The Role of Chronic Endometritis in The Etiopathogenesis of Adenomyosis

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Abstract

Objective: The aim of our study is to examine the relationship between adenomyosis and chronic endometritis and to discuss its possible effects on pathogenesis. Design: Prospective analysis of previous patients’ pathology specimens Setting: A tertiary university hospital’s department of obstetrics and gynecology. Patients: Patients who underwent hysterectomy at were divided into two groups according to the presence or absence of adenomyosis. A propensity score matching analysis was performed to minimize selection bias in patient groups. A total of 146, 73 patients in each group, were included in the study. Methods: The previous specimens of the patients were re-evaluated with the CD38 immunohistochemistry staining method. A positive diagnosis of CE was made in the presence of plasma cells. In particular, basal endometrial thickness was measured in endomyometrial transition zones. Main outcome measures and Results: The adenomyosis group was significantly younger than the group without adenomyosis (47.14 ± 4.24 vs. 50.36 ± 7.02, p = 0.012). 17 (11.6%) patients in the adenomyosis group were diagnosed with chronic endometritis, while 7 (4.8%) patients in the control group were diagnosed with chronic endometritis, and a statistically significant difference was found (p<0.05). Basal endometrium could be measured in a total of 112 (76.7%) patients, while basal endometrial loss was observed in 34 (23.3%) patients. Chronic endometritis was found in 16 (47%) of the patients with basal endometrial loss. The baseline endometrial thickness of 112 (76.7%) of the patients could be measured, but only 8 (7.1%) of them had chronic endometritis. There was a statistically significant difference between the groups (p<0.001). In multivariate analysis, there was a statistically significant relationship between basal endometrial loss and CE. Conclusion(s): A significant relationship was observed between adenomyosis and chronic endometritis.

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Study funding/competing interest: Ankara University Scientific Research Projects

Key Words: Chronic endometritis, Adenomyosis, Inflammation, Heavy Menstrual Bleeding, Infertility

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Introduction

Uterine adenomyosis is a benign gynecological disease characterized by the presence of endometrial glands and stroma within the myometrium (1). Although there is ongoing debate over the exact reason, it is generally accepted that adenomyosis occurs when the basal endometrium grows and invaginates into the myometrium (1, 2). Adenomyosis and endometriosis are disorders that share common pathogenetic mechanisms and primarily originate from the basal endometrial layer (3).

Chronic endometritis (CE) is a persistent inflammation of the endometrium that is often overlooked by gynecologists due to its subtle symptoms and challenging diagnostic process. CE can manifest without any symptoms or with unclear and nonspecific signs (4). Studies clearly confirm the correlation between endometriosis and chronic endometritis (5, 6). Although diagnostic criteria are a matter of debate, inflammation in the endometrial layer may play a role in the development of endometriosis, especially when archimetriosis theory and TIAR mechanisms are evaluated (3). It is likely that the same relationship will be observed with adenomyosis (3).

Recent studies have shown a link between CE and reproductive failures such as recurrent implantation failures, recurrent miscarriages, and unexplained infertility after IVF-ET (7). The causative relationship between CE and embryo implantation failure is a topic of debate. However, there are findings that indicate CE has an adverse effect on reproductive outcomes (7, 8). Adenomyosis is also correlated with recurrent pregnancy loss (9, 10). The correlation with adenomyosis was not investigated, unlike that with endometriosis. The aim of our study is to examine the relationship between adenomyosis and chronic endometritis and to discuss its possible effects on pathogenesis.

Methods

This clinical study was conducted using pathology specimens from patients who underwent hysterectomy at Ankara University, Department of Gynecology and Obstetrics, between January 2018 and December 2022. A total of 543 patients’ clinical and pathological data were obtained through a re-examination of the pathology materials. Patient data including age, pathology results, demographic information, and medical history were collected from the database and patient files following institutional review board approval. The study was approved by the Ankara University Clinical Research Ethics Committee (Ethics no: I1-03-20).

Patient Selection

Patients with a history of antibiotic or anti-inflammatory treatment in the last 3 months, active genital tract infections, and preneoplastic or neoplastic lesions were ineligible for the study. Additionally, patients diagnosed with endometriosis and those lacking sufficient data from the file and database were also excluded.

The age range of the patients was limited to 25-65 years. They were divided into two groups based on the presence or absence of adenomyosis. A propensity score matching analysis was conducted to minimize selection bias in patient groups with and without adenomyosis. The propensity score was developed using a multivariate logistic regression model, which included factors such as age at surgery, body mass index, presence of fibroids, history of previous uterine surgery, parity, and use of tamoxifen. The analysis was
performed based on each patient’s estimated propensity score with a 1-to-1 match tolerance set at 0.01 unchanged. After the propensity score matching process, a total of 146 patients - 73 from each group - were successfully matched (Figure 1).

**Pathological Examination and Immunohistochemistry**

The Department of Pathology at Ankara University re-evaluated the previous patient specimens using the CD38 immunohistochemistry staining method. A pathologist performed histological examinations without knowledge of the clinical findings. Five micro sections were stained with hematoxylin and eosin to mark areas of adenomyosis, and measure basal endometrial thickness in the endo-myometrial transition zones. The blocks representing the histopathological findings were selected and 4-micron sections were taken for immunohistochemical staining using p38 antibody as a secondary antibody in Ventana automatic staining device. Plasma cells stained with p38 were counted per unit area (11). Chronic endometritis was diagnosed in the presence of 5 or more plasma cells in the area (11, 12).

**Statistical Methods**

Descriptive statistics were presented as mean ± standard deviation, and nominal variables as number of cases and ratio (%). The normality of distribution was assessed using Shapiro-Wilk test. For groups with a normal distribution, the T-test was used to determine differences in means, while the Mann Whitney test was employed for non-normally distributed data. Nominal variables were assessed using Pearson Chi-Square or Fisher’s exact test.

Age, multiparity, previous uterine surgery, BMI, use of tamoxifen, presence of uterine fibroids identified as risk factors for adenomyosis were defined as potential confounders (13, 14), and the groups were determined by controlling the propensity scores for imbalances related to these variables. Matching was achieved by allowing the closest match to be selected. If a unique match wasn’t available, replacement was allowed, indicating the use of a method like nearest neighbor matching with replacement. Trend score matching was conducted using R for Statistical Computing Software (v.4.0.4). To examine the independent effect of each variable on the risk of CE, univariate analyses were performed using age, body mass index, multiparity, fibroids, basal endometrial loss, presence of adenomyosis, and logistic regression analysis. Parameters with a p-value below 0.1 in univariate analysis were included in multivariate analysis. Results with p<0.05 were considered statistically significant. In patients where baseline endometrial thickness could be evaluated, receiver operating characteristic analysis was performed to evaluate the relationship with chronic endometritis. Statistical Package for Social Sciences (SPSS) for Windows version 26.0 (Chicago, IL, USA) program was used for statistical analysis.

**Results**

A total of 146 patients, with 73 in each group, were included in the study after propensity score matching. Table 1 displays the demographic variables of the groups. The mean age of the general population was 48.75 ± 6 years and the mean BMI was 28.71 ± 4.87. The group in which adenomyosis was detected in the pathology examination was significantly younger than the group in which adenomyosis was not detected (47.14 ± 4.24 vs. 50.36 ± 7.02, p = 0.012). Although preoperative Hb value was lower in the adenomyosis group 17 (23.28%) patients and in the control group 7 (9.59%) patients were diagnosed with chronic endometritis, and a statistically significant difference was detected (p<0.05).

Basal endometrial thickness was assessed in the pathology specimens of the patients as shown in Figure 2. The baseline endometrium measurement was possible for a total of 112 patients (76.7%), revealing basal endometrial loss in 34 individuals (23.3%). Among those with baseline endometrium loss, chronic endometritis was found in 16 cases (47%). Out of the evaluated patients whose basal endometrial thickness could be measured, only 8 individuals (7.1%) showed chronic endometritis. Difference between groups was significant (p<0.001). The relationship between basal endometrial thickness and chronic endometritis was examined. Firstly, with univariate logistic regression analysis, the effect of basal endometrial thickness on chronic
endometritis was found to be statistically significant (p = 0.031), and it was observed that an increase in this variable significantly reduced the likelihood of chronic endometritis. A receiver operating characteristic (ROC) analysis was performed to evaluate whether there was a threshold value for the thickness of the basal endometrium to detect chronic endometritis. The sensitivity and specificity values of 0.15 mm basal endometrial thickness calculated in the ROC analysis are 83.3% and 86.9%, respectively (area under the curve: 0.888, 95% CI 0.798-0.977; p < 0.001) (Figure 3).

Univariate and multivariate regression analysis was performed to identify risk factors associated with chronic endometritis (Table 2). While there was a statistically significant relationship between CE and adenomyosis and basal endometrium loss in univariate analysis, this relationship was observed only between basal endometrium loss and CE in multivariate analysis.

Discussion

In our study, we investigated the potential connection between adenomyosis and chronic endometritis. Additionally, we aimed to assess the basal endometrial thickness and its correlation with CE. Our study stands out as one of the few that directly explores the link between adenomyosis and CE. Furthermore, it adds value to existing literature by examining how thinning and loss of the basal endometrial layer relate to CE. Many of the studies have noted this strong correlation between the chronic endometritis and endometriosis, showing different prevalence rates in various groups of the population. For example, according to a study by Cicinelli et al., the incidence of CE in patients with endometriosis was found to be significantly higher compared to the control group without endometriosis (42.3% vs. 15.4%) (4). This epidemiological link is further supported by Takebayashi et al., who documented a higher rate of concurrent CE among women diagnosed with endometriosis (52.94% vs. 27.02%) (5). Only one study has thoroughly examined the relationship between adenomyosis and chronic endometritis. This particular study primarily focuses on distinguishing between focal and diffuse adenomyosis groups (15). In 2023, Hiraoka et al. highlighted this deficiency in a systematic review, emphasizing that while the relationships between CE and recurrent implantation failure and/or recurrent early pregnancy were examined, the direct relationship between adenomyosis and CE was not investigated (16). In our study, we aimed to fill a research gap by investigating the association between adenomyosis and chronic endometritis. Our findings suggest that the coexistence of CE and adenomyosis may be more than coincidental and may imply a pathophysiological interaction that requires further investigation.

Based on our results, we cannot claim a direct cause-effect relationship between CE and adenomyosis. However, considering the pathogenesis of adenomyosis, it is possible that CE plays a role in the development of basal endometrium damage and inflammation through the TIAR mechanism. CE may occur as a result of intrauterine microbial colonization and/or tissue inflammatory reactions (17-20). Many authors have written in the past few years that they think there is a link between endometriosis and chronic inflammation (6, 21). In particular, these studies formulated the hypothesis that there are at least two distinct phases in the development of endometriosis. Intrauterine microorganisms may play a crucial role in the development of endometriosis. When microorganisms initially stimulate pathogen recognition receptors, it triggers the activation of proinflammatory pathways. Toll-like receptors not only respond to molecular patterns associated with various external pathogens but also activate a wide range of endogenous pro-inflammatory molecular pathways. This increased expression of pro-inflammatory molecules then leads to Nuclear Transcription Factor-κB-dependent sterile inflammation as a subsequent process. Thus, after the first wave of Toll-like receptor activation comes a second significant wave of sterile inflammation (21). Sterile inflammation is believed to have a significant impact on the development of endometriosis. However, there is limited data available regarding the connection between intrauterine infection and the development of CE in women with adenomyosis (15). A recent study in Japan found that the occurrence of intrauterine infection was 2.0% in women with focal adenomyosis and 10.9% in those with diffuse adenomyosis (15, 22). This information supports the idea that uterine infection may play a role in the development of CE in women with adenomyosis, such as endometriosis. A characteristic pattern of uterine contraction has been observed in women with CE, which differs significantly from that seen in women without CE at different stages of the menstrual cycle (23). This unique peristalsis pattern also supports the TIAR mechanism and the role of CE in the
development of adenomyosis. Additionally, the presence of distinctly different microbiotic populations in the uterus of patients with endometriosis and adenomyosis, as compared to healthy individuals, further bolsters this theory (24).

Recent studies by various researchers have indicated that chronic endometritis can interfere with both spontaneous and assisted reproductive technology-induced pregnancies, leading to infertility, ART failures, abortion, and other obstetric complications (25-28). Additionally, implantation failure and increased abortion rates observed in adenomyosis patients may be linked to the high prevalence of concomitant chronic endometritis (7, 28, 29). The notable presence of CE in adenomyotic patients as well as those with unexplained infertility and recurrent implantation failure suggests that antibiotic therapy could potentially have a positive impact on pregnancy outcomes (25, 27).

The highly heterogenous diagnostic criteria for chronic endometritis make their precise definition very difficult in clinical routine, particularly due to subtle and inconspicuous manifestations. While hysteroscopy and endometrial sampling are often used for diagnosis, their effectiveness can vary based on the skills of the surgeon and tissue sample quality. In our study, we mitigated such uncertainties by analyzing previously obtained hysterectomy specimens, which provided a definitive histological diagnosis and allowed us to explore the true extent of the association between CE and adenomyosis without the confounding effects of differential diagnosis variation. It is noteworthy that there are at least seven different diagnostic criteria for CE with varying prevalences in the literature (13). To minimize uncertainty associated with this condition, we identified the presence of five plasma cells and conducted patient analysis using immunohistochemistry. We also used propensity score matching to eliminate confounding factors when creating groups. We did not observe any significant differences in known risk factors for adenomyosis between groups. However, the age of hysterectomy in the adenomyosis group was significantly younger, consistent with the literature. We think that this may be due to the negative impact of adenomyosis on the patient’s life (30).

Our study utilized propensity score matching, excluded patients with endometriosis, and considered adenomyosis risk factors when creating groups to reveal the possible effect of CE more clearly. Additionally, we evaluated the presence of basal endometrium in the adenomyotic area under light microscopy in H&E stainings and measured its thickness. After adjusting for BMI, and presence of adenomyosis in hysterectomy samples; we found that loss of basal endometrium was roughly 10 times more likely in cases where CE was present. Additionally, a significant inverse correlation with CE was observed in patients in whom baseline endometrial thickness could be evaluated. In conclusion, this study reveals that the effect of baseline endometrial thickness variable on CE is both statistically significant and clinically important. These findings could indicate using basal endometrial thickness as a potential biomarker in future studies. This supports our idea that CE contributes to the TIAR mechanism, particularly through damage to the basal endometrium.

The therapeutic implications of chronic endometritis and adenomyosis are especially important here. Since CE can be cured with antibiotics (25), it is important that this condition be detected in patients with adenomyosis, for these patients might improve some of their symptoms. In this case, if antibiotic treatment helps to achieve reduction in the inflammatory component relevant to adenomyosis, then its positive effect on women suffering from adenomyosis will be observed. Quality of life for women and increased chances of conception would be the goals. Besides, the knowledge including CE as a contributing factor to adenomyosis creates a need for the routine screening of CE during the uterine abnormalities. Therefore, this technique could be utilized prior to the occurrence of the health complications and may also be associated with the treatment of related reproductive challenges such as infertility or implantation failure.

The results of our research revealed a correlation between endometritis and adenomyosis. It is essential to acknowledge the limitations that may affect the broad applicability of our findings. A significant limitation is the small size of our sample, which could potentially compromise the precision of our findings and may not provide a comprehensive representation of the entire population. Furthermore since our study was retrospective there’s a chance of bias in how participants were selected. Additionally diagnosing adenomyosis and CE was based on examining specimens, which could introduce bias in diagnosis and might exclude milder cases that didn’t require surgery. This also means we couldn’t evaluate women who may have had these
conditions but didn’t undergo hysterectomy. Another limitation is the lack of long term data on how these conditions progress or respond to treatment. Future studies, with cohorts would help confirm our findings and address these limitations effectively.

The noted association of chronic endometritis and adenomyosis seems to open up a variety of research opportunities consecutively. Prospecting longitudinal studies that can collect information about the development of these diseases and therapeutic outcomes over time would be useful to uncover the nature complications and the efficacy of the treatments. Also, increasing the pool size and with more groups for instance, will further the generalization of the results. Genetic studies in association with epigenetic analyses might be key to unmask the interactive cue between chronic endometritis and adenomyosis. The studies including KRAS and other adenomyosis-related mutations in conjunction with CE might give a clue to the occurrence and development of these disorders. Another area of potential breakthroughs comes from research into non-invasive diagnostic means, including advanced imaging techniques, which could dramatically change the early identification of CE and adenomyosis, thereby possibly leading to more effective treatment algorithms. Exploring the way these factors alter pregnancy complications like abnormal placentation and preeclampsia during pregnancy is one of the distinguishing features of maternal-fetal medicine. Basically, interdisciplinary research that is the integration of the clinical, molecular and epidemiological teaching methodology is expected to have a definitive impact on our knowledge and support for the chronic endometritis and adenomyosis management.

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Authors’ Roles

BA: Conceptualization, Methodology, Writing-Original draft preparation. CCÔ: Conceptualization, Methodology YES: Conceptualization, Writing- Reviewing and Editing, Formal analysis. BO: Visualization, Investigation. MS: Writing- Reviewing and Editing, Supervision. BB: Investigation, Supervision. RA: Investigation, Supervision. CSA: Investigation, Supervision.

Conflict of interest

The authors declare no conflict of interest.

References


Figure Legends

Figure 1. Presentation of the patient selection phase

Figure 2. Evaluation of basal endometrium thickness by microscopy

Figure 3. Receiver operating characteristics (ROC) curve for prediction of chronic endometritis using baseline endometrial thickness measurement

Sensitivity and specificity values were 83.3% and 86.9%, respectively (area under the curve: 0.888, 95% CI 0.798-0.977; p < 0.001)
Jan 2016- Dec 2022
543 Subjects

- Patients with a history of antibiotic or anti-inflammatory treatment in the last 3 months,
- Active genital tract infection
- Preneoplastic or neoplastic lesions,
- Presence of endometriosis,
- <25 or >65 years were excluded.

292 patients were available for analysis

73 patients in the adenomyosis group.

73 patients in the control group.
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