

Expression of laminin-1 and matrix metalloproteinase-9 in benign and malignant endometrium

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Background/aim: Laminin-1 and matrix metalloproteinase (MMP)-9 may play roles in the progression from benign to malignant endometrium, so we aimed to investigate their levels of expression in these tissues.

Materials and methods: This case-control study was conducted at a tertiary care center between January 2014 and December 2016. Paraffin blocks of 50 specimens of benign endometrium with proliferative (n = 20), secretory (n = 11), and atrophic (n = 5) endometrium; simple endometrial hyperplasia without atypia (n = 12); and endometrial polyp (n = 2) histology and 49 specimens of malignant endometrium with endometrioid (n = 40), serous (n = 7), clear cell (n = 1), and undifferentiated (n = 1) types were immunostained with laminin-1 and MMP-9 antibodies and assessed for basement membrane continuity for laminin-1 and the percentage and intensity of MMP-9 expression in epithelial cytoplasm.

Results: Laminin-1 continuity in the basement membrane was higher in benign (92%) compared to malignant (16.3%) endometrium (p < 0.0001) without any difference between the subgroups within each group (p > 0.05). All atrophic endometria and endometrial polyps and 23.5% of low grade endometrioid and none of the other endometrial cancers showed uninterrupted basement membrane staining with laminin-1. All cases in malignant endometrium expressed MMP-9 with either low or high immunoreactivity while none of the cases in benign endometrium showed a high staining with MMP-9 (p < 0.01). Proliferative and hyperplastic endometrium together with grade 1 endometrioid cancer expressed MMP-9 better than the atrophic endometrium (p < 0.05). The immunoreactivity with MMP-9 increased gradually from secretory to hyperplastic endometrium and serous carcinoma (p < 0.05). MMP-9 expression in all types of cancers except grade 1 endometrioid and clear cell compared to proliferative endometrium was significantly higher (p < 0.05) and increased from proliferative to grade 2 endometrioid, grade 3 endometrioid, serous and undifferentiated endometrial carcinoma.

Conclusion: Gradual increments in MMP-9 expression and basement membrane laminin-1 discontinuity may indicate progression from normal to hyperplastic and to low- and high-grade cancerous endometrium.

Key words: Endometrium, endometrial neoplasms, endometrial hyperplasia, immunohistochemistry, laminin, matrix metalloproteinase 9

1. Introduction

In order to be able to achieve host tissue invasion and metastasize distantly, endometrial cancer cells have to attach to basement membrane laminin, leading to production of proteolytic enzymes degrading the extracellular matrix and migration across the basement membrane [1]. Laminin, the main noncollagenous glycoprotein and major constituent of all basement membranes with collagen IV, exists in multiple isoforms, among which laminin-1 ($\alpha 1\beta 1\gamma 1$) appears to be almost limitedly expressed in epithelial basement membranes [2]. In tumor invasion and metastasis, the key event,

interaction of cancerous cells with laminin, is moderated by nonintegrin and integrin receptors of laminin, the expressions of which are altered in cancer [3]. Laminin receptor 1, which is a nonintegrin-type 67 kDa receptor of laminins (most studies are on laminin-1), is highly involved in tumor cell dissemination induced by laminin [3].

When compared to laminin-1-non-adherent cells, the cells adherent to laminin-1 were associated with reduction in apoptosis and increased activity in proliferation [4]. In addition, haptotactic, chemotactic, and migratory roles of laminin for tumor cells have also been reported [5,6].

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Moreover, tumor cell invasion is also promoted by laminin through induction of proteases, which degrade various extracellular matrix components [7,8]. Human cancer cell lines of cervix (SiHa) and breast (MCF-7) cultured on a surface coated with laminin-1 have been shown to express matrix metalloproteinase-9 (MMP-9) [9,10], a 92-kDa member of the MMP family, which are calcium- and zinc-dependent metalloproteinases implicated in degradation of the extracellular matrix in normal as well as pathological conditions [11]. MMP-9 expression has been found to be modified under normal physiological states of the uterus as well as in uterine pathologies like infertility, myoma uteri, endometriosis, and breakthrough or dysfunctional bleeding [12-21]. MMP-9 appears to play important roles in various cancer types, including endometrial cancer [22-26].

These observations indicate the need for future studies on the link between epithelial basement membrane integrity and tumor behavior in normal and malignant endometrial tissues using antibodies to basement membrane protein laminin-1 and its degrading enzyme, MMP-9.

The aim of the present study was to examine the patterns of laminin-1 and MMP-9 expression immunohistochemically in benign versus malignant endometrium, and to determine whether observable alterations might be of value in characterizing the conversion of benign endometrium to malignant endometrium.

2. Materials and methods

2.1. Clinical data collection and handling of tissues

This case-control study was conducted at University of Health Sciences, Konya Training and Research Hospital between January 2014 and December 2016. After approval was granted by Necmettin Erbakan University Faculty of Medicine Institutional Review Board (IRB) on 15.11.2013 with the approval number of 2013-517, a total of 99 samples with the histological diagnoses of normal endometrium or benign endometrial pathologies (n = 50) and endometrial adenocarcinomas (n = 49) were obtained from the department of pathology. We purposely excluded the cases with atypical endometrial hyperplasia to include cases having exclusively benign or malignant features. The calculation of sample sizes in the present study were based on the studies by Soini et al. [22] and Di Nezza et al. [23], in which sample sizes of 29 had been shown to be sufficient. The human normal endometrial tissues had been obtained by probe-guided curettage during routine diagnostic and therapeutic procedures performed in the Department of Obstetrics and Gynecology. The human endometrial cancer tissues had been collected during oncologic surgery at the Gynecological Oncology Clinic of

this department between 2014 and 2016. No chemotherapy or radiotherapy had been given to these patients before the surgical procedures including initial peritoneal washings followed by total abdominal or laparoscopic hysterectomies and bilateral salpingo-oophorectomies, as well as pelvic-paraortic dissections of lymph nodes and cytoreductions when necessary. In addition to the multiple paraffin blocks, the clinical data and pathologic reports of the patients were also available from archives of the Obstetrics and Gynecology and Pathology departments.

2.2. Histopathological evaluation

Paraffin-embedded tissues of ninety-nine patients were used to obtain histologic sections stained with hematoxylin and eosin, which were rereviewed by a single pathologist (Y.Ü.). Noyes' criteria were used in histologic dating [27]. The 1994 World Health Organization classification system was used in the classification of hyperplasia [28]. Biopsy specimens of the endometrium from patients 24 to 73 years of age and expressing no hyperplasia with atypia, apparent inflammation, or malignancy were examined; twenty were proliferative, eleven were secretory, and five were atrophic (from menopausal women lacking hormonal stimulation) endometrium, and twelve were simple hyperplasia without atypia (all from unopposed estrogen stimulation), whereas two were endometrial polyps. The endometrial cancers from the women between 45 and 84 years of age were endometrioid (n = 20, n = 14, and n = 6 for grade 1, grade 2, and grade 3, respectively), serous (n = 7), clear cell (n = 1), and undifferentiated (n = 1) histological types.

The grading scheme of International Federation of Gynecology and Obstetrics (FIGO) was used while grading the endometrioid type of endometrial cancer [29]. Endometrioid grade 1 or 2 cancers were defined as "low grade" and endometrioid grade 3 or nonendometrioid ones (clear cell, serous, and undifferentiated) as "high grade", as proposed by International Society of Gynecological Pathologists [30].

2.3. Laminin-1 and matrix metalloproteinase-9 immunolocalization

The biotin-streptavidin indirect triple method was used for the immunohistochemical examination. Blocks embedded in paraffin were cut into tissue sections of 3 µm in thickness and put onto positively charged adhesion slides. The slides were immunohistochemically stained with rabbit polyclonal antibody to laminin-1 (RB-082-A1; Thermo Scientific, Fremont, CA, USA) diluted 1:100 and rabbit polyclonal antibody to MMP-9 (RB-9234-P; Thermo Scientific, Fremont, CA, USA) diluted 1:50 using an automated Leica BOND-MAX immunohistochemical device (Leica Microsystems, Cambridge, UK). The staining process was conducted using BOND Polymer Refine Detection kits (Leica Microsystems, Cambridge, UK) in accordance with the manufacturer's instructions. Paraffin

sections from the specimens were firstly deparaffinized and then rehydrated in alcohol and water with BOND Wash Solution (Leica Microsystems, Cambridge, UK) according to standard protocols. Antigenic masking was eliminated with BOND Epitope Retrieval Solution (Leica Microsystems, Cambridge, UK). The endogenous peroxidase activity was inhibited using peroxide blocking agent (Leica Microsystems, Cambridge, UK). Primary MMP-9 and laminin-1 antibodies were diluted. After washing, the antibodies were diluted again. The color reaction was performed with 3,3'-diaminobenzidine tetrahydrochloride (DAB). After washing, visualization of the sections was conducted with DAB and Mayer's hematoxylin stain was used for counterstaining. Finally, distilled washing was automatically performed in the device, and the stained preparations were closed with a Leica CV5030 automatic closing device (Leica Microsystems, Cambridge, UK).

2.4. Evaluation of the slides

The slides of fifty tissues from normal endometrium or benign lesions of the endometrium and forty-nine tissues from malignant endometrium were examined immunohistochemically for the expression of laminin-1 in the basement membrane and of MMP-9 expression in the cell cytoplasm. The slides were evaluated with a light microscope (model BX51TF, Olympus, Tokyo, Japan) under 400× magnification by a single author (Y.Ü.) blinded to the clinical data of the patients.

Laminin-1 expression was evaluated as to whether it was stained throughout the basement membranes in a linear, uninterrupted, and continuous pattern or the staining was interrupted, being incomplete and absent in areas or in total. Only cytoplasmic and/or stromal staining was regarded as nonstaining.

The staining with MMP-9 was evaluated with two semiquantitative arbitrary scales. The first scale was used to categorize the percentage of epithelial cells with positive cytoplasmic staining as 0% (0), 1% to 10% (I), 11% to 50% (II), or 51% to 100% (III). The second scale was used to categorize the intensity of this staining as nonstaining (-), weak (+), moderate (++), or strong staining (+++). The histoscore was determined by multiplying the two values. The histoscore values from 0 to 9 were grouped to denote negative (0), low (1–5), and high (6–9) expression as described in the study by Jiraskova et al. [31]. The properties of staining for benign endometrium were compared with those for cancerous lesions of the endometrium.

2.5. Statistical analysis

SPSS Statistical Software for Windows, version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. The immunohistochemical data were presented as frequencies and percentages for categorical variables and analyzed with the Fisher's exact χ^2 test. For comparison of continuous variables which were expressed as mean \pm

standard deviation (SD), the unpaired two tailed Student's t-test or the Mann-Whitney U test (in the case of skewed data) was used. p-values below 0.05 were considered statistically significant.

3. Results

In this immunohistochemical study, laminin-1 and MMP-9 expression levels were evaluated in benign and malignant endometrium tissue samples by using rabbit polyclonal antibodies to laminin-1 and MMP-9 antigens, respectively. The mean ages of patients with a normal endometrium and with endometrial cancer were statistically different (46.7 ± 8.5 and 61.6 ± 8.3 years, respectively) ($p < 0.0001$).

In most of the benign endometrial tissues (92%) but in only 16.3% of the malignant endometrial lesions, linear and continuous staining of laminin-1 was evident in the basement membranes ($p < 0.0001$). Stained epithelium was surrounded by a narrow basement membrane band in the proliferative endometrium (Figure 1A). Distinct laminin positivity as a tortuous band was also evident in the endometrial glands of secretory endometrium stained with laminin-1. The basement membrane was distinctly visible in stained cases of simple endometrial hyperplasia without atypia. No significant difference was evident between subgroups within each group of benign and malignant endometria ($p > 0.05$). While the basement membranes of all atrophic endometria and endometrial polyps were stained with laminin-1 in a distinct linear, uninterrupted, and continuous manner beneath the epithelium, none of the high grade endometrioid (Figure 1B), serous, clear cell, or undifferentiated endometrial carcinomas expressed laminin-1 in their basement membranes. In 25% of grade 1 endometrioid endometrial carcinomas, a well-defined basement membrane was observed in the subepithelium. However, the basement membrane structure was incomplete in fifteen cases (75%) of grade 1 endometrioid endometrial carcinomas. In 21.4% of grade 2 endometrioid endometrial carcinomas, the areas of invasion were encircled by a continuous basement membrane, while no basement membrane was observed in invasion areas of 78.6% of grade 2 endometrioid endometrial carcinomas. Foci of intra- and intercellular staining for laminin were seen in one of the serous-type endometrial carcinomas (Figure 1C). The higher expression of laminin-1 in benign endometrium was observed in proliferative, secretory, and atrophic endometria together with simple endometrial hyperplasia without atypia against serous and all grades of endometrioid endometrial carcinomas ($p < 0.05$). However, the basement membranes of endometrial polypoid lesions expressed laminin-1 higher than only those of grade 3 endometrioid and serous carcinomas ($p < 0.05$). The results are detailed in Table 1.

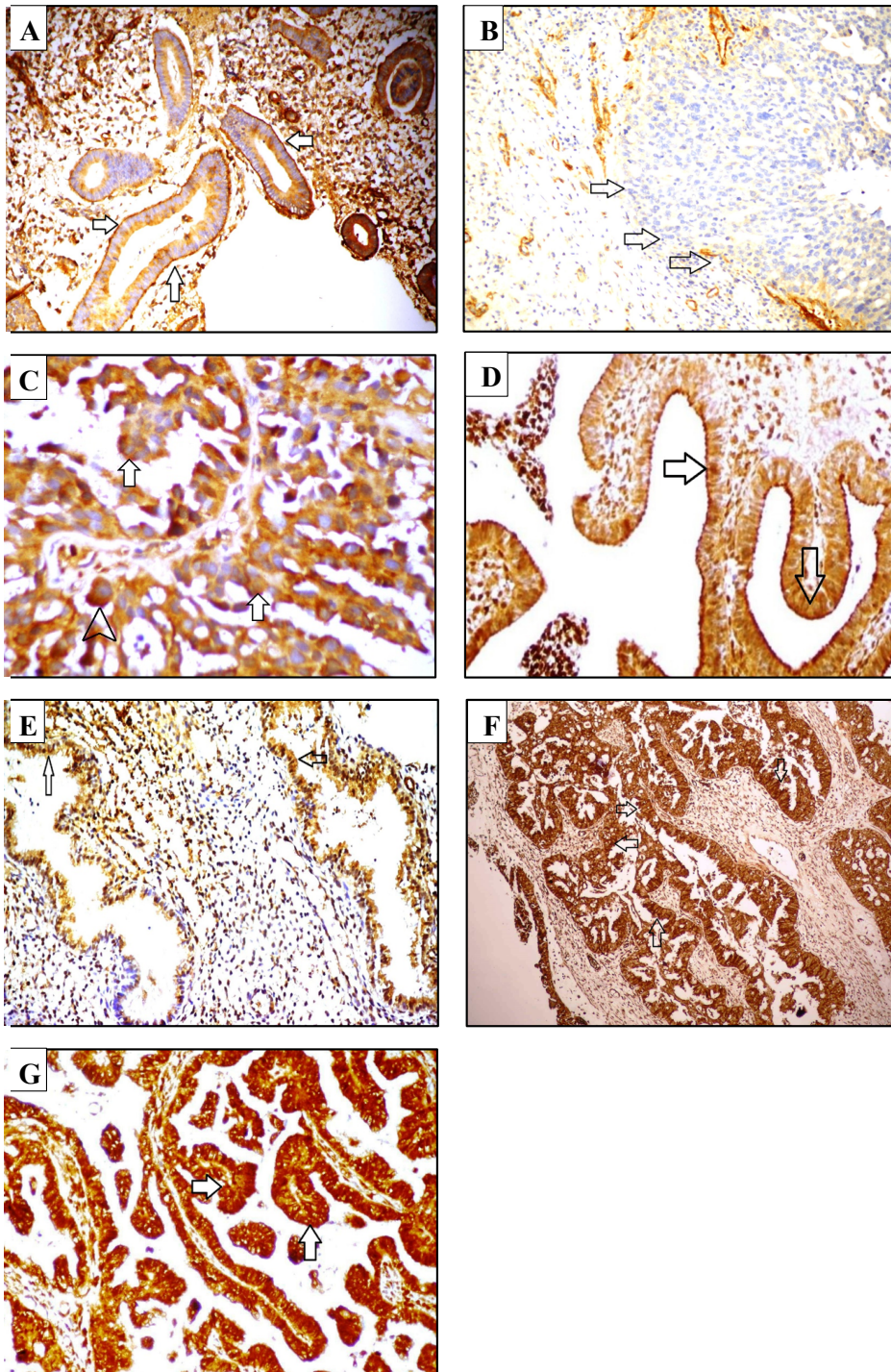


Figure 1. Examples of laminin-1 and matrix metalloproteinase (MMP)-9 immunohistochemical staining. (A) Proliferative endometrium showing continuous linear basement membrane laminin-1 immunopositivity (arrows). (B) Endometrioid-type endometrial carcinoma (grade 3) with basement membrane laminin-1 immunonegativity (arrows). (C) Serous-type endometrial carcinoma with cytoplasmic (arrowhead) and intercellular laminin-1 immunopositivity (arrows). (D) Simple endometrial hyperplasia without atypia showing moderate (++) cytoplasmic MMP-9 immunopositivity (arrows) in 11% to 50% (II) of cells. (E) Normal secretory endometrium with weak (+) cytoplasmic MMP-9 immunopositivity (arrows) in 11% to 50% (II) of cells. (F) Endometrioid-type endometrial carcinoma (grade 2) with moderate (++) cytoplasmic MMP-9 immunopositivity (arrows) in 11% to 50% (II) of cells. (G) Serous-type endometrial carcinoma showing strong (+++) cytoplasmic MMP-9 immunopositivity (arrows) in 51% to 100% (III) of cells. [original magnifications: 100× (A); 200× (B); 200× (C); 150× (D); 100× (E); 150× (F); 150× (G)].

Table 1. Immunostaining for laminin-1 in epithelial basement membranes of benign and malignant endometrium.

Type of tissue	Number of cases	Staining for laminin-1	
		Discontinuous/ defective or absent	Continuous
Benign endometrium	50	4 (8)	46 (92) [*]
Proliferative	20	1 (5)	19 (95) ^{†,‡,§,‡}
Secretory	11	2 (18.2)	9 (81.8) ^{†,‡,§,‡}
Atrophic	5	0	5 (100) ^{†,‡,§,‡}
Simple hyperplasia without atypia	12	1 (8.3)	11 (91.7) ^{†,‡,§,‡}
Endometrial polyp	2	0	2 (100) ^{§,‡}
Malignant endometrium	49	41 (83.7)	8 (16.3)
Grade 1 endometrioid	20	15 (75)	5 (25)
Grade 2 endometrioid	14	11 (78.6)	3 (21.4)
Grade 3 endometrioid	6	6 (100)	0
Endometrial serous	7	7 (100)	0
Endometrial clear cell	1	1 (100)	0
Undifferentiated endometrial	1	1 (100)	0

Results are expressed as the number (percentage)

^{*} Significance when compared with the group of malignant endometrium, $p < 0.0001$

[†] Significance when compared with the group of grade 1 endometrioid endometrial carcinoma, $p < 0.01$

[‡] Significance when compared with the group of grade 2 endometrioid endometrial carcinoma, $p < 0.01$

[§] Significance when compared with the group of grade 3 endometrioid endometrial carcinoma, $p < 0.05$

[‡] Significance when compared with the group of endometrial serous carcinoma, $p < 0.05$

In general, in all tissue types, a good correlation was observed between the percentages of cells stained positive with MMP-9 and the staining intensities of them evaluated with the two semiquantitative scales, as detailed. Based on histoscore on MMP-9 expression, all stained cases in benign endometrium showed a low immunoreactivity (78%), while the rate of nonstaining with MMP-9 (22%) was statistically higher than that for malignant endometrium (0%) ($p < 0.01$). In malignant endometrium, while 26.5% of slides expressed MMP-9 with a high immunoreactivity, none of the slides in benign endometrium showed a high staining with MMP-9 ($p < 0.01$). Benign and malignant endometrial lesions did not differ from each other with respect to low staining with MMP-9 (78% and 73.5%, respectively) ($p > 0.05$). The details are given in Table 2.

When compared to the proliferative endometrium, the rate of nonstaining with MMP-9 was higher (10% versus 80%, respectively) and the rate of low immunoreactivity with MMP-9 was lower (90% versus 20%, respectively) for the atrophic endometrium ($p < 0.05$) (Table 2). Low immunoreactivity was visualized in the cytoplasm of the only case of atrophic endometrium staining positive with MMP-9. All cases of simple endometrial hyperplasia without atypia showed low immunostaining with MMP-9

(Figure 1D), which was statistically higher than that for atrophic (20%) and secretory endometrium (63.6%) ($p < 0.05$). The results are detailed in Table 2.

Nonstaining with MMP-9 was highest in the atrophic endometrium (80%), which significantly differed from the serous and all grades of endometrioid endometrial carcinomas (0%) ($p < 0.05$). The secretory endometrium also showed a significantly higher percentage of nonstaining with MMP-9 (36.4%) compared to grade 1 and 2 endometrioid carcinomas (0%) ($p < 0.05$). MMP-9 expression in the stained cases of secretory endometrium was low (Figure 1E). A significantly higher percentage of slides expressed MMP-9 with a low immunoreactivity in grade 1 (90%) but not in grade 2 (71.4%) (Figure 1F) and grade 3 (66.7%) endometrioid carcinoma when compared with atrophic endometrium (20%) ($p < 0.05$). A significantly higher percentage of slides showed high immunoreactivity with MMP-9 in grade 2 (28.6%), grade 3 (33.3%), endometrial serous (57.1%), and undifferentiated endometrial carcinomas (100%) when compared to the proliferative endometrium (0%) ($p < 0.05$). Additionally, high immunoreactivity with MMP-9 was also significantly better in endometrial serous carcinoma when compared to the secretory endometrium and simple endometrial

Table 2. Matrix metalloproteinase-9 expression in epithelial cytoplasm evaluated by histoscores (staining intensity \times percentage of positive cells).

Type of tissue	Number of cases	Histoscore for MMP-9		
		Negative	Low	High
Benign endometrium	50	11 (22) [†]	39 (78)	0 [*]
Proliferative	20	2 (10) [†]	18 (90) [†]	0
Secretory	11	4 (36.4)	7 (63.6)	0
Atrophic	5	4 (80)	1 (20)	0
Simple hyperplasia without atypia	12	0 ^{†,‡}	12 (100) ^{†,‡}	0
Endometrial polyp	2	1 (50)	1 (50)	0
Malignant endometrium	49	0	36 (73.5)	13 (26.5)
Grade 1 endometrioid	20	0 ^{†,‡}	18 (90) [†]	2 (10)
Grade 2 endometrioid	14	0 ^{†,‡}	10 (71.4)	4 (28.6) [†]
Grade 3 endometrioid	6	0 [†]	4 (66.7)	2 (33.3) [†]
Endometrial serous	7	0 [†]	3 (42.9) ^{†,§,¶}	4 (57.1) ^{†,§,¶}
Endometrial clear cell	1	0	1 (100)	0
Undifferentiated endometrial	1	0	0	1 (100) [†]

Results are expressed as the number (percentage)

The histoscore values from 0 to 9 were grouped to indicate negative (0), low (1–5) and high (6–9) expression

^{*} Significance when compared with the group of malignant endometrium, $p < 0.01$

[†] Significance when compared with the group of atrophic endometrium, $p < 0.05$

[‡] Significance when compared with the group of secretory endometrium, $p < 0.05$

[§] Significance when compared with the group of proliferative endometrium, $p < 0.05$

[¶] Significance when compared with the group of simple endometrial hyperplasia without atypia, $p < 0.01$

[•] Significance when compared with the group of grade 1 endometrioid endometrial carcinoma, $p < 0.05$

hyperplasia without atypia (0%) ($p < 0.05$) (Table 2). Between the subgroups of malignant endometrium, only endometrial serous carcinoma showed a significant difference from grade 1 endometrioid endometrial carcinoma with its higher immunoreactivity with MMP-9 ($p < 0.05$) (Figure 1G). The details are given in Table 2.

4. Discussion

Without any difference between the subgroups within each group, the basement membrane laminin-1 staining was mostly continuous in benign endometrium in contrast to malignant endometrium in which it was usually defective or discontinuous. While the cases in benign endometrium either did not stain or stained low with MMP-9, all cases in malignant endometrium expressed MMP-9 with either low or high immunoreactivity. A better expression of MMP-9 was observed in proliferative and hyperplastic endometrium together with grade 1 endometrioid cancer compared to the atrophic endometrium. The immunoreactivity with MMP-9 increased gradually from secretory to hyperplastic endometrium and serous carcinoma while its expression in all types of cancers

except grade 1 endometrioid and clear cell compared to proliferative endometrium was significantly higher and increased from proliferative to grade 2, 3 endometrioid, serous, and undifferentiated endometrial carcinoma.

In functioning (proliferative, secretory), atrophic, and hyperplastic (simple endometrial hyperplasia without atypia, endometrial polyp) endometrium, a distinct basement membrane was visualized in the subepithelium and around the glands of the endometrium in nearly all tissues examined (46 out of 50) as narrow, continuous bands. In concordance with the results reported by Stenback et al. [32], Furness et al. [33], and Tanaka et al. [34], our observations with normal endometrium emphasize that the basement membranes are synthesized during the reproductive cycle of the endometrium, beginning from as early as the proliferative phase, and continues after the cessation of cell renewal and growth in the atrophic senile endometrium, which lacks constant hormonal stimulation. Although they were rarely seen in our tissues with normal epithelium (4 out of 50, all were either proliferative and secretory endometrium or simple

endometrial hyperplasia without atypia), small defects and focal attenuation in laminin staining, which was also observed in the study by Vogel et al., have been associated with acute or chronic inflammation in these areas [35]. Similar to our findings, in the reports by Vogel et al. [35] and Bulletti et al. [36], endometrial hyperplasias had basement membranes that were generally intact but defective in some areas. A comparable result for endometrial polyps was also reported by Vogel et al. [35].

A distinct continuous basement membrane was evident only in almost a quarter (8 out of 34) of the low-grade endometrial cancers (endometrioid histological type) but in none of the high-grade cancers of the endometrium (grade 3 endometrioid, clear, serous, and undifferentiated). It has previously been reported that an intact basement membrane is rarely associated with carcinoma and having a basement membrane or not is the major discrepancy between the tumors with benign and malignant behavior [37]. Our demonstration of progressive loss of laminin-1 membranes with increasing degrees of anaplasia is similar to the findings reported by other workers [32,35,36] and it has been proposed to have resulted from either decreased production and deposition or increased degradation by the tumorous cells [32]. Bulletti et al. [36] linked this degradation with acquisition of enzymatic degradation activity based on previous studies [37,38]. The logic of progressive proliferation of endometrium from normal to hyperplastic and later to neoplastic state is based on a vicious cycle consisting of disappearance of the components of the basement membrane in glandular epithelial cells leading to increased permeability of the basement membrane to estrogen influx, endometrial proliferation and increased vasculature of the surface area, abnormal endometrial proliferation, resulting in hyperplasia and sometimes in endometrial adenocarcinoma, and in this way again the loss of basement membrane integrity [36].

It has been also commented that the finding of laminin containing basement membrane-like material in undifferentiated endometrial carcinoma tissues itself, which was also observed in one of our cases of serous carcinoma, might have been implicated by their limited ability left to produce basement membrane proteins [32]. Cytoplasmic accumulation of laminin in two cases of endometrioid carcinomas was interpreted as a sporadic abnormality in its synthesis and release by Vogel et al. [35].

In our study, MMP-9 was not expressed in most of the postmenopausal atrophic endometrium compared to the premenopausal proliferative endometrium. Similarly, in Laird et al.'s study, MMP-9 concentrations in premenopausal women were better than those in menopausal ones, which has been suggested as probably a reflection of atrophic endometrium associated with

decreased secretory activity [39]. Nonchanging MMP-9 concentrations firstly in proliferative and later in secretory phases of the cycle were also a consistent finding between our study and the study by Laird et al. [39]. However, there are also some other reports suggesting that MMP-9 concentration diminishes during the early period of the secretory phase and subsequently increases late in the menstrual cycle with the beginning of menstruation [40,41]. One of the interesting results of our study was a significant increment in the expression of MMP-9 from nonfunctioning atrophic endometrium towards normal functioning proliferative endometrium, endometrium with hyperplasia (simple endometrial hyperplasia without atypia) or grade I endometrioid carcinoma. For MMP-9 expression, a significant gradual increment from proliferative endometrium towards grade 2 endometrioid, grade 3 endometrioid, serous and undifferentiated endometrial carcinoma together with a steady increase from secretory to hyperplastic endometrium and serous carcinoma were also evident. The findings of a previous study by Amalinei et al. [42] are consistent with ours.

In accordance with the findings of previous studies [24,39,42-44], our investigation also revealed that the expression of MMP-9 is upregulated in carcinomatous endometrium compared to benign endometrium. We noted that the expression of MMP-9 did not vary between the various histologic subtypes of endometrial cancer studied. This was just the opposite of the finding reported by Monaghan et al., whose study revealed significantly higher MMP-9 expression in endometrioid tumors versus serous ones [9]. Probably, with our too small serous endometrial cancer cases, a statistically significant difference for MMP-9 staining could not be reached compared to endometrioid type cancer (7 vs 40). However, similar to our findings, another study, by Graeslinn et al., did not reveal a difference in MMP-9 expression according to the histologic subtype [45]. Although increased production of MMP-9 has been linked to advanced grade in some reports [23,25,43,46], no such correlation was reported by Lopata et al. [24]. In our study, the histological grades of endometrioid endometrial carcinoma did not differ within themselves, but grade 1 endometrioid which is a low-grade cancer expressed MMP-9 less intensely than serous carcinoma which is a high-grade cancer.

To the best of our knowledge, this is the first immunohistochemical study investigating the possible roles of laminin-1 and MMP-9 together in transformation from the premalignant to malignant condition in the same pathological samples of benign, hyperplastic, and malignant endometrium. Uninterrupted, continuous expression of laminin-1 in the basement membranes of most of the benign endometrial tissues compared to

discontinuous/defective or absent staining in basement membranes of the malignant endometrium was accompanied by increasing expression of MMP-9 from normal to hyperplastic endometrium and to cancerous lesions. This may be associated with the activation of an altered pathway of signal transduction by laminin-1 through a 67 kDa nonintegrin receptor of laminin or integrins on tumor cells, which results in basement membrane dissolution with collagenolysis by MMP-9 while normal tissue is becoming neoplastic [3]. In a study by Maity et al., a cell line of human cervical cancer (SiHa) cultured on a surface coated with laminin-1 induced the activation and expression of MMP-9 [47]. In another study, by Pal et al., a cell line of human breast carcinoma (MCF-7) cultured on a surface coated with laminin-1 also led to upregulation in MMP-9 expression together with diminished expression of tissue inhibitor of metalloproteinases-1 [10]. Both studies indicated that binding of these cell lines to laminin-1, probably by $\alpha 2\beta 1$ integrin, induces signaling including focal adhesion kinase, phosphatidylinositol-3-kinase, extracellular signal regulated kinase, and nuclear factor-kappaB followed by upregulation of MMP-9 and cell migration [10,47]. Such a pathophysiological mechanism in tumor metastasis may also be valid for cancers of the endometrium and therefore similar investigations are warranted on them.

The basement membrane which separates the vascular endothelium and connective tissue from epithelium constitutes a barrier to the metastasis of tumor cells. For movement of tumor cells through the extracellular matrix to metastasize, its loss or remodeling is required. All components of extracellular matrix including the basement membrane can be degraded by active proteases including MMP-9 in vitro and they are frequently expressed at sites where the extracellular matrix is cleaved [48], as it was observed in our study. It has been shown that the staining of laminin-1 (also known as laminin-111) which is a large trimeric basement membrane glycoprotein is often discontinuous in breast tumors [49,50]. It was proposed that some cryptic sites that have biological activity may be exposed by the proteolytic cleavage of structural proteins. Four laminin-1-derived synthetic peptides that are active in malignancy

have been described and three of them were shown to promote tumor growth by using different mechanism and cellular receptors [51].

This study has some limitations. The clinical-demographic data and endometrial biopsy indications of whole study population is lacking. Secondly, the small sizes of some histologic types of normal and malignant endometrium could have led to the observation of some statistically insignificant results. Thirdly, it would be interesting to evaluate endometrial hyperplasia with atypia and see whether laminin retention/loss and MMP expression are also changed in these tissues. It would be also great if we could evaluate the possible relation between the expression properties of laminin-1 and MMP-9 and the FIGO stages of forty-nine malignant cases together with their survival status through prognostic data.

5. Conclusion

Disease progression-related increase in MMP-9 and progressive loss of laminin-1 with increasing tumor grade may be involved in the progression from normal to hyperplastic and to low- and high-grade cancerous endometrium. On the molecular level, this transition may be implicated by the activation of cells by laminin-1 via altered signaling pathways, resulting in the induction of MMP-9-related basement membrane degradation, which needs to be clarified with further studies.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

Approval for this study was granted by Necmettin Erbakan University Faculty of Medicine Institutional Review Board (IRB) in 15.11.2013 with an approval number of 2013-517.

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