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Review article

Endometriosis and possible inflammation markers

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ABSTRACT

Inflammation plays an important role in the pathogenesis of endometriosis. Infiltration of peritoneal macrophages and local proinflammatory mediators in the peritoneal microenvironment affect ovarian function and pelvic anatomy leading to the symptoms and signs of endometriosis. The identification of a noninvasive marker for endometriosis will facilitate early diagnosis and treatment of this disease. This review provides an overview of local microenvironmental inflammation and systemic inflammation biomarkers in endometriosis.

Introduction

Endometriosis is considered a chronic inflammatory disease. It is defined as the presence of endometrial stroma and glands outside the uterine cavity. The prevalence of endometriosis in women of reproductive age is about 10%.[1] Although the exact etiology of endometriosis is still controversial, Sampson's retrograde menstruation theory is a widely accepted explanation for endometriosis.[2] In order to ectopically implant on the peritoneal cavity, refluxed endometrial tissue processes through attachment, acute inflammation, macrophage infiltration, tissue remodeling, and neovascularization. Inflammation can lead to an anatomic disorder and affect ovarian function, which plays an important role in the pathogenesis of endometriosis. Infiltration of peritoneal macrophages and local proinflammatory mediators in the peritoneal microenvironment affect the symptoms and signs of women with endometriosis. This review provides an overview of local microenvironmental inflammation and systemic inflammation biomarkers in endometriosis.

Local microenvironment inflammation in endometriosis

Endometriosis is a pelvic inflammatory process with altered immune surveillance in the local peritoneal microenvironment. The endometriosis-associated inflammatory responses are dependent on increased activated macrophages and their secreted cytokines in peritoneal fluid. A local inflammatory microenvironment will sustain the growth and maintenance of endometriosis through endometrial-peritoneal adhesion, invasion, angiogenesis, and proliferation. The inflammation process in endometriosis further causes pelvic pain and infertility, two major symptoms of endometriosis.

Cyclooxygenase-2 in endometriosis

Local proinflammatory mediators, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, will activate nuclear factor κB (NF-κB) and hypoxia inducible factor (HIF)-1α signaling pathways, and then increase cyclooxygenase (COX)-2 expression in endometriosis.[3] These cytokines act as autocrine and/or paracrine signals to regulate local immune and inflammatory responses. COX is a rate-limiting enzyme in the biosynthesis of proinflammatory prostaglandins (PGs). COX-1 is constitutively expressed, and its primary function is housekeeping. COX-2 is an inducible enzyme which is usually absent under physiologic conditions, but rapidly expressed after stimulation by cytokines and proinflammatory agents in various pathological conditions such as endometriosis.[4,5]

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In peritoneal macrophages, COX-2 expression was significantly increased in women with endometriosis, whereas expression of COX-1 was only increased in severe stages of endometriosis.[4] The expression of COX-2 in peritoneal macrophages is associated with PG levels in peritoneal fluid and the severity of endometriosis. Treatment with IL-1β, TNF-α, or PGE_2 induced a significant increase of COX-2 expression in peritoneal macrophages in disease-free women. However, these proinflammatory agents failed to induce COX expression in peritoneal macrophages in women with endometriosis. Since distinct patterns of COX-2 expression in peritoneal macrophages were found under the exposure of microenvironmental stimulants in peritoneal fluid, the failure of proinflammatory factors to stimulate COX-2 expression in peritoneal macrophages from women with endometriosis may be due to the desensitization of active macrophages (expressed CD25 cell antigen) under long-term stimulation in peritoneal fluid.

COX-2 is overexpressed in glandular epithelia and stroma of ectopic endometriotic implants.[5,6] In addition, increased COX-2 expression is found in eutopic endometria cells from women with endometriosis compared with disease-free women. Increased COX-2 and COX-2-derived PGE_2 production regulate cell survival, migration, and invasion of ectopic endometriotic tissues.[6] The proinflammatory cytokines in the peritoneal microenvironment will stimulate the implantation of ectopic endometriotic tissues. Elevation of COX-2 expression in normal and endometriotic stromal cells is regulated by proinflammatory cytokines such as IL-1β[5] or PGE_2 itself (in an autocrine manner),[7] primarily due to increased COX-2 mRNA stability. In ectopic endometriotic stromal cells, IL-1β not only increases mRNA stability but also upregulates COX-2 promoter activity (transcriptional regulation), which is mediated via mitogen-activated protein kinase (MAPK)-dependent signaling pathways through binding to the cAMP-responding element site of a COX-2 promoter. Downregulation of microRNAs (miR199a and miR16) also lead to increased COX-2 translation.[3] In addition, chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) regulates a subset of genes involved in endometrial stroma cell decidualization and plays a role in controlling the expression of inflammatory cytokines. Downregulation of COUP-TFII in endometriotic stromal cells mediated by proinflammatory cytokines including IL-1β, TNF-α, and transforming growth factor (TGF)-β1 via microRNA-302a results in COX-2 upregulation.[8] These promote an inflammatory microenvironment for the development of endometriosis.

Endometriosis is also considered as an estrogen-dependent inflammatory disease.[7] Inflammation and estrogen are lined in a positive feedback cycle which enhances the expression of aromatase, COX-2, and local estrogen production in ectopic endometriotic lesions. Estrogen receptor β (ERβ) in endometriotic tissue is increased and mediates the induction of COX-2 expression by estradiol.[7] A lack of ERβ promoter methylation is associated with increased ERβ activity in endometriotic stromal cells, and hypermethylation of a CpG island at the promoter region silences ERβ gene expression in eutopic endometrial stromal cells. Increased ERβ in endometriotic stromal cells suppressed the expressions of ERα and progesterone receptors, leading to inflammation, progesterone resistance, and deficient inactivation of estradiol in endometriotic tissue. Similar epigenetic changes in endometriotic tissue are found in the orphan nuclear receptor steroidogenic factor (SF) 1, which mediates PGE_2-dependent induction of estradiol production.[7] In the MCF-7 human mammary adenocarcinoma cell line, hypoxic treatment induces activation of HIF-1α and upregulates inflammatory response genes such as COX2 expression.[9] In our recent study,[10] treatment of endometrial stromal cells with hypoxia induced ERβ expression. By contrast, knockdown of HIF-1α abolished hypoxia-induced ERβ expression and increased ERα expression.[10] It further provides important information regarding how hypoxia mediates estrogen action via different ER expressions which may induce inflammatory responses in endometriosis.

Prostaglandins in endometriosis

Elevated PGE_2 and $PGF_{2\alpha}$ in peritoneal fluid due to the overexpression of COX-2 may contribute to the presence of symptoms of endometriosis such as dysmenorrhea and infertility.[4] PGE_2 is a polypotent eicosanoid that regulates many pathophysiological processes in the development of endometriosis, including cell proliferation, antiapoptosis, immune suppression, and angiogenesis.[11] Due to its inherent nature, although PGE_2 production is higher in epithelial cells than in the stromal cells of endometriotic tissues, endometriotic stromal cells have more potential to migrate and invade than epithelial cells.[6] Several proinflammatory cytokines induce COX-2 expression in peritoneal macrophages and endometriotic stromal cells, leading to increased PGE_2 level in peritoneal fluid. However, the short half-lives of COX-2 and PGs cannot explain their continuous overexpression in both peritoneal macrophages and ectopic endometriotic stromal cells from patients with endometriosis. Two positive feedback loops, COX-2-PGE_2-estrogen in ectopic endometriotic stromal cells and COX-2-PGE_2-proinflammatory cytokines such as IL-1β, TNF-α, and even PGE_2 secreted by peritoneal macrophages will constantly keep an elevated PGE_2 concentration in the peritoneal fluid of patients with endometriosis as a self-supported survival system to the growth of endometriosis.[11,12]

Endometriosis is an estrogen-dependent disease. Two committed steps required for *de novo* synthesis of estrogen are steroidogenic acute regulatory protein (StAR) and aromatase. PGE_2-induced StAR expression, which transports cholesterol across the mitochondrial membrane to the inner mitochondrial leaflet, is restricted in ectopic endometriotic stromal cells.[13–15] Similar to the mechanism seen in StAR regulation, the induction of aromatase expression by PGE_2 occurs only in ectopic endometriotic stromal cells, and is mediated via binding to EP2/EP4 receptor-coupled signaling pathways.[16] Therefore, selective inhibition of EP2/EP4 receptors is considered as potential nonsteroidal therapy for women with endometriosis in human endometriotic cells.[17]

Furthermore, estrogen *per se* is not a mitogen. The mitogenic effect of estrogen is mediated by the upregulation of fibroblast growth factor (FGF)-9.[18] FGF-9 is an endometrial stromal growth factor which binds to its high-affinity receptor, especially FGFR2IIIC, which activates Ras-Raf-MEK-ERK and phospholipase C-calcium-mTOR pathways.[19,20] The induction of FGF-9 expression by PGE_2 to induce endometriotic cell proliferation is biphasic via binding to different EP receptors. PGE_2-induced FGF-9 expression and estrogen biosynthesis via the EP2 receptor coupled PKA signaling pathway is a delayed response to PGE_2 (after 24 hours). Only in ectopic endometriotic stromal cells, the induction of FGF-9 expression by PGE_2 is mediated via the EP3 receptor-activated PKCσ signaling pathway in an estrogen-independent manner.[21] This upregulation of FGF-9 via the EP3 receptor signaling pathway through Gq-protein activation is the acute response to PGE_2 (within 12 hours). It activates the downstream signaling cascade to phosphorylate the transcription factor Elk-1, then binds to the FGF-9 promoter and initiates its transcription.

Immune dysfunction such as hyperactive peritoneal macrophage with poor phagocytic ability is considered as an important factor in the development of endometriosis. The phagocytic capacity of peritoneal macrophages is mediated by at least two mechanisms. The first mechanism is the secretion and activation of matrix metalloproteinases (MMPs) to break down the extracellular matrix, and the second is the expression of scavenger receptors on

the macrophages to enhance the uptake and degradation of cell debris. Decreased expression and the enzymatic activity of MMP-9 by peritoneal macrophages were seen in women with endometriosis, which failed to destroy the basement membrane and break down endometriotic tissues.[22] The suppression of peritoneal macrophage MMP-9 expression was demonstrated after incubation by the peritoneal fluid derived from cells of women with endometriosis. PGE_2, elevated in the peritoneal fluid of women with endometriosis, effectively decreased peritoneal macrophage MMP-9 expression via the EP2/EP4 receptor-dependent PKA signaling pathway.

Reduced scavenger receptor expression also results in decreased macrophage phagocytic function. The known scavenger receptors participating in the macrophage phagocytosis include Class A and Class B scavenger receptors. The SR-BIII (known as CD36) expression and phagocytic ability were reduced in peritoneal macrophages derived from women with endometriosis compared with cells from disease-free women.[23] PGE_2 inhibited CD36-dependent phagocytosis of peritoneal macrophages via the EP2 receptor-dependent signaling pathway.[24] Ectopic expression of CD36 rescued the PGE_2-inhibited phagocytic ability of peritoneal macrophages. In an animal study, this inhibiting effect increased the number and size of endometriotic lesions *in vivo*. Similar expressions in the levels of annexin A2 in peritoneal macrophages were found in endometriosis.[25] Therefore, PGE_2-downregulated annexin A2, MMP-9, and CD36 expressions, which impairs the first and second mechanisms of phagocytic capability of macrophages, facilitates the survival of ectopic endometriotic tissues. Inhibiting PGE_2 signaling may help to develop novel strategies against endometriosis.

Leptin in endometriosis

Leptin is a multifunctional hormone with immunoregulatory, proinflammatory, and angiogenic effects and plays an important role in controlling reproductive functions and the development of endometriosis. Leptin is a prognostic factor of ovarian responsiveness after hyperstimulation. Elevated leptin concentrations may modulate embryo quality and be associated with poor outcome of *in vitro* fertilization/intracytoplasmic sperm injection cycle.[26] In our previous study,[27] there was a negative correlation between follicular fluid leptin and the flow index of ovarian stroma in the control group. Although there were nonsignificant increased serum leptin values in the endometriosis group, loss of the above correlation was found in women after laparoscopic ovarian cystectomy for endometriomas.

Leptin modulates inflammatory processes and immune responses.[28] Endometriosis is considered as a good example of a chronic inflammatory disease. Although direct association of leptin with pelvic inflammation and local immune response has not so far been elucidated, leptin may modulate the functions of peritoneal macrophages and aberrant gene expression of stromal cells, which might contribute to the pathophysiological process of endometriosis. Leptin mRNA was undetectable or was present in only a minute amount in eutopic endometrium.[29] By contrast, there was a marked increase in leptin mRNA and protein expression in ectopic endometriotic lesions of patients with endometriosis. The leptin receptor mRNA levels were suppressed, and were associated with the severity of endometriosis. Administration of leptin stimulated its own mRNA expression in ectopic endometriotic stromal cells but decreased leptin receptor mRNA expression. In addition, leptin significantly enhanced both eutopic and ectopic endometrial stromal cell proliferation. Hypoxic stress is associated with the growth and survival of endometriotic lesions. We also found the mRNA and protein levels of HIF-1α, a master transcription factor that mediates the hypoxic effect, were greater in ectopic endometriotic tissue.[30] HIF-1α could bind to a leptin promoter and then induce its activity after hypoxia treatment. Therefore, our data demonstrate that aberrant expression of leptin in ectopic endometriotic stromal cells is induced, at least in part, by elevated level of HIF-1α in these cells, and the mitogenic and auto-augmentation effects of leptin may contribute to the pathogenesis of endometriosis.

Leptin is a cytokine which promotes CD4+ T helper I cell proliferation, macrophage phagocytosis, and the secretion of inflammatory cytokines.[28] Concentration of peritoneal fluid leptin was found to be increased in patients with endometriosis.[31] Increased peritoneal fluid leptin in endometriosis patients may affect local inflammatory/immune reactions.[32] Peritoneal fluid leptin showed a negative correlation with IL-1β, a classical inflammatory cytokine, and interferon-gamma, a major T helper cell type I cytokine. It also showed the existence of a positive correlation with the percentage of $CD3_+$ pan-T cells and infiltration of $CD4_+$ T helper cells. Elevated leptin levels in peritoneal fluid may contribute to the pathological process of endometriosis through the activation of peritoneal macrophages. In our study, treatment of peritoneal macrophages with leptin induced COX-2 expression.[31] Increased production of $PGF_{2\alpha}$ by peritoneal macrophages was found after leptin stimulation in women with endometriosis. It affects the presence of symptoms, such as menstrual pain, of patients with endometriosis. These lines of evidence support that peritoneal fluid leptin may play a role in endometriosis-associated chronic pelvic pain, but is not involved in the pathophysiology of endometriosis-associated infertility.[33]

NF-κB in endometriosis

NF-κB, a proinflammatory transcription factor, plays an important role in both physiological immunity and pathological inflammation. Peritoneal oxidative stress, secondary to iron overload in the peritoneal cavity during retrograde menstruation, may impair cellular function by increasing proinflammatory gene expression through the regulation of NF-κB activation.[34] The activation of NF-κB involves macrophage migration inhibitory factor (MIF) gene expression in ectopic endometrial cells in response to IL-1β.[35] Therefore, various inflammatory factors activate NF-κB, and NF-κB further stimulates the synthesis of proinflammatory cytokines to form an autoregulatory loop. Constitutive activation of NF-κB, involving p65- and p50-containing dimmers, in endometriotic lesions and peritoneal macrophages from patients with endometriosis promotes inflammation, invasion, angiogenesis, and cell proliferation, and inhibits apoptosis of endometriotic cells.[36] The suppressed progesterone receptor (PR) and increased COX-2 levels is contributed to progesterone resistance and inflammation. PR-A and PR-B in the uterus play an anti-inflammatory role that antagonizes NF-κB activation and COX-2 expression.[37] PR levels, particularly the PR-B isoform, are significantly decreased in endometriotic stromal cells, which is associated with a high ERβ-to-ERα ratio.[38] This aberrant increased ERβ-to-ERα ratio in endometriotic stromal cells is also regulated by hypoxia,[10] which may be associated with elevated NF-κB activation. Hypoxia-induced HIF-1α expression is blocked by NF-κB inhibition under hypoxic conditions at the translational level in cancer cells.[39] The alteration of physiologic cyclic NF-κB-p65 subunit activation pattern in women with endometriosis is concurrent with progesterone resistance that could affect the implantation window and lead to infertility.[40] Some drugs with NF-κB inhibitory properties are effective in suppressing the above endometriosis-associated changes and reduce the expression of COX-2, which is a promising therapeutic potential for endometriosis.[41]

Peritoneal proinflammatory cytokines and chemokines in endometriosis

The levels of cytokines and chemokines, such as IL-1β, TNF-α, IL-6, and IL-8 secreted by peritoneal macrophages and ectopic endometriotic lesions were abnormally increased in peritoneal fluid.[42] These elevated peritoneal factors induce COX-2 expression and trigger PGE_2 production, which forms a positive feedback loop to enhance the development of endometriosis. IL-1β is a major proinflammatory cytokine that is overproduced by endometriosis-derived peritoneal macrophages and is elevated in the peritoneal fluid of patients with endometriosis. As we have discussed in the previous sections, although IL-1β failed to induce COX-2 expression in peritoneal macrophages from women with endometriosis, IL-1β enhances significant COX-2 expression in peritoneal macrophages from disease-free women, and eutopic and ectopic endometrial stromal cells.[4,5] Especially, IL-1β upregulates both COX-2 mRNA stability and promoter activity in ectopic endometriotic stromal cells. Similarly, IL-1β-induced COX-2 expression upregulates cell migration and the invasion ability of endometrioma-derived ectopic endometrial mesenchymal stem cells.[43]

TNF-α mediates inflammation by inducing inflammatory mediators IL-6, IL-8, granulocyte macrophage–colony-stimulating factor, and monocyte chemoattractant protein (MCP)-1 secretion in the endometrium.[44] TNF-α can enhance endometrial epithelial cell proliferation in the ectopic environment of women with endometriosis. TNF-α-induced COX-2 overexpression in the eutopic endometrium of women with endometriosis is through NF-κB activation, which may play a critical role in the pathophysiology of endometriosis formation.[45] Prolonged stimulation with TNF-α will induce partial methylation in the promoter region of PR-B with concomitant reduction of PR-B expression in immortalized epithelial-like endometriotic cells that is contributed to progesterone resistance.[46]

TGF-β may be involved in the initiation of menstruation via vasoconstriction, scarless postmenstrual regeneration of endometrium, and establishment and/or maintenance of endometriosis.[47] The levels of TGF-β, secreted by the endometrial cells and macrophages, are increased in the peritoneal fluid of endometriosis. TGF-β significantly induces the expression of macrophage colony-stimulating factor receptor encoded by the c-fms gene and enhances endometrial epithelial cell transmesothelial invasion.[48] However, until now, more studies regarding TGF-β in the pathogenesis of endometriosis are needed.

The activation of protease-activated receptor 2 (PAR2), as an important mediator of inflammation activated by enzymes from neutrophils and mast cells, stimulates proliferation and IL-6 and IL-8 secretion of endometriotic stromal cells through the involvement of MAPKs.[49] IL-6 expression is highly associated with angiogenesis and migration. Inflammatory stimuli, IL-1β and TNF-α, first induce activin-A protein expression in endometrioma stromal cells. It further increases the expression of IL-6 and PAR-2 mRNA expression, and enhances the proliferation of endometrioma stromal cells.[50] Additionally, TGF-β1 directly increases the expression of PAR-2 and increases IL-6 secretion in endometriotic stromal cells.[51] IL-8 induces chemotaxis of neutrophils and is also a potent angiogenic agent. IL-8 may act as an autocrine growth factor in the endometrium promoting the vicious circle of endometrial cell attachment, cell growth, and further self-stimulation in the pathogenesis of endometriosis.[52] In contrast to higher levels of leptin in early stages of endometriosis, elevated peritoneal fluid IL-8 levels in women with endometriosis is correlated with the severity of the disease.[53] Sex steroids stimulate the chemokine IL-8 expression in endometrial endothelial cells from women with endometriosis.[54] IL-8 increases endometrial stromal cell metalloproteinase activity and invasive capability that degrade extracellular matrix to help the endometrial cells invade the peritoneum and to develop an endometriotic lesion.[55]

Macrophage-derived factors, such as migration inhibitory factor (MIF) and MCP-1, can promote angiogenesis in the development of endometriosis.[56] MIF, with its potent proinflammatory and angiogenic properties, is markedly elevated in lesions from the early stage of endometriosis, particularly in endometrial implants with highly vascularized, active forms of the disease.[57] The overproduction of IL-1β in endometriosis upregulates the expression of MIF in endometrial stromal cells and ectopic endometrial cells via NF-κB nuclear translocation that shows the immunomodulatory properties of MIF in endometriosis-associated inflammation, ectopic cell growth, angiogenesis, and tissue remodeling.[35,58] MIF acts directly on ectopic endometrial cells to stimulate the synthesis of COX-2 and the production of PGE_2 that triggers the coordinate activation of multiple enzymes in the PGE_2 biosynthesis pathway.[59] MIF, interacting with ectopic endometrial cells, further markedly upregulates the secretion of major angiogenic factors including vascular endothelial growth factor (VEGF), IL-8, and MCP-1 expression via CD44, CD74, and MAPK signaling pathways.[60]

The peritoneal fluid level of MCP-1 is positively correlated with leptin level, especially in the early stages of endometriosis, which may play a role in the pathogenesis of endometriosis-associated infertility.[61] The levels of VEGF are significantly increased in the endometrial stromal cells of women with endometriosis in the presence of MCP-1 chemokine and/or estradiol.[62] In addition, the stimulation of MCP-1 expression by sex hormones in the endometrial endothelial cells in women with endometriosis may involve recruiting mononuclear cells and contributing to the inflammatory aspect of endometriosis.[63]

Systemic inflammation biomarkers in endometriosis

Endometriosis may be asymptomatic or associated with nonspecific symptoms. This causes delays in the early diagnosis and treatment time of endometriosis by about 8–12 years.[64] Conventional laparoscopic surgery, even mini-laparoscopic surgical technique, is indicated for women with endometriosis because it is less invasive and causes less postoperative pain.[65,66] Although laparoscopy is the gold choice for the definitive diagnosis of endometriosis, noninvasive methods are needed for preoperative and follow-up evaluation. Ultrasound is an adequate noninvasive approach to detect ovarian endometrioma,[67] but is hard to rule out peritoneal endometriosis and adhesion. A reliable single biomarker or panel of biomarkers may help to diagnose endometriosis earlier and reduce unnecessary operation.[68] However, to date, it still cannot identify suitable peripheral biomarkers for clinical use.[69]

There are different kinds of peripheral biomarkers, including cytokines, antibodies, cell populations, glycoproteins, cell adhesion, growth factors, proteomics, hormones, angiogenesis, and apoptosis, proposed for clinical use in the diagnosis of endometriosis. There has been various conflicting differences among these studies. These conflicting results may be due to patient numbers, the phase of menstrual cycle, the type of endometriosis, and selection of an appropriate control group.[69] For example, our cytokine study found elevated levels of IL-4 in the serum of women with endometriosis, and those were reduced to normal after medical and surgical treatment.[70] This suggests that altered cytokine secretion of T-helper cell type 1 and 2 plays a role in immunologic responses to endometriosis. But another study showed no changes in serum IL-2, IL-4, or IL-10 levels.[71] Conflicting results were also found in biomarkers of cell adhesion, which is important in cell–cell interaction. Our previous study showed significantly elevated serum levels of soluble intercellular adhesion molecule-1 in women with

endometriosis,[72] whereas another study failed to find the difference.[73] Furthermore, leptin plays a role in inflammatory processes and immune responses.[28] Leptin links proinflammatory T helper 1 immune responses, and induces the expression of intercellular adhesion molecule 1 and matrix metalloproteinases. Matarese et al[74] found that serum and peritoneal fluid leptin levels were significantly increased in patients with endometriosis. However, our study showed elevated leptin concentrations in peritoneal fluid of endometriosis,[31] but without significant change in serum levels.[27]

The most commonly used biomarker for the detection of endometriosis is cancer antigen CA125, which is elevated in the serum of patients with endometriosis but lacks sensitivity and specificity. It is necessary to pay attention to the elevated fluctuations in CA125 during menstruation to prevent unnecessary diagnostic procedures on unaffected women with a similar presentation as women with endometriosis.[75] Serum CA125 test alone may be beneficial in diagnosing advanced stages of endometriosis and in monitoring treatment.[76,77] Preoperative CA125 levels higher than 65 IU/mL helps to decide preoperative bowel preparation for patients with a high risk of severe pelvic adhesion, with a sensitivity of 76%, a specificity of 71%, a positive predictive value of 76%, and a negative predictive value of 93.2%.[78] In addition, a panel of biomarkers may be more reliable with increased sensitivity and specificity in the screening for endometriosis.[68] Mihalyi et al[79] reported that a panel of six selected biomarkers (plasma concentrations of IL-6, IL-8, TNF-α, high-sensitivity C-reactive protein, and cancer antigens CA125 and CA19-9) during the secretory phase or during menstruation can diagnose both minimal-mild and moderate-severe endometriosis with high sensitivity and clinically acceptable specificity.[79] The other serum biomarker combination of MCP-1, MIF, leptin, and CA-125 improved the diagnostic capability to 73% of patients, with 94% overall accuracy.[80,81]

Recently, microRNAs appear to be potent regulators of gene expression at the post-transcriptional level in the development of endometriosis, including those associated with hypoxia, inflammation, proliferation, angiogenesis, apoptosis, and extracellular matrix remodeling.[3] Endometriosis-associated microRNAs may be secreted from tissues into the bloodstream and be identified in the serum. Thus, they may be attractive candidates of novel diagnostic biomarkers for endometriosis. For example, in our recent study, elevation of miR20a in endometriotic stromal cells is secondary to HIF-1α-dependent transcriptional upregulation.[82] It induces the expression of several MAPK-regulated angiogenic genes and enhances PGE_2-induced FGF-9 expression. Thus, miR20a may be an important modulator in the development of endometriosis and can be considered as a potential biomarker for the diagnosis of endometriosis. The use of technologies, including genomics, proteomics, and metabolomics, in the future may help to identify potential novel biomarkers with high sensitivity and specificity for clinical use in the differential diagnosis of endometriosis and as a noninvasive diagnostic test to shorten the period between onset of symptoms and diagnosis of endometriosis.

Conclusion

Inflammatory factors play an important role in the pathophysiology of endometriosis, which will interact locally and systemically to affect symptoms and may be biomarkers for diagnosis or the target of treatment. Although treatment with COX inhibitors can improve the symptoms and signs of women with endometriosis, short half-life and unfavorable side effects affect the compliance of the treatment. Endometriosis-focused basic research can help to provide better diagnosis, treatment, and a way of prevention. However, there is disappointment with the slow progress of these developments associated with the opaqueness of clinical endometriosis trials.[83] Further studies are needed to clarify the potential mechanism of inflammation in the development of endometriosis and to develop a suitable preventive or therapeutic strategy for this disease.

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References

1. Olive DL, Schwartz LB. Endometriosis. *N Engl J Med*. 1993;328:1759–1769.
2. Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol*. 1927;14:442–469.
3. Teague EM, Print CG, Hull ML. The role of microRNAs in endometriosis and associated reproductive conditions. *Hum Reprod Update*. 2010;16:142–165.
4. Wu MH, Sun HS, Lin CC, et al. Distinct mechanisms regulate cyclooxygenase-1 and -2 in peritoneal macrophages of women with and without endometriosis. *Mol Hum Reprod*. 2002;8:1103–1110.
5. Wu MH, Wang CA, Lin CC, et al. Distinct regulation of cyclooxygenase-2 by interleukin-1beta in normal and endometriotic stromal cells. *J Clin Endocrinol Metab*. 2005;90:286–295.
6. Banu SK, Lee J, Speights Jr VO, Starzinski-Powitz A, Arosh JA. Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms. *Endocrinology*. 2008;149:1180–1189.
7. Bulun SE. Endometriosis. *N Engl J Med*. 2009;360:268–279.
8. Lin SC, Li YH, Wu MH, et al. Suppression of COUP-TFII by proinflammatory cytokines contributes to the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. 2014;99:e427–437.
9. Tafani M, Russo A, Di Vito M, et al. Up-regulation of pro-inflammatory genes as adaptation to hypoxia in MCF-7 cells and in human mammary invasive carcinoma microenvironment. *Cancer Sci*. 2010;101:1014–1023.
10. Wu MH, Lu CW, Chang FM, Tsai SJ. Estrogen receptor expression affected by hypoxia inducible factor-1alpha in stromal cells from patients with endometriosis. *Taiwan J Obstet Gynecol*. 2012;51:50–54.
11. Wu MH, Lu CW, Chuang PC, Tsai SJ. Prostaglandin E2: the master of endometriosis? *Exp Biol Med (Maywood)*. 2010;235:668–677.
12. Wu MH, Shoji Y, Chuang PC, Tsai SJ. Endometriosis: disease pathophysiology and the role of prostaglandins. *Expert Rev Mol Med*. 2007;9:1–20.
13. Tsai SJ, Wu MH, Lin CC, Sun HS, Chen HM. Regulation of steroidogenic acute regulatory protein expression and progesterone production in endometriotic stromal cells. *J Clin Endocrinol Metab*. 2001;86:5765–5773.
14. Sun HS, Hsiao KY, Hsu CC, Wu MH, Tsai SJ. Transactivation of steroidogenic acute regulatory protein in human endometriotic stromal cells is mediated by the prostaglandin EP2 receptor. *Endocrinology*. 2003;144:3934–3942.
15. Hsu CC, Lu CW, Huang BM, Wu MH, Tsai SJ. Cyclic adenosine 3',5'-monophosphate response element-binding protein and CCAAT/enhancer-binding protein mediate prostaglandin E2-induced steroidogenic acute regulatory protein expression in endometriotic stromal cells. *Am J Pathol*. 2008;173:433–441.
16. Yang S, Fang Z, Suzuki T, et al. Regulation of aromatase P450 expression in endometriotic and endometrial stromal cells by CCAAT/enhancer binding proteins (C/EBPs): decreased C/EBPbeta in endometriosis is associated with overexpression of aromatase. *J Clin Endocrinol Metab*. 2002;87:2336–2345.
17. Lee J, Banu SK, Subbarao T, Starzinski-Powitz A, Arosh JA. Selective inhibition of prostaglandin E2 receptors EP2 and EP4 inhibits invasion of human immortalized endometriotic epithelial and stromal cells through suppression of metalloproteinases. *Mol Cell Endocrinol*. 2011;332:306–313.
18. Wing LY, Chuang PC, Wu MH, Chen HM, Tsai SJ. Expression and mitogenic effect of fibroblast growth factor-9 in human endometriotic implant is regulated by aberrant production of estrogen. *J Clin Endocrinol Metab*. 2003;88:5547–5554.
19. Tsai SJ, Wu MH, Chen HM, Chuang PC, Wing LY. Fibroblast growth factor-9 is an endometrial stromal growth factor. *Endocrinology*. 2002;143:2715–2721.
20. Wing LY, Chen HM, Chuang PC, Wu MH, Tsai SJ. The mammalian target of rapamycin-p70 ribosomal S6 kinase but not phosphatidylinositol 3-kinase-Akt signaling is responsible for fibroblast growth factor-9-induced cell proliferation. *J Biol Chem*. 2005;280:19937–19947.
21. Chuang PC, Sun HS, Chen TM, Tsai SJ. Prostaglandin E2 induces fibroblast growth factor 9 via EP3-dependent protein kinase C delta and Elk-1 signaling. *Mol Cell Biol*. 2006;26:8281–8292.

22. Wu MH, Shoji Y, Wu MC, et al. Suppression of matrix metalloproteinase-9 by prostaglandin E(2) in peritoneal macrophage is associated with severity of endometriosis. *Am J Pathol*. 2005;167:1061–1069.
23. Chuang PC, Lin YJ, Wu MH, et al. Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. *Am J Pathol*. 2010;176:850–860.
24. Chuang PC, Wu MH, Shoji Y, Tsai SJ. Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. *J Pathol*. 2009;219:232–241.
25. Wu MH, Chuang PC, Lin YJ, Tsai SJ. Suppression of annexin A2 by prostaglandin E(2) impairs phagocytic ability of peritoneal macrophages in women with endometriosis. *Hum Reprod*. 2013;28:1045–1053.
26. Anifandis G, Koutselini E, Stefanidis I, et al. Serum and follicular fluid leptin levels are correlated with human embryo quality. *Reproduction*. 2005;130:917–921.
27. Wu MH, Tsai SJ, Pan HA, Hsiao KY, Chang FM. Three-dimensional power Doppler imaging of ovarian stromal blood flow in women with endometriosis undergoing in vitro fertilization. *Ultrasound Obstet Gynecol*. 2003;21:480–485.
28. La Cava A, Alviggi C, Matarese G. Unraveling the multiple roles of leptin in inflammation and autoimmunity. *J Mol Med*. 2004;82:4–11.
29. Wu MH, Chuang PC, Chen HM, Lin CC, Tsai SJ. Increased leptin expression in endometriosis cells is associated with endometrial stromal cell proliferation and leptin gene up-regulation. *Mol Hum Reprod*. 2002;8:456–464.
30. Wu MH, Chen KF, Lin SC, Lgu CW, Tsai SJ. Aberrant expression of leptin in human endometriotic stromal cells is induced by elevated levels of hypoxia inducible factor-1alpha. *Am J Pathol*. 2007;170:590–598.
31. Wu MH, Huang MF, Chang FM, Tsai SJ. Leptin on peritoneal macrophages of patients with endometriosis. *Am J Reprod Immunol*. 2010;63:214–221.
32. Milewski L, Barcz E, Dziunycz P, et al. Association of leptin with inflammatory cytokines and lymphocyte subpopulations in peritoneal fluid of patients with endometriosis. *J Reprod Immunol*. 2008;79:111–117.
33. Bedaiwy MA, Falcone T, Goldberg JM, et al. Peritoneal fluid leptin is associated with chronic pelvic pain but not infertility in endometriosis patients. *Hum Reprod*. 2006;21:788–791.
34. Lousse JC, Van Langendonckt A, Defrere S, et al. Peritoneal endometriosis is an inflammatory disease. *Front Biosci (Elite Ed)*. 2012;4:23–40.
35. Veillat V, Lavoie CH, Metz CN, et al. Involvement of nuclear factor-kappaB in macrophage migration inhibitory factor gene transcription up-regulation induced by interleukin-1 beta in ectopic endometrial cells. *Fertil Steril*. 2009;91:2148–2156.
36. Gonzalez-Ramos R, Van Langendonckt A, Defrere S, et al. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. *Fertil Steril*. 2010;94:1985–1994.
37. Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. *Endocrinology*. 2008;149:1190–1204.
38. Bulun SE, Cheng YH, Pavone ME, et al. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin Reprod Med*. 2010;28:36–43.
39. Nam SY, Ko YS, Jung J, et al. A hypoxia-dependent upregulation of hypoxia-inducible factor-1 by nuclear factor-kappaB promotes gastric tumour growth and angiogenesis. *Br J Cancer*. 2011;104:166–174.
40. Gonzalez-Ramos R, Rocco J, Rojas C, et al. Physiologic activation of nuclear factor kappa-B in the endometrium during the menstrual cycle is altered in endometriosis patients. *Fertil Steril*. 2012;97:645–651.
41. Zheng Y, Liu X, Guo SW. Therapeutic potential of andrographolide for treating endometriosis. *Hum Reprod*. 2012;27:1300–1313.
42. Wu MY, Ho HN. The role of cytokines in endometriosis. *Am J Reprod Immunol*. 2003;49:285–296.
43. Kao AP, Wang KH, Long CY, et al. Interleukin-1beta induces cyclooxygenase-2 expression and promotes the invasive ability of human mesenchymal stem cells derived from ovarian endometrioma. *Fertil Steril*. 2011;96, 678–684 e1.
44. Grund EM, Kagan D, Tran CA, et al. Tumor necrosis factor-alpha regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase kinase, p38, and nuclear factor kappaB in human endometriotic epithelial cells. *Mol Pharmacol*. 2008;73:1394–1404.
45. Kim YA, Kim JY, Kim MR, et al. Tumor necrosis factor-alpha-induced cyclooxygenase-2 overexpression in eutopic endometrium of women with endometriosis by stromal cell culture through nuclear factor-kappaB activation. *J Reprod Med*. 2009;54:625–630.
46. Wu Y, Starzinski-Powitz A, Guo SW. Prolonged stimulation with tumor necrosis factor-alpha induced partial methylation at PR-B promoter in immortalized epithelial-like endometriotic cells. *Fertil Steril*. 2008;90:234–237.
47. Omwandho CO, Konrad L, Halis G, Oehmke F, Tinneberg HR. Role of TGF-betas in normal human endometrium and endometriosis. *Hum Reprod*. 2010;25:101–109.
48. Liu YG, Tekmal RR, Binkley PA, et al. Induction of endometrial epithelial cell invasion and c-fms expression by transforming growth factor beta. *Mol Hum Reprod*. 2009;15:665–673.
49. Hirota Y, Osuga Y, Hirata T, et al. Activation of protease-activated receptor 2 stimulates proliferation and interleukin (IL)-6 and IL-8 secretion of endometriotic stromal cells. *Hum Reprod*. 2005;20:3547–3553.
50. Yoshino O, Izumi G, Shi J, et al. Activin-A is induced by interleukin-1beta and tumor necrosis factor-alpha and enhances the mRNA expression of interleukin-6 and protease-activated receptor-2 and proliferation of stromal cells from endometrioma. *Fertil Steril*. 2011;96:118–121.
51. Saito A, Osuga Y, Yoshino O, et al. TGF-beta1 induces proteinase-activated receptor 2 (PAR2) expression in endometriotic stromal cells and stimulates PAR2 activation-induced secretion of IL-6. *Hum Reprod*. 2011;26:1892–1898.
52. Arici A. Local cytokines in endometrial tissue: the role of interleukin-8 in the pathogenesis of endometriosis. *Ann N Y Acad Sci*. 2002;955:101–109. discussion 118, 396–406.
53. Malhotra N, Karmakar D, Tripathi V, Luthra K, Kumar S. Correlation of angiogenic cytokines-leptin and IL-8 in stage, type and presentation of endometriosis. *Gynecol Endocrinol*. 2012;28:224–227.
54. Luk J, Seval Y, Kayisli UA, et al. Regulation of interleukin-8 expression in human endometrial endothelial cells: a potential mechanism for the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. 2005;90:1805–1811.
55. Mulayim N, Savlu A, Guzeloglu–Kayisli O, Kayisli UA, Arici A. Regulation of endometrial stromal cell matrix metalloproteinase activity and invasiveness by interleukin-8. *Fertil Steril*. 2004;81(Suppl 1):904–911.
56. Akoum A, Kong J, Metz C, Beaumont MC. Spontaneous and stimulated secretion of monocyte chemotactic protein-1 and macrophage migration inhibitory factor by peritoneal macrophages in women with and without endometriosis. *Fertil Steril*. 2002;77:989–994.
57. Kats R, Metz CN, Akoum A. Macrophage migration inhibitory factor is markedly expressed in active and early-stage endometriotic lesions. *J Clin Endocrinol Metab*. 2002;87:883–889.
58. Cao WG, Morin M, Metz C, Maheux R, Akoum A. Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukin 1, beta involving the nuclear transcription factor NFkappaB. *Biol Reprod*. 2005;73:565–570.
59. Carli C, Metz CN, Al-Abed Y, Naccache PH, Akoum A. Up-regulation of cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic cells by macrophage migration inhibitory factor: involvement of novel kinase signaling pathways. *Endocrinology*. 2009;150:3128–3137.
60. Veillat V, Carli C, Metz CN, et al. Macrophage migration inhibitory factor elicits an angiogenic phenotype in human ectopic endometrial cells and triggers the production of major angiogenic factors via CD44, CD74, and MAPK signaling pathways. *J Clin Endocrinol Metab*. 2010;95:e403–412.
61. Tao Y, Zhang Q, Huang W, et al. The peritoneal leptin, MCP-1 and TNF-alpha in the pathogenesis of endometriosis-associated infertility. *Am J Reprod Immunol*. 2011;65:403–406.
62. Lin J, Gu Y. Effect of monocyte chemoattractant protein-1 and estradiol on the secretion of vascular endothelial growth factor in endometrial stromal cells in vitro. *Fertil Steril*. 2005;84:1793–1796.
63. Luk J, Seval Y, Ulukus M, et al. Regulation of monocyte chemotactic protein-1 expression in human endometrial endothelial cells by sex steroids: a potential mechanism for leukocyte recruitment in endometriosis. *Reprod Sci*. 2010;17:278–287.
64. Hadfield R, Mardon H, Barlow D, Kennedy S. Delay in the diagnosis of endometriosis: a survey of women from the USA and the UK. *Hum Reprod*. 1996;11:878–880.
65. Nomura H, Okuda K, Saito N, et al. Mini-laparoscopic surgery versus conventional laparoscopic surgery for patients with endometriosis. *Gynecol Minim Invasive Ther*. 2013;2:85–88.
66. Takebayashi A, Shimizu Y, Takahashi A, et al. Comparison of the outcome of in vitro fertilization after laparoscopic laser ablation surgery versus laparoscopic cystectomy for endometrioma. *Gynecol Minim Invasive Ther*. 2013;2:27–29.
67. Wu MH, Cheng CY, Chang FM. Ultrasonographic assessment of ovarian endometrioma. *J Med Ultrasound*. 2008;16:241–248.
68. May KE, Conduit-Hulbert SA, Villar J, et al. Peripheral biomarkers of endometriosis: a systematic review. *Hum Reprod Update*. 2010;16:651–674.
69. Yen CF, Wang HS, Lee CL, Liao SK. Roles of integrin-linked kinase in cell signaling and its perspectives as a therapeutic target. *Gynecol Minim Invasive Ther*. 2014;3:67–72.
70. Hsu CC, Yang BC, Wu MH, Huang KE. Enhanced interleukin-4 expression in patients with endometriosis. *Fertil Steril*. 1997;67:1059–1064.
71. Hassa H, Tanir HM, Tekin B, Kirilmaz SD, Sahin Mutlu F. Cytokine and immune cell levels in peritoneal fluid and peripheral blood of women with early- and late-staged endometriosis. *Arch Gynecol Obstet*. 2009;279:891–895.
72. Wu MH, Yang BC, Hsu CC, Lee YC, Huang KE. The expression of soluble intercellular adhesion molecule-1 in endometriosis. *Fertil Steril*. 1998;70:1139–1142.
73. De Placido G, Alviggi C, Di Palma G, et al. Serum concentrations of soluble human leukocyte class I antigens and of the soluble intercellular adhesion molecule-1 in endometriosis: relationship with stage and non-pigmented peritoneal lesions. *Hum Reprod*. 1998;13:3206–3210.
74. Matarese G, Alviggi C, Sanna V, et al. Increased leptin levels in serum and peritoneal fluid of patients with pelvic endometriosis. *J Clin Endocrinol Metab*. 2000;85:2483–2487.
75. Kafali H, Artuc H, Demir N. Use of CA125 fluctuation during the menstrual cycle as a tool in the clinical diagnosis of endometriosis; a preliminary report. *Eur J Obstet Gynecol Reprod Biol*. 2004;116:85–88.
76. Maiorana A, Cicerone C, Niceta M, Alio L. Evaluation of serum CA 125 levels in patients with pelvic pain related to endometriosis. *Int J Biol Markers*. 2007;22:200–202.

77. Matalliotakis IM, Arici A, Goumenou AG, et al. Comparison of the effects of leuprorelin acetate and danazol treatments on serum CA-125 levels in women with endometriosis. *Int J Fertil Womens Med*. 2004;49:75–78.
78. Cheng YM, Wang ST, Chou CY. Serum CA-125 in preoperative patients at high risk for endometriosis. *Obstet Gynecol*. 2002;99:375–380.
79. Mihalyi A, Gevaert O, Kyama CM, et al. Non-invasive diagnosis of endometriosis based on a combined analysis of six plasma biomarkers. *Hum Reprod*. 2010;25:654–664.
80. Seeber B, Sammel MD, Fan X, et al. Panel of markers can accurately predict endometriosis in a subset of patients. *Fertil Steril*. 2008;89:1073–1081.
81. Seeber B, Sammel MD, Fan X, et al. Proteomic analysis of serum yields six candidate proteins that are differentially regulated in a subset of women with endometriosis. *Fertil Steril*. 2010;93:2137–2144.
82. Lin SC, Wang CC, Wu MH, et al. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. *J Clin Endocrinol Metab*. 2012;97:e1515–1523.
83. Guo SW. Keep the pressure on for more transparency of clinical trials on endometriosis. *Gynecol Minim Invasive Ther*. 2013;2:73–74.