Induction of the epithelial-mesenchymal transition in the endometrium by chronic endometritis in infertile patients

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Abstract

Background

The purpose of the present study was to evaluate the relationship between chronic endometritis (CE) and the epithelial-mesenchymal transition (EMT) in the eutopic endometrium of infertile patients in the implantation phase.

Methods

Endometrial biopsy specimens from 74 infertility patients were enrolled. The presence of CE was investigated by immunostaining for CD138. Immunohistochemical staining for E-cadherin, N-cadherin, Slug, and Snail was performed, and expression profiles were statistically analyzed according to the presence of CE. When loss of E-cadherin expression and/or positive N-cadherin expression was detected, the specimen was considered EMT-positive. EMT-positive cases were also statistically analyzed according to the presence of CE. Patients' characteristics were compared between the EMT-positive and EMT-negative groups. Logistic regression analysis was also performed with variables including age, body mass index (BMI), gravidity, parity, and each factor causing infertility to examine the independent effect of each variable on EMT-positive status.

Results

Loss of E-cadherin expression, N-cadherin expression and EMT-positive were significant in CE patients ($p = 0.0037$, 0.0039 and < 0.0001, respectively). Slug, cytoplasmic Snail, and nuclear Snail expression were significant in CE patients ($p = 0.0008$, 0.0004 and 0.028, respectively). Differences were detected in unexplained infertility and CE between EMT-positive and EMT-negative cases. Unexplained infertility and CE were identified as variables related to EMT-positive status on logistic analysis.

Conclusion

The EMT was frequent in the eutopic endometrium in infertile patients with CE. Since the EMT is associated with unexplained infertility and CE, the EMT appears to be involved in altered mechanisms of implantation.

Background

Chronic endometritis (CE) is a persistent chronic inflammatory process of the endometrium. CE is defined as infiltration of plasma cells in the endometrial stroma [1-4], and immunostaining for CD138, a plasma cell marker, has been reported to be a useful method for demonstrating plasma cells in the endometrial stroma [2, 5]. CE is usually asymptomatic or presents only with subtle symptoms, including abnormal

uterine bleeding, dyspareunia, leucorrhea, and pelvic pain [1-3]. However, recent studies have focused on the association between CE and various gynecological conditions, and they have shown that CE has a positive relationship with infertility and implantation failure [6-8]. CE has also been shown to modify the function of endometrial cells and affect deciuda [9-12].

The epithelial-mesenchymal transition (EMT) is a process by which polarized epithelial cells lose polarity and intercellular contraction and acquire mesenchymal cell motility [13]. It is well known that the EMT plays crucial roles not only in normal embryological development, but also in several pathological conditions such as wound healing, fibrosis, and cancer development[12]. Focusing on the endometrium during the implantation phase, it has been shown that the mesenchymal-epithelial transition (MET), which is the reverse process to that of EMT, is induced in the endometrium and promotes the acceptance of embryos to the uterus [14, 15]. On the other hand, it has been found that the EMT also plays an important role in implantation [16, 17]. To date, no studies have evaluated the status of the endometrial EMT in the implantation phase in humans.

Thus, the occurrence of the EMT with or without CE was analyzed in infertile patients to elucidate the pathophysiology of CE causing impaired implantation. In addition, the effect of the EMT on infertility was evaluated by examining which infertility cause was associated with the occurrence of the EMT in the endometrium.

Methods

Ethics

This study conformed to the Clinical Research Guideline of Shiga University of Medical Science and was approved by the research ethics committee. Written, informed consent to participate in this study was obtained from all patients. This study included only patients over the age of 20.

Patients and endometrial sampling

A total of 74 infertile patients referred to the Department of Obstetrics and Gynecology of Shiga University of Medical Science from April 2015 to March 2018 were enrolled. They were all free from immune diseases, cancer, and diseases increasing susceptibility to infections, and they were not taking anti-inflammatory drugs or corticosteroids. Patients given 7 or more days of antibiotics for the treatment of CE before endometrial sampling were excluded. The ovulation day was determined by a urine ovulation test and ultrasonography, and the endometrium was collected 7-9 days after ovulation. On the day of endometrial sampling, the patients' blood was sampled to measure serum levels of estradiol (E_2) and progesterone (P_4). Patients with E_2 <50 pg/mL and P4 <8 ng/mL were excluded. Endometrial tissue obtained by the first curettage from each patient was used in this study. Clinical information including

age, gravidity, parity, body mass index(BMI), serum levels of E_2 and P_4, and cause of infertility were obtained from the clinical records.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks of the resected and biopsied specimens were cut into 3-μm-thick sections, deparaffinized, and rehydrated. Each section was used for immunostaining. Immunohistochemical analyses were performed using an autostainer (Benchmark XT system; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. The primary antibodies used in this study were as follows: a mouse monoclonal antibody against CD138 (clone B-A38, Cell Marque, CA, USA); a mouse monoclonal antibody against E-cadherin (clone 36, Roche Diagnostics); a mouse monoclonal antibody against N-cadherin (clone 6G11, DAKO Japan Co. Ltd., Kyoto, Japan); a rabbit polyclonal antibody against Slug (ab38551, Abcam, Cambridge, UK); and a rabbit polyclonal antibody against Snail (ab180714, Abcam).

Analyses of immunostaining

In this study, the number of CD138-immunoreactive cells was counted under high-power fields (magnification x 400) in 10 non-overlapping, random endometrial stromal areas in all resected and biopsied endometrial tissues. CE was diagnosed when one or more CD138-positive plasma cells were detected in the endometrial stroma [18, 19].

Membranous immunoreactivity for E- and N-cadherin was evaluated as positive staining. When one or more cell clusters were not stained with E-cadherin in the epithelium, it was considered loss of E-cadherin. When one or more cell clusters were stained with N-cadherin in the epithelium, it was considered N-cadherin-positive.

Nuclear immunoreactivity for Slug was evaluated as positive staining, and positive staining in the endometrial glandular cells and stromal cells was analyzed separately. Moreover, nuclear or cytoplasmic immunoreactivity for Snail was evaluated as positive staining, and both of them were analyzed separately.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5 (GraphPad Software Inc., La Jolla, CA). Each dataset was checked for a normal distribution using the Kolmogorov-Smirnov test, and Student's *t*-test or the non-parametric Mann-Whitney U test was used to test for significance depending on the distribution

pattern. The significance of differences in loss of E-cadherin expression, N-cadherin expression, loss of E-cadherin expression and/or N-cadherin expression, stromal Slug expression, cytoplasmic Snail expression, nuclear Snail expression, and both stromal Slug and cytoplasmic Snail expressions between the CE-positive group and CE-negative group was examined using Fisher analysis.

When loss of E-cadherin expression and/or positive N-cadherin expression was detected, the specimen was designated EMT-positive. Patients' characteristics including age, gravidity, parity, BMI, E_2 and P_4 levels, cause of infertility, and CE were compared between the EMT-positive and EMT-negative groups.

In addition, since this was a retrospective study, multivariate logistic regression analysis was performed for 8 explanatory variables, including 7 infertility factors (male factor, tubal factor, endometriosis, ovarian factor, fertilization failure, immune factor and unexplained infertility) and CE with respect to the objective variable, EMT-positive. SSPS statistics version 25 was used for this analysis. Odds ratios and P values were calculated. A significant difference was defined as a P value <0.05 in all analyses.

Results

CE was identified in 40 of 74 cases (54.1%). Age, gravidity, parity, BMI, and E_2 and P_4 levels were not different between the CE and Non-CE groups (Tables I). Regarding causes of infertility, among ovarian factor, tubal factor, endometriosis, male factor, fertilization failure, immune factor, and unexplained fertility, ovarian factor was higher in the Non-CE group, and unexplained infertility tended to be higher in the CE group, although there were no differences in other factors between the groups (Tables I).

Comparison of cadherin expression profiles between the CE and Non-CE groups

Table summarizes the immunohistochemical results of the CE and Non-CE groups. Loss of E-cadherin expression in the endometrial glands was observed in 14 of 40 and 2 of 34 cases in the CE and Non-CE groups, respectively (p = 0.0037) (Fig. 1A, B). N-cadherin-positive glandular cells were seen in 22 and 7 cases in the CE and Non-CE groups, respectively (p = 0.0039) (Fig. 1C, D). Loss of E-cadherin expression and/or N-cadherin expression was observed in 26 and 8 cases in the CE and Non-CE groups, respectively (p = 0.0005).

Comparison of EMT marker expressions between the CE and Non-CE groups

Table summarizes the associations between CE and expression profiles of Slug and Snail. Slug expression in the stromal cells was seen in 39 of 40 and 23 of 34 cases in the CE and Non-CE groups, respectively (p = 0.0008) (Fig. 1E, F). No cases showing Slug expression in the endometrial glandular cells were present in both groups. Cytoplasmic Snail expression was noted in 31 and 12 cases in the CE and Non-CE groups, respectively (p = 0.004) (Fig. 1G, H), and nuclear Snail expression was seen in 6 and 0 cases, respectively (p = 0.028). Both stromal Slug and cytoplasmic Snail expressions were seen in 30 and 8 cases, respectively (p < 0.0001).

Analysis of the variables related to EMT-positive status

Table summarizes patients' characteristics in the EMT-positive and EMT-negative groups. Age, gravidity, parity, BMI, E_2 and P_4 levels were not different between the EMT-positive and EMT-negative groups. Regarding causes of infertility, among ovarian factor, tubal factor, endometriosis, male factor, fertilization failure, anti-sperm antibody, and unexplained fertility, only unexplained infertility was higher in the EMT-positive group; there were no differences in the other factors between the groups. Regarding gynecological disease, among uterine fibroids, adenomyosis, endometrial polyps, and CE, only CE was higher in the EMT-positive group.

Unexplained infertility and CE were identified as variables related to EMT-positive on multivariate logistic analysis (Table)

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Discussion

In the present study, the effect of CE on the endometrial EMT in infertile patients was investigated, and it was found that the EMT occurred frequently in CE patients, and EMT-related transcription factor was also induced in CE patients at a high frequency.

The risk factors for EMT were also investigated among the characteristics of infertile patients, and it was found that CE was associated with the highest risk for the induction of the EMT. Thus, CE is closely related to the induction of the EMT in the implantation phase endometrium of infertile patients. To the best of our knowledge, this is the first report on the impact of CE on the occurrence of the EMT in the endometrium in the world.

One of the key functions of the endometrium is for embryos to implant and be nourished for the establishment and maintenance of pregnancy. The process of embryo implantation has long been classified into three phases: apposition, attachment, and penetration [20–22]. Apposition is defined as unstable adhesion of the blastocyst to the endometrial surface when blastocysts and the endometrial apical surface face each other. During this stage, the trophoblasts come close to the luminal epithelium. Then, trophoblasts begin to invade the endometrial surface, called the attachment phase. At this stage, the embryo and endometrium begin cross talk. A local increase in stromal vascular permeability at the blastocyst attachment site occurs, and rapid morphological changes in the endometrium are initiated. Penetration is a phase that involves the invasion of the embryo into the stromal area through the luminal epithelium to establish a closer relationship with the mother. It has been reported that the EMT and the MET are involved at the time of this attachment or penetration [14, 16]. Decidualization is the process by which the fibroblast-like endometrial stromal cells differentiate into polygonal epithelial-like cells during implantation [23, 24]. Zhang et al. showed that decidualization induced the MET by showing downregulated Snail and upregulated E-cadherin in mouse models [15]. E-cadherin has also been reported to be expressed in the mouse uterus at the start of implantation [16, 17]. It is expressed in stroma

before the trophoblastic cell invasion. On the other hand, some reports suggested that the EMT occurred in vivo and in vitro during the implantation process. The upregulation of N-cadherin and vimentin with downregulation of E-cadherin and cytokeratin was shown in the in vitro implantation model. Increased expressions of N-cadherin and TWIST2 (twist family basic helix-loop-helix transcription factor 2: a key transcription factor for Epithelial-mesenchymal transition) were detected at the implantation sites of trophoblasts. The immunohistochemical analysis for TWIST2 suggested that it was extensively expressed in endometrial glandular epithelium and luminal epithelium at implantation sites. The implantation rate of embryos was lower after the administration of TWIST2-siRNA interference, which prevented the EMT via decreased TWIST2 expression[17]. These findings suggest that the EMT is involved in receptivity of the uterine endometrium to the embryo. Thus, the endometrial cell possesses the ability to transform between epithelial and mesenchymal phenotypes by undergoing timely switches between the EMT and the MET and develop healthy gland and stromal structure for successful implantation of embryos[24]. CE is known to cause impaired implantation [6, 7]. In the present study, it was shown that CE frequently induces the EMT in the endometrium at the implantation phase. Implantation might be impaired due to abnormal EMT induction by CE or due to the presence of such an environment that induces an abnormal EMT.

In the present study, unexplained infertility was shown to be associated with the induction of the EMT in the endometrium. The EMT was found in 68% (13/19) of unexplained infertility cases. This rate is higher than that found in CE patients (65%, 26/40), and it was also significantly higher compared with infertility patients with other than unexplained infertility (21/55, P = 0.033). Patients with unexplained infertility include a high proportion of patients diagnosed with implantation failure and endometriosis [26–28]. The high frequency of the EMT in unexplained infertility suggests that the EMT may be associated with implantation failure and endometriosis.

The present study found no relationship between CE and endometriosis in infertility patients. In our previous report, we showed that patients with endometriosis had a high frequency of CE in resected uterine specimens. Endometriosis was diagnosed in the present study when endometriosis was detected by laparoscopy or laparotomy in the past three years or ovarian chocolate cysts were detected by ultrasonography or MRI. Since laparoscopic or laparotomy was not performed for all patients, a definitive diagnosis of early endometriosis was not obtained. This might be a cause of the difference between the present study and the previous study.

Endometrial cancer and endometriosis, as well as CE, are known to be frequent in infertile patients [4–8, 29–32]. Considering that CE is frequently found in infertile patients, CE, by triggering the EMT, might be the cause of the increased prevalence of endometrial cancer and endometriosis in infertile patients. The EMT is thought to be a central process of cancer invasion and metastasis, and some recent studies demonstrated the association between endometriosis and the EMT [33–36]. Moreover, regardless of the factors that induce the EMT in stem cells (or non-stem cells), genetic or epigenetic changes caused by endogenous or external stimuli establish a partial EMT and make the hybrid (epithelial-mesenchymal) phenotype stable [37, 38]. Whether the EMT or its reverse process the MET enables cells to obtain

pluripotency still remains controversial [39], it is necessary to study whether CE affects the development of endometrial cancer or endometriosis through the EMT in the future.

Conclusion

This is the first report to demonstrate the association between CE and the endometrial EMT. The infertility caused by CE may be due to its high EMT frequency and its effect on endometrial function during implantation.

Abbreviations

BMI: body mass index, CE: Chronic endometritis, EMT: epithelial-mesenchymal transition, E_2: estradiol, MET: mesenchymal-epithelial transition, P_4: progesterone, TWIST2: twist family basic helix-loop-helix transcription factor 2

Declarations

Ethics approval and consent to participate: This research was approved by the Ethics Committee of Shiga University of Medical Science (IRB-ethics committee approval number, R2014-090).

Consent for publication: Informed written consent was obtained for publication.

Availability of data and materials: We can provide the raw data. The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests: No author has any conflict of interest to disclose.

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Authors' contributions

Conceived the study: MI, FK; designed the experiments: MI; performed the experiment: MI, AT; analyzed the data: MI, FK; contributed reagents/materials/analysis tools: AT, FK, JK, TH, KT; wrote the manuscript: MI, FK. Final approval: KT, TM.

All authors read and approved the final manuscript.

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References

1. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observation. Obstet Gynecol. 1981;58:176–84.
2. Kitaya K, Yasuo T. Immunohistochemical and clinicopathological characterization of chronic endometritis. Am J Reprod Immunol. 2011;66:410–5.
3. Crum CP, Egawa K, Fenoglio CM, Richart RM. Chronic endometritis: the role of immunohistochemistry in the detection of plasma cells. Am J Obstet Gynecol. 1983;147:812–5.
4. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, et al. Review: Chronic endometritis and its effect on reproduction. J Obstet Gynaecol Res. 2019;45:951–60. doi:10.1111/jog.13937.
5. Kitaya K, Yasuo T. Inter-observer and intra-observer variability in immunohistochemical detection of endometrial stromal plasmacytes in chronic endometritis. Exp Ther Med. 2013;5:485–8.
6. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF. Hum Reprod. 2015;30:323–30.
7. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril. 2010;93:437–41.
8. Polisseni F, Bambirra EA, Camargos AF. Detection of chronic endometritis by diagnostic hysteroscopy in asymptomatic infertile patients. Gynecol Obstet Invest. 2003;55:205–10.
9. Matteo M, Cicinelli E, Greco P, et al. Abnormal pattern of lymphocyte subpopulations in the endometrium of infertile women with chronic endometritis. Am J Reprod Immunol. 2009;61:322–9.
10. Mishra K, Wadhwa N, Guleria K, Agarwal S. ER, PR and Ki-67 expression status in granulomatous and chronic non-specific endometritis. J Obstet Gynaecol Res. 2008;34:371–8.
11. Wu D, Kimura F, Zheng L, et al. Chronic endometritis modifies decidualization in human endometrial stromal cells. Reprod Biol Endocrinol. 2017;15:16.
12. Kaku S, Kubo T, Kimura F, Nakakumra A, Kirazawa J, Morimune A, et al. Relationship of chronic endometritis with chronic deciduitis in cases of miscarriage. BMC Women's Health. Jun 1; 20: 114.
13. Barriere G, Fici P, Gallerani G, Fabbri F, Rigaud M. Epithelial mesenchymal transition: a double-edged sword. Clin Transl Med. 2015;4:14.
14. Paria BC, Zhao X, Das SK, Dey SK, Yoshinaga K. Zonula occludens-1 and E-cadherin are coordinately expressed in the mouse uterus with the initiation of implantation and decidualization. Dev Biol. 1999;208:488–501.
15. Zhang XH, Liang X, Liang XH, Wang TS, Qi QR, Deng WB, et al. The mesenchymal-epithelial transition during in vitro decidualization. Reprod Sci. 2013:354–60.
16. Gou J, Hu T, Li L, Xue L, Zhao X, Yi T, et al. Role of epithelial-mesenchymal transition regulated by twist basic helix-loop-helix transcription factor 2 (Twist2) in embryo implantation in mice. Reprod Fertil Dev. 2019;31:932–40.

17. Uchida H, Maruyama T, Nishikawa-Uchida S, Oda H, Miyazaki K, Yamasaki A, et al. Studies using an in vitro model show evidence of involvement of epithelial-mesenchymal transition of human endometrial epithelial cells in human embryo implantation. J Biol Chem. 2012;287:4441–50.
18. Kasius JC, Broekmans FJ, Sie-Go DM, Bourgain C, Eijkemans MJ, Fauser BC, Devroey P, Fatemi HM. The reliability of the histological diagnosis of endometritis in asymptomatic IVF cases: a multicenter observer study. Hum Reprod. 2012;27:153–8.
19. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. The association between endometriosis and chronic endometritis. PLoS ONE. 2014;9:e88354.
20. Edwards RG. Human implantation: the last barrier in assisted reproduction technologies? Reprod Biomed Online. 2007;14:5–22.
21. Fitzgerald JS, Poehlmann TG, Schleussner E, Markert UR. Trophoblast invasion: the role of intracellular cytokine signalling via signal transducer and activator of transcription 3 (STAT3). Hum Reprod Update. 2008;14:335–44.
22. Kim SM, Kim JS. A Review of Mechanisms of Implantation. Dev Reprod. 2017;:351–359.
23. Sharma S, Godbole G, Modi D. Decidual Control of Trophoblast Invasion. Am J Reprod Immunol. 2016;75:341–50.
24. King A. Uterine leukocytes and decidualization.Hum Reprod Update. 2000;6:28–36.
25. Bilyk O, Coatham M, Jewer M, Postovit LM. Epithelial-to-Mesenchymal Transition in the Female Reproductive Tract: From Normal Functioning to Disease Pathology. Front Oncol. 2017;7:145.
26. Kanda Y, Ikeda M, Ishikawa M, Sakakibara H, Hirahara F. Laparoscopy for the treatment of unexplained infertility. Reprod Med Biol. 2006;5:59–64.
27. Gleicher N, Barad D. Unexplained infertility: does it really exist? Hum Reprod. 2006;21:1951–5.
28. Cicinelli E, Matteo M, Trojano G, Mitola PC, Tinelli R, Vitagliano A, et al. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. Am J Reprod Immunol. 2018;79.
29. Lundberg FE, Iliadou AN, Rodriguez-Wallberg K, Gemzell-Danielsson K, Johansson ALV. The risk of breast and gynecological cancer in women with a diagnosis of infertility: a nationwide population-based study. Eur J Epidemiol. 2019;34:499–507.
30. Yang HP, Cook LS, Weiderpass E, Adami HO, Anderson KE, Cai H, et al. Infertility and incident endometrial cancer risk: a pooled analysis from the epidemiology of endometrial cancer consortium (E2C2). Br J Cancer. 2015;112:925–33.
31. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet. 2010;376(9742):730–8.
32. Prescott J, Farland LV, Tobias DK, Gaskins AJ, Spiegelman D, Chavarro JE, et al. A prospective cohort study of endometriosis and subsequent risk of infertility. Hum Reprod. 2016;31:1475–82.
33. López-Novoa JM, Nieto MA. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol Med. 2009;1:303–14.

34. Zhou C, Liu J, Tang Y, Liang X. Inflammation linking EMT and cancer stem cells. Oral Oncol. 2012;48:1068–75.
35. Proestling K, Birner P, Gamperl S, Nirtl N, Marton E, Yerlikaya G, et al. Enhanced epithelial to mesenchymal transition (EMT) and upregulated MYC in ectopic lesions contribute independently to endometriosis. Reprod Biol Endocrinol. 2015;13:75.
36. Bartley J, Jülicher A, Hotz B, Mechsner S, Hotz H. Epithelial to mesenchymal transition (EMT) seems to be regulated differently in endometriosis and the endometrium. Arch Gynecol Obstet. 2014;289:871–81.
37. Denisov EV, Perelmuter VM. A fixed partial epithelial-mesenchymal transition (EMT) triggers carcinogenesis, whereas asymmetrical division of hybrid EMT cells drives cancer progression. Hepatology. 2018;68:807–10.
38. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene. 2020;29:4741–51.
39. Chen T, You Y, Jiang H, Wang ZZ. Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. J Cell Physiol. 2017;232:3261–72.

Tables

Table . Patients' characteristics in the CE and Non-CE groups

	CE (40 cases)	Non-CE (34 cases)	*p* value
Age (y.o)	37.08	36.35	0.43
Gravidity	0.63	0.62	0.97
Paritiy	0.3	0.29	0.96
Serum level of estradiol	135	135.9	0.95
Serum level of prgesterone	14.69	15.58	0.55
Infertiity cause			
Ovarian factor	0	5	0.017
Tubal factor	9	10	0.6
Endometriosis	10	9	0.79
Male factor	9	9	0.6
Fatilization failure	4	1	0.37
Immune factor	0	0	1
Unexplained	14	5	0.063
Gynecological disease			
Uterine fibroid	11	6	0.41
Adnoemyosis	0	1	0.46
Endometrial polyp	5	3	0.72

CE: chronic endometritis

Table . Association between chronic endometritis and cadherin expression profiles in the specimens

	CE (40 cases)	Non-CE (34 cases)	*p* value
Loss of E-cadherin expression	14	2	0.0037
N-cadherin expression	22	7	0.0039
Loss of E-cadherin expression and/or N-cadherin expression	26	8	0.0005

CE, chronic endometritis

Table . Associations between chronic endometritis and EMT marker expression in the specimens

	CE (40 cases)	Non-CE (34 cases)	*p* value
Stromal Slug expression	39	23	0.0008
Cytoplasmic Snail expression	31	12	0.0004
Nuclear Snail expression	6	0	0.028
Both stromal Slug + Cyto Snail	30	8	<0.0001

CE, chronic endometritis; EMT, epithelial-mesenchymal transition.

Table . Patients' characteristics in the EMT-positive and EMT-negative groups

	EMT+ (34cases)	EMT- (40 cases)	*p* value
Age	37.59	36.03	0.087
Gravidity	0.68	0.58	0.65
Paritiy	0.32	0.28	0.67
Serum level of estradiol	129.1	140.7	0.47
Serum level of prgesterone	15.21	14.99	0.88
Infertiity cause			
Ovarian factor	1	5	0.21
Tubal factor	6	12	0.28
Endometriosis	11	6	0.78
Male factor	5	11	0.78
Fatilization failure	2	3	1
Anti-sperm antibody	0	1	1
Unknown	13	6	0.033
Gynecological disease			
Uterine fibroid	8	10	1

Adnoemyosis	0	1	1
Endometrial polyp	5	6	1
Chronic endometritis	26	14	0.0005

EMT: epithelial-mesenchymal transition.

Table . Logistic regression analysis for the EMT

Variable	*p* value	Odds ratio	95% CI
Chronic endometritis	0.002	5.3	1.83-15.4
Unexplained infertility	0.046	3.72	1.03-13.5

EMT: epithelial-mesenchymal transition.

Figures

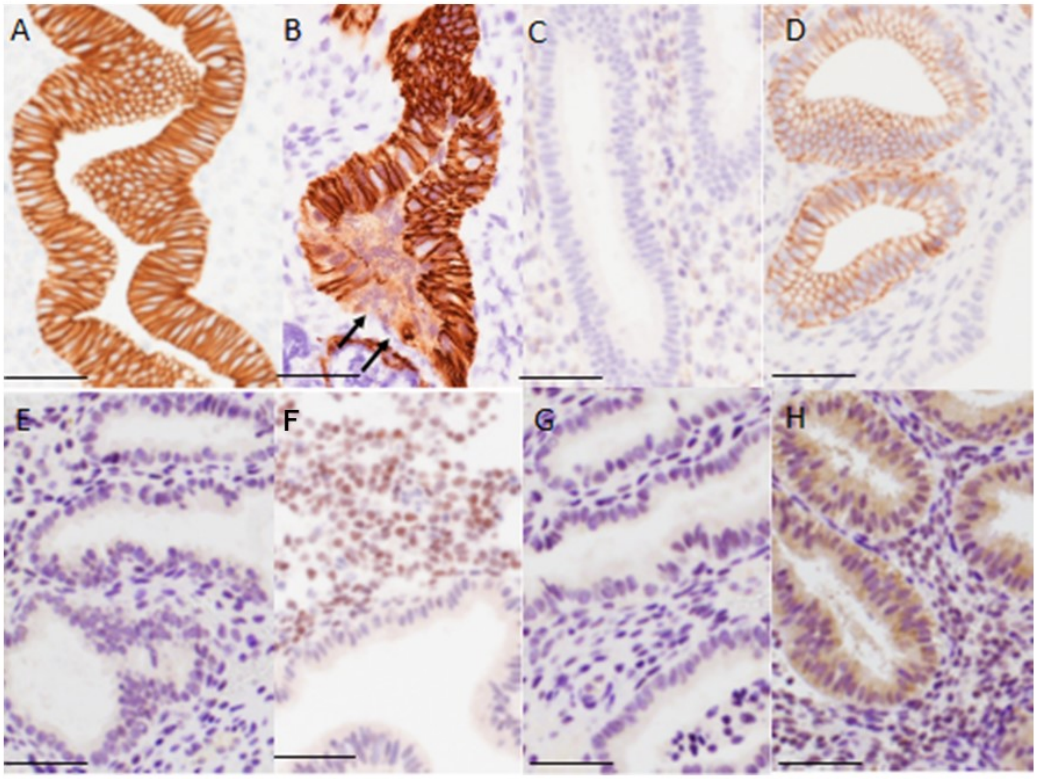

Figure 1

Immunohistochemical results of the specimens. (A) E-cadherin was detected in the glandular cells in endometrial specimens. (B) A cluster of E-cadherin-negative in endometrial glandular cells was observed (arrows). When such a finding was detected, it was judged as loss of E-cadherin. (C) N-cadherin was not detected in the glandular cells in endometrial specimens. (D) N-cadherin-positive glandular cells was present in endometrial glandular cells. When the staining was observed, it was judged as N-cadherin positive. (E) Slug was not expressed in stromal cells of the endometrium. (F) Slug is expressed in the nuclei of endometria stromal cells. When the staining was observed, it was judged as slug positive. (G) Snail expression was not noted in the glandular cells of the endometrium. (H) Cytoplasmic Snail expression was noted in the glandular cells of the endometrium. When the staining was observed, it was judged as Snail positive. Bar is 100μm.