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Original Article

The deep infiltrating endometriosis tissue has lower T-cadherin, E-cadherin, progesterone receptor and oestrogen receptor than endometrioma tissue

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ABSTRACT

Objective: To compare the T-cadherin, E-cadherin, PR and ER staining levels of deep infiltrating endometriosis (DIE) tissue, ovarian endometriomas and normal endometrial tissues.
Materials and methods: DIE tissue of 24 cases, endometrioma of 30 cases and normal endometrial tissues of 30 cases were examined. T-cadherin, E-cadherin, ER-α and PR-α staining levels of DIE, endometrioma tissues and endometrial tissues were compared immunohistochemically. *H*-score was calculated to compare the expression of T-cadherin, E-cadherin, ER-α, PR-α in IHC staining based on the percentage of cells stained at each intensity level.
Results: T-cadherin, E-cadherin, ER and PR H-score were found lowest in DIE tissue and the highest in endometrial tissue ($p < 0.0001$, <0.0001, <0.0001 and < 0.0001, respectively). In correlation analysis, a positive correlation was found between T-cadherin, E-cadherin, PR and ER H-score ($p < 0.0001$ for each). No correlation was found between age, body mass index (BMI), visual analog scale (VAS) score, CA125, endometrioma size and the severity of dysmenorrhea, dyspareunia and dystonia ($p > 0.05$).
Conclusion: T-cadherin, E-cadherin, ER and PR *H*-score were found lowest in DIE tissue, the highest in endometrium tissue. The finding of lower expression of PR-α in endometriotic nodule in our study may be related to decrease in progesterone effect which could not inhibit the decrease in the expression of T-cadherin and E-cadherin, thus the invasiveness of DIE tissue. These findings suggest that DIE tissue and ovarian endometrioma tissues have a different biology.
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Introduction

Endometriosis affects 7–10% of women of reproductive age [1,2]. The prevalence of endometriosis increases up to 40–50% in patients with infertility and pelvic pain [3–5]. Endometriosis is categorised into three classes as peritoneal, ovarian and deep infiltrating endometriosis (DIE) by the American Society of Reproductive Medicine (ASRM) [6]. DIE is diagnosed when the depth of endometriotic foci exceed the peritoneal surface by more than 5 mm [7]. Deep infiltrating endometriosis can affect the bladder, ureter, vagina or uterosacral ligaments. It is believed that each of the three classes of lesions have a separate pathogenesis [8]. Different genetic mutations were found in DIE tissue from peritoneal and ovarian endometriosis lesions [9].

Although endometriosis is a benign disease, it shows features similar to malignant diseases such as cellular invasion, metastasis to lymph nodes and distant organs, abnormal morphology, migration towards nerve bundles, unregulated cell proliferation, neoangiogenesis, DNA aneuploidy and loss of heterozygosity [10]. The epithelial–mesenchymal transition (EMT) is one of the most important events in cancer invasion [11]. Similar to cancer invasion, the EMT process is thought to play a role in the pathogenesis of pelvic endometriosis [12].

The cadherin family is a transmembrane glycoprotein family involved in cellular events such as cell–cell adhesion, cell recognition and signalling [13]. There is serious evidence that cadherin molecules play a role in tumour development, invasion and metastasis [14–16]. For instance, E-cadherin down-regulation has

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been associated with a poor prognosis in prostate cancer [17]. In addition, E-cadherin is a tumour suppressor molecule that is important in the EMT process [18]. T-cadherin is structurally different from other cadherin molecules [19]. Like E-cadherin, T-cadherin is thought to be a tumour suppressor molecule. In previous studies, T-cadherin down-regulation has been shown in lung, ovarian, bladder, cervical and prostate cancers. It has been shown that the invasive potential and growth rate of cells in breast cancer slows down with T-cadherin overexpression [20].

In the literature examining T-cadherin level in endometriosis tissue, T-cadherin level was found to be low in endometriosis tissue [21]. It was shown that the expression of E-cadherin was decreased in DIE tissue [22]. In fact, in samples taken from invasive parts of DIE tissue close to the rectum, E-cadherin levels were significantly lower in glands located at the front of the lesions [23].

In healthy endometrial tissue, oestrogen receptors (ER) and progesterone receptors (PR) are densely expressed on endometrial cells, similar to endometrial cells in endometriosis tissue. However, the density of these receptors is less than in healthy endometrial tissue [23]. Drugs containing progesterone are frequently used in the medical treatment of endometriosis. Especially in individuals with high endometriosis stage and DIE tissue, a significant proportion of patients do not respond to progesterone treatment [24]. As a cause of this unresponsiveness, it is thought that there may be mechanisms such as cells having a small number of progesterone receptors that create resistance to progesterone, and abnormalities in post-receptor mechanisms [25,26]. Deficient methylation of the ERβ promoter results in pathological overexpression of oestrogen receptor β (ER-β) in endometriotic stromal cells. High levels of ER-β suppress oestrogen receptor -α (ER-α) expression. A severely high ER-β to ER-α ratio in endometriotic stromal cells is associated with suppressed progesterone receptor contributing to progesterone resistance [27]. Studies examining DIE tissue in the literature suggest that nodule tissue is biologically different from ovarian endometrioma tissue in many ways. Progesterone was shown to suppress the EMT process in vitro studies [28–32]. Progesterone was also shown to inhibit the EMT processes in endometrium cancer tissue. [30], and increase the expression of E-cadherin in Ishikawa cells [33].

In the single study that investigated the T-cadherine levels in endometriosis tissue in literature it was reported that the decreased T-cadherine expression may be linked to progesterone resistance but noticed that it was a subject of future studies [21].

Although not consistently proven, it is thought that the two phenotypes of endometriosis, the endometrioma and DIE, may differ in clinical behaviour such that DIE may respond suboptimal to medical treatment [8,34]. Thus, differences in biology may influence the treatment options for DIE. Despite the thought that the biology of these two may differ studies on this difference are limited [35]. In the present study, we tried to elucidate a part of this difference which may potentially affect the treatment of choice in future taking into consideration that targeted therapies will find its place in treatment of endometriosis.

In the present study we aimed to investigate the relationship of T-cadherin, E-cadherin, progesterone receptor-α (PR-α) ve ER-α levels and their receptors in endometrioma, DIE tissue and normal endometriosis tissues.

Material and methods

Ethical approval

Ethical approval was obtained from the Non-invasive Research Ethics Committee of Kütahya Health Sciences University, Kütahya, Turkey (Research number: 2020/07-10).

Patients and tissue sample collection

In this case–control study, subjects who underwent endometriosis surgery at Kütahya Health Sciences University Evliya Çelebi Training and Research Hospital and Söke Private Egemed Hospital in Turkey between July 2016 and July 2020 constituted the study group and patients who had endometrial sampling before myomectomy constituted the control group. 90 patients were enrolled however six women were excluded due to flaws in records. Twenty-four cases in which DIE tissue was examined were included in the 1st group, 30 cases in which ovarian endometrioma tissue was examined were included in the 2nd group, and 30 cases in which endometrial sampling tissue was examined (after which there was no intraoperative endometriosis performed before abdominal myomectomy) were included in the 3rd group.

As the inclusion criteria for the study, women who did not use an intrauterine device and did not receive hormonal treatment for the last 3 months were accepted. Subjects with malignancy, those receiving immunosuppressive therapy, and those using drugs such as gonadotropin-releasing hormone agonist or danazol effective on ER and PR were not included in the study. Demographic data of the cases (age, preoperative, visual analog scale (VAS) score in cases with nodules) and surgical information (endometriosis stage according to ASRM classification, endometrioma diameter, DIE tissue diameter in cases with DIE, presence of adhesion) were recorded. T-cadherin, E-cadherin, ER-α and PR-α staining levels of groups were compared immunohistochemically.

In this study, endometriosis was defined as the presence of endometrial glands and stroma-like lesions outside of the uterus [2].

DIE diagnosis was made according to criteria of histopathological fibrous/muscular infiltration of organs and anatomical structures containing endometrial tissue below the peritoneum, regardless of the depth of infiltration [36].

DIE was grafted beneath the peritoneum in five different sites in the pelvic area: upper and lower parts of both uterosacral ligaments close to the ureters [10] and rectovaginal septum [7]. Sections taken from the DIE tissue were taken from the side facing the bowel segment for the rectovaginal nodule. Sections for other nodules were taken from the opposite side of the uterus.

Immunohistochemical examination (IHC)

All tissue samples were fixed with 10% formaldehyde solution and embedded in paraffin blocks. Blocks that contained placenta samples representing the diagnosis were chosen. All blocks were sectioned with 4 mm thickness. Sections were deparaffinized in xylene solution and rehydrated in decreasing concentrations of ethanol. After a washing step in phosphate buffer solution (PBS), sections were incubated in 3% H_2O_2 solution for 10 min to inhibit endogenous peroxidase activity. Sections were boiled in 0.06% trypsin/EDTA (0.25 mM) solution at 850 W for 5 min and then at 350 W for 5 min in a microwave. Then, sections were incubated with primary antibodies at 4C for a 24 h period. The primary antibodies used were monoclonal anti-T-cadherin (CDH13) antibody (Sigma–Aldrich Corp., USA, 1:100 dilution), anti-E-cadherin antibody (Sigma–Aldrich Corp., USA, 1:100 dilution), anti-ER-α antibody (Sigma–Aldrich Corp., USA, 1:100 dilution) and anti-PR-α antibody (Sigma–Aldrich Corp., USA, 1:100 dilution). Antibodies were boiled with citrate buffer solution in a microwave oven. All sections were washed with PBS and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for a 60 min period. Diaminobenzidine chromogen was then added, and Mayer haematoxylin was used for counterstaining.

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Imaging analysis

An Olympus BX53 light microscope was used for evaluation, and each section was photographed with an Olympos DP27 digital camera. Five high magnification views were randomly selected in each immunohistochemical section. According to the proportion of positive cells and staining intensity of positive cells, we made a semi-quantitative score grade. The staining intensity level scoring was described as nonstaining for 0 points, faint staining for 1 point, moderate staining for 2 points and strong staining for 3 points. Immunoreactivity was assessed using a four-tiered scale: 0–5% (0), 6–20% (+1), 21 50% (+2), 51–100% (+3). *H*-score was calculated to compare the expression of T-cadherin in IHC staining based on the percentage of cells stained at each intensity level, ranging from 0 for negative staining to 3 for the most intense staining using the following formula: *H*-score = (% cells with intensity level 1 * 1) + (% cells with intensity level 2 * 2) + (% cells with intensity level 3 * 3) [37].

Statistical analysis

For data analysis, the Statistical Package for the Social Sciences (SPSS), version 21.0 (SPSS Inc., Chicago, IL) and R statistical computing software (version 3.6.1, https://www.r-project.org/) were used. Data are presented as mean ± SD and median [25th percentile; 75th percentile]. Conformity to normal distribution was evaluated with the Kolmogorov–Smirnov test or Shapiro–Wilk tests. Differences of continuous variables between groups were evaluated with ANOVA or Kruskal Wallis test. In post-hoc analysis Tukey's test was used. Relationships between categorical data and groups were analysed by Chi–Square test. Correlation between variables was evaluated using Pearson or Spearman's correlation coefficient. A value of $p < 0.05$ was considered statistically significant.

Results

Mean age of the 84 women included in the study was 34.39 ∓ 7.18 years. There was no significant difference in age of the groups in terms of body mass index (BMI) data ($p > 0.05$). T-cadherin, E-cadherin, ER and PR H-score were lowest in DIE tissue and highest in endometrial tissue ($p < 0.0001$, <0.0001, <0.0001, <0.0001, respectively). Demographics, T-cadherin, E-cadherin, progesterone receptor and oestrogen receptor H-score levels of groups are given in Table 1. Additionally, T-cadherin, E-cadherin, progesterone receptor and oestrogen receptor H-score of groups are given in Fig. 1.

Endometrioma size was 7.06 ∓ 2.56 cm. There was no difference in CA125 levels between the DIE tissue group and the endometrioma group (70.29 ∓ 44.76 vs 66.56 ∓ 37.35; $p = 0.807$). There was no difference between the DIE tissue group and the endometrioma group in terms of visual analog scale (VAS) in the preoperative period (8 [6–10] vs 8 [6–10]; $p = 0.292$).

A positive correlation was found between T-cadherin, E-cadherin, PR and ER H-score in correlation analysis ($p < 0.0001$ for each). No correlation was found between age, BMI, VAS score, CA125, endometrioma size and severity of dysmenorrhea, dyspareunia or dystonia ($p > 0.05$). Correlation analyses of T-cadherin, E-cadherin, PR and ER H-score and demographic, clinical parameters of women were given in Table 2.

T-cadherin, E-cadherin, PR and ER staining levels of the groups in terms of immunohistochemistry are given in Fig. 2.

Discussion

Endometriosis is a heterogeneous disease including peritoneal, ovarian and deep infiltrating endometriosis. It is likely that the biology of these lesions and therefore the treatment modalities to be applied are different. Therefore, it is very important to understand well the characteristics of the lesions. In the present study, there was no significant difference in terms of demographic data of the groups. T-cadherin, E-cadherin, ER and PR H-score were lowest in DIE tissue and highest in endometrial tissue. A positive correlation was found between T-cadherin, E-cadherin, PR-α and ER-α H-score in correlation analysis. However, no correlation was found between age, BMI, VAS score, CA125, endometrioma size and severity of dysmenorrhea, dyspareunia or dystonia.

Solares JG and et al. examined rectovaginal DIE tissue of 17 DIE cases. Samples taken from the closest (most invasive) part of the tissues to the rectum and the middle (less invasive) part were examined immunohistochemically in terms of beta-catenin, E-cadherin, N-cadherin, matrix metalloproteinase-9 (MMP-9), nerve growth factor (NGF) and nerve fibre density (NFD). In sections taken from more invasive parts of tissues that were close to the rectum, thinning and increased proliferation rates were shown in the endometrial glands. Also, it was shown that while N-cadherin and MMP-9 levels increased, E-cadherin staining decreased [23]. In various studies, it has been shown that NFD is

Table 1
Demographic, T-cadherin, E-cadherin, progesterone receptor and oestrogen receptor H-score levels of groups.

	DIE tissue (n = 24)	Endometrioma (n = 30)	Endometrium (n = 30)	*P value*
Age (years)[a]	35,62 ∓ 7,56 35 [31,5–39,5]	32,60 ∓ 9,06 23,15 [26–39]	35,20 ∓ 3,87 36 [33–38]	0.231
BMI (kg/m2)[a]	26,89 ∓ 4,68 28,05 [22,15–30,25]	24,88 ∓ 4,24 35 [21,5–29,1]	26,34 ∓ 4,46 25,94 [22,95-30,04]	0.227
T-cadherin H-score[a]	26,21 ∓ 28,92 20 [10–30]	121,47 ∓ 57,82 120 [70–160]	200,17 ∓ 78,48 220 [180–270]	<0.0001*
E-cadherin H-score[a]	25,00 ∓ 20,43 20 [10–35]	121,00 ∓ 59,50 105 80–170]	246,00 ∓ 70,00 280 [200–300]	<0.0001*
PR H-score[b]	42,92 ∓ 40,80 30 [10–90]	137,67 ∓ 68,36 105 [80–160]	294,33 ∓ 45,46 300 [290–300]	<0.0001*
ER H-score[b]	42,08 ∓ 40,54 25 [10–70]	120,00 ∓ 72,25 100 [60–160]	277,33 ∓ 32,16 295 [260–300]	<0.0001*

DIE: deep infiltrating endometriosis; BMI: body mass index; PR: progesterone receptor; ER: oestrogen receptor.
Data are presented as mean ± SD, median [interquartile range] or number (percentage).
All of the pairwise comparisons of T-cadherin, E-cadherin, PR and ER H-score between groups were statistically significant (<0.0001 for all).
Statistically, significant comparisons were marked with *.
[a] ANOVA test.
[b] Kruskal Wallis test.

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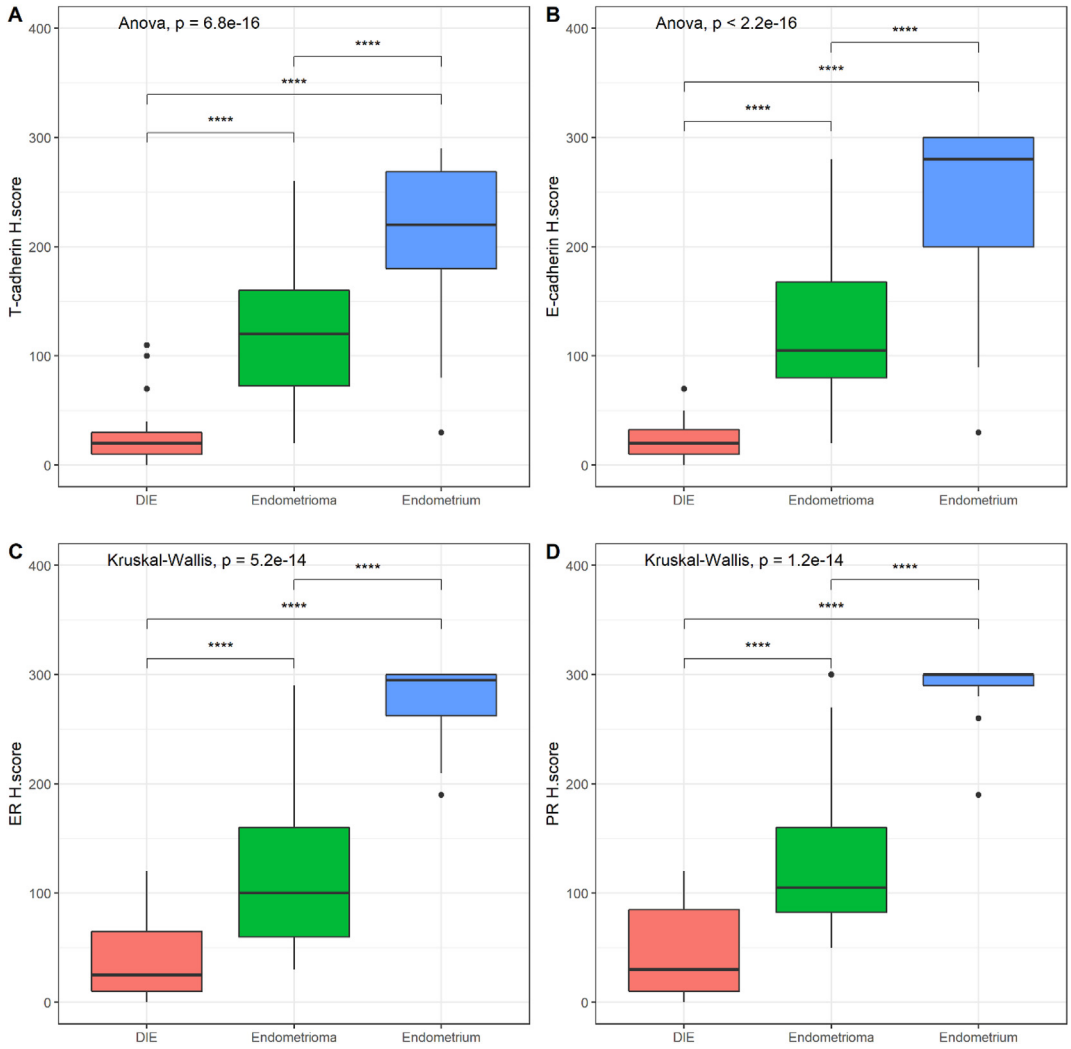


Fig. 1. Comparison of the endometrial gland and stromal cells T-cadherin, E-cadherin, ER and PR H-scores among groups (DIE: deep infiltrating endometriosis; ER: oestrogen receptor, PR: progesterone receptor). A) T-cadherin H.scores of tissues; B) E-cadherin H.scores of tissues; C) ER H.Scores of tissues; D) PR H.Scores of tissues.

Table 2
Correlation analyses of T-cadherin, E-cadherin, PR and ER H-score and demographic, clinical parameters of women.

		T-cadherin H-score	E-cadherin H-score	ER H-score	PR H-score
T-cadherin H-score	Correlation Coefficient	1000	0,855[a]	0,713[a]	0,772[a]
	p value		<0.0001	<0.0001	<0.0001
E-cadherin H-score	Correlation Coefficient	0,855[a]	1000	0,818[a]	0,828[a]
	p value	<0.0001		<0.0001	<0.0001
ER H-score	Correlation Coefficient	0,713[a]	0,818[a]	1000	0,907[a]
	p value	<0.0001	<0.0001		<0.0001
PR H-score	Correlation Coefficient	0,772[a]	0,828[a]	0,907[a]	1000
	p value	<0.0001	<0.0001	<0.0001	
Age	Correlation Coefficient	−0,104	−0,047	−0,011	−0,062
	p value	0,346	0673	0,921	0575
BMI	Correlation Coefficient	−0,070	−0,024	−0,004	−0,043
	p value	0,528	0825	0,971	0699
VAS score	Correlation Coefficient	−0,109	0035	−0,130	−0,051
	p value	0,431	0801	0,348	0715
CA125	Correlation Coefficient	0,014	0118	0,123	−0,074
	p value	0,922	0394	0,375	0597
Endometrioma size	Correlation Coefficient	0,018	0162	0,226	0082
	p value	0,926	0400	0,237	0673

ER: oestrogen receptor; PR: progesterone receptor; BMI: body mass index; VAS: visual analog scale.
[a] Correlation is significant at the 0.01 level (Spearman's correlation test).

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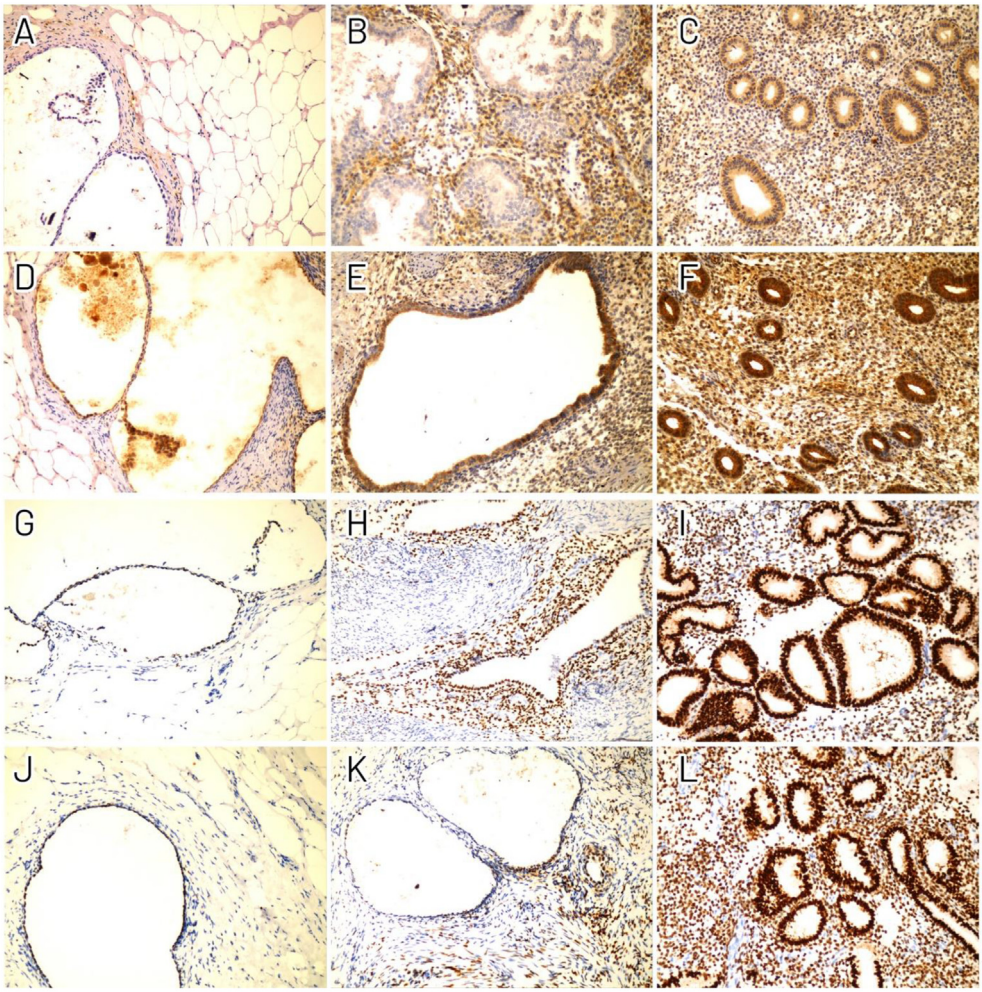


Fig. 2. In immunohistological examination, T-cadherin staining in endometrial gland and stromal cells in A) Deep infiltrating endometriosis (DIE) tissue showed low or no staining in endometrial gland and stromal cells, B) Endometrioma tissue showed moderate staining in endometrial gland and stromal cells, C) Normal endometrium tissue showing high staining was observed in endometrial gland and stromal cells; E-cadherin staining in endometrial gland and stromal cells in D) DIE tissue showed low or no staining in endometrial gland and stromal cells, E) Endometrioma tissue showed moderate staining in endometrial gland and stromal cells, F) Normal endometrium tissue showing high staining was observed in endometrial gland and stromal cells; PR staining in endometrial gland and stromal cells; G) DIE tissue showed low or no nuclear staining in endometrial gland and stromal cells, H) Endometrioma tissue showed moderate nuclear staining in endometrial gland and stromal cells, I) Normal endometrium tissue showing high nuclear staining was observed in endometrial gland and stromal cells; ER staining in endometrial gland and stromal cells; J) DIE tissue showed low or no nuclear staining in endometrial gland and stromal cells, K) Endometrioma tissue showed moderate nuclear staining in endometrial gland and stromal cells, L) Normal endometrium tissue showing high nuclear staining was observed in endometrial gland and stromal cells (×200).

increased in DIE cases compared to peritoneal and ovarian endometriosis cases [26,37–39]. In a study conducted by Donnez et al. in a baboon model, it was shown that E-cadherin expression is reduced in the invasive part of the DIE tissue [22]. However, Orellana et al. found E-cadherin expression in the DIE tissue invasive part to be similar to the non-invasive part in the baboon model [40]. In the only study examining T-cadherin level in endometriosis tissue, T-cadherin level was found to be low in endometriosis tissue. In the same study, 40 endometriosis tissues were examined, and it was not clearly stated how many of these tissues were DIE tissues. In the cell culture phase of the study, it was shown that T-cadherin inhibits invasion and migration of endometrial stromal cells in endometriosis [21]. In the current study, E-cadherin level was found to be low in DIE tissue, in accordance with the literature. As additional information for the literature, T-cadherin and E-cadherin levels were found to be less than endometrioma tissue in DIE tissue. Generally, T-cadherin and E-cadherin levels of endometriosis lesions were found to be lower than normal endometrial tissue.

Progesterone containing drugs are frequently used in the medical treatment of endometriosis. In particular, a significant proportion of patients with high endometriosis stage and DIE tissue do not respond to progesterone treatment [24]. As the cause of this unresponsiveness, it is thought that there may be mechanisms, such as cells having a small number of progesterone receptors that creates resistance to progesterone, and abnormalities in post-receptor mechanisms [25,26]. While there is a reduction in the size of ovarian endometriomas with hormonal treatment [41,42], the resistance of DIE tissue to medical treatments suggests that the biology of these two tissues is different [24,43]. Studies show that oestrogen and progesterone receptors in endometriosis tissue are less than in normal endometrial tissue [44]. Deficient methylation of the ER-β promoter results in pathological overexpression of ER-β in endometriotic stromal cells. High levels of ER-β suppress ER-α expression. A severely high ERβ-to-ERα ratio in endometriotic stromal cells is associated with suppressed progesterone receptor contributing to progesterone resistance [27]. In the present study we found decreased levels of ER-α and PR receptors in

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endometriosis tissues but more pronounced in DIE tissue similar to previous reports. Also, in the present study, PR and ER levels were found to be lower in both DIE tissue and endometrioma tissue than in normal endometrium.

Zanatta et al. found that expression of ER-α (in 16 of 18 patients), PR (in 17 of 18 patients), and PR-B (17 of 18 patients) was moderate to strong in the glands and stroma of nodules during both phases in rectosigmoid DIE tissue. However, in that study, DIE tissue, ovarian endometrioma and eutopic endometrium tissues were not compared [45]. Donnez et al. found cytokeratin and vimentin content, as well as ER and PR content, to be significantly lower in both types of lesion when compared with eutopic endometrium in a study in which 52 peritoneal endometriotic implants and 68 DIE tissue of rectovaginal septum were examined. Vimentin immunoreactivity in the epithelium, as well as the ER and PR content, were significantly lower in DIE tissue when compared with black peritoneal lesions [46]. Liu et al. found that E-cadherin levels were the lowest in DIE tissue in the control group in their study, in which they examined 25 endometriomas, 20 DIE tissue and 25 eutopic endometrial tissues. They found the highest ER-beta staining level in the ovarian endometrioma group and the lowest in the eutopic endometrium tissue. In contrast, they found the highest PR-B staining level in eutopic endometrium tissue and the lowest in DIE tissue [35]. In the current study, similar to the study conducted by Liu et al. the highest PR level was found in the normal endometrium group and the lowest in the DIE tissue. The staining order of ER levels in tissues was different from the study conducted by Liu et al. This difference may be due to assessment of ER-α in the present study versus ER-β staining in the study conducted by Liu et al. In addition, a positive correlation was found between the staining levels of T-cadherin, E-cadherin and ER-α and PR-α levels in the current study. The fact that T-cadherin and E-cadherin staining levels and ER-α and PR-α levels were lower in ovarian endometrioma and normal endometrial tissues than in DIE tissue, which is the most severe form of endometriosis, suggests that these molecules and receptors may have an effect on the invasive character.

To the best of our knowledge, our study is the first in literature that comparatively examines the tissue levels of T-cadherin, E-cadherin, ER and PR in DIE, ovarian endometriomas and eutopic endometrial tissue.

We examined the tissue levels of these molecules using immunohistochemical staining which only depicts the receptor and molecular density but not the function. This may be considered one important flaw of our method.

In further studies in DIE tissue and endometrioma tissue, overexpression or knockout of T-cadherin and E-cadherin may indicate whether they are part of the complex mechanisms that regulate invasion. Also, functional studies evaluating the amount of PR and ER-α and post-receptor mechanisms would contribute to understanding the biology of DIE tissue. In the present study, although there was a correlation between T-cadherin, E-cadherin, PR and ER staining levels, the functional relationship between these molecules needs to be explained. In future cell and tissue culture studies dealing with PR, ER, T-cadherin and E–cadherin expressions, it could be shown that progesterone may supress the EMT process by upregulating the cadherin expression mechanisms.

Conclusion

In conclusion, we found T-cadherin, E-cadherin, ER and PR H-scores to be lowest in DIE tissue and highest in normal endometrial tissue. A positive correlation was found between T-cadherin, E-cadherin, PR and ER H-scores in correlation analysis. These findings suggest that endometriotic nodules and ovarian endometrioma tissues have a different biology. In the present study, lower PR-α, ER-α, T-cadherin and E–cadherin expressions and lower progesterone effect that could not inhibit the decrease in cadherin expressions may explain the invasiveness of DIE tissue.

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Declaration of competing interest

No authors have any conflict of interest to report.

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