Decreased Fertility in Women with Cesarean Scar Syndrome Is Associated with Chronic Inflammation in the Uterine Cavity.

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Decreased Fertility in Women with Cesarean Scar Syndrome Is Associated with Chronic Inflammation in the Uterine Cavity

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Chronic inflammation in cesarean scar defect contributes to secondary infertility in women with cesarean scar syndrom; however, it remains unclear about the situation of inflammation in uterine cavity in women with cesarean scar syndrome. This ambidirectional cohort study aimed to explore the effect of inflammation in the uterine cavities of women with cesarean scar syndrome on infertility at a single university hospital. The frequency of chronic endometritis in infertile patients was retrospectively compared between the cesarean scar syndrome group and non-cesarean scar syndrome group. The frequency of endometriosis was also investigated in patients with cesarean scar syndrome who underwent laparoscopy. The level of tumor necrosis factor-α and interleukin-1β in the uterine cavity was prospectively evaluated in the cesarean scar syndrome group and in women with a history of cesarean section (control group) using an enzyme-linked immunosorbent assay. There was a significant difference in the incidence of chronic endometritis between the cesarean scar syndrome and non-cesarean scar syndrome groups (65.8% and 46.0%, respectively, $p = 0.0315$). Endometriosis was detected in 51 (70%) patients with laparoscopy. Tumor necrosis factor-α and interleukin-1β levels in the cesarean scar syndrome group were significantly higher than those in the control group ($p = 0.0002$ and $p = 0.0217$, respectively). Our findings suggest that one cause of secondary infertility in women with cesarean scar syndrome is embryo implantation failure-associated chronic endometritis, endometriosis, and chronic inflammation in the uterine cavity.

Keywords: cesarean section; chronic; endometritis; infertility; scar
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Introduction

In recent years, the rate of cesarean section has been increasing, and consequently, there is a growing concern about secondary infertility related to cesarean scar syndrome (CSS) (Morris 1996; Tsuji et al. 2015). CSS is defined as abnormal postmenstrual bleeding, dysmenorrhea, chronic pelvic pain, and infertility due to cesarean scar defect (CSD) (Morris 1996; Tsuji et al. 2015). Impaired fertility in women with CSS is believed to be due to the CSD-associated blood retention, which affects sperm quality and interrupts sperm penetration or implantation (Florio et al. 2012; Kremer et al. 2019; Donnez 2020). Unfortunately, in cases of CSS, treatment with *in vitro* fertilization-embryo transfer (IVF-ET) is ineffective (Tsuji et al. 2015).

Adenomyosis in CSD area was histologically detected in 21% of 38 patients with CSS (Donnez 2020). Similarly, our previous report revealed that the frequency of adenomyosis in CSD area was 43% of patients (Higuchi et al. 2022); however, the area of endometriosis was not large, and it remains unclear whether small lesions can affect fertility. Iatrogenic adenomyosis under cesarean scar was reported in 28% of patients who underwent hysterectomy with a history of cesarean section (Morris 1995). Inflammatory infiltration with fibrosis and distortion of the lower uterine segment was associated with complaints of pain, such as dyspareunia (Morris 1995). Our previous report also demonstrated that there was chronic inflammation in CSD area, which may contribute to secondary infertility in women with CSS (Higuchi et al. 2022). However, there was a lack of evidence regarding inflammation and its role in the uterine cavity.

Chronic inflammation in the uterine cavity is hypothe-

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sized to contribute to infertility in women with CSS, as the lower part of the uterus involved in CSD formation was connected to the uterine cavity. Recent studies have demonstrated that chronic endometritis (CE) is associated with implantation failure (Park et al. 2016; Kimura et al. 2019). CE is defined as mild persistent inflammation in the endometrium without fever and lower abdominal pain, unlike acute endometritis. CE is diagnosed through CD138 immunostaining of the endometrium (Kimura et al. 2019). Our laboratory has previously revealed an association between endometriosis and CE by CD138 immunostaining (Takebayashi et al. 2014). This study investigated CE and endometriosis in patients with CSS. In addition, inflammatory cytokine analysis was conducted to evaluate inflammation in the uterine cavity.

Materials and Methods

Patients and ethical approval

All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments.

The CE study in infertility women was a retrospective case-control study conducted at the Shiga University of Medical Science (Fig. 1). Patients who were diagnosed with infertility and underwent a CE examination between January 2020 and December 2021 were enrolled. The exclusion criteria were as follows: (1) patients who underwent hormonal therapy and antibiotic treatment before the examination, and (2) patients with an endometrial polyp, submucous myoma, infection, and cervical neoplasia. The participants were divided into two groups: the CSS group and the non-CSS group. The endometriosis study in women with CSS was also a retrospective case-control study conducted at the Shiga University of Medical Science (Fig. 1). The aim of this study was to evaluate the frequency of endometriosis in patients with CSS. Medical records were investigated for patients who underwent hysteroscopic surgery with laparoscopy from September 2014 to January 2021. These retrospective studies were approved by the ethical committee of Shiga University of Medical Science (approval number R2021-019). The information on conducting the study was made public, and the opportunity for refusal was guaranteed as much as possible through an opt-out option, as suggested by the Ethics Committee.

The cytokine analysis study in the uterine cavity was performed prospectively from April 2020 to April 2021 at the Shiga University of Medical Science (Fig. 1). In this analysis, the patients with regular menstrual cycles and a history of a cesarean section but without hypermenorrhea, prolonged menstruation, or abnormal uterine bleeding were designated as controls. This study was approved by the ethical committee of Shiga University of Medical Science (approval number R2019-316). Informed consent was obtained from all participants before examination using consent forms.

Surgical procedure

Our surgical technique for infertility due to CSS has been reported previously (Tsuji et al. 2018, 2020). Briefly, hysteroscopic surgery was performed under laparoscopy. The hysteroscopic procedure uses inferior edge resection

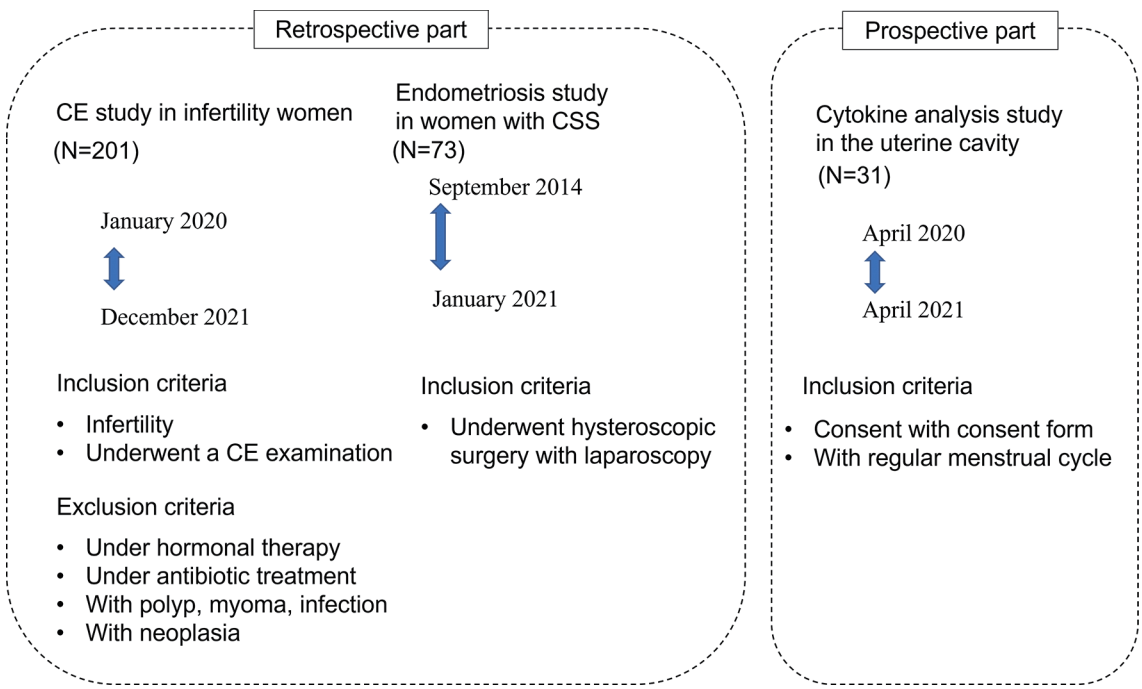

Fig. 1. A schema for the ambidirectional cohort study.
CSS, cesarean scar syndrome; CE, chronic endometritis.

and superficial cauterization on the CSD area without excision. The purpose of simultaneous laparoscopy and hysteroscopic surgery is to monitor accidental perforation during cauterization and to treat other causes of infertility, such as endometriosis. Through this technique, we also confirmed the presence of endometriosis in the pelvic cavity in all cases.

Immunohistochemistry and diagnosis of chronic endometritis

Immunohistochemical examination of plasma cells was performed by staining CD138, following previous reports from our laboratory (Takebayashi et al. 2014; Wu et al. 2017; Kaku et al. 2020). Briefly, after deparaffinization and rehydration, antigen retrieval was performed on cells attached to the slide using pre-heated tris-ethylenediaminetetraacetic acid (EDTA) (pH 9.0) and boiled at 98C° for 10 minutes. Monoclonal mouse anti-human CD138 antibody (M7228; DAKO, Glostrup, Denmark) was used as the primary antibody. After immunostaining for 30 minutes at room temperature, cells attached to the slide were incubated with horseradish peroxidase-conjugated secondary antibody (MAX-PO; Nichirei Corp., Tokyo, Japan) for 30 minutes at room temperature and immersed in diaminobenzidine (VECTOR SK-4100; VECTASTAIN, Vector Laboratories, Newark, CA, USA). Cells on the slide were counterstained with Mayer's hematoxylin solution, followed by dehydration and mounting.

The number of CD138-positive plasma cells was counted under a light microscope [high-power field (HPF), 400 × magnification] in 10 non-overlapping random stromal areas. Because epithelial cells can also be stained by CD138 antibody, we did not count the number of CD138-positive cells when these cells were detected in epithelial areas. Based on the criteria used in our previous report and others, CE was diagnosed when at least one CD138-positive plasma cell was detected in the endometrial stroma area in 10 random HPFs (Kannar et al. 2012; Takebayashi et al. 2014; Kabodmehri et al. 2022).

Sample collection and evaluation of tumor necrosis factor-α and interleukin-1β in the uterine cavity

The inflammatory state of the uterus was evaluated by measuring tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in saline delivered into the uterine cavity, following a previously described procedure (Inagaki et al. 2003). Briefly, a flexible infant feeding tube was inserted into the uterine cavity. About 4 mL of sterile saline was infused through the feeding tube and immediately aspirated. Samples were centrifuged at 3,500 rpm for 10 minutes, and the supernatants were stored at −80 °C until cytokine analysis. All samples were collected between the 9th and 14th days of the menstrual cycle from women with CSS or non-CSS after the acquisition of informed consent. The samples were sent to SRL Inc. (Tokyo, Japan) for analysis of TNF-α and IL-1β by enzyme-linked immunosorbent assay (ELISA). The minimum detectable value of IL-1β was 10 pg/mL, and it was recorded as 10 pg/mL when the measurement value was < 10 pg/mL.

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 7 (GraphPad Software, Inc., San Diego, CA, USA). Data were analyzed for data distribution using the D'Agostino-Pearson test. Normally distributed data are described as mean ± standard deviation, while non-normally distributed data are presented as medians (interquartile range). Comparisons between the CSS and control groups were conducted using an unpaired two-tailed t-test or Mann-Whitney U test for parametric and non-parametric data, respectively. Categorical data were analyzed using Fisher's exact test. Statistical significance was defined as $p < 0.05$.

Results

In the CE study in infertility women, two hundred and one patients were enrolled in the CE study, with 38 and 163 patients in the CSS and non-CSS groups, respectively. There was no significant difference among the participants in terms of age. However, significant differences in gravidity, parity, and the number of cesarean sections between the CSS and non-CSS groups were observed (Table 1). Overall, 25 (65.8 %) patients and 75 (46.0 %) patients were diagnosed with CE (Fig. 2) in the CSS and non-CSS groups, respectively (Table 2). A significant difference was found in the frequency of CE between the CSS and non-CSS groups ($p = 0.0315$). The number of CD138 positive cells was 2 (0-4.25) and 0 (0-3) in the CSS and non-CSS groups, respectively. However, no inter-group significant

Table 1. Background details of the participants in the chronic endometritis study.

	CSS group	Non-CSS group	*p*-value
Number	38	163	
Age, median (IQR) (years)	38 (34-43)	37 (33-40)	n.s.
Gravidity, median (IQR)	2 (1-3)	0 (0-1)	< 0.0001
Parity, median (IQR)	1 (1-2)	0 (0-1)	< 0.0001
The number of C/S, median (IQR)	1 (1-2)	0 (0-0)	< 0.0001

CSS, cesarean scar syndrome; C/S, cesarean section; n.s., not significant; IQR, interquartile range.

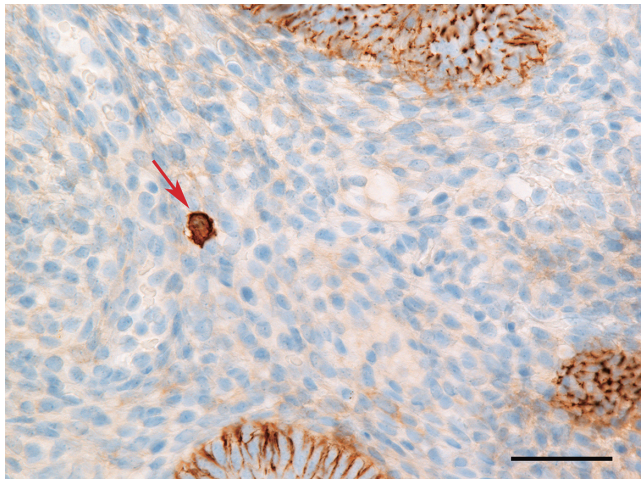

Fig. 2. Immunohistochemistry for CD138 in the endometrium.
The red arrow indicates CD138-positive plasma cells in the stromal area. CD138 antibody also stains epithelial cells in the endometrium. The scale bar is 50 μm.

difference was found in the number of CD138 ($p = 0.1096$). Furthermore, we compared the frequency of CE between infertile women with and without a history of cesarean section in the non-CSS group; however, there was no significant difference between them (30.7% and 47.3%, respectively).

In the endometriosis study in women with CSS, we collected the data of 73 patients who underwent hysteroscopic surgery under laparoscopy and the data revealed that endometriosis was detected in 51 (70%) patients. According to medical record, 45 (88%) patients were classified into the minimal and mild groups (stages 1-2) based on the revised American Society for Reproductive Medicine classification.

In the cytokine analysis study in the uterine cavity, we recruited 31 patients for inflammatory cytokine analysis, which consisted of 20 patients in the CSS group and 11 in the control group. No significant difference was observed in terms of the baseline characteristics between the CSS and non-CSS groups (Table 3). ELISA analysis revealed a TNF-α level of 0.88 (0.43-2.37) pg/mL in the CSS group and 0.15 (0.15-0.27) pg/mL in the control group. IL-1β level was 17 (10-44.7) pg/mL in the CSS group and 10 (10-10) pg/mL in the control group. A significant difference was observed between the two groups for both TNF-α and IL-1β levels ($p = 0.0002$ and $p = 0.0217$, respectively) (Fig. 3).

Discussion

To the best of our knowledge, this study is the first report to reveal the association between CSS and CE by studying CD138-positive cells. This study also first shows that the inflammatory cytokine levels in the CSS group were higher than those in the control group based on TNF-α and IL-1β measurement by ELISA. Furthermore, endometriosis was detected laparoscopically in 70% of the women with CSS.

In the last decade, there has been growing evidence showing that CE leads to infertility (Park et al. 2016; Wu et al. 2017; Cicinelli et al. 2018; Kimura et al. 2019). Although there is no consensus on the diagnosis of CE, it is commonly recognized as a chronic inflammatory condition defined as the presence of plasma cells in the endometrium. Implantation failure is considered to be the cause of infertility in patients with CE. Therefore, even if a good-quality embryo is transferred to patients with CSS, there is a low chance of a successful conception.

Generally, the prevalence of CE ranges from 8% to 72% (Kimura et al. 2019). In this study, a total of 65.8% of patients with CSS were diagnosed with CE, which is a relatively high frequency. Moreover, the frequency of CE in the non-CSS group was also high. We believe that this was because all participants in the non-CSS group were infertile. According to the comparison about the frequency of CE between infertile women with and without a history of

Table 2. Comparison in chronic endometritis ratio between two groups.

	CSS group	Non-CSS group	*p*-value
Number of CE cases	25 (65.8%)	75 (46.0%)	0.0315

CSS, cesarean scar syndrome; CE, chronic endometritis.

Table 3. Background details of the participants for the cytokine analysis study.

	CSS group	Non-CSS group	*p*-value
Number	20	11	
Age, median (IQR) (years)	39 (32-40)	33 (30-39)	n.s.
Gravidity, median (IQR)	1.5 (1-2)	2 (1-2)	n.s.
Parity, median (IQR)	1 (1-1)	1 (1-1)	n.s.
The number of C/S, median (IQR)	1 (1-1)	1 (1-1)	n.s.

CSS, cesarean scar syndrome; C/S, cesarean section; n.s., not significant; IQR, interquartile range.

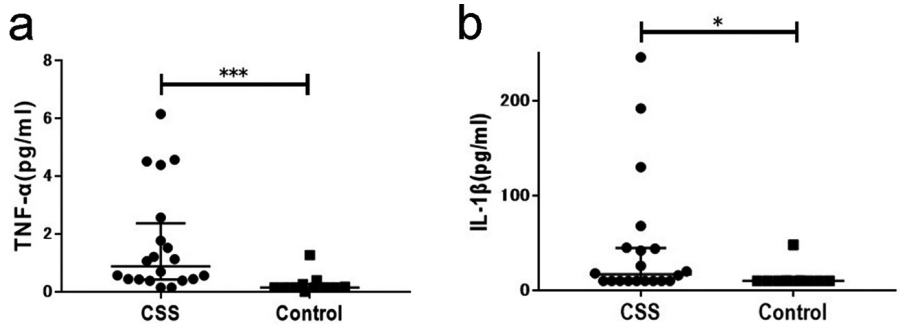

Fig. 3. The concentration of proinflammatory cytokine in sterile saline infused into the uterine cavity. a) TNF-α (tumor necrosis factor-α). b) IL-1β (interleukin-1β). *$p < 0.05$; ***$p < 0.005$. CSS, cesarean scar syndrome.

cesarean section in the non-CSS group, the cause of CE may be no relevant to the opening of the uterine cavity at cesarean section but caused by other factors. Implantation failure due to CE can lead to the reduced ability of CSS patients to conceive. This hypothesis is consistent with the finding that patients with CSS cannot become pregnant efficiently, even after the aspiration of the fluid retained in the uterus.

Chronic inflammation in the uterine cavity was identified in patients with CSS by measuring proinflammatory cytokines. A previous report demonstrated that TNF-α levels were markedly higher in menstrual effluents of women with CE (Tortorella et al. 2014). This finding is consistent with our results which showed that TNF-α and IL-1β levels in patients with CSS were higher than those in the control group.

The prevalence of endometriosis is approximately 10% in women of reproductive age, and about 30% to 50% of women with endometriosis have infertility (Zondervan et al. 2020; Muhaidat et al. 2021; Smolarz et al. 2021). However, this study showed that 70% of women with CSS were laparoscopically diagnosed with endometriosis. This discrepancy suggests that CSS is an endometriosis-related disease. An association between CE and endometriosis has also been revealed previously (Takebayashi et al. 2014). If the underlying etiology is CE, infertility in women with CSS should be successfully treated with antibiotics alone (Kimura et al. 2019; Cicinelli et al. 2021). However, there was no report of the successful treatment for CSS-associated infertility by antibiotics. In contrast, there were plenty of reports of successful surgical treatment for CSD in infertile women with CSS (Gubbini et al. 2011; Tanimura et al. 2015; Tsuji et al. 2020). These facts suggest that CSD may generate CE in the uterine cavity.

This study had a few limitations. First, it remains unknown why women with CSS develop CE. This study revealed that the frequency of CE in women with CSS was higher than that in patients with infertility but not CSS under the same diagnostic procedure. Secondly, TNF-α was also detected in ascites, especially in women with endometriosis (Koga et al. 2000; Hanada et al. 2018). Therefore, it is uncertain whether TNF-α and IL-1β were produced in the uterine cavity or whether TNF-α came from the peritoneal cavity with endometriosis. Thirdly, this study does not demonstrate endometriosis in non-CSS patients because they did not undergo laparoscopy.

A previous report demonstrated that infertility in women with CSS was associated with CSD, as the persistence of menstrual blood had a negative impact on sperm quality and eventually interfered with embryo implantation (Florio et al. 2012). This study may add one cause of secondary infertility in women with CSS: embryo implantation failure due to CE and chronic inflammation in the pelvis, such as endometriosis. These findings would help treat secondary infertility caused by CSS.

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Conflict of Interest

The authors declare no conflict of interest.

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