Reproductive Sciences
20(12) 1465-1470

DOI: 10.1177/1933719113488452
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The Role of Placental Protein 14 in the Pathogenesis of Endometriosis

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Abstract

Placental protein 14 (PP-14) is the principal secretory phase product of endometrium and has been shown to inhibit cell immune function. But its role in the pathogenesis of endometriosis is controversy. The objective of this study is to determine the concentrations of PP-14 in peritoneal fluid (PF) and serum and PP-14 protein expression in endometriotic lesions in women with ovarian endometriosis (n = 75) when compared to women without endometriosis (n = 49) between day 7 and day 20 of their menstrual cycle. Concentrations of PP-14 in PF and serum as well as PP-14 protein expression in endometriotic lesions in women with and without endometriosis were evaluated by using enzyme-linked immunosorbent assay and immunohistochemical staining, respectively. Serum PP-14 concentrations were significantly increased in women with endometriosis (7.5 $\pm$ 1.4 ng/mL) compared to those in women without endometriosis (5.8 $\pm$ 0.9 ng/mL; $P < .05$) and statistically decreased after surgery and further reduced by using gonadotropin-releasing hormone agonist therapy ($P < .05$). However, the concentrations of PP-14 in PF did not reach a significant difference between women with and without endometriosis ($P > .05$). In women with endometriosis, scores of PP-14 protein expression in the lesions (n = 50, 2.2 [0 $\sim$ 5.8]) were significantly correlated with serum PP-14 concentrations (n = 50, 7.6 $\pm$ 1.3 ng/mL; $P < .01$). Our results suggest that PP-14 may play an important role in the pathogenesis of endometriosis.

Keywords

endometriosis, placental protein 14, serum, peritoneal fluid, endometrium

Introduction

Causes of endometriosis remain unclear, but a defective immune response contributing to the implantation and development of retrograde endometrial cells from fallopian tube during menstruation within the peritoneal cavity is well accepted.[1-3] Placental protein 14 (PP-14), which is also known as glycodelin and progesterone-dependent endometrial protein, is a 42 000 MW glycoprotein that exhibits potential immunosuppressive activity.[4] It has been shown that PP-14 is a highly menstrual cycle-dependent protein, and its high serum PP-14 concentrations occur only in the late luteal and early follicular phases of the menstrual cycle.[5] Obviously, PP-14, which is a T-cell inhibitor and produced by endometrium itself,[6,7] may contribute to the implantation of retrograde endometrium and the formation of endometriotic lesions.

However, the current available data on PP-14 concentrations in peritoneal fluid (PF) and serum are conflicting; some authors found no differences in PP-14 concentrations between women with and without endometriosis either in PF[8] or in serum,[9] whereas others reported increased concentrations of PP-14 in serum from women with deep-infiltrating, ovarian, and advanced endometriosis[10-12] and in PF from women with peritoneal endometriosis.[10] Moreover, PP-14 is highly expressed in endometriosis lesions,[13] but *PP-14* gene expression in ectopic endometrium is lower than that in eutopic and normal endometrium.[14] These findings suggest that the role of PP-14 in the pathogenesis of endometriosis and its associated clinical importance are still questioned. Obviously, further research into the correlations of PP-14 expression in endometriotic lesions and PP-14 concentrations in PF and serum in women with endometriosis are reasonable.

Cancer antigen (CA) 125, a tumor-associated antigen secreted by coelomic epithelium, is a well-known biomarker for endometriosis and helpful in daily clinical practice when endometriosis is suspected. However, elevated serum CA-125 concentrations have been mostly demonstrated in women with advanced and ovarian endometriosis.[15-17] Therefore, in this study we aimed to determine the concentrations of PP-14 and CA-125 in serum and PF of women with ovarian endometriosis when compared to women without endometriosis, the correlations of PP-14 expression in endometriotic lesions, and PP-14 concentrations in serum and PF of women with endometriosis.

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Materials and Methods

Patients

A total of 124 consecutive patients undergoing laparoscopic surgery at the Women's Hospital between July 2010 and September 2012 participated in this study. Gynecological indications for laparoscopy included the evaluation of infertility and pelvic mass or elective tubal sterilization. Of the 124 patients, 75 patients (75 of 124) were diagnosed with endometriosis by laparoscopic and histological examinations, and 49 patients (49 of 124) had no evidence of endometriosis. In patients with endometriosis, the primary indication for surgery was pelvic mass (70 of 75) and infertility (5 of 75). In patients without endometriosis, the primary indication for surgery was infertility (30 of 50), tubal sterilization (13 of 50), and pelvic mass (7 of 50). Of the 7 patients without endometriosis who had a pelvic mass, 5 had simple mesosalpinx cyst (cystic diameter, <5 cm), and 2 had simple parovarian cyst (cystic diameter, <5 cm). All women participating in the study were confirmed to have normal ovulatory menstrual cycles and to have not taken nonsteroidal anti-inflammatory drugs and hormonal medication for at least 3 months before the time of surgery. After surgery, all women with endometriosis were followed monthly at the endometriosis clinic and treated with gonadotropin-releasing hormone agonist (GnRHa) for 6 months. Each patient gave informed consent for participation in the study. The institutional review board of Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang gave ethical permission for this study.

The day of the menstrual cycle was established from the patient's menstrual history and basic body temperature and verified randomly by histologic examination of the endometrium. In order to avoid the possible influence of endometrial PP-14 on their serum concentrations, all the patients underwent laparoscopic surgery between day 7 and day 20 of the menstrual cycle.[5,10] At surgery, the pelvis was assessed for the presence or absence of endometriosis, and the endometriosis was scored according to a modification of the revised American Fertility Society (rAFS, 1997) staging system that ranged from I to IV (III = 35; IV = 40).[18] In all cases, endometriosis was confirmed histologically. Control patients were women in whom no visible evidence of pelvic disease was found and who had no history of endometriosis.

A blood sample was taken immediately before laparoscopy, and the PF was aspirated at the beginning of the procedure. After surgery, a blood sample was also collected from women with endometriosis. All samples were centrifuged, and the PF and serum samples were frozen at −20°C until assayed. During the surgery, a biopsy for endometrial tissue was obtained from the study patients and ovarian endometriotic lesions were collected from women with ovarian endometriosis. All biopsied endometrial samples were examined under microscope, and their histology was dated according to the general classifications of Noyes et al.[19]

Immunoassay and Immunohistochemical Staining

Concentrations of PP-14 and CA-125 in PF and serum were measured by enzyme-linked immunosorbent assay (ELISA) using a human PP-14 ELISA kit (HM10076, Bio-swamp, Shanghai, China) and a human CA-125 ELISA kit (HM10776, Bio-swamp, Shanghai, China), respectively, according to the manufacturer's instruction. The intraassay and interassay coefficients for both PP-14 and CA-125 were <10%. For each sample, duplicate sera were assayed.

Immunohistochemical staining for PP-14 was performed using 5-mm paraffin sections on the selected slides. A standard immunohistochemical technique was used.[20-22] After blocking endogenous peroxides and proteins, primary antibody (ab17247, PP-14: 1:50; Abcam Corp, Cambridge, United Kingdom) was applied for 2 hours at room temperature. The standard streptavidin–biotin indirect method was used. Diaminobenzidine was used as chromogen (GK346810; Novocastra, United Kingdom). Human late secretory endometrium was used as a positive control. Negative controls were incubated with normal goat serum (X 0907; Dako, Glostrup, Denmark) instead of the primary antibody.

Scoring for PP-14 Protein Expression

The expression of PP-14 was classified according to the following grading system as described by Huang et al.[20,23] Scores that correspond to the percentages of staining cells were defined as follows: 0 for no documented positive staining cell; 1 for the 25% positive staining cells; 2 for >25% and 50%; and 3 for >50%. Moreover, in term of intensity of the stain, the following scores were designated: 0 for no documented stains; 1 for weak; 2 for intermediate; and 3 for strong. A value of immunostaining scores for PP-14 expression was represented as the sum of the percentage score and the intensity score, and the expression of PP-14 was finally defined as follows: no expression (−) for a score of <2; weak expression (+) for a score of >2 and 4; and strong expression (++) for a score of 5 or 6.

Statistical Analysis

We used the Statistical Package for the Social Sciences version 11.0 to perform statistical analyses. Nonparametric data were described as median (range) and parametric data as mean (±standard deviation). The Mann-Whitney U test was used for the comparison of their age between groups. The Unpaired t test was used for the comparisons of PP-14 and CA-125 concentrations in serum and PF at surgery between the groups. The 1-way analysis of variance was used for the comparisons of PP-14 and CA-125 concentrations in serum after surgery between the groups. The Spearman analysis was used to analyze the correlations between the levels of PP-14 expression in endometriotic lesions and the concentrations of PP-14 in PF and serum. $P < .05$ was considered statistically significant.

Table 1. Patient's Age and Concentrations of PP-14 and CA-125 in Serum.

Variable	Endometriosis (n = 75)	No Endometriosis (n = 49)	*P* Value
Age at operation, years[a]	34 (26-43)	33 (28-41)	>.05
Serum CA-125, U/mL[b]	33.6 ± 6.2	25.1 ± 3.9	<.05
Serum PP-14, ng/mL[b]	7.5 ± 1.4	5.8 ± 0.9	<.05

Abbreviations: CA-125, cancer antigen 125; PP-14, placental protein 14.
[a] Median.
[b] Mean.

Table 2. Concentrations of PP-14 and CA-125 in PF From Women With and Without Endometriosis.[a]

Peritoneal Fluid	Endometriosis (n = 19)	No Endometriosis (n = 17)	*P* Value
CA-125, U/mL	337.6 ± 71.2	333.7 ± 69.9	>.05
PP-14, ng/mL	57.5 ± 13.3	54.9 ± 12.1	>.05

Abbreviations: CA-125, cancer antigen 125; PF, peritoneal fluid; PP-14, placental protein 14; SD, standard deviation.
[a] Mean ± SD.

Table 3. Serum CA-125 and PP-14 Concentrations in Women With Endometriosis Using GnRHa Therapy.[a]

Variable	Serum PP-14 (ng/mL)	*P* Value	Serum CA-125 (U/mL)	*P* Value
At surgery (n = 75)	7.5 ± 1.4	–	33.6 ± 6.2	–
After surgery (n = 75)	6.0 ± 1.0	<.05[b]	26.3 ± 4.0	<.05[b]
GnRHa 3 months (n = 48)[c]	5.1 ± 0.8	<.01	23.7 ± 3.7	<.01
GnRHa 6 months (n = 39)	4.3 ± 0.5	<.01	19.1 ± 2.2	<.01

Abbreviations: CA-125, cancer antigen 125; GnRHa, gonadotropin-releasing hormone agonist; PP-14, placental protein 14; SD, standard deviation.
[a] Mean ± SD.
[b] After surgery and GnRHa therapy versus at surgery.
[c] After 3 months of GnRHa therapy.

Results

Blood samples were taken from all the study participants. However, both eutopic endometrial tissues and ovarian endometriotic lesions were sampled only from 50 of the 75 women with ovarian endometriosis. Also, control endometrial tissues were sampled only from 20 of the 49 women without endometriosis. All women with ovarian endometriosis were in the stage III and stage IV of the disease. There was no significant difference in the age between women with endometriosis and women without endometriosis ($P > .05$; Table 1). Concentrations of both CA-125 and PP-14 in serum were significantly higher in women with endometriosis than those in women without endometriosis ($P < .05$; see Table 1). However, either CA-125 or PP-14 concentrations in PF did not reach statistical significance between the endometriosis group and the control group ($P > .05$; Table 2). After surgery, serum CA-125 and PP-14 concentrations were both significantly reduced and further decreased when women with endometriosis were treated with GnRHa ($P < .05$; Table 3). Using Spearman analysis, a significant correlation between CA-125 and PP-14 concentrations was found in both PF and serum of women with and without endometriosis ($P < .05$).

The PP-14 expression could be detected in 42 (84.0%) of 50 ovarian endometriotic lesions and 3 (6.0%) of 50 eutopic endometrial tissues from women with endometriosis and in 1 (5.0%) of 20 control endometrial tissues from women without endometriosis, respectively. Under microscopy, we found that 1 endometrial tissue from women without endometriosis that showed very weak positive PP-14 staining was in the mid-secretory phase of the menstrual cycle (Figure 1E). Three eutopic endometrial tissues from women with ovarian endometriosis all showed very weak positive PP-14 staining and were in the early secretory phase of the menstrual cycle (Figure 1C and 1D). In eutopic and control endometrium, expression of PP-14 showed similar localization patterns, and both were located in the epithelial cells of the endometrial glands and had very little PP-14 staining in the stromal cells (Figure 1C-E). In ovarian endometriotic implants, PP-14 was expressed in the epithelial cells, and very little PP-14-positive staining appeared in the glandular epithelium and the interstitial cells (Figure 1A and 1B). Of the 42 ovarian endometriotic implants with PP-14-positive staining, 4 showed strong expression, 22 showed weak expression, and 22 showed no expression. Scores of PP-14 protein expression in lesions were significantly correlated with serum PP-14 concentrations in women with ovarian endometriosis ($r = .385$, $P = .006$; Figure 2).

Discussion

Our results showed that serum PP-14 concentrations were increased in women with ovarian endometriosis when compared to women without endometriosis and correlated with PP-14 expression levels in endometriotic lesions. However, our results did not find any significant differences in PP-14 levels in PF between women with and without endometriosis. These results suggest that PP-14 secreted by ovarian endometriotic lesions may contribute to the plasma, leading to increased concentrations of PP-14 in serum of women with endometriosis.[8] Because PP-14 can inhibit natural killer (NK) cell cytotoxicity function and regulate type 1 (Th1) helper T cells and type 2 (Th2) helper T cells immune responses differently,[24,25] it is implied that PP-14 may play a role in the pathogenesis of endometriosis.[26]

The PP-14 is the principal secretory phase product of endometrial epithelial cells,[27] and its serum PP-14 levels reflect endometrial cycle phase, which are in concert with progesterone levels.[5,27,28] In healthy women, serum PP-14 concentrations start to rise at the mid-luteal phase and begin to fall at the very early mid-follicular phase of the menstrual cycle.[5,10,27,28] It is indicated that low endometrial protein PP-14 levels occur between the mid-follicular and mid-luteal phases of the menstrual cycle. Our study patients recruited between day 7 and day

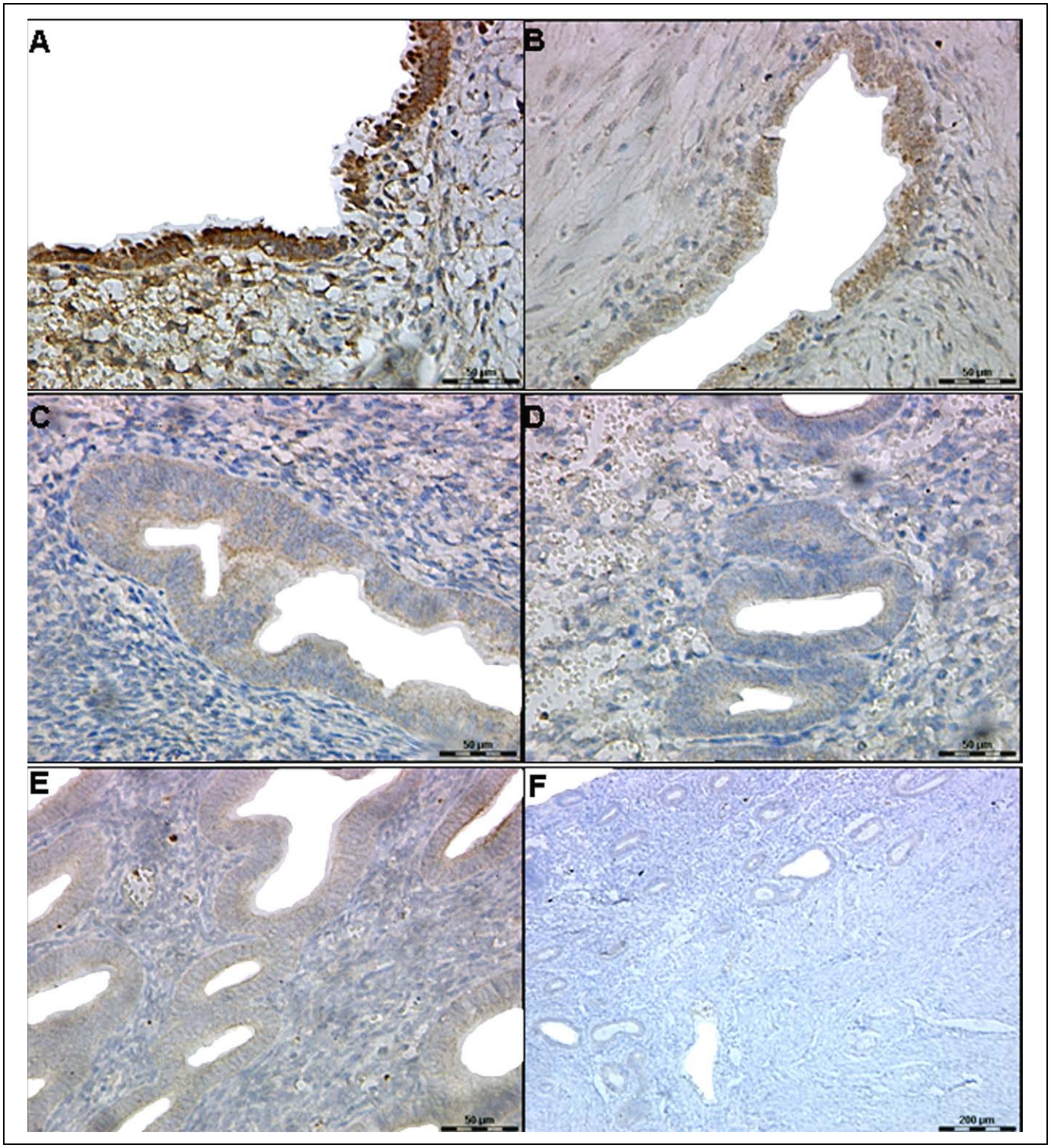

Figure 1. Expression of PP-14 in endometriotic lesions and in endometriosis and control endometrium. A, Strong PP-14-immunoreactive staining in ovarian endometriotic lesions. B, Weak PP-14-immunoreactive staining in ovarian endometriotic lesions. C and D, Very weak PP-14-immunoreactive staining in endometriosis endometrium. E, Very weak PP-14-immunoreactive staining in control endometrium. F, Negative PP-14-immunoreactive staining in control endometrium. Scale bars represent 50 µm (original magnification ×400) in (A), (B), (C), (D), and (E); and 200 µm (original magnification ×100) in (F). PP-14 indicates placental protein 14.

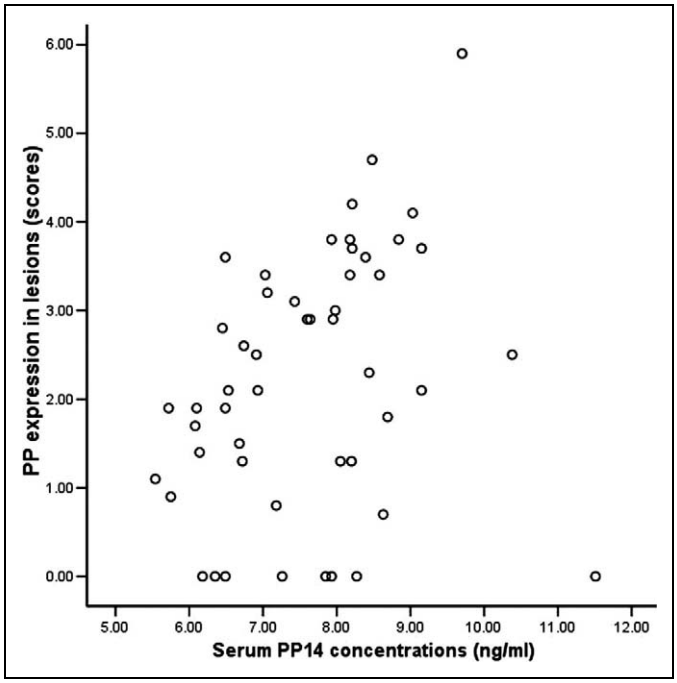

Figure 2. Association of PP-14 protein expression levels in endometriotic lesions and serum PP-14 concentrations in women with ovarian endometriosis. PP-14 indicates placental protein 14.

20 of their menstrual cycle were in the low-level cycle stage of endometrial PP-14 production. Moreover, PP-14 in endometriosis endometrium shows lower levels when compared with normal endometrium.[29] It is apparent that PP-14 protein produced by the lesions causes increased serum PP-14 concentrations in women with endometriosis.

It has been shown that PP-14 protein expression in both endometriosis and normal endometrium can be detectable after day 22 of the menstrual cycle.[29] However, our study found 1 normal endometrium with very weak positive PP-14 staining on the day 20 of menstrual cycle. But this normal endometrium had high secretory changes. Other 3 endometriosis endometria with very weak PP-14-immunoreactive staining were all at the very end of the early secretory cycle phase. Although endometriosis endometrium is quite different from the normal endometrium,[15,30] yet, the exact mechanism of why these 3 endometriosis endometria showed very weak PP-14-immunoreactive staining still needs to be investigated.

The PP-14 protein is mainly expressed in endometriotic lesions with secretory response, but it can be expressed in the lesions with proliferative and atrophic changes.[13] In our study, PP-14 protein expression could be detected as long as the implants appeared epithelial cells. Obviously, the epithelial cells within the lesions may be the main origin of increased serum PP-14 concentrations in women with ovarian endometriosis.

Our results also showed that serum PP-14 and CA-125 concentrations were both decreased after surgery and further reduced after using GnRHa therapy. Moreover, serum PP-14 concentrations correlated with serum CA-125 levels in women with and without endometriosis. It is suggested that PP-14, like CA-125, may be useful for monitoring the treatment efficacy of endometriosis.[10,11]

In summary, our results showed that serum PP-14 concentrations were increased in women with ovarian endometriosis and decreased after surgery and further reduced using GnRHa therapy. Moreover, serum PP-14 concentrations were correlated with PP-14 expression in endometriotic lesions in women with endometriosis and with serum CA-125 levels in women with and without endometriosis. These results only suggest that PP-14 may play an important role in the pathogenesis of endometriosis and may be useful for endometriosis clinical practice. Further studies with a large sample are needed.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We appreciate the financial support of the Nature Science Foundation of Zhejiang province (Y2110181, Y2110128), and Shanghai Pujiang Program Fund (10PJ1409000).

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