Pelvic pain in women with ovarian endometrioma is mostly associated with coexisting peritoneal lesions

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STUDY QUESTION: Is the occurrence of pelvic pain in women with ovarian endometrioma associated with coexisting peritoneal lesions (PLs)?

SUMMARY ANSWER: Pelvic pain in women with ovarian endometrioma is usually associated with coexisting PLs. An increased tissue inflammatory reaction with elevated prostaglandin (PG) production may be responsible for the generation of pain.

WHAT IS KNOWN ALREADY: Severe pelvic pain in women with ovarian endometrioma is reported to be associated with deeply infiltrating endometriosis. However, information on pelvic pain in women with ovarian endometriosis with and without coexistent peritoneal superficial lesions is limited.

STUDY DESIGN, SIZE AND DURATION: Retrospective clinical study with case-controlled biological research using prospectively collected tissue samples derived from women with and without endometriosis and their retrospective evaluation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We performed a retrospective cohort study conducted in 2988 cases who had laparoscopic surgery for indications of ectopic pregnancy, tubal infertility and other benign gynecologic diseases. We analyzed the occurrence of pelvic pain in the cases with ovarian endometrioma according to the distribution of coexisting PLs and pattern of intrapelvic adhesions. Inflammatory reaction of eutopic and ectopic endometria was measured by immunoreaction to macrophage marker, CD68. The tissue expression of cyclooxygenase (COX) 2 was examined by immunohistochemistry and tissue concentrations of PG F2α were measured by ELISA.

MAIN RESULTS AND THE ROLE OF CHANCE: Among the 2988 surgical cases, 350 (11.7%) were found to have ovarian endometrioma at laparoscopy. Coexisting PLs were present in 269 of these women and in this group 85.4% of cases experienced pelvic pain and 14.6% had no pain. In contrast, among the 81 women with ovarian endometrioma only, 38.3% cases experienced pelvic pain and 61.7% cases had no pain and the difference between the groups was statistically significant (P < 0.01). The infiltration of CD68-immunoreactive macrophages was significantly higher in the eutopic and ectopic endometria of women with peritoneal endometriosis than in ovarian endometrioma. The tissue expression of COX2 and levels of PGF2α were significantly higher in both the eutopic and ectopic endometria derived from women with peritoneal endometriosis than in similar tissues derived from women with ovarian endometrioma.

LIMITATIONS, REASONS FOR CAUTIONS: Lack of evaluation in the detection of general or disseminated deeply infiltrating endometriosis in the pelvic cavity could be a bias or limitation in this study. Further multicenter prospective studies are needed to strengthen our current findings.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings may provide some new insights to understand the physiopathology of pelvic pain in women with ovarian cystic endometriosis and may hint at proper surgical manipulation to prevent the recurrence of pelvic pain in these women.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by Grants-in-Aid for Scientific Research from the...
Introduction

Endometriosis is an estrogen-dependent chronic inflammatory disease affecting 6--10% of women of childbearing age (Giudice and Kao, 2004). Endometriosis is histologically characterized into three types: peritoneal superficial endometriosis, ovarian endometrioma and deeply infiltrating endometriosis (DIE) and is associated with infertility and a variable degree of pelvic pain (Fauconnier and Chapron, 2005; de Ziegler et al., 2010). Different mechanisms have been proposed to explain the relationship that exists between endometriosis and pelvic pain such as tissue inflammatory reaction, production of prostaglandins (PGs) with consequent uterine contraction and nerve entrapment within lesions (Anaf et al., 2000; Khan et al., 2004a; Berkley et al., 2005; Miura et al., 2006). For DIE, the entrapment of nerves within the endometriotic nodules represents a possible mechanism explaining pain (Anaf et al., 2000). For peritoneal superficial endometriosis and ovarian endometrioma, bleeding within the lesion, tissue inflammatory reaction, production of PGE2/PGF2α and associated intra-pelvic adhesion may explain their association with pain (Khan et al., 2004a; Miura et al., 2006; Khan et al., 2009). The severity of pain in women with endometriosis is associated with color appearance of peritoneal lesions (PLs), depth of infiltration, anatomic location of lesions and intensity of inflammatory reaction with resultant pelvic adhesions (Fauconnier et al., 2002, 2005; Khan et al., 2004a).

The relationship between ovarian endometrioma [chocolate cyst (CC)] and painful symptoms is not well established and is still controversial (Fauconnier and Chapron, 2005). This pain could be due to the presence of endometrioma itself or due to coexisting PLs or associated pelvic adhesions. Currently, little is known about the mechanisms by which pain might be associated with ovarian endometrioma. Therefore, first of all, we aim to retrospectively review the recorded files of laparoscopic surgery and analyze the possible association of pelvic pain in women with ovarian endometrioma who had coexisting PLs or without any visible PLs. Secondly, we plan to investigate the mechanistic basis of pelvic pain using prospectively collected biopsy specimens derived from women with peritoneal endometriosis, ovarian endometrioma and women without endometriosis during laparoscopic surgery.

Materials and Methods

We prospectively collected biopsy specimens from the eutopic and ectopic endometria of 15 women with pelvic endometriosis, 22 women with ovarian endometrioma and 10 control women with dermoid cysts without any evidence of PLs. Ten biopsy specimens were collected from red lesions and 15 from black lesions. Twelve women with ovarian endometrioma who had coexisting PLs (red, black or mixed) (CC + PL group) and 10 women had only ovarian endometrioma without any coexisting visible PLs (CC-only group). Besides tissue sampling from 10 control women, we also collected biopsy specimens from unaffected normal peritoneum from six women each with and without endometriosis. The distribution of biopsy samples based on menstrual cycle in these two groups of women is as follows: for CC + PL group, proliferative phase \((n = 4)\), secretory phase \((n = 6)\) and menstrual phase \((n = 2)\); for CC-only group, proliferative phase \((n = 3)\), secretory phase \((n = 5)\) and menstrual phase \((n = 2)\). All collected biopsy specimens were prepared for formalin-fixed paraffin-embedded tissue blocks for subsequent histopathological and immunohistochemical study. All biopsy specimens were collected in accordance with the guidelines of the Declaration of Helsinki and with the approval by the Institutional Review Board of Nagasaki University. An informed consent was obtained from all women.

Immunohistochemistry

Immunohistochemical analysis was performed to immunolocalize CD68, a macrophage (Mφ) marker and cyclooxygenase 2 (COX2), a rate-limiting enzyme for the production of PGs in the eutopic and ectopic endometria derived from women with and without endometriosis. The following first
antibodies were used for immunohistochemistry: (i) CD68 (KP1), a mouse monoclonal antibody from Dako, Denmark and a 1:50 dilution was used, (ii) COX2 (sc-1745), a goat polyclonal antibody from Santa Cruz Biotechnology and a 1:100 dilution was used. Non-immune mouse immunoglobulin G1 antibody in 1:50 dilution was used as a negative control. The details of the immunohistochemical procedure are described elsewhere (Khan et al., 2004a; Miura et al., 2006).

The CD68 immunoreactive spots (brown spots) were counted in five different fields of one section (×200 magnification) by light microscopy and expressed as the mean M±SEM number per field in one specimen.

Quantification of COX2-immunostained cells by quantitative-histogram score
The immunoreactivity of COX2-stained gland cells, stromal cells and endometrioma cyst wall was quantified by a computer-analyzed modified method of quantitative-histogram (Q-H) scores as described previously (Khan et al., 2003, 2005a; Ishimaru et al., 2004). The Q-H score was calculated using the following formula: Q-H score = Σ Pi, (I + 1), where I is the staining intensity graded as 0 = no staining, 1 = weak, 2 = moderate and 3 = strong and Pi is the percentage of cells stained at each intensity. We calculated the mean Q-H scores of five different fields of one section by light microscopy at moderate magnification (×200).

Measurement of PGF2α in tissue samples
A fraction of biopsy specimens from PLs/cyst walls and autologous eutopic endometria were homogenized in a homogenizing buffer using a Polytron homogenizer (Kinematics, Luzern, Switzerland). The respective tissue suspension was centrifuged at 400 g for 5 min to obtain the supernatant and stored at −80 °C for the subsequent measurement of prostaglandin F2α (PGF2α). The tissue concentrations of PGF2α in the homogenized supernatant of respective samples were measured in duplicate using a commercially available sandwich enzyme-linked immunosorbent assay (Quantikine; R&D System, Minneapolis, MN, USA) according to the manufacturer’s instructions. The protein concentration of samples was measured by the R&D System, Minneapolis, MN, USA) according to the manufacturer’s instructions. The concentration of samples was measured by the Bradford method (1976) to standardize PGF2α levels. The antibodies used in PGF2α determination do not cross-react with other cytokines. The lower limit of detection was >6.78 pg/ml for PGF2α. Both the intra-assay and inter-assay coefficients of variation were <10% for this assay. The tissue concentrations of PGF2α were expressed as pg/μg protein.

Statistical analysis
All results are expressed as either the mean ± SEM or mean ± SD or medians. The clinical characteristics of the subjects were compared with one-way analysis of variance and the χ² test for any difference between groups. Any difference in M±SEM numbers or Q-H scores of PGF2α between two groups was analyzed by the non-parametric Mann–Whitney U-test. For comparisons among groups, the Kruskal–Wallis test was used to assess the differences. A box plot analysis of tissue levels of PGF2α was performed using the medians and inter-quartile range (IQR). A value of P < 0.05 was considered statistically significant.

Results
During the period between September 1982 and April 2008, we could search 2988 cases that had had laparoscopic surgery for a variable indication as mentioned in the section Materials and methods. Ovarian endometrioma was found in 350 cases (11.7%) at laparoscopy and the clinical characteristics of these are shown in Table I. Most women were 20–29 (31.4%) or 30–39 (51.7%) years of age. Left-sided endometrioma was more common than either right-sided or bilateral ovarian endometrioma.

The association of coexisting PLs in women with ovarian endometrioma was as follows: no lesions (23%), red lesions (8.4%), black lesions (23.5%), white lesions (5.3%) and mixed lesions (39.7%) (Table I). The distribution of associated intra-pelvic adhesions was as follows: no lesions (23%), red lesions (8.4%), black lesions (23.5%), white lesions (5.3%) and mixed lesions (39.7%) (Table I). The distribution of associated intra-pelvic adhesions was as follows: no adhesion (44.0%), filmy adhesion (26.8%), and bilateral (29.1%).

Table I Clinical characteristics of 350 cases with ovarian endometrioma that were detected among 2988 cases of laparoscopic surgery during the period of 1982–2008.

<table>
<thead>
<tr>
<th>Distribution according to age (years)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>3 (0.85)</td>
</tr>
<tr>
<td>20–29</td>
<td>110 (31.4)</td>
</tr>
<tr>
<td>30–39</td>
<td>181 (51.7)</td>
</tr>
<tr>
<td>40–49</td>
<td>51 (14.6)</td>
</tr>
<tr>
<td>50–59</td>
<td>5 (1.4)</td>
</tr>
<tr>
<td>Distribution according to size (cm)</td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>8 (2.3)</td>
</tr>
<tr>
<td>&gt;3–10</td>
<td>185 (52.8)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>133 (38.0)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>3 (0.85)</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Left sided</td>
<td>154 (44.0)</td>
</tr>
<tr>
<td>Right sided</td>
<td>94 (26.8)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>102 (29.1)</td>
</tr>
<tr>
<td>Revised ARSM staging</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>6 (1.7)</td>
</tr>
<tr>
<td>Stage III</td>
<td>151 (43.1)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>193 (55.2)</td>
</tr>
<tr>
<td>Phases of menstrual cycle (P/S/M/A)</td>
<td>87/212/36/15</td>
</tr>
<tr>
<td>Coexisting PLs</td>
<td></td>
</tr>
<tr>
<td>No lesion</td>
<td>81 (23.1)</td>
</tr>
<tr>
<td>Red lesions</td>
<td>29 (8.4)</td>
</tr>
<tr>
<td>Black lesions</td>
<td>83 (23.5)</td>
</tr>
<tr>
<td>White lesions</td>
<td>18 (5.3)</td>
</tr>
<tr>
<td>Mixed lesions</td>
<td>139 (39.7)</td>
</tr>
<tr>
<td>Pattern of pelvic adhesion</td>
<td></td>
</tr>
<tr>
<td>No adhesion</td>
<td>55 (15.7)</td>
</tr>
<tr>
<td>Filmy adhesion</td>
<td>216 (61.8)</td>
</tr>
<tr>
<td>Dense adhesion</td>
<td>79 (22.5)</td>
</tr>
</tbody>
</table>

P, proliferative phase; S, secretory phase; M, menstrual phase; A, amenorrhea.
variable PLs (data not shown).

Metrioma who had no adhesion complained of pain due to coexisting and dense adhesion, 86.9% (Table III). Most of the women with endometrioma according to pattern of pelvic red lesions (88.2%), black lesion (85.5%) and mixed lesion (87.7%).

Table II Association of pain in 350 cases with ovarian endometrioma with and without coexisting PLs.

<table>
<thead>
<tr>
<th>Cases with ovarian endometrioma without PLs (n = 81)</th>
<th>Cases with ovarian endometrioma with PLs (n = 269)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No complain of pain, n (%)</td>
<td>50 (61.7)</td>
</tr>
<tr>
<td>Complain of pain, n (%)</td>
<td>31 (38.3)</td>
</tr>
<tr>
<td>39 (14.6)</td>
<td>230 (85.4)*</td>
</tr>
</tbody>
</table>

Pain was defined by the presence of menstrual pain, dysmenorrhea and chronic pelvic pain either alone or in combination. *P < 0.01 versus cases without PLs by the χ² test.

Table III Association of pain in 350 cases with ovarian endometrioma according to pattern of pelvic adhesion.

<table>
<thead>
<tr>
<th>No adhesion (n = 55)</th>
<th>Filmy adhesion (n = 216)</th>
<th>Dense adhesion (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No complain of pain, n (%)</td>
<td>29 (52.2)</td>
<td>44 (20.6)</td>
</tr>
<tr>
<td>Complain of pain, n (%)</td>
<td>26 (46.8)</td>
<td>172 (79.6)</td>
</tr>
</tbody>
</table>

Pain was defined by the presence of menstrual pain, dysmenorrhea and chronic pelvic pain either alone or in combination.

Pain symptoms based on the size of the ovarian endometrioma (<5 versus >5 cm), we found no significant difference between these two groups of endometrioma (46.2 versus 53.8%, respectively).

The association of pain in women with ovarian endometrioma was more remarkable when these cases were coexistent with dominant red lesions (88.2%), black lesion (85.5%) and mixed lesion (87.7%) compared with white lesions (63.6%) (data not shown). The incidence of pain in women with endometrioma according to pattern of pelvic adhesion was as follows: no adhesion, 46.8%; filmy adhesion, 79.6% and dense adhesion, 86.9% (Table III). Most of the women with endometrioma who had no adhesion complained of pain due to coexisting variable PLs (data not shown).

Tissue infiltration of CD68-immunostained Mφ in the eutopic and ectopic endometria

We found a differential infiltration of CD68-positive infiltration of Mφ in the PLs, cyst wall and their corresponding eutopic endometria, and unaffected normal peritoneum (Fig. 1A). The infiltration of Mφ was significantly higher in the red lesions and corresponding eutopic endometria compared with biopsy samples derived from the black lesion/ectopic endometria, cyst wall of CC/ectopic endometria or to samples of eutopic endometria derived from control women (Fig. 1B). The amount of Mφ was significantly higher in the eutopic endometria derived from women with black lesions and with CC + coexistent PL than in similar tissues derived from control women and women with CC only (Fig. 1B). No difference in the Mφ infiltration was found among black lesions, cyst wall of CC + PL and cyst wall of CC-only group. As a negative control, unaffected normal peritoneum derived from women with and without endometriosis was examined and found that normal peritoneum also displayed minimal inflammatory reaction without showing any significant difference between women with and without endometriosis (Fig. 1A and B).

Immunoeexpression of COX2 in the eutopic and ectopic endometria

The immunostaining of COX2 was found in both glandular epithelial cells and stromal cells and cyst wall lining cells. The immunoeexpression of COX2 was the strongest in the red lesion, moderate in the black lesion, cyst wall of CC who had coexisting PLs and weak in the cyst wall derived from CC only cases (Fig. 2A, lower column). A similar strong immunoeexpression of COX2 was found in the corresponding eutopic endometria derived from women containing red lesions, black lesions and from women with CC + PL but COX2 expression appears to be weak in the eutopic endometrium derived from CC only cases (Fig. 2A, upper column). The tissue expression of COX2 in the eutopic endometria derived from control women was similar to that in CC-only group (data not shown).

Q-H scores of COX2 immunoreaction

When we combined the Q-H scores of COX2 immunoreactivity in gland cells and stromal cells, we found a significantly higher Q-H scores of COX2 in the red lesions than in either black lesions or in the cyst walls of CC with or without coexisting PLs (Fig. 2B). Although Q-H scores of COX2 immunoreexpression were similar in the eutopic endometria of women containing red lesions, black lesions or cyst wall + PLs, these Q-H scores of COX2 were significantly higher than in the corresponding eutopic endometria derived from CC-only women or control women (Fig. 2B). The Q-H scores of COX2 in the cyst wall of CC/ectopic endometria derived from women who had coexisting PLs were also significantly higher compared with corresponding tissues derived from CC-only women (Fig. 2B).

We analyzed immunoreexpression and Q-H scores of COX2 in the eutopic endometria derived from women with CC who had coexisting PLs or did not based on the phases of the menstrual cycle. We found maximum immunoreexpression/Q-H scores of COX2 during the menstrual phase, intermediate expression in the secretory phase and less expression in the proliferative phase of these two groups of women (Fig. 3A and B). We could not find any significant difference in COX2 expression among phases of the menstrual cycle, may be due to the less number of cases in each phase. However, the Q-H scores of COX2 expression were found to be higher in the endometria derived from the CC + PL group than in CC-only group during any phase of the menstrual cycle (Fig. 3B).

Tissue levels of PGF2α in the eutopic and ectopic endometria

The tissue levels of PGF2α were significantly higher in the eutopic endometria derived from women with red lesions, black lesions and from women with CC who had coexisting PL than in control women or in CC-only women (P < 0.05 versus control women or CC-only women, white box, Fig. 4). Although no apparent difference in PGF2α levels was found among black lesions, cyst wall of CC that
had PL and cyst wall of CC without coexistent PL, a significantly higher PGF2α level was found in red lesions than in other three groups of lesions (P < 0.05 versus each of other lesion, hatched box, Fig. 4).

Similar to the immunoexpression of COX2, we found a similar pattern of PGF2α tissue levels in the eutopic endometria and cyst walls derived from women with CC + PL and CC-only women when we distributed the findings according to the phases of the menstrual cycle (Fig. 5A and B).

**Discussion**

In this retrospective cohort study using the collective data during the past 25 years, we demonstrated that women with only ovarian endometriosis experience less pelvic pain and most of the pain manifestations in women with CCs are mostly associated with variable coexisting PLs. When we extended our study to explain the mechanistic basis of pain in women with peritoneal and ovarian endometriosis, we found that tissue inflammatory reaction, expression of COX2 and tissue levels of PGF2α were remarkably higher in PLs and their autologous eutopic endometria derived from women with pelvic endometriosis than in tissue samples derived from women with only CCs. An interesting finding in our current study was that tissue inflammation, COX2 immunoexpression and levels of PGF2α was higher in the cyst walls and corresponding eutopic endometria of women with CC who had different coexisting PLs when compared with similar tissues derived from women harboring only CCs without any visible evidence of PLs in their pelvic cavity.
The information regarding the association of pelvic pain in women with ovarian endometrioma is scanty and is still controversial (Berkley et al., 2005). Most recently, it has been demonstrated that severe pelvic pain in women with cystic endometrioma is mostly associated with deeply infiltrating endometriosis (Chapron et al., 2012). We could not analyze the association with DIE or severity of pain in our current study, because information was not enough on either DIE or severity of pain in the old recorded files of laparoscopic surgery. This could be a bias in our study when analyzing the association between pelvic pain and ovarian endometriosis.

Studies that found an association between ovarian endometrioma and pelvic pain were performed using univariate analysis (Fedele et al., 1992; Muzii et al., 1997). However, the methodological approach was poor to establish the fact that ovarian endometrioma are frequently associated with other PLs (Redwine, 1999; Chapron et al., 2009), which themselves could cause pain. In studies that used multivariate analysis, results were less clear with pain symptoms apparently not correlated with the presence of ovarian endometrioma (Koninckx et al., 1991; Porpora et al., 1999). In this controversial situation, we demonstrated for the first time that pelvic pain in women with ovarian endometrioma is mostly associated with coexisting PLs.

In fact, we found that women with ovarian cystic endometriosis who had variable PLs in their pelvic cavity complained of higher occurrence of pelvic pain (85.4%) than in women without coexisting visible...
The incidence of no complaint of pain was higher among women with CC whose pelvic cavity was free of PLs (61.7%) than in women who had coexistent PLs (14.6%).

When we investigated the association between the occurrence of pain symptoms and size of ovarian endometrioma (>5 versus >5 cm), we did not find any significant difference in pain manifestations between these two groups of women. This indicates that the occurrence of pain in these women is independent of the size of ovarian endometrioma. This could be due to the fact that both of these two groups of women with ovarian endometrioma (>5 and >5 cm size) had variable patterns of coexisting PLs.

In clinical practice, women with ovarian endometriosis are presented with different PLs of pelvic endometriosis and with variable pattern of inflammation-induced pelvic adhesions. The associated intra-pelvic adhesion could be a cause of pain symptoms in addition to PLs. As a matter of fact, we found that the association of pain in women with ovarian endometriosis was higher in women having filmy or dense adhesion when compared with cases with no adhesion (Table III). A proportion of cases with no adhesion also complained of pelvic pain. Most of the cases without any pelvic adhesion were coexistent with a variable distribution of PLs. This indicates that women with ovarian endometrioma may complain of pain due to associated PLs irrespective of the presence of intra-pelvic adhesion.

We previously demonstrated that the tissue activity of PLs, as evidenced by the higher tissue infiltration of macrophages (Mφ) and the production of different cytokines/growth factors, was significantly higher in red lesions, specially in blood-filled opaque red lesions, than in other transparent/translucent PLs or in cyst walls of ovarian endometriosis (Khan et al., 2004a,b). The tissue infiltrations of Mφ in the endometria and peritoneal fluid content of Mφ were found to be significantly higher during the late secretory phase or during the menstrual phase in women with peritoneal endometriosis than in control women (Khan et al., 2004a, 2005b). These findings of intrauterine or pelvic inflammation may promote the occurrence of pelvic pain during the perimenstrual period. Macrophages and eutopic/ectopic endometrial cells have been reported to be the

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**Figure 3** Immunolocalization of COX2 in the biopsy specimens of the eutopic endometrium derived from women with ovarian endometrioma (CC) + coexisting PLs (CC + PL group, upper column of A) and women with CC only without any coexisting PLs (CC-only group, lower column of A) based on the phases of menstrual cycle (A). The Q-H scores of COX2 in the eutopic endometria were found to be higher during the menstrual phase than in either the proliferative phase or secretory phase of the menstrual cycle (B). These menstrual phase-dependent findings of COX2 expression were more prominent in women with CC + PL than in CC-only women (B). The results are expressed as the mean ± SEM. Five different fields were measured in each section by light microscopy at moderate magnification (×200).
predominant cell types exhibiting increased expression of COX2 and production of PGE2/PGF2α (Benedetto, 1989; Herath et al., 2006). In our current study we found an increased tissue infiltration of macrophages, increased tissue expression of COX2 and consequently increased tissue concentration of PGF2α in different PLs and corresponding eutopic endometria comparing with cyst walls/eutopic endometria of women who did not harbor any PL in their pelvic cavity. This may enhance the PGF2α-induced uterine contraction and may explain the mechanistic basis of increased prevalence of pelvic pain in women with ovarian endometrioma who had coexistent variable PLs.

The association between higher production of PGs and severity of pelvic pain or between application of PGE2/PGF2α and dose-dependent increase in intrauterine pressure secondary to uterine contraction has been demonstrated (Dawood et al., 1984; Koike et al., 1992; Dittrich et al., 2009). In parallel with the higher Mφ infiltration, both PGE2 and PGF2α levels in endometrial tissues were found to be maximum during the menstrual phase, intermediate in the secretory phase and less visible during the periovulatory phase of the menstrual cycle. This enhanced uterine contraction during the menstrual phase was more frequently identified in women with endometriosis than in control women (Kido et al., 2007). These previously published reports of uterine contraction and our current findings may explain the role of COX2/PGF2α in the generation of higher incidence of pelvic pain in women with CC + PL than in women with only CC without any coexisting visible PL.

In conclusion, our retrospective analysis of cases with ovarian endometrioma and prospective biological investigation indicated that women with only ovarian endometrioma experience less pain and pelvic pain in women with ovarian endometrioma is mostly associated with coexisting PLs. An increased tissue inflammatory reaction with elevated PG production may be responsible for the generation of pain in these women. Lack of evaluation in the detection of general

**Figure 4** Tissue concentrations of prostaglandin F2α (PGF2α) in the eutopic (white box) and ectopic endometria (hatched box) of control women, women with peritoneal endometriosis and women with ovarian endometrioma (CC). The tissue concentration of PGF2α was significantly higher in the eutopic endometria derived from women containing red lesions, black lesions and from women with CC + coexisting PLs (CC + PL group) than in the control women and in CC-only women. Although no difference in tissue levels of PGF2α was observed among black lesions, cyst wall of CC + PL and cyst wall of CC-only group, the PGF2α level in red lesions was significantly higher than in other lesions. Boxes represent the distance (IQR) between the first (25%) and third (75%) quartiles and the horizontal lines in the boxes represent median values. *P < 0.05 versus CC-only group/control women; **P < 0.05 versus other lesions.
or disseminated DIE in the pelvic cavity could be a bias or limitation in this study. Further multicenter prospective studies are needed to strengthen our current findings.

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Authors’ roles

K.N.K. was involved in concept, study design, experiments, data analysis and manuscript draft; M.K., A.F., K.H. and A.M. contributed equally to operative procedure and sample collection; M.N. and H.M. was equally involved in draft advice.

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Conflict of interest

None declared.

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