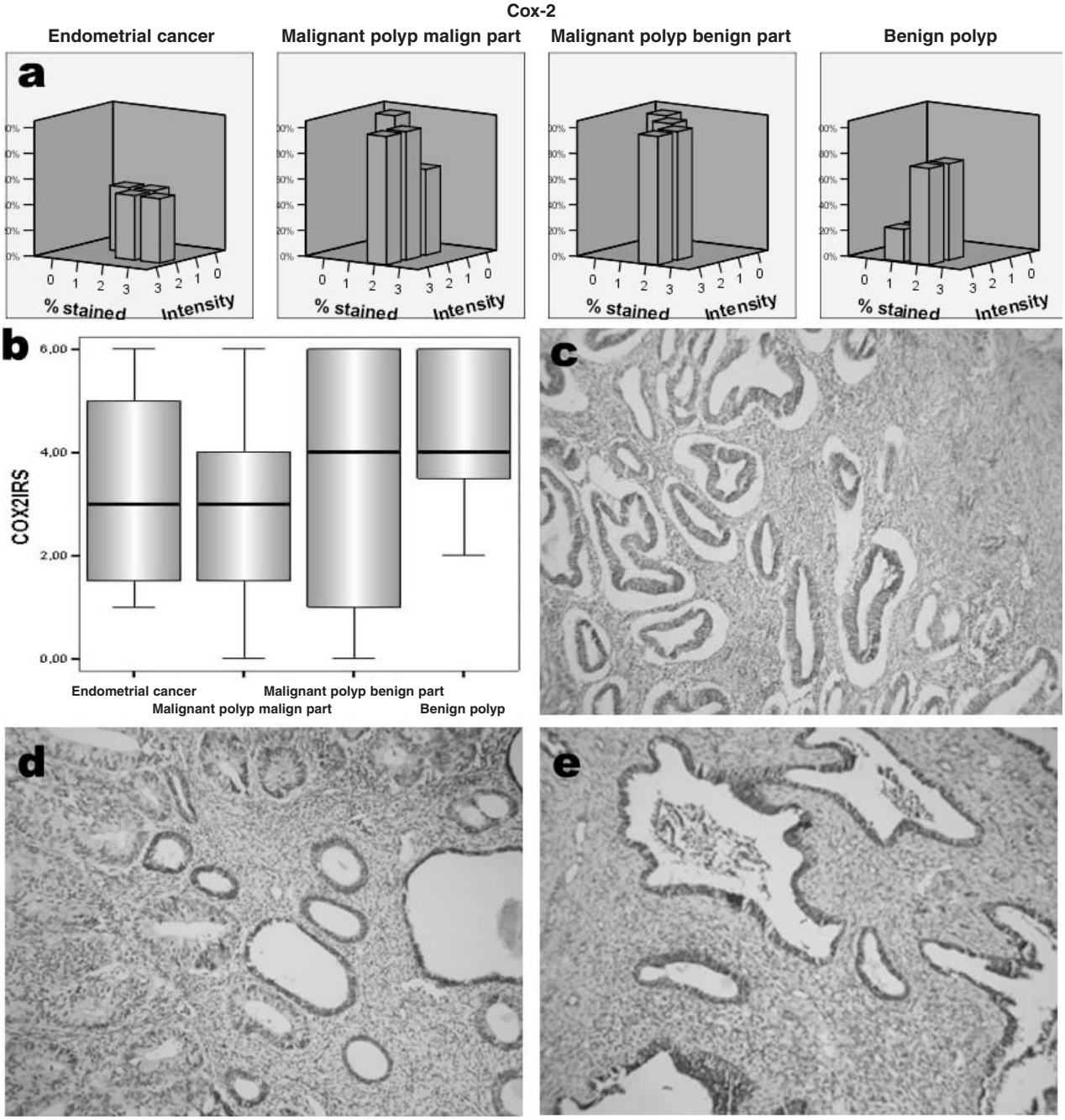

Figure 1. — a), b) Cox-2 staining intensity, percentage of stained cells and IRS in endometrial cancer not associated with polyps (ECNAP), malignant endometrial polyps (MEP) and benign endometrial polyps (0: no staining, 1: weak, 2: moderate, 3: strong/0: no staining, 1: < 10%, 2: 11-50%, 3: 51-80%, 4: > 80%). c) Immunohistochemical expression of COX-2 in ECNAP, d. in MEP and e. benign polyp is moderate, moderate and strong, respectively.

COX-2 expression is reported to be increased in endometrial carcinoma and it is proposed that COX-2 inhibits apoptosis, enhances metastases and angiogenesis [12, 13, 20-22]. However, it is not known whether COX-2 expression is a late or an early step in the development of endometrial carcinogenesis [13]. COX-2 expression was found to be similar in benign polyps, MEP, and Stage IA endometrial carcinoma in our study. Orejuela *et al.* compared expression of COX-2 in biopsy samples of endometrial cancer, endometrial hyperplasia, and normal endometria and found no statistically significant increase in COX-2 expression in the endometrial cancer cases or endometrial hyperplasia samples [23]. They have also reported that COX-2 expression was noticeably greater in the superficial layer of normal epithelium. Interestingly, immunohistochemical expression of COX-2 was remarkable in benign endometrial polyps in our study. In contrast to these findings, Nasir *et al.* reported that COX-2

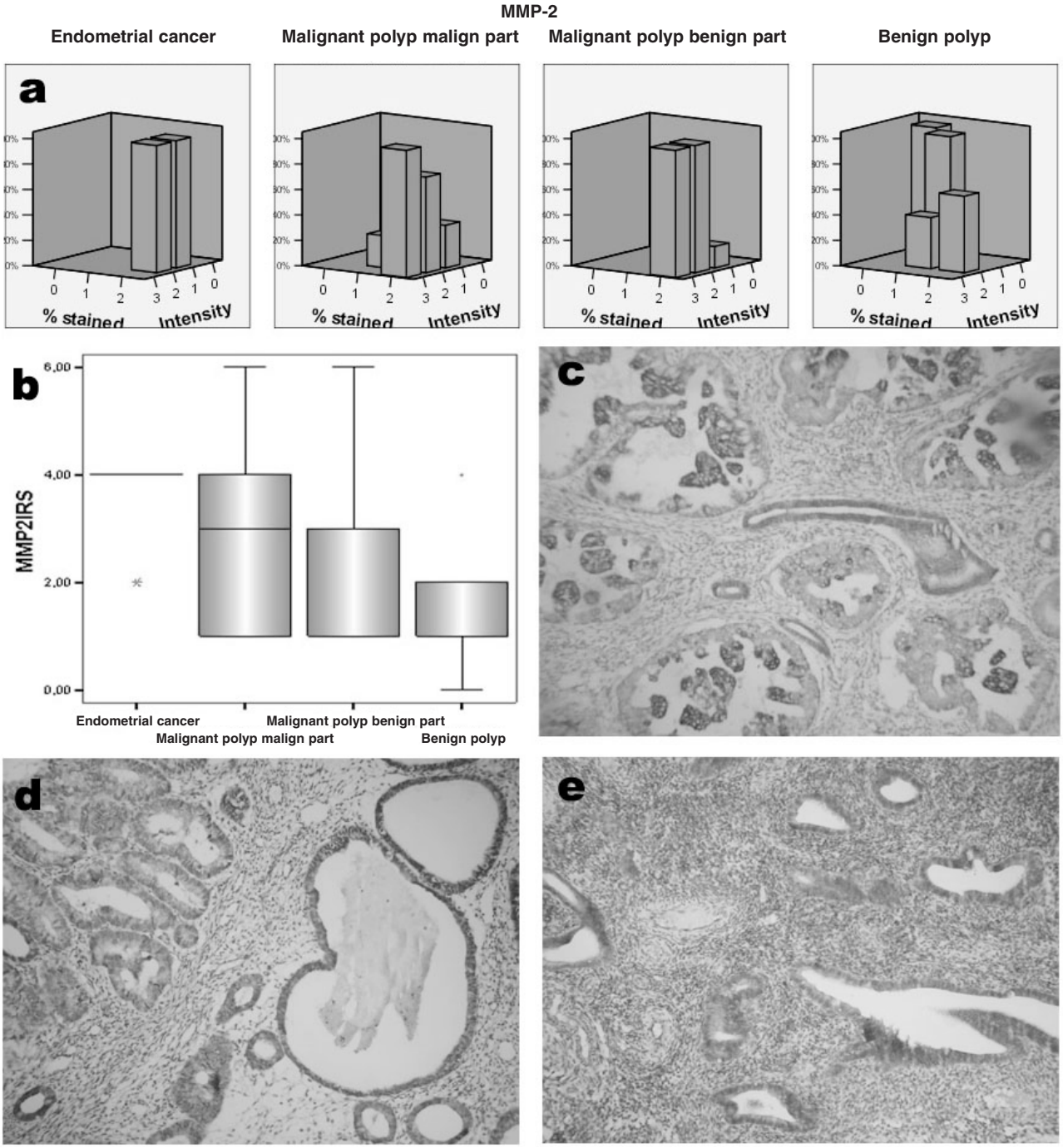

Figure 2. — a), b) MMP-2 staining intensity, percentage of stained cells and IRS in endometrial cancer not associated with polyps (ECNAP), malignant endometrial polyps (MEP) and benign endometrial polyps (0: no staining, 1: weak, 2: moderate, 3: strong/0: no staining, 1: < 10%, 2: 11-50%, 3: 51-80%, 4: > 80%). c) Immunohistochemical expression of MMP-2 in ECNAP, d) in MEP and e. benign polyp is strong, moderate and weak, respectively.

expression increases as the severity of the disorder changes from endometrial hyperplasia to invasive endometrial cancer [24]. Further, studies are needed to evaluate the expression of COX-2 and to clarify its role in the process of endometrial hyperpasia and early-stage endometrium cancer.

Upregulation of MMP-2 and MMP-9 in endometrial cancer have been associated with increased myometrial invasion, higher grade, metastasis and poor prognosis in previous reports [7, 8, 25]. Serous carcinomas usually arise in polyps and have a worse prognosis than other type of carcinomas, but it is not known whether similar types of carcinomas that begin in a polyp are different from carcinomas not associated with polyps [17, 26]. IRS

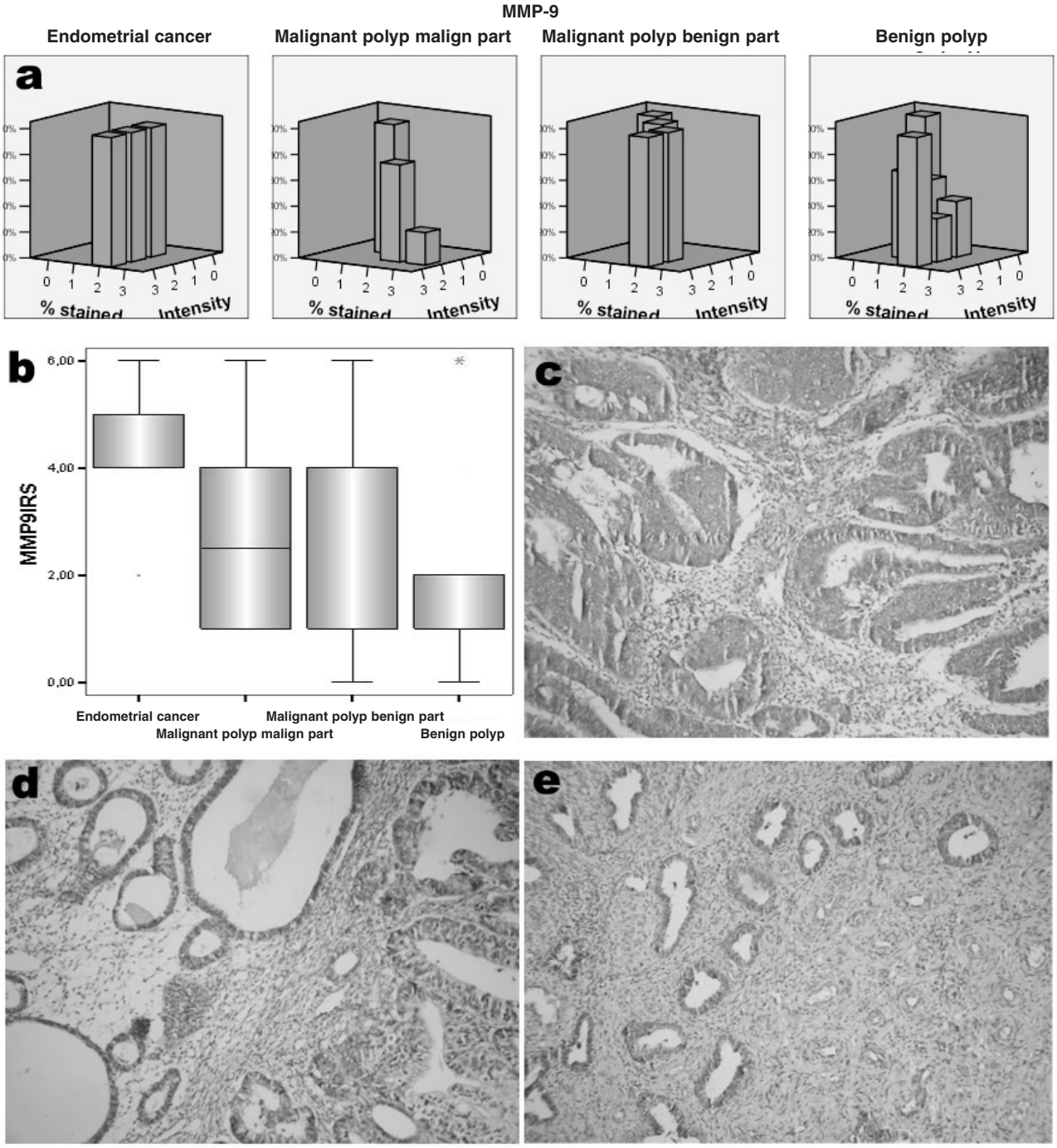

Figure 3. — a), b) MMP-9 staining intensity, percentage of stained cells and IRS in endometrial cancer not associated with polyps (ECNAP), malignant endometrial polyps (MEP) and benign endometrial polyps (0: no staining, 1: weak, 2: moderate, 3: strong/0: no staining, 1: < 10%, 2: 11-50%, 3: 51-80%, 4: > 80%).c. Immunohistochemical expression of MMP-9 in ECNAP, d. in MEP and e. benign polyp is strong, moderate and weak, respectively.

of MMP-2 and MMP-9 increased from benign endometrial polyps to endometrial cancer. Immunohistochemical expression of MMP-2 and MMP-9 in MEP were moderate between benign polyps and endometrial cancer in the present study, suggesting that early endometrioid endometrial cancer developing in a polyp may be more indolent. Immunohistochemical expression of MMP-2 and MMP-9 was not statistically different in benign and malignant sections within a polyp, indicating that invasiveness potential is not increased in malignant parts of polyps in early-stage endometrium cancer. However, gelatinases which play a role in tumor progression are overexpressed in ECNAP.

In conclusion, we found that MMP-2 and MMP-9 are

overexpressed in early-stage endometrial cancer. However, immunohistochemical expression of COX was not different. COX-2 may be involved in the pathogenesis of endometrium cancer in later stages of tumoral development. Similar immunohistochemical expression of MMP-2, MMP-9 and COX-2 within a polyp and with benign polyps may indicate an immunohistochemically indolent characteristic of MEP.

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