

Chapter 5

Oxidative Stress and the Pathogenesis of Endometriosis

Introduction

Endometriosis is a complicated disease with an ambiguous etiology. It affects 10% of all women in the general population and is an important causative factor in 40% of all cases of female infertility and in 60% of all cases of female chronic pelvic pain.¹

Endometriosis occurs in women of reproductive age and in postmenopausal women secondary to the use of exogenous estrogen. Generally, the disease is characterized by the presence of endometrial glands and stroma outside the endometrium and uterine muscle. It is diagnosed primarily by laparoscopy, the current gold standard. Although laparoscopy attempts to diagnose endometriotic lesions at various stages, the procedure is unable to detect subtle endometriotic lesions and therefore cannot render an early diagnosis.² As a result, the current prevalence of endometriosis is most likely an underestimation. A nonsurgical method of diagnosing endometriosis would have many benefits, including the possibility of early diagnosis, sensitivity to a wider range of endometriotic stages, and the provision of a non-invasive option for treatment or prevention. The most anticipated noninvasive option for diagnosis is the analysis of serum and peritoneal markers. Of particular interest is the identification of markers that indicate elevated levels of oxidative stress (OS) and altered immune function in the follicular and peritoneal environments.

The relationship between endometriosis and reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals is currently being evaluated as a possible source for serum and peritoneal fluid markers. Damage caused by ROS produces an inflammatory cascade that gives rise to a plethora of serum and peritoneal fluid markers such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), interleukin 8

(IL-8), interleukin 1 (IL-1) beta, and serum paraoxonase-1 (PON-1). In this chapter, we will further discuss the influence of ROS on endometriosis as well as summarize what the latest research shows on the use of these markers as a noninvasive option for diagnosing endometriosis.

Endometriosis: Theories of Etiology

Although the etiology of endometriosis is uncertain, four main hypotheses have been circulated as plausible causes: 1) Sampson's theory of retrograde menstruation, 2) celomic metaplasia and induction theories (an extension of the celomic metaplasia theory), 3) the embryonic rest theory, and 4) lymphatic and vascular metastasis theories.³ The most widely accepted hypothesis is the theory of implantation, also known as Sampson's theory. It proposes that the disorder arises due to retrograde menstruation of endometrial tissue into the peritoneal cavity through patent fallopian tubes.⁴ Women with endometriosis have been found to have larger amounts of menstrual reflux of both blood and endometrial tissue than women without the disorder.⁵ The anatomical distribution of endometriotic lesions supports the idea of retrograde reflux and subsequent peritoneal implantation. Flow of peritoneal fluid is arrested or repetitive in the peritoneal cavity in four main places: the pouch of Douglas at the rectosigmoid level, the cecum and ileocecum junction, the superior portion of the sigmoid mesocolon, and the right paracolic gutter.⁶ These areas are consequently the main areas where ectopic endometriotic lesions are generally found.⁶

A number of animal studies support Sampson's theory, including one in which menstrual endometrium was injected into the retroperitoneal space of four baboons, all of which subsequently developed endometriosis.⁷ In 1950 a study conducted by TeLinde and Scott also supported

Sampson's theory. The results of the study demonstrated that 50% of monkeys developed endometriosis after their menstrual flow was diverted into the peritoneal cavity.⁸

Sampson's theory provides and explains three requirements for the establishment of endometriosis.⁴ The first requirement is retrograde menstruation through the fallopian tubes. In fact, 76% to 90% of all women with patent fallopian tubes have some degree of retrograde menstruation;³ however, not all develop endometriosis. The second requirement is the presence of viable refluxed cells in the peritoneal cavity. Mungyer *et al* found endometrial cells in peritoneal fluid of women with endometriosis after performing endometrial lavage, and the cells stayed viable in culture for up to two months.⁹ The third requirement necessitates that refluxed endometrial cells adhere to the peritoneal epithelium where they implant and proliferate.³ In order for the implant to survive, a blood supply must be established. Oxidative stress contributes to angiogenesis in ectopic endometrial implants by increasing VEGF production.¹⁰ This effect is partially mediated by glycodelin—a glycoprotein with increased expression caused by OS. Glycodelin acts as an autocrine factor that augments VEGF expression within ectopic endometrial tissue.¹⁰

Some researchers believe that endometriosis also has a genetic component. This belief is based on the fact that women with first-degree relatives who have endometriosis have a high incidence of the disease themselves. This is especially true with maternal inheritance patterns. One of every 10 women with severe endometriosis has a first-degree relative with clinical manifestation of endometriosis.

Investigating Role of Oxidative Stress in Endometriosis

Oxidative stress is a cause of female infertility as it affects ovulation, fertilization, and embryo development and implantation. It occurs when there is an imbalance between levels of oxidants and antioxidants. Usually, OS is a product of ROS overproduction rather than low antioxidant levels, both of which have been found in women with endometriosis.^{11, 12} Elevated ROS levels in oviductal fluid might have adverse effects, impairing oocyte and spermatozoa viability and fertilization and embryo transport within the oviduct. Oxidative stress is known to occur when neutrophils and macrophages become activated—as in pro-inflammatory states, for example—which further amplifies ROS production in the oviductal fluid.¹³ A substantial increase in ROS produc-

tion might result in oxidative damage to sperm plasma and acrosomal membranes, impairing their motility and hindering the ability of spermatozoa to bind to and penetrate an oocyte. DNA damage secondary to OS may lead to failed fertilization, reduced embryo quality, failure of pregnancy, and spontaneous abortion.

To date, no true cause-and-effect relationship has been established between OS and endometriosis. Two studies determined a positive association,^{11, 14} whereas others have reported no association.^{15, 16} It is difficult to establish a definitive conclusion in regards to the association between OS and endometriosis based on these studies. Jackson *et al* investigated this relationship by evaluating women undergoing laparoscopy for suspected endometriosis. The serum from these women was measured for four biomarkers of OS and antioxidant levels. The biomarkers that were selected were presumed to measure the main targets in the biochemical pathways involved in OS: (i) thiobarbituric acid-reacting substances (TBARS), (ii) 8-F2-isoprostane, (iii) fat-soluble antioxidants, and (iv) paraoxonase activity. The study adjusted for the following potential confounding factors: age, body mass index, smoking status, hormone use in the past 12 months, gravidity, serum vitamin E levels, and serum estradiol and total serum lipid levels. The authors reported a weak association between TBARS, a measure of overall OS, and endometriosis. However, at the time of serum collection, no agent was added to prevent auto-oxidation (AO) from occurring. This may have altered the levels of oxidants and AO. Furthermore, the type of biospecimen used in this study (serum versus peritoneal fluid) served to further limit the study.

In the peritoneal fluid, OS is initiated in the inflammatory cells with cellular debris acting as a substrate, and products of OS are transported to the serum. Peritoneal fluid from patients with endometriosis has been shown to exhibit inadequate antioxidant defenses, including low total antioxidant capacity (TAC) and significantly reduced levels of individual antioxidant enzymes such as superoxide dismutase (SOD).^{11, 17} Statistically, it has been found that infertile women with endometriosis have lower concentrations of SOD than fertile controls. Despite the various associations between OS in peritoneal fluid and endometriosis, many studies have failed to demonstrate a difference in ROS, nitric oxide (NO), lipid peroxide, and antioxidant levels in the peritoneal fluid of women with endometriosis and fertile controls.^{16, 18} This may be due to the fact that only stable enzymes and byproducts of OS have been observed at the time when endometriosis is diagnosed. Another possible explanation might be that

focal OS is not great enough to increase total ROS levels in peritoneal fluid. On the other hand, a study done by Murphy *et al* demonstrated that peritoneal fluid has significantly lower levels of vitamin E, an antioxidant, than the serum. This suggests that the peritoneal cavity has less AO protection, resulting in more susceptibility to OS. This belief can be further supported by a 2007 study by Mier-Cabrera *et al*. They reported the effect of vitamin C and E supplementation on peripheral OS markers such as plasma levels of malondialdehyde (MDA) and lipid hydroperoxides (LOOHs) in women with endometriosis. The authors found that levels of MDA and LOOHs were significantly lower following therapeutic intervention with vitamin C and E supplementation, although supplementation with vitamin C and E did not have any effect on pregnancy rates after therapy.¹⁹

Researchers have noted that the mitochondrial gene in endometriotic tissue becomes rearranged after its deletion. Ectopic and eutopic endometrium have exhibited differential gene expression, including 904 differentially expressed genes and differential expression of the glutathione-S-transferase gene family, which is involved in glutathione antioxidant metabolism. Cell proliferation and angiogenesis are cellular responses to OS that also may be determined by differential gene expression.²⁰ Also, free radicals such as NO and hydrogen peroxide activate transcriptional factor, nuclear factor κ B, and activator protein1, which mediate the expression of cell adhesion molecules involved in cell-cell and cell-tissue binding.²¹ Expression of cell adhesion molecules is a very important phenomenon for the initiation and progression of the adhesion process between ectopic endometrial and mesothelium tissue.

ROS and Antioxidants

Reactive oxygen species such as superoxide anion, hydrogen peroxide, and the hydroxyl radical are types of oxidants that can attack biomolecules such as lipids, proteins, and nucleic acids. Antioxidants, on the other hand, can help prevent that damage. There are two types: enzymatic antioxidants such as catalase and SOD and non-enzymatic antioxidants such as vitamin C and glutathione.²² Antioxidants stabilize ROS by donating electrons to oxygen-based free radicals.²³ In the follicular fluid of healthy patients, antioxidants protect oocytes from ROS damage. In endometriosis, ROS production increases due to stimulated peritoneal fluid mononuclear cells and macrophages.²⁴ In some instances, the peritoneal fluid in women with endometriosis contains increased concentrations of NO and inducible nitric oxide synthase (iNOS)

activity. Abnormally elevated NO concentrations, as generated by activated macrophages, can counteract fertility by altering: the composition of the peritoneal fluid environment, processes of ovulation, gamete transport, sperm oocyte interaction, fertilization, and early embryonic development.²⁵ It has been shown that the peritoneal fluid nitrite and nitrate content is higher in women with endometriosis. Interferon-alpha and interferon-gamma with lipopolysaccharide (LPS) can activate macrophages in the endometriotic peritoneal fluid and increase iNOS and NO production. Levels of oxidatively modified lipid-protein complexes, which are strong chemotaxins for monocytes and inducers of cytokine secretion in the peritoneal fluid of women with endometriosis, are also increased.¹³

Nitric oxide regulates endometrial stromal edema production, which is an important step for endometrial growth during the menstrual cycle, embryo implantation, and uterine contraction. In healthy fertile women, contractions in the subendometrial myometrium vary with the phases of menstrual cycle, but in women with endometriosis, uterine hyperperistalsis and dysperistalsis have been observed.¹³ Excessive NO production might disturb uterine contractions and tubal function, impairing fertilization and implantation in the process, and lead to spontaneous abortion and compromised fecundity.

For uterine receptivity, integrin alpha V beta 3 is the best adhesive molecule marker. Its levels and those of eNOS have the same expression patterns throughout the menstrual cycle.¹³ Both are located predominantly in endometrial glandular epithelium. In cases of endometriosis, however, when eNOS expression in glandular and luminal epithelium increases, integrin alpha V beta 3 expression decreases.¹³ The prominent increase in eNOS during the mid-luteal phase and the decrease in integrin alpha V beta 3 lead to implantation difficulties in endometriosis. Since NO can induce endometrial cell apoptosis, high NO levels in the endometrium may also impair embryo implantation and development.¹³

The endometrium of women with endometriosis has been described to have elevated levels of NO and NOS²⁵ and increased expression of NOS. Altered NOS expression may affect endometrial receptivity and hinder embryo implantation. Deviations in endothelial NOS gene expression also may induce endometrial angiogenesis, thereby facilitating the development of endometriosis.²⁵

Increased expression of manganese and copper/zinc SOD (defensive enzymes) has been seen in the endometrium of women with endometriosis and adenomyosis throughout the menstrual cycle.¹³ In addition, aberrant

expressions of glutathione peroxidase and xanthine oxidase have been found in both eutopic and ectopic endometrium.¹³ This change in antioxidant enzyme levels may lead to significant OS in endometriosis.

Circulating levels of OS from other sources, such as the endometrium and ectopic endometrial implants, may also contribute to the pathogenesis of endometriosis. The endometria of patients with endometriosis have an increased lipid-protein complex modification resulting in high lipid peroxide concentrations.^{11,17} The epitopes that are produced as a result of lipid peroxidation have been found in macrophage-enriched areas of both the endometrium and endometriosis implants.²⁶ One study showed that high levels of various antioxidants inhibit the proliferation of endometrial stromal cells and that moderate levels of OS promote endometrial stromal cell proliferation. It also was found that the highest tested level of OS inhibits proliferation. This can be attributed to the biphasic dose-response to OS in which only moderate doses of ROS instigate growth/proliferation, whereas higher doses cannot, due to direct cytotoxic effects and higher rates of apoptosis [Foyouzi, 2004 #6].

Serum paraoxonase-1 is a high-density lipoprotein (HDL)-associated enzyme that prevents oxidative modification of low-density lipoprotein (LDL). A study conducted by Verit *et al.* in 2008 compared serum PON-1 activity in women with endometriosis with that of healthy controls.²⁷ Serum PON-1 activity, LOOH levels, serum triglyceride (TG), total cholesterol (TC), HDL, and LDL levels were measured. PON-1 activity was significantly lower and LOOH levels were significantly higher in women with moderate to severe endometriosis than in women with mild endometriosis and controls. Also, lower PON-1 activity was observed in women with mild endometriosis compared with controls. A significant negative correlation was found between PON-1 activity and stage of the disease. PON-1 activity and HDL levels were decreased, whereas levels of LOOH, TG, TC, and LDL were higher in all women with endometriosis than in controls. Reduced serum PON-1 activity and increased LOOH levels are pieces of evidence suggesting that OS plays a role in the pathophysiology of endometriosis. PON-1 activity can be used indirectly to detect endometriosis, but an official diagnosis requires histopathologic confirmation.

Role of Iron

Erythrocytes from retrograde menstruation yield the pro-inflammatory factors hemoglobin and heme, which contain the redox-generating iron molecule.²⁸ The presence

of iron¹²—as well as macrophages¹³ and environmental contaminants such as polychlorinated biphenyls²⁹—may disrupt the balance between ROS and antioxidants in the peritoneal fluid. Iron-overload provokes iron-mediated damage, oxidative injury, and inflammation, leading to pathogenesis of endometriosis.

In the case of endometriosis, the source of iron overload in the pelvic cavity is a result of pelvic erythrocyte lysis. Under normal conditions; the pelvic cavity has protective mechanisms to counteract the reflux of erythrocytes. However, it has been suggested that women with endometriosis have strained peritoneal protective mechanisms due to one of two hypotheses: (1) abundance of reflex or (2) defective AO capacity. The result is iron accumulation. Studies indicate that blood from bleeding lesions in the ectopic endometrium contribute significantly to the abundant erythrocyte population (5).³⁰ Experimental mouse model studies mimicking retrograde menstruation conditions and lesion bleeding have confirmed the origin of iron overload in the pelvic cavity. In one study, endometriosis was induced in nude mice by injection. Iron deposits similar to those found in human endometriosis were observed in lesions that had been induced with injected menstrual effluent or endometrial cells with erythrocytes.^{5,31,32} Peritoneal macrophages phagocytize a number of erythrocytes entering the pelvic cavity. Hemoxygenase 1 (HO-1) metabolizes hemoglobin and releases iron. Iron is incorporated into the macrophages as ferritin or is released into peritoneal fluid where it binds with Tf, an iron transporter.

Defrere *et al.* demonstrated that ectopic endometrial cells can incorporate Tf and metabolize it into ferritin.³¹ This concept was further demonstrated by Mizuuchi *et al.* who studied the expression of transferrin receptor (TfR) by endometrial cells.³³ Numerous studies and murine endometriosis models all have observed the presence of iron conglomerates in endometriotic lesions. Additionally, ectopic lesions in the peritoneal cavity release Hp. Hemoglobin released by erythrocytes binds Hp to form Hb-Hp complex. This complex is endocytosed by macrophages, which become saturated with Hp, thereby signifying strain on peritoneal protective mechanisms.

In endometriosis, the number of peritoneal macrophages is increased and they are also more highly activated, resulting in chronic inflammation. Oxidative injury occurs when iron is continuously delivered to peritoneal macrophages, preventing ferritin from storing and sequestering iron. Consequently, iron generates free radicals and disrupts the balance between ROS production and antioxidant defense, and OS ensues.

In 2003, Wagener *et al.* studied the HO-1 detoxification system to demonstrate the association between iron overload and subsequent OS contributing to endometriosis. Heme plays a critical role in a wide variety of enzymes and also enhances gene expression. In respect to endometriosis, the concentration of HO-1, a heme-degrading enzyme, is low due to poor expression by macrophages and mesothelial cells. HO-1 functions to degrade heme and generate CO, bilirubin, and ferritin. These degradation products serve as a defense mechanism by detoxifying and protecting against adverse effects of oxidative stress. Consequently, low HO-1 concentrations seen in endometriosis result in an impaired detoxifying system and subsequent OS.

Oxidative stress might cause local damage to the peritoneal mesothelium.^{29,34} Normally, the mesothelium lining serves as a protective barrier to the adhesion of menstrual endometrial fragments. However, because of its fragile state, the mesothelium can easily be disrupted in the presence of OS, resulting in adhesion sites on the surface. Demir *et al.* showed that the menstrual effluent factor iron-binding protein Hb is harmful to mesothelium.³⁵ This supports the above theory since iron is a known factor that induces OS, causing macromolecular oxidative damage, tissue injury, and chronic inflammation.³⁶

Iron overload further contributes to the development of endometriosis by promoting epithelial cell proliferation. Defere *et al.* created a murine endometriosis model to study the effect of iron overload on ectopic endometrium.³¹ The study demonstrated how an erythrocyte injection increased the proliferative activity of epithelial cells in endometriotic lesion whereas desferrioxamine (DFO) administration drastically inhibited it.

Reactive oxygen species affect the regulation of the transcriptional factor, NF- κ B.³⁷ NF- κ B is responsible for the expression of proinflammatory cytokines, growth factor, angiogenic factor, adhesion molecules, and inducible enzymes iNOS and COX-2.³⁸ These products all play a role in the development of endometriosis by inducing endometrial fragment adhesion, proliferation, and neovascularization.³⁹ A study by Lousse *et al.* found that NF- κ B activity in peritoneal macrophages from patients with endometriosis was significantly higher than that in controls (Figure 5-1).⁴⁰

Role of TNF- α in Pathogenesis of Endometriosis

Tumor necrosis factor-alpha (TNF- α), a pleiotropic cytokine, is produced and activated by a number of cell types including, but not limited to, neutrophils,

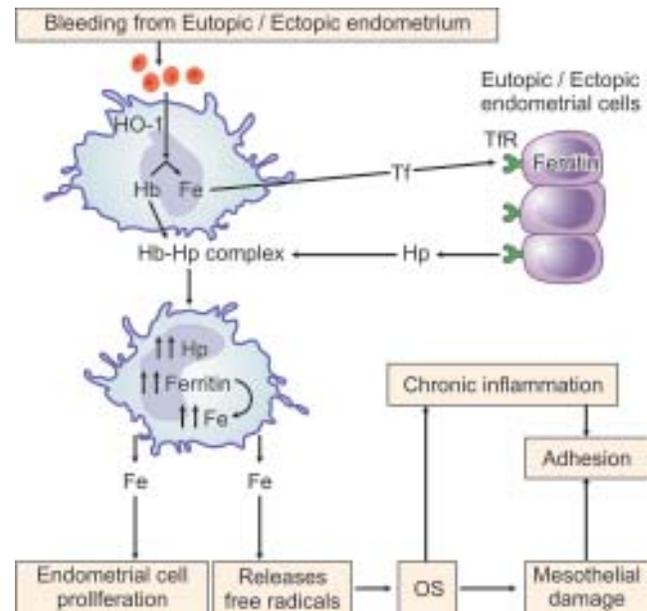


FIGURE 5-1: Chronic Inflammation and Adhesions in Endometriosis. Bleeding from eutopic and ectopic endometrium causes release of erythrocytes into the peritoneal cavity. Peritoneal macrophages phagocytose erythrocytes. HO-1 metabolizes Hb component of erythrocytes and subsequently releases Fe. Fe is transported via Tf to bind TfR on eutopic and ectopic endometrial cells. Once bound, it is incorporated into ferritin. Eutopic and ectopic endometrial cells synthesize Hp. Hb is released from the peritoneal macrophage to bind Hp, forming the Hb-Hp complex. Macrophages phagocytose the Hb-Hp complex to become saturated, thereby impairing the peritoneal protective mechanism, resulting in increased Hp, ferritin and Fe. The consequent release of Fe from saturated macrophages results in chronic inflammation and OS, ultimately leading to adhesions. HO-1: heme oxygenase-1; Hb: hemoglobin; Fe: iron; Tf: transferrin; TfR: transferrin receptor; Hp: haptoglobin; Hb-Hp complex: hemoglobin-haptoglobin complex; OS: oxidative stress

lymphocytes, and macrophages. TNF- α is a major pro-inflammatory cytokine known to impair glutathione (GSH) production by several mechanisms, creating an environment conducive to the development of OS. This pathogenic cycle of GSH disturbances and enhanced TNF- α production may be active in the female reproductive tract in endometriosis. An *in vitro* study investigating endometriosis-associated infertility has shown that spermatozoa quality decreases following incubation with TNF- α in a dose- and time-dependent manner.⁴¹

The actions of TNF- α include the activation of Th cells,⁴² upregulation of metalloproteinases in concert with IL-1,⁴³ instigation of angiogenic and cytotoxic effects on targets in concert with IL-1 and IL-6, attraction of neutrophils and stimulation of neutrophil adhesion to

endothelial cells, and the production of IL-1, oxidants and PGE2.⁴³ Tumor necrosis factor-alpha secretion is stimulated by IL-1 and bacterial endotoxin.⁴³ When mediated by IL-8, TNF- α has been known to promote the growth of endometriotic cells.⁴⁴ Elevated levels of peritoneal fluid TNF- α have been associated with endometriosis.^{45,46} Rana, 1996 #60]^{47,48} in comparison with women who do not have the disease or women with idiopathic infertility.⁴³ Higher concentrations of TNF- α receptors (TNFR), both sTNFR-1 and sTNFR-II, have been found in the peritoneal fluid of endometriotic patients as well.⁴³ Tumor necrosis factor-alpha has not been associated with the severity or stage of the disease.⁴⁹ However, a lower frequency of TNF- α 1031 c polymorphism in the promoter region of the TNF- α gene was found in the most severe cases of endometriosis in a Japanese study, suggesting that the polymorphism has a protective mechanism.⁵⁰ Peritoneal fluid TNF- α , along with IL-6, was found by Bedaiwy and colleagues to be both a sensitive and specific marker for diagnosing individuals with and without the disease—at a level of 15 pg/mL, the sensitivity was 100% and the specificity was 89%; at a level of 20 pg/mL, the sensitivity was 96% and the specificity was 95%.⁴⁹

Interleukin 6

Interleukin 6 (IL-6) not only regulates cytokine secretion, but also plays an important role in implantation events and endometrial cell growth regulation.⁴² Interleukin 6 is produced in monocytes, macrophages, endothelial cells, vascular smooth-muscle cells, and endometrial epithelial stromal cells (51). IL-6 and other inflammatory cytokines have been suggested to contribute to the maintenance of peritoneal endometriosis.⁵²

A study conducted by Sharpe-Timms *et al.* sought to demonstrate the relationship between IL-6 and endometriosis. Endometriotic tissue is a biochemically active tissue that secretes and synthesizes numerous proteins. Of interest are the endometriosis proteins (Endo), in particular Endo-I, which is a unique form of haptoglobin requiring IL-6 for maximal expression. Haptoglobin (Hp) is predominantly synthesized by the liver in response to inflammation or injury;⁵³ however, a variety of other tissues have been demonstrated to synthesize Hp,^{54,55-62} including endometriotic lesions. Endo-I differentiates itself from hepatic Hp in that it is secreted in a glycosylated form.⁵⁴ The alteration in the pattern of protein glycosylation initiates the phagocytic process and allows Endo-I to bind to peritoneal macrophages,⁶³ thereby initiating the immune

response seen in women with endometriosis. When bound to peritoneal macrophages, Endo-I blocks macrophage phagocytic capacity by interfering with adherence. Altered macrophage function elicits production of inflammatory mediators, such as IL-1, IL-6, and TNF-alpha.⁶⁴⁻⁶⁶ In turn, these cytokines function to upregulate the expression of Endo-I, creating a positive feed-forward loop between endometriotic haptoglobin and IL-6.⁶⁷ Interleukin 1 (IL-1) in peritoneal fluid instigates IL-6 production, and therefore exacerbates the inflammatory effects of IL-6.⁴³ The concentration of IL-6 in peritoneal fluid was found to be significantly higher in women with endometriosis than in control subjects⁶⁸ and could be used to differentiate between women with and without the disease with a high specificity (67%) and sensitivity (90%).⁴⁹ Levels of IL-6 are also significantly higher in women with a large number of implants.⁶⁹ Interleukin 6 has also been found to be elevated in the serum of women with endometriosis,⁷⁰ as IL-6 is produced by both eutopic and ectopic endometrium. However, using serum levels as an independent tool has limited value in predicting the disease.⁷¹

Role of Leptin in Endometriosis

Leptin is considered a class I cytokine due to its role in cell growth and maturation.⁷² It is produced mainly in adipose tissue, but also in human ovarian follicles (both granulosa and cumulus cells), placenta, stomach, and skeletal muscle. Leptin receptors are found in a plethora of tissues including endothelial cells,⁷³ T cells,⁷⁴ and endometrium.⁷⁵ Leptin expression is inhibited by testosterone and increased by ovarian sex steroids.⁷⁶

Although leptin helps regulate food intake and plays a role in energy balance and hematopoiesis, peritoneal fluid levels have been positively correlated with stage III and IV endometriosis⁷² and chronic co-morbid pelvic pain.⁷¹ However, no correlation was found with leptin levels and infertility associated with endometriosis⁷¹ or ovarian endometriosis.⁷² A possible explanation of the difference is that leptin is free to diffuse into the peritoneal fluid in peritoneal endometriosis, whereas it is sequestered in the cystic fluid of ovarian endometriomata.⁷⁷ Leptin has been found to promote neoangiogenic activity by up-regulation of VEGF.⁷⁸ The cytokine also promotes the invasion of the extracellular matrix by ectopic endometriotic stromal cells via increased expression of matrix metalloproteinases,⁷⁶ bcl2, and intercellular adhesion molecule.⁷⁶ Leptin is produced during the acute phase inflammatory response and acts as a c reactive protein and IL-1 beta during systemic inflammation and fever.⁷⁹ Furthermore, levels of

leptin significantly increase in response to acute infection and sepsis and have an instigating effect on CD4+ T cell lymphocyte proliferation, macrophage phagocytosis, and IL-1 and TNF- α (both inflammatory cytokines) secretion.⁷⁶ Hypoxia inducible factor-1 α (HIF-1 α), working in conjunction with pro-inflammatory cytokines such as IL-1 α and prostaglandins, increases levels of leptin in ectopic endometriotic stromal cells due to hypoxic stress (<1% O₂) in the peritoneal cavity.⁸⁰

Conclusion

Oxidative stress plays an integral role in the pathogenesis of endometriosis resulting from increased free radical generation and/or decreased levels of scavenging antioxidants. Whether there is a cause–effect relationship between free radical excess and the pathophysiology of these conditions or a temporal one remains to be demonstrated. Regardless, it appears reasonable to investigate the role of antioxidant agents in both the prevention and treatment of endometriosis. They may help ameliorate the extent of lesions and help reduce the severity of symptoms and any subsequent complications that develop [Alkan, 2006 #74]. Thus, the identification of OS markers or markers of altered immune function such as IL-6, TNF-alpha, IL-8, IL-1 beta and PON-1 in the serum and peritoneal fluid as a noninvasive option for diagnosing the disease and gauging its severity is important but is still investigational. Further, iron overload contributes to the development of endometriosis by promoting epithelial cell proliferation, and the role DFO plays in inhibiting lesion growth represents an exciting new avenue of research.

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