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Occurrence of chronic endometritis in different types of human adenomyosis

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Abstract

Purpose: Human adenomyosis has an adverse effect on female fertility. Exact mechanistic basis is still unclear. We investigated the occurrence of chronic endometritis (CE) in different types of human adenomyosis.

Methods: This is a prospective non-randomized observational study enrolling patients with focal (n = 30), diffuse (n = 26), intrinsic (n = 23), and extrinsic (n = 10) adenomyosis. Endometrial biopsy samples were collected from hysterectomy specimens. Immunohistochemical analysis was performed using antibody against CD68 (Mφ marker) with biopsy samples of intrinsic/extrinsic adenomyosis and CD138 (Syndecan-1), a marker of plasma cells, in all biopsy samples.

Results: In GnRHa-untreated groups, a higher trend in the occurrence of CE, as characterized by infiltration of ≥1 plasma cells in endometrial stroma, was found in women with focal (58.8%, p = 0.0849) and diffuse adenomyosis (60.0%, p = 0.0841) comparing to control women (10.0%). In women with focal adenomyosis, ipsilateral side showed a significantly higher occurrence of CE (58.8%) than on the contralateral side (11.7%) (p = 0.043). Tissue infiltration of macrophages in endometria was significantly higher in intrinsic than in extrinsic adenomyosis (p = 0.03) without showing any significant difference in the occurrence of CE between these two variants of adenomyosis.

Conclusion: A variable occurrence of CE in different types of adenomyosis may be involved in adverse reproductive outcome.

KEYWORDS
CD138, CD68, extrinsic adenomyosis, focal/diffuse adenomyosis, intrinsic adenomyosis

1 | INTRODUCTION

Uterine adenomyosis is a benign gynecological disease characterized by the presence of endometrial glands and stroma within the myometrium.[1,2] Although exact pathogenesis is still controversial, it is a common understanding that adenomyosis develops as a down-growth and invagination of the basalis endometrium into the myometrium.[2-4] With the advent of recent imaging modalities, diagnosis of adenomyosis is based on magnetic resonance imaging (MRI) and transvaginal ultrasonography and occurs most likely during the fourth and fifth decades of life and after the completion of childbearing activity. However, an increase in the prevalence of

adenomyosis in the past few years has been observed in younger women of reproductive age.[1,5] Decreased quality of life due to severe painful symptoms/abnormal uterine bleeding and/or subfertility/infertility are the main problems in women with adenomyosis and warrants proper treatment.[6,7]

Adenomyosis appears in different configuration such as diffuse, focal and rare cases of cystic adenomyoma and is better detected by MRI.[1,8,9] Clinically, diffuse dispersion of numerous foci of endometrial glands and stroma within the myometrium is considered as diffuse adenomyosis and circumscribed nodular aggregates as observed on either anterior or posterior wall of the uterus is considered as focal adenomyosis.[10] Recently, the classical hypothesis of adenomyosis supporting origin from basalis endometrium is criticized by a Japanese group and proposed four subtypes of adenomyosis based on their clinical experience and assessment by MRI/histology.[11] Among them, intrinsic adenomyosis (subtype I) is considered as a product of direct endometrial invasion involving inner-mid myometrium and extrinsic adenomyosis (subtype II) as endometriotic lesion coming from outside involving outer myometrium. Unlike intrinsic adenomyosis, extrinsic adenomyosis was found to be mostly coexistent with deep infiltrating endometriosis (DIE), a term recently recognized as deep endometriosis.[11,12]

Adenomyosis is linked to infertility but the exact mechanism behind this relationship is still unclear. A number of factors have been proposed as follows: (i) Abnormal utero-tubal sperm transport secondary to intrauterine abnormalities and increased uterine peristalsis. (ii) Altered endometrial function and receptivity due to abnormal endometrial steroid metabolism, increased inflammatory response, defective intra-uterine oxidative stress environment, and/or impairment of implantation.[13,14] (iii) Mostly recently, an axonemal alteration in the apical endometria of women with adenomyosis appears to be involved in infertility.[15] Impact of adenomyosis on ART outcome is not fully understood, as data are scarce and there are contradictions within the available evidence.[16,17] Adenomyosis has been associated with a higher prevalence of miscarriage and with a generally worse perinatal outcomes.[18,19] A multicenter cohort study in Japan reported higher incidence of uterine infection in patients with diffuse adenomyosis without investigating concurrent occurrence of chronic endometritis (CE) in these women.[20] Although information on intrauterine infection in women with adenomyosis is limited, we postulate that similar to endometriosis, a variable prevalence of CE might occur in different types of adenomyosis in response to uterine infection and if occurs, this may further clarify the negative fertility outcome in these women.

Endometritis is an infectious and inflammatory disorder of the endometrium and can manifest in the form of either acute endometritis (AE) or CE.[21,22] Acute endometritis, as characterized by micro-abscess formation and increased neutrophil infiltration in the endometrium, is an overt clinical condition manifesting fever, pelvic pain, and vaginal discharge. In contrast, CE is mostly asymptomatic and sometimes shows subtle symptoms such as pelvic discomfort, spotting, and leucorrhea. Therefore, CE is often unnoticed by patients and/or ignored by gynecologists.[23] CE is characterized by endometrial superficial edematous change, focal or diffuse hyperemia, endometrial micropolyps, and stromal plasmacyte infiltration.[24,25] Comparing to hysteroscopic and histological diagnosis, immunohistochemical staining with CD138 allows simple and reliable identification of plasma cells in the endometrial tissue and is gaining much more popularity.[22-26]

Recent studies have shown a correlation between CE and reproductive failures such as recurrent implantation failures after IVF-ET, recurrent miscarriage, and unexplained infertility.[27,28] The major cause of CE is microbial infection in the uterine cavity. This is supported by the fact that treatment with antibiotics is effective to eliminate plasma cells in the affected patients.[27,29,30] Although it is controversial about the causality between CE and embryo implantation failure, reports suggest that CE negatively affects reproductive outcome. Based on our serial works on uterine infection and endometriosis over the last decade, we reported involvement of intrauterine microbial colonization (IUMC) and occurrence of CE in women with endometriosis.[31] A variable rate of CE occurrence in endometriosis and its association with adverse reproductive outcome have been reported in different studies.[31-36] However, information on the occurrence of CE in women with different types of adenomyosis is unknown.

Therefore, we investigated the following issues using biopsy samples derived from hysterectomy specimens of control women and women with different types of adenomyosis: (1) Occurrence rate of CE in the endometria collected from control women and gonadotropin-releasing hormone agonist (GnRHa)-treated and -untreated women with focal and diffuse adenomyosis. (2) Tissue infiltration of macrophages in the endometria collected from control women and women with intrinsic and extrinsic adenomyosis and coexistent DIE lesions. (3) Occurrence rate of CE in the endometria collected from women with intrinsic and extrinsic adenomyosis. (4) Finally, we discussed the cause-effect of CE on adverse reproductive outcome in women with adenomyosis.

2 | MATERIALS AND METHODS

2.1 | Patients

This is a prospective non-randomized observational study. The patients in this study were women of reproductive age. We collected biopsy samples from control women and women with different types of adenomyosis for immunohistochemical analysis. All paraffin-embedded tissue sections for the current study were derived from the same tissues blocks prepared from the biopsy samples of women with and without adenomyosis that we used for our two recent studies.[12,37]

2.2 | Collection of biopsy samples

Between March 2015 and December 2017, full-thickness (from the endometrium to the myometrium) biopsy specimens were

collected after hysterectomy from 10 control women with uterine myoma, 30 women with focal adenomyosis, and 26 women with diffuse adenomyosis. The collected uteri were transported to the laboratory in DMEM/F12 media (GIBCO) on ice under sterile conditions. Biopsy specimens were collected from the anterior wall and posterior wall for the cases with diffuse adenomyosis and from contralateral side (side opposite the lesion), ipsilateral side (lesion side) for the cases with focal adenomyosis. The surgical procedures for focal and diffuse adenomyosis are described elsewhere.[37] A proportion of women with focal and diffuse adenomyosis received GnRHa treatment for a variable period of 3–6 months before surgery. GnRHa treatment was indicated for a variable complaint of abnormal genital bleeding, hypermenorrhea, or anemia with or without associated complaint of dysmenorrhea or pelvic pain. During the same period, full-thickness biopsy specimens were collected after hysterectomy from 23 women with intrinsic adenomyosis (subtype I) and 8 women with extrinsic adenomyosis (subtype II) with concurrent biopsy samples from coexistent DIE. As a conservative surgery, biopsy specimens were collected after adenomyomectomy from two women with extrinsic adenomyosis. All 23 women with intrinsic and a total of 10 women with extrinsic adenomyosis did not receive any hormonal medication before surgery. Control women and women with different adenomyosis were age-matched and were operated on for either of total hysterectomy or conservative surgery.

The diagnosis of uterine myoma and focal/diffuse adenomyosis was made clinically by transvaginal USG and MRI and confirmed by histology.[37] The MRI and histologic diagnosis of intrinsic and extrinsic adenomyosis was made based on the reported criteria.[11] A T2-weighted MRI photograph of each type of adenomyosis is shown in Figure 1. The anatomic location of adenomyosis and quality of each collected biopsy specimens were retrospectively reviewed and confirmed by MRI, video image, and tissue observation (K.N.K., A.F., A.K.). The coexistent DIE was diagnosed by MRI and during surgery as infiltrating endometriosis (>5 mm) into either of recto-vaginal septum, utero-sacral ligament, sigmoid colon or rectum. The detailed anatomical location of each DIE and surgical approaches are described elsewhere.[12] All biopsy specimens were collected in accordance with the guidelines of the Declaration of Helsinki and were approved by the Institutional Review Board of our University (IRB No. 16005). A written informed consent was obtained from all the women.

2.3 | Antibodies used

We performed immunohistochemical analysis of target antigen in the serial section of biopsies using the following antibodies and dilutions: (1) CD68 for macrophages (Mφ) in intact tissues. CD68 (KP1), a mouse monoclonal antibody (1:50) was derived from Dako. CD68

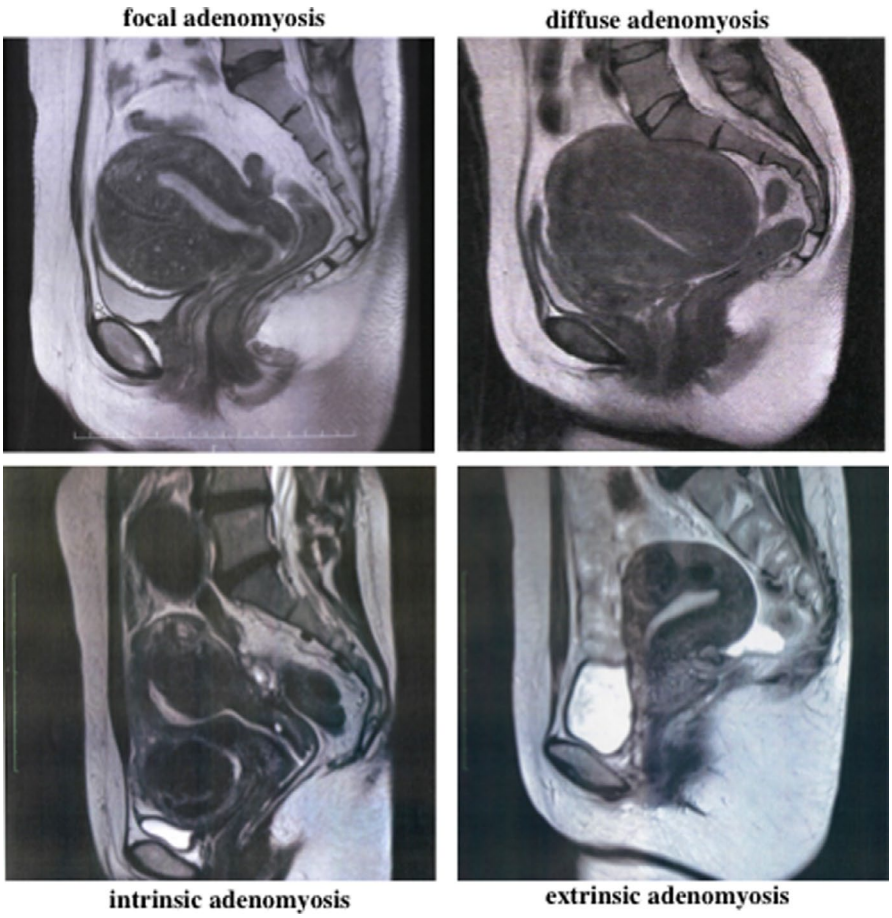

FIGURE 1 T2-weighted magnetic resonance images (sagittal section) of focal adenomyosis (upper left panel), diffuse adenomyosis (upper right panel), intrinsic adenomyosis (lower left panel), and extrinsic adenomyosis (lower right panel) are shown. An explanation of each subtype of adenomyosis is described in the text

antigen (clone KP1), which we used for our current study as a marker of matured and activated Mφ, is a glycosylated trans-membrane glycoprotein that is mainly located in lysosomes. (2) Anti-Syndecan-1 antibody [1:200, mouse monoclonal (B-A38) to Syndecan (CD138), ab34164, Abcam] was used to immunolocalize plasma cells. (3) Non-immune immunoglobulin (Ig) G1 (1:50), a mouse monoclonal antibody from Dako, was used as a negative control.

2.4 | Immunohistochemistry

The detailed procedures of immunohistochemical staining were followed as we described previously.[38-40] We used at least three slides per biopsy for immunohistochemical analysis.

The immunoreactive CD68 spots were counted in five different fields of one section (×200 magnification) by light microscopy and expressed as the mean Mφ number per field in one specimen. CD138 (Syndecan-1)-stained plasma cells were counted in 10 different fields of one section by light microscopy at moderate magnification (×200). By means of the criteria proposed by Kiviat et al.,[21] we defined CE by the presence of ≥1 plasma cells only in the endometrial stroma and the absence of neutrophils (also called plasma cell endometritis). Biopsy specimens from plasmacytoma were used as positive control for plasma cells as we described previously.[31] The histological diagnosis of CE was performed by an independent investigator (KNK) and was confirmed by an expert histopathologist (MN).

2.5 | Statistical analysis

All results are expressed as either mean ± SD, median and interquartile range (IQR). The clinical characteristics of the patients were compared with one-way analysis of variance. Mann-Whitney *U*-test was used to analyze any difference between two groups. For non-parametric comparisons among groups, the Kruskal-Wallis test was used. Categorical variables were compared using chi-square (χ^2) test or Fisher exact test where appropriate. The distribution of macrophages according to groups was expressed using the box and whisker plots with the medians and IQR. A value of $p < 0.05$ was considered to be statistically significant. Data analysis was conducted using SAS software version 9.4 (SAS Institute Inc.).

3 | RESULTS

Thirteen women in focal adenomyosis group and 11 women in diffuse adenomyosis group received GnRHa treatment before surgery. The remaining 17 women with focal adenomyosis and 15 women with diffuse adenomyosis received no hormonal treatment. One woman in focal adenomyosis and four women in diffuse adenomyosis had coexistent endometriosis. One woman in focal and six women in diffuse adenomyosis had coexistent uterine myoma. The detailed clinical profiles of women with focal and diffuse adenomyosis are described elsewhere.[37] During the same study period we could collect biopsy samples from 23 women with intrinsic adenomyosis and 10 women with extrinsic adenomyosis based on the diagnostic criteria.[11,12] All women with intrinsic and extrinsic adenomyosis received no hormonal medication within 6 months before surgery. The clinical background and MRI, surgical, and histologic findings of these women with intrinsic and extrinsic adenomyosis are reported elswhere.[12]

3.1 | CD138 (Syndecan-1)-positive plasma cell infiltration in focal and diffuse adenomyosis

The immunolocalization CD138 (Syndecan-1)-stained plasma cells in endometria derived from contralateral side/ipsilateral side of GnRHa-untreated women with focal adenomyosis (upper row) and anterior/posterior wall of diffuse adenomyosis (lower row) are shown in Figure 2. The corresponding slides of positive controls (plasmacytoma) and negative controls are shown on the right two panels of Figure 2. CD138-stained plasma cells were observed in the stromal compartment of endometria without any presence of neutrophils. In cases with plasma cell infiltration, we could detect diffuse distribution of plasma cells in at least 4 of 10 non-overlapping fields of CD138-stained slides.

Chi-square test indicated a higher tendency in the occurrence rate of CE, as characterized by infiltration of ≥1 plasma cells in endometria of women with focal adenomyosis (10/17, 58.8%, $p = 0.084$) and diffuse adenomyosis (9/15, 60.0%, $p = 0.084$) comparing to control women (1/10, 10.0%) (Table 1). GnRHa treatment did not further increase the rate of CE occurrence in these two groups of women (Table 1).

When we analyzed CD138-stained plasma cells in endometria collected from GnRHa-untreated women with focal adenomyosis, we found that occurrence rate of CE was significantly higher on the ipsilateral side (10/17, 58.8%) than on the contralateral side (2/17, 11.7%) of focal adenomyosis ($p = 0.043$, χ^2 test) (Table 2). The distribution of CD138-stained plasma cells in the endometria of contralateral side and ipsilateral side of GnRHa-untreated women with focal adenomyosis is shown in Table 3. A statistically significant difference in the infiltration of CD138-stained plasma cells in endometria was found between these two groups ($p = 0.011$). In our separate analysis, we did find any significant difference in the occurrence rate of CE between anterior wall (6/15, 40.0%) and posterior wall (9/15, 60.0%) of GnRHa-untreated women with diffuse adenomyosis ($p = 0.526$) (Table 4).

3.2 | CD68-positive Mφ infiltration in intrinsic/extrinsic adenomyosis and coexistent DIE

We examined CD68-positive Mφ infiltration in the endometria of intrinsic and extrinsic adenomyosis and their coexistent DIE lesions. Tissue infiltration of Mφas shown by CD68-positive brown spots is

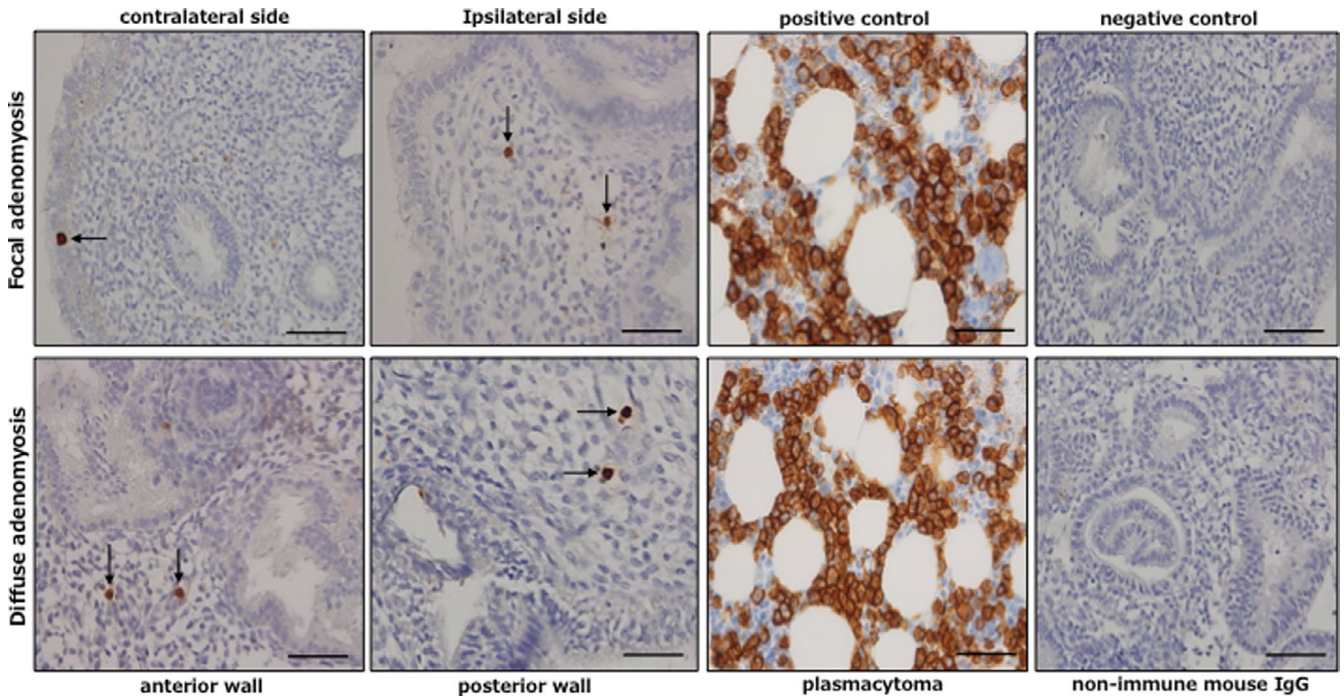

FIGURE 2 Immunohistochemical staining of CD138 (Syndecan-1) in the endometria derived from ipsilateral side and contralateral side of focal adenomyosis (upper low) and anterior wall and posterior wall of diffuse adenomyosis (lower row). Arrows indicate the CD138-stained plasma cells in endometrial stroma of each slide. CD138-stained plasma cells in biopsy samples derived from cases with plasmacytoma was used as a positive control (second column from right) and non-immune mouse IgG staining was used for negative control (right column). Scale bar = 50 μm (for slides in left two columns) and Scale bar = 100 μm (for slides in right two columns)

TABLE 1 Occurrence rate of chronic endometritis in GnRHa-treated and -untreated women with focal and diffuse adenomyosis

	Controls	Focal adenomyosis	Diffuse adenomyosis
GnRHa (−), *n* (%)	1/10 (10.0)[a]	10/17 (58.8)[b]	9/15 (60.0)[c]
GnRHa (+), *n* (%)		8/13 (61.5)[d]	7/11 (63.6)[e]

Note: Data were analyzed by chi-squared test.

$p = 0.084$, a vs. b; $p = 0.084$, a vs. c; $p = 0.972$, b vs. c; $p = 0.959$, d vs. e.

GnRHa (−), gonadotropin-releasing hormone agonist-untreated women.

GnRHa (+), gonadotropin-releasing hormone agonist-treated women.

TABLE 2 Occurrence rate of chronic endometritis in GnRHa-untreated women with focal adenomyosis

	Contralateral side	Ipsilateral side	*p* Value
Cases ($n = 17$), *n* (%)	2/17 (11.7)	10/17 (58.8)	0.043

Note: Data were analyzed by chi-squared test.

TABLE 3 Distribution of CD138-stained plasma cells in endometria of contralateral and ipsilateral side of GnRHa-untreated women with focal adenomyosis.

	Contralateral side ($n = 17$)	Ipsilateral side ($n = 17$)	*p* Value
Mean ± SD	0.47 ± 1.5	4.2 ± 4.1	0.011
Median	0	3	
Range	0–6	0–10	

Note: Mann-Whitney *U*-test showed significant differences in the infiltration of CD138-stained plasma cells in endometria between groups.

TABLE 4 Occurrence rate of chronic endometritis in GnRHa-untreated women with diffuse adenomyosis

	Anterior all	Posterior wall	p Value
Cases ($n = 15$), *n* (%)	6/15 (40.0)	9/15 (60.0)	0.526

Note: Data were analyzed by chi-squared test.

shown in the endometria (upper row) and their coexistent DIE lesions (lower row) derived from two representative cases each of intrinsic adenomyosis (left two columns) and extrinsic adenomyosis (right two columns) (Figure 3A). We found that tissue infiltration of Mφ was significantly higher in the endometria collected from women with intrinsic adenomyosis comparing to that of extrinsic adenomyosis ($p = 0.03$) or control women ($p < 0.05$) (Figure 3B). There was no remarkable difference in the pattern of Mφ infiltration in the DIE lesions coexistent with either intrinsic or extrinsic adenomyosis (Figure 3B).

3.3 | CD138 (Syndecan-1)-positive plasma cell infiltration in intrinsic and extrinsic adenomyosis

The immunolocalization CD138 (Syndecan-1)-stained plasma cells in the endometria derived from two representative cases (upper and lower rows) of intrinsic adenomyosis (left column) and extrinsic adenomyosis (right column) are shown in Figure 4. CD138-stained plasma cells were observed in the stromal compartment of endometria without any presence of neutrophils. Although a higher prevalence of CE was observed in intrinsic adenomyosis, chi-square test indicated an insignificant difference in the occurrence rate of CE between intrinsic adenomyosis (14/23, 60.8%) and extrinsic adenomyosis (4/10, 40.0%) ($p = 0.536$) (Table 5).

3.4 | Distribution of CD138 (Syndecan-1)-positive plasma cells in endometrial stroma

Table 6 shows the distribution in the numbers of CD138-stained plasma cells in respective endometria collected from GnRHa-untreated women with focal adenomyosis, diffuse adenomyosis, intrinsic adenomyosis, and extrinsic adenomyosis. Except one case with intrinsic adenomyosis, all other cases harbored two or more CD138-stained plasma cells in endometrial stroma. Mann-Whitney *U*-test and Kruskal-Wallis test between groups and among groups indicated no significant differences in the distribution of CD138-stained plasma cells in endometria derived from women with different types of adenomyosis (Table 6).

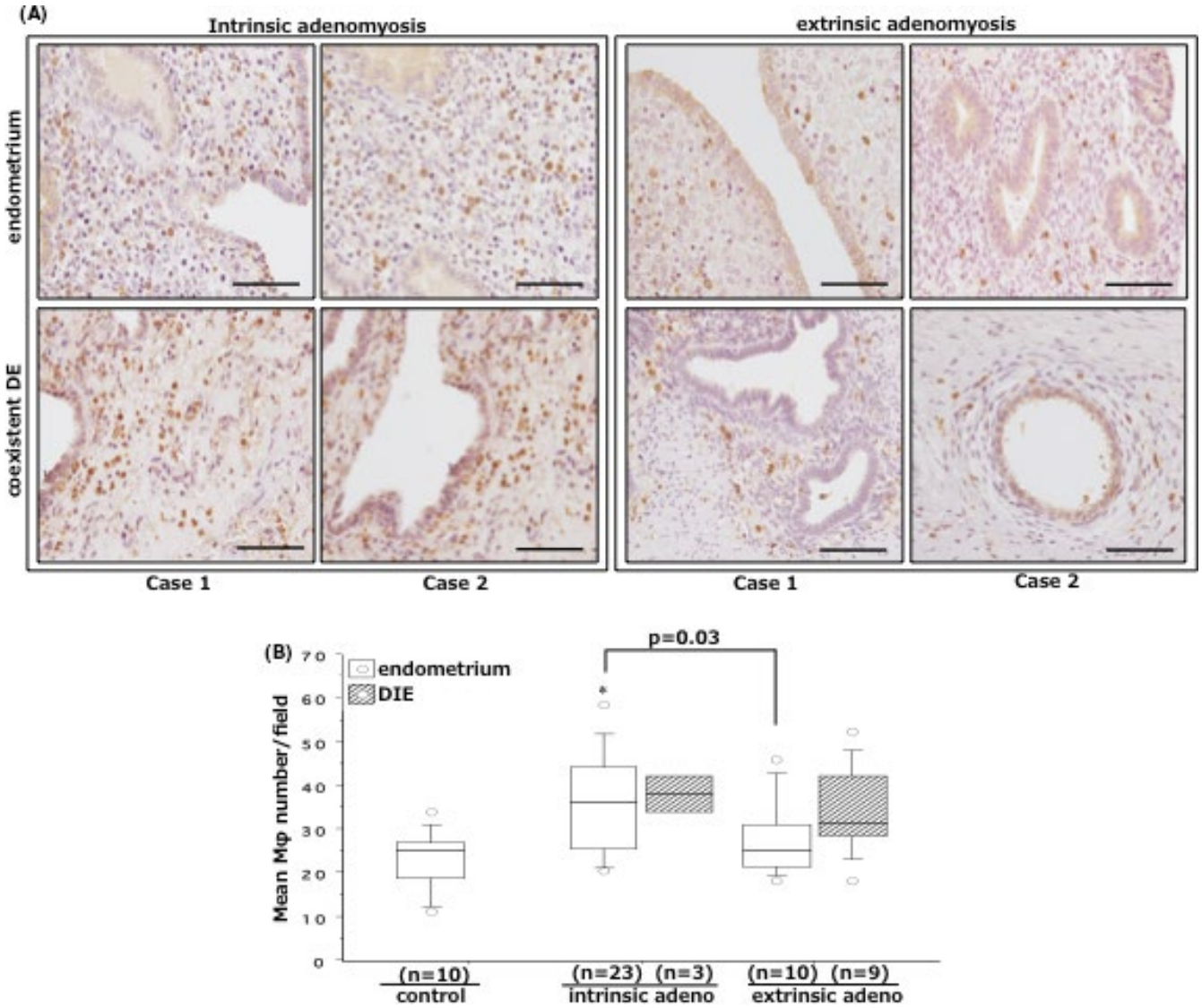

FIGURE 3 (A) Immunohistochemical analysis of CD68-stained macrophages (Mφ) in the endometria (upper row) and in biopsy samples of coexistent deep infiltrating endometriosis (DIE) lesions (lower row) collected from two representative cases of intrinsic adenomyosis (left two columns) and extrinsic adenomyosis (right two columns). (B) Tissue infiltration of Mφ in the endometria (white box) was significantly higher in women with intrinsic adenomyosis comparing to control women (*$p < 0.05$) or women with extrinsic adenomyosis ($p = 0.03$). No significance difference in mean Mφ number was observed between DIE lesions (hatched box) coexistent with intrinsic and extrinsic adenomyosis. The boxes represent the interquartile ranges and horizontal lines in the boxes represent median values. Scale bar = 50 μm for each slide

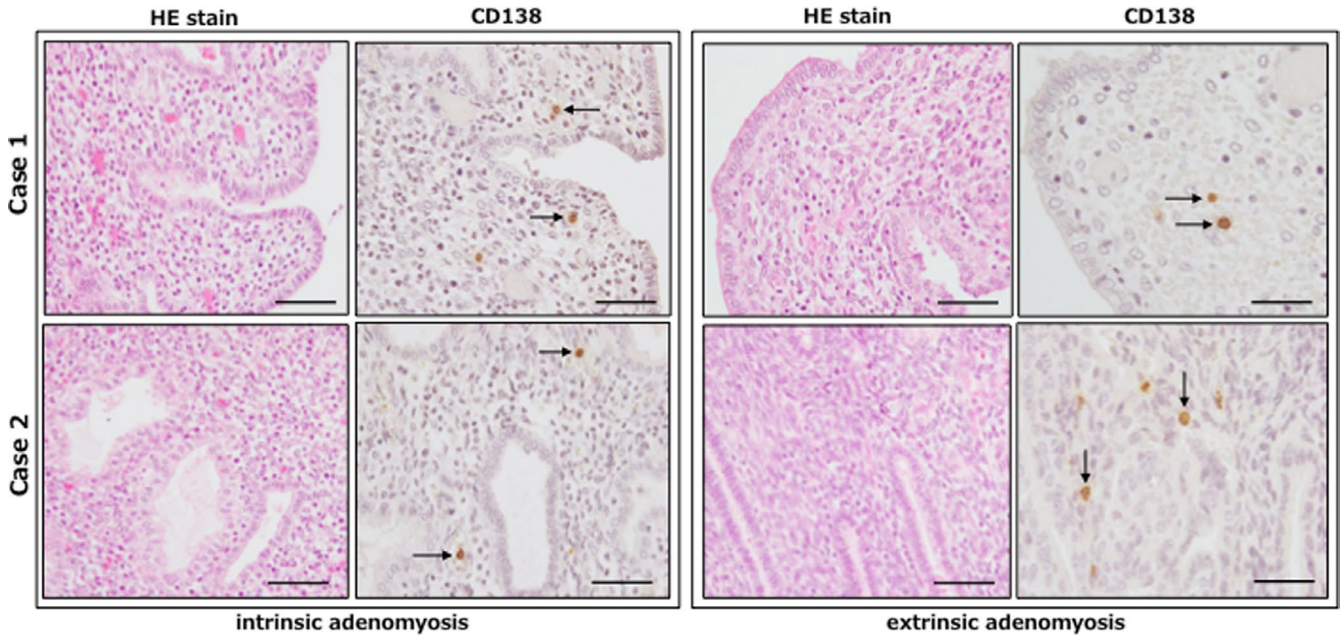

FIGURE 4 Hematoxylin and eosin staining (HE stain) and immunohistochemical staining of CD138 (Syndecan-1) in the endometria derived from two representative cases of intrinsic adenomyosis (left columns) and extrinsic adenomyosis (right columns). Arrows indicate the CD138-stained plasma cells in endometrial stroma of each side. Scale bar = 50 μm for each slide

TABLE 5 Occurrence rate of chronic endometritis in women with intrinsic and extrinsic adenomyosis regardless of the presence of deep infiltrating endometriosis

	Intrinsic adenomyosis ($n = 23$)	Extrinsic adenomyosis ($n = 10$)	*p* Value
Cases, *n* (%)	14/23 (60.8)	4/10 (40.0)	0.536

Note: Data were analyzed by chi-squared test.

4 | DISCUSSION

Our current study provides the first piece of clinical evidence that a variable rate of CE indeed occurs in women with different types of adenomyosis. We found that comparing to control women, endometrial biopsy samples collected from women with focal and diffuse adenomyosis displayed a higher tendency in the occurrence of CE. GnRHa treatment failed to further increase the occurrence rate of CE that was observed in GnRHa-untreated cases. When we analyzed biopsy samples of focal adenomyosis, we found that the occurrence rate of CE was significantly higher on the ipsilateral side (lesion side) comparing to contralateral side (lesion free side). Further analysis with two separate variants of adenomyosis (intrinsic and extrinsic) provided us new information that while tissue inflammatory reaction in endometria was significantly stronger in intrinsic adenomyosis (involving inner to mid-myometrium) comparing to extrinsic adenomyosis (involving outer myometrium), no significant difference was observed in the occurrence rate of CE between these two variants of adenomyosis.

Over the last decade, studies have disclosed the potential association between poor reproductive outcome and endometritis, particularly CE.[22-27] However, most of these studies demonstrated an incidental association between poor fertility outcome and CE.

TABLE 6 Distribution of CD138-stained plasma cells in endometria derived from GnRHa-untreated women with different types of adenomyosis

	Focal ($n = 10$)	Diffuse ($n = 9$)	Intrinsic ($n = 14$)	Extrinsic ($n = 4$)
Mean ± SD	6.2 ± 2.7	6.4 ± 2.4	4.8 ± 2.2	4.7 ± 2.7
Median	5.6	6.0	5.0	4.5
Range	2–10	2–10	1–8	2–8

Note: Mann-Whitney *U*-test and Kruskal-Wallis test indicated no significant differences in CD138-stained plasma cells in endometria between groups and among groups.

Abbreviation: GnRHa, gonadotropin-releasing hormone agonist.

Among different proposed factors that may contribute to adverse reproductive outcome in women with endometriosis and adenomyosis,[13,33,35] occurrence of CE and its persistence could be an additional factor that may worsen the receptivity environment of endometrial bed in women with adenomyosis. Successful endometrial receptivity, implantation, and deep placentation are prerequisite factors for successful pregnancy and its maintenance. Similar to endometriosis, a variable occurrence of CE in different types of adenomyosis may distort the functional and structural integrity of endometrium. More recently, an in vitro study demonstrated that decidual reaction of endometrial stromal cells, collected from women suffering from CE,

was impaired by reducing secretion of prolactin (PRL) and insulin-like growth factor binding protein 1 (IGFBP1).[41]

Several studies including ours revealed that CE is the result of IUMC and/or tissue inflammatory reaction.[31,41-43] If this is true, then occurrence of CE could be a pathological process secondary to uterine infection. However, information on the association between intrauterine infection and occurrence of CE in women with adenomyosis is limited. A recent study from Japan demonstrated uterine infection with a prevalence of 2.0% in women with focal adenomyosis and 10.9% in women with diffuse adenomyosis.[20] The association between *Haemophilus influenzae* infection and CE associated with adenomyosis has been reported elsewhere.[44] All these scant information may support a possible role of uterine infection in the occurrence of CE in women with adenomyosis similar to endometriosis. Future prospective studies on intrauterine infection in women with adenomyosis are warranted.

We previously demonstrated biological differences between intrinsic and extrinsic adenomyosis and confirmed a close histological and biological relevance between extrinsic adenomyosis and coexistent DIE by tissue analysis of ER, PGR, and fibrosis.[12] Here, we came to learn that endometria of women with intrinsic adenomyosis harbor significantly more macrophages (Mφ) comparing to extrinsic type. In contrast, similar tissue inflammatory reaction was observed in DIE lesions coexistent with either intrinsic or extrinsic adenomyosis. It is reasonable to explain that a persistent tissue injury and stress reaction in the endometria in response to intrinsic adenomyosis may produce more chemokines to recruit more Mφ in the endometria of these women.[12] The lesions of extrinsic adenomyosis on the outer myometrium are apart from endometria and do not exhibit repeated tissue injury and stress reaction in their endometria. The infiltrating lesions of DIE in pelvis are associated with severe inflammation, adhesion and clinical manifestations.[45] This may explain a non-significant difference in Mφ infiltration in these DIE lesions coexistent with intrinsic and extrinsic adenomyosis. The association of primary infertility in women with focal adenomyosis on the outer myometrium (FAOM, so-called extrinsic adenomyosis) may be the result of pelvic inflammation induced by coexisting DIE.[46] Even the inflammatory reaction was stronger in the endometria of women with intrinsic adenomyosis, we did not find any difference in the occurrence rate of CE between intrinsic and extrinsic adenomyosis. This may be explained by the similar tissue inflammatory reaction in their coexistent DIE lesions as we demonstrated here and/or similar pattern of uterine infection in these two variants of adenomyosis. Further experiments are indeed needed to address this unclear issue.

There are currently a number of different criteria used in the literature to diagnose upper genital tract inflammation including CE. These include the presence of five or more neutrophils per high power field (HPF) and one or more plasma cells within endometrial stroma per low power field,[21] two or more than two plasma cells per HPF,[47] and even the presence of a single plasma cell in the entire specimen.[48] In our current study we defined CE by the infiltration of one or more plasma cells per HPF in endometrial stroma which is supported by different old and recent literatures.[21,22,27,49] In fact, we could identify a wide range of CD138-stained plasma cells (1–10) with a median value of 4.5–6.0 in endometria collected from different types of adenomyosis (Table 6). Our findings may support the agreement by many experts that multiple endometrial stromal plasma cells are required for histopathological diagnosis of CE. However, the clinical significance of single or 10 plasma cells visualized by CD138 in our study remains unknown. Most recent report by McQueen et al. indicated that plasma cells induce variable changes in stromal cells and proposed to add these findings to redefine CE.[49] Our ongoing study on the possible changes of endometrial stromal cells based on the number of plasma cells may address this unclear issue.

If CE is the result of uterine infection, some questions may arise now: "how should we explain the mechanistic basis of CE in response to uterine infection and what could be the consequences of CE after its occurrence in women with endometriosis or adenomyosis"? As a first-line defense against microbes, increased tissue infiltration of Mφ in the endometrium and LPS-stimulated secretion of interleukin-8 (IL-8) (a chemokine for neutrophils) by Mφ/stromal cells could be involved in the accumulation of neutrophils in the endometrium and produce a state of AE.[50,51] Different ligands of intrauterine bacteria including LPS stimulates Mφ for the secretion of B lymphocyte stimulator (BLyS, a soluble ligand of TNF cytokine family). BLyS is a prominent factor for differentiation and activation of B1 (thymus-dependent) and B2 (thymus-independent) variants of B lymphocytes and their differentiation into plasma cells and accumulation/survival in the endometrium resulting in CE.[52,53] The infiltration of plasma cells in the endometria and the interaction between BLyS and BCMA (receptor for BLyS) may further result in autoantibody production (IgG/IgM) by plasma cells. This may produce an equivocal autoimmune condition in women with adenomyosis and/or endometriosis. With this background information, we can presume that microbial infection in uterine cavity triggers immune responses and ultimately contributes to the occurrence of CE. A diagrammatic presentation to clarify the association between uterine infection and occurrence of CE is shown in Figure 5.

The consequences of CE could be explained as follows: (1) Delayed differentiation of endometrium in mid-secretory phase as was observed in infertile women with CE.[54] (2) Endometria of women with CE overexpress anti-apoptotic genes (*BCL2*/*BAX*), cell proliferation marker (Ki-67), and ovarian steroid receptors (ER/PGR).[55,56] This indicates that the occurrence of CE may worsen the disease condition such as endometriosis or adenomyosis. (3) Expression profiles of genes potentially associated with embryo receptivity (*IL11*, *CCL4*, *IGF1*) and decidualization (*PRL*, *IGFBP1*) are down-regulated in the endometria of women with CE resulting in poor pregnancy outcome.[41,55] (4) Plasma cells in endometria express high levels of IgG or IgM with a predominance of IgG2 subclass.[51] The excessive mucosal antibodies in women with CE may have negative impact on the embryo implantation process.[57] All these information collectively indicate that utilizing different pathways, CE might be involved in poor fertility outcome in women with adenomyosis and/or endometriosis.

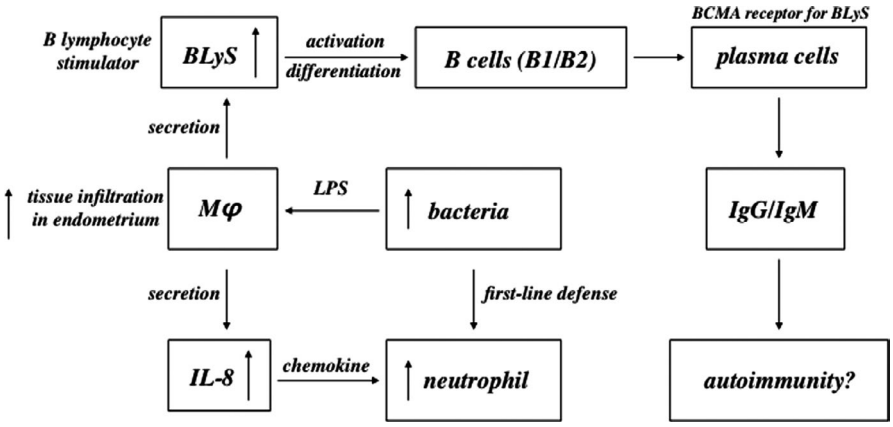

FIGURE 5 Shows a diagrammatic representation to explain the interaction among bacterial infection, immune cells, and occurrence of acute/chronic endometritis. One of the bacterial ligands such as LPS stimulates Mφ to secrete IL-8 which recruits neutrophils in endometrium and results in the occurrence of acute endometritis. Similarly, LPS stimulates Mφ, produces B lymphocyte stimulator (BLyS), involves in the differentiation/activation of B1 (thymus-dependent) and B2 (thymus-independent) variants of B lymphocytes (B cells), their differentiation into plasma cells and contributes to the occurrence of chronic endometritis. The accumulation/survival of plasma cells in the endometria and the interaction between BLyS and BCMA (receptor for BLyS) may further result in autoantibody production (IgG/IgM) by plasma cells (Refs 53, 57). This cascade may produce an equivocal autoimmune condition in women with adenomyosis and/or endometriosis. Mφ, macrophages; IL-8, interleukin-8; LPS, lipopolysaccharide; BCMA, B cell membrane-associated receptor; IgG, immunoglobulin G; IgM, immunoglobulin M

Recently it has been claimed that in addition to infectious etiology of CE, the concept of non-infectious etiology such as impaired inflammatory state of the endometrium (IISE) may contribute to detective endometrial inflammation and could be the cause of CE.[58] In fact, a compelling body of evidence demonstrates that transient, repeated, and persistent IISE may be involved in major gynecological and obstetrical disorders such as endometrial polyp, unexplained infertility, miscarriage, placenta-related pathology, and endometrial cancer.[58] While immunohistochemical analysis remains the gold standard to confirm CE, hysteroscopy can play a key role in IISE assessment. Until now, it has been well recognized that early diagnosis and treatment with suitable antibiotics can resolve CE and improve fertility outcome. Although optimal diagnostic and therapeutic tools for IISE are yet to be determined, minimal effective anti-inflammatory regimens appear promising for therapeutic IISE management.[58] Given the alarming epidemiology of chronic inflammation, non-microbial inflammation of the endometrium deserves further investigation to clarify its role in CE and/or in other obstetric and gynecological disorders.

There are some limitations in our current study. (1) These findings are limited by the cross-sectional nature of the study and we used only histochemistry and immunohistochemistry. (2) We represented our data of Mφ and plasma cells in biopsy samples derived from the previously used tissue blocks rather than fresh biopsy samples. (3) This is a non-randomized observational study and not a prospective randomized case-control study. Future studies with large sample size are required to confirm the association between CE and adenomyosis with and without complain of infertility.

Finally, we conclude that a variable prevalence of CE occurred in different types of adenomyosis that may be involved in adverse reproductive outcome. Our current findings and discussion may open the new avenue to investigate the exact mechanistic basis and cause-effect relationship between CE and adenomyosis and its consequence on fertility outcome. Future studies may strengthen the validity of our current study as well as may address the nature and role of plasma cells and activated macrophages in adenomyosis and/or endometriosis.

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CONFLICT OF INTEREST

All authors (Khan KN, Fujishita A, Ogawa K, Koshiba A, Mori T, Itoh K, Nakashima M, Kitawaki J) declare that they currently retain position as an officer, student, or advisor of Kyoto Prefectural University of Medicine in Kyoto and Saiseikai Nagasaki Hospital/Nagasaki University in Nagasaki and they have financial relationship with these organizations unrelated to this article. Dr. Khaleque N. Khan received research funding from the Japan Society for the Promotion of Science (JSPS) (Grant Nos. 24592474, 15K10675, 18K09268).

HUMAN RIGHTS STATEMENTS AND ETHICAL COMMITTEE APPROVAL

All human biopsy specimens were collected in accordance with the guidelines of the Declaration of Helsinki and were approved by the Ethics Committee and Institutional Review Board of our University (IRB No. 16005).

INFORMED CONSENT

A written informed consent was obtained from all women.

ANIMAL STUDIES

Not applicable.

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