Oxidative Stress and its Role in Endometriosis—Mechanistic and Therapeutic Implications

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Introduction

Endometriosis is a common gynecological disorder that affects about 2 to 22% of women in their reproductive years and between 35 to 50% of women diagnosed with infertility.\(^1\) It is also diagnosed in 10 to 70% of women with pelvic pain.\(^2\)

Endometriosis is finding of uterine and endometrial tissue as well as menstrual effluent outside of the uterus. The location of implantation of such tissue is typically in the peritoneal cavity, but has also been found within the pleural cavity, liver, kidney, glutal muscles, and bladder.\(^3\) The disease itself is diagnosed through laparoscopy, the actual gold standard. Laparoscopic
procedures can also be used in conjunction with other surgical procedures for the treatment of endometriosis.5 There are many factors that can play a role in the generation of endometriosis including genetics. A study published in 2005 looked at familial endometriosis in groups of sisters that had been surgically diagnosed with the disease. From this study, it was concluded that a new genetic linkage for the disease was found on chromosome 10q26.5 Another possible linkage was found on 20p13 among other chromosomes that requires additional studies.5 Evolution of better diagnostic methods and treatments could evolve through the understanding of abnormalities at these loci.5

An additional method to diagnose endometriosis may be to assess the level of oxidative stress, a core mechanism implicated in the pathophysiology of the disease, within the serum and the peritoneal fluid. However, the effects of reactive oxygen species (ROS) and their implications in endometriosis are uncertain based on experiments done by different groups. ROS such as superoxide radical, hydrogen peroxide, hydroxyl radical, and peroxynitrate, can be measured. Through reactions with cellular components, these ROS will produce molecules such as lysophosphatidylcholine6 and malondialdehyde,7 which are products of lipid peroxidation. Elevated levels of these 2 molecules have been reported in women with endometriosis.

This chapter will focus on different markers that are elevated in oxidative stress and their contribution to the progression of endometriosis. The role of various ROS along with the roles of iron, nitric oxide, and cytokines will be described. The newest clinical research, along with innovative treatments for this disease will also be evaluated.

Etiology of Endometriosis

The etiology of endometriosis is not currently addressed by only one theory. Rather four separate theories have been hypothesized to simulate the disease process. These include: (1) Sampson’s implantation theory (2) Coelomic metaplasia and induction theories (3) The embryonic rest theory, and (4) Lymphatic and vascular metastasis theories.8 The coelomic metaplasia theory introduced by Meyer proposes that endometriosis originates due to reversible changes of the epithelial lining in the peritoneal cavity. The metaplasia may occur due to infectious, hormonal, or other stimuli.8 The induction theory, a broadening of the coelomic metaplasia theory proposes endogenous biochemical and immunological factors as inducers of differentiation of previously undifferentiated cells into endometrial tissue.8 This theory was proved experimentally in rabbits and in vitro human cells.8 The embryonic rest theory states that cell rests of Mullerian origin differentiate into endometrium due to a particular stimulus.8 The lymphatic metastasis theory states that endometrial cells spread through the blood and lymphatic pathways.8

Sampson’s theory, hypothesized in 1927, is the main accepted one within the scientific community. The theory has its basis in three major elements: retrograde menstruation (the reflux of endometrial and uterine tissue along with menstrual effluent from the uterus through the fallopian tube and into the peritoneal cavity where implantation occurs),8 the presence of viable cells within this retrograde menstruation,8 and a third of these viable cells must have the ability to then implant and form peritoneal lesions. For proper implantation to occur there needs to be a blood supply to the lesion which is one of the findings that has been implicated in women with the disease.9

Role of Oxidative Stress in Endometriosis (Figure 37.1)

Imbalance Between ROS and Antioxidants

Although the different causes of endometriosis have not been proven yet, there does seem to be an implication for oxidative stress in the manifestation and progression of the disease process. Oxidative stress occurs due to an imbalance between reactive oxygen species and antioxidants. ROS are molecules that have an unpaired electron and that stabilize themselves by extracting electrons from different molecules in the body, like lipids, nucleic acids, and proteins. Antioxidants are a defense mechanism created by the body to neutralize the ROS.

Measurement of Oxidative Stress Markers

The ROS target polyunsaturated fatty acids of cell membranes in the peritoneal cavity. This results in lipid peroxidation, of which breakdown products are the measurable oxidative stress markers malondialdehyde (MDA)7 and lysophosphatidylcholine. Both products are seen as antigenic to the body causing activation of an immune response.5,7 The autoantibodies along with the products of lipid peroxidation are indicators of oxidative stress.

Cellular markers such as heat shock protein 70B’ (HSP70B’) are increased in the disease as well.10 Typically, HSP are present to maintain proper protein synthesis and protect it from cellular stress. Circulating HSP70B’ has been found to be implicated in cellular stress along with pathogenesis of endometriosis.10
Elevated Macrophages and Inflammatory Activity

The endometrial tissue and menstrual effluent found in the peritoneal cavity are seen as antigenic, activating macrophages. Macrophage number and activity in the peritoneal fluid (PF) have been found to be increased during endometriosis, which causes increased phagocytosis of the antigens as well as release of ROS.

Role of NF-κB Transcription Factor

Along with increased activity of macrophages, it has been found that transcription factor nuclear factor kappa B (NF-κB) is upregulated during the endometriosis process. NF-κB can further increase the proinflammatory state and cause activation of many genes that induce the progression of the disease. NF-κB elevation was found to be due to elevated iron levels, which will be detailed later in this chapter, and can also be released by macrophages, which were found by Lousse et al to have the inert ability to secrete this transcription factor. NF-κB can then bind to DNA and cause transcription of genes that code for cytokines, growth factors, angiogenic factors, adhesion molecules, and inducible enzymes such as nitric oxide synthase and cyclooxygenase. One of the main types of adhesion molecules that can be activated through this process is intercellular adhesion molecule-1 (ICAM-1). ICAM-1 mRNA and protein have been found to be elevated in ectopic endometrial cells. In a study by Gonzalez-Ramos et al, levels of ICAM-1 were studied in black and red endometriosis lesions, the latter being the implants that have the greatest proliferative capacity and it was found that they had the largest expression of ICAM-1. Constitutive activation of the transcription factor NF-κB was also found in the red lesions. Since red lesions are thought to occur at earlier stages of endometriosis, measuring and targeting NF-κB levels may be an early diagnostic measure that could also be targeted by therapy to decrease the progression of the disease.

Implantation and Angiogenesis Factors

ROS have also been found to attack the fragile mesothelium which typically warrants against adhesion of
Oxidative Stress and its Role in Endometriosis

Antioxidants and Different Treatment Options in Endometriosis

Antioxidants are important in neutralizing the effects of ROS and they can be enzymatic or non-enzymatic. Enzymatic antioxidants include catalase and glutathione peroxidase. The non-enzymatic antioxidants include several vitamins along with glutathione. When the levels of these antioxidants along with total antioxidant levels were tested in the PF of women with endometriosis associated infertility, it was found that all were diminished significantly. The impairment of the antioxidant system decreases the ability to protect the PF from the effects of ROS.

One of the newer fields of treatment that is being tested is the role of oral antioxidants for treatment of endometriosis. In a study by Mier-Cabrera et al, daily antioxidant intake was found to be significantly decreased in women with endometriosis (WEN) compared to women without endometriosis (WWE). Four months after a high-antioxidant diet, plasma vitamin C levels had doubled with vitamins A and E increasing as well. Glutathione peroxidase and superoxide dismutase levels increased two months into the study. Oxidative stress markers of lipid hydroperoxides and malondialdehyde were decreased three months into the study. This new therapeutic measure has shown that administration of a high antioxidant diet could have the ability to be successful in treating endometriosis.

An additional therapeutic measure to treat endometriosis associated infertility is the drug pentoxifylline. Several studies have shown contrasting results. In a study published in 2008, pentoxifylline was administered following surgery for a total of six months. Though results were not significant, it was found that there was a 28% pregnancy rate in women receiving treatment versus 14% in women who received the placebo. Another study by Kamencic et al, demonstrated the efficiency of pentoxifylline in the management of post-operative pain. It is essential that additional studies are completed and evaluated as to the therapeutic aspects of the drug.

Role of Cytokines

Cytokines play a major role in endometriosis, as they are released into the PF and contribute to the regulation of leukocytes and act on ectopic endometrium, resulting in tissue and cell damage. Sources of cytokines include peritoneal macrophages, lymphocytes, ectopic endometrial implants, and mesothelial cells of the peritoneum.

These cytokines contribute to tissue remodeling and implantation of cells or tissue within the endometrium. Thus, increased PF cytokine levels can be used as specific markers in the diagnosis of endometriosis.

Chemoattractant cytokines, such as interleukin-8 (IL-8) and regulated on activation normal T cell expressed and secreted (RANTES), assist macrophage recruitment into the peritoneal cavity. Hsu et al found that there is a shift of T cells toward Th2 (which produce cell-mediated immunity suppressant cytokines), suggesting that maybe this imbalance contributes to the defective immunological defense in endometriosis.

IL-1 influences the activation of T cells and the differentiation of B cells and has also been found to be implicated in the implantation of ectopic endometrium.

IL-6 is an important cytokine that helps regulate the activity of other cytokines, activates B-cell stimulation and is also important in reproduction, i.e. folliculogenesis, steroid hormone synthesis, implantation and it monitors growth of endometrial cells. Rier et al demonstrated rising levels of IL-6 in the PF with increasing severity of endometriosis. This suggests the possibility of using IL-6 level as a marker for different stages of endometriosis. Another study showed that PF IL-6 levels were higher in women with minimal-moderate (MM) endometriosis than in controls, but that these levels were lower in women with moderate-severe (MS) endometriosis. Carbohydrate antigen-125 (CA 125) was found to be elevated in MS endometriosis, suggesting that
IL-6 is a reliable marker for MM endometriosis and CA 125 is better for more severe cases. Several other studies have also shown that the level of PF IL-6 is up-regulated in women with endometriosis and can be used as an effective, non-invasive marker for the diagnosis of endometriosis. However, the use of serum levels still have controversial value in diagnosis.

IL-8 is a small polypeptide involved in the chemotaxis of neutrophils and in angiogenesis. Arici et al showed that IL-8 levels were elevated in the PF of women with endometriosis, and that the levels increased with the severity of the disease. It was also found to stimulate attachment of the endometrial cells to the extracellular matrix protein, fibronectin, suggesting its important role in the endometrial implant adhesion. It has been studied that the serum concentrations of IL-8 in ovarian endometriosis patients were higher than in patients with benign ovarian cysts and that epithelial IL-8 levels were higher in women with endometriosis when compared to normal women during the proliferative phase. These findings show that IL-8 plays an important role in the pathogenesis of endometriosis and can be detected and used as a reliable marker for diagnosis.

IL-12 is a cytokine important for the regulation of natural killer (NK) cell activity, proliferation of T cells, and increasing the cytotoxicity of NK cells. Levels of IL-12 are low but detectable in the PF of women with and without endometriosis, suggesting that IL-12 levels may not be of predictive value for the diagnosis of endometriosis. However, a study has shown that the addition of IL-12 effectively interfered with the implantation of endometrium (surface and weight of lesions) in ectopic sites in a murine model of endometriosis. This shows the possible therapeutic value of cytokine administration in the management of the development of endometriosis.

Tumor necrosis factor α (TNF-α) is a pleiotropic cytokine produced and stimulated by neutrophils, macrophages, lymphocytes, NK cells, among others. It is a pro-inflammatory cytokine used to activate Th cells and to promote endometrial proliferation and shedding. TNF-α disrupts glutathione production, causing increased susceptibility to ROS production within the female reproductive tract. A study found that spermatozoa quality decreased when TNF-α was added in a dose- and time-dependent manner, and this decrease in sperm quality affected endometriosis-associated infertility. In addition to activation of Th cells, TNF-α is also involved in angiogenesis and cytotoxicity, tumor progression, the up-regulation of mettallomatrix proteins in collaboration with IL-1, recruitment of neutrophils to endothelium, and the production of IL-1, oxidants, and PGE2. TNF-α has also been found to increase the expression of IL-6 in endometriotic stromal cells via the NF-κB pathway. PF TNF-α levels are increased in patients with endometriosis, and have been shown to increase with increased severity of the disease but not with its stage. Treatment of endometriosis could include the inhibition of Th1 cytokines such as TNF-α. Many substances are currently being tested to study the suppression of endometriosis: hCG, GnRH analogs and danazol have been found to attenuate cytokine production and suppress endometriotic TNF-α gene expression in vitro.

**Role of Iron**

Iron is an essential element incorporated in a wide variety of molecules throughout the body, including hemoglobin. However, overproduction and increased levels of iron in the PF due to retrograde menstruation may be a cause of oxidative stress.

Endometrial cells have been quantitatively found in the peritoneal cavity of 59 to 79% of menstruating women, but only some develop endometriosis which may be attributed to the impairment or efficiency of protective mechanisms against elevated iron levels. These include macrophage maintenance of iron homeostasis, haptoglobin sequestration of hemoglobin, and production of the hemopexin-heme complex (Figure 37.2).

Macrophages play a huge role in the homeostasis of iron levels and have the ability to phagocytize the erythrocytes, releasing hemoglobin and heme.
Subsequently, these molecules will be broken down into iron, ferritin, carbon monoxide, and bilirubin through an enzyme called heme oxygenase 1 (HO-1).\textsuperscript{56} Hemoglobin (Hb) released from erythrocytes can also be bound by haptoglobin (Hp), a scavenger protein.\textsuperscript{57} The Hb-Hp complex is then recognized by a scavenger receptor CD163 on the surface of macrophages and phagocytized.\textsuperscript{57}

Finally, any free floating heme released during metabolism of hemoglobin will be bound to hemopexin which has antioxidant capacity.\textsuperscript{58,59}

In women with endometriosis, it has been found that there is an elevated level of iron within the PF compared to controls.\textsuperscript{60-62} The increased levels of iron within the PF may occur in part due to increased amount of erythrocytes from retrograde menstruation or due to peritoneal lesion bleeding.\textsuperscript{63, 64} This, coupled with the decreased protective mechanisms, may account for the development of endometriosis.

Ferritin, an antioxidant, sequesters iron and allows there to be a decreased amount of the element available to produce oxidative stress via the Fenton reaction.\textsuperscript{65} However, due to the elevated amount of erythrocytes found in the peritoneal cavity of women with endometriosis, the ferritin sequestration system becomes overwhelmed quickly, causing there to be release of iron into the peritoneal cavity, which can then participate in the Fenton reaction generating hydroxyl radical, and inducing oxidative stress.\textsuperscript{66}

Another ROS-antioxidant imbalance cause is the decreased amount of bilirubin, a potent antioxidant that is produced by heme oxygenase (HO) breakdown of heme.\textsuperscript{56} Although HO-1 levels were found to be elevated in certain parts of the peritoneal cavity, the majority of the cells that constitute the peritoneal cavity in endometriosis, the macrophages, do not express the enzyme in endometriosis patients.\textsuperscript{67} Decreased expression of the enzyme does not allow the final byproduct of heme breakdown, bilirubin, to form, and its antioxidant capacity is missing in these women.\textsuperscript{67}

Overall, iron overload does cause there to be elevated proliferation of endometrial lesions and progression of the disease, which means that this mechanism should have therapeutic targets. One such target is the iron chelator, desferrioxamine DFO, which has shown to decrease endometrial proliferation and progression of the disease. By decreasing levels of iron, the levels of oxidative stress resulting from free iron in the peritoneal cavity can also be controlled.\textsuperscript{54}

However, in high concentrations, NO can have damaging effects on the gametes, embryos, and oviductal function.\textsuperscript{69}

It has been hypothesized that interleukin 10 (IL-10), increased during earlier stages of endometriosis, may stimulate the release of NO by macrophages.\textsuperscript{70}

NO synthase, the enzyme that ultimately produces NO through conversion of L-arginine to L-citrulline, can be found in three forms.\textsuperscript{71} These three forms include the neuronal form NOS1, the inducible form NOS2 (iNOS), and the endothelial form NOS3 (eNOS).\textsuperscript{71} Macrophages isolated from women with endometriosis associated infertility had higher iNOS activity and subsequently released higher NO levels than endometriosis free controls.\textsuperscript{69} The peritoneal macrophages have the ability to move from the peritoneal cavity to other parts of the female reproductive system including the fallopian tubes where fertilization takes place and where their increased capacity to produce nitric oxide can then cause greater risk of infertility.\textsuperscript{69}

Some studies have shown the localization of the different NOS in the endometrium of control women and women with adenomyosis.\textsuperscript{72} Levels of eNOS were found to be high in the luminal and glandular epithelium of the endometrium of controls and in the eutopic endometrium of adenomyosis subjects, while levels of iNOS were found to be elevated in stromal lesions of endometrium, in control endometrium and in the eutopic endometrium of adenomyosis subjects. This pattern of expression was found only to be in the secretory stage of the menstrual cycle for both sets of women. However, in the ectopic endometrium of patients with adenomyosis, both enzymes were found to be high throughout the entire menstrual cycle (including the proliferative phase).\textsuperscript{72}

NO released in high levels has the ability to react with superoxide radical forming a potent ROS called peroxynitrate that has a sustained half life.\textsuperscript{73} Nitrotyrosine, a product that forms when peroxynitrate reacts with the amino acid tyrosine was found to be elevated during the proliferative and secretory phases in the eutopic endometrium of women with adenomyosis.\textsuperscript{72} Peroxynitrate, like any ROS, can cause progression of the disease through damage to other molecules. NO levels can also be increased by activation of NF-kB due to increased expression of the gene coding for NOS.\textsuperscript{13}

The levels of NOS and NO are also correlated with the levels of the reproductive hormones estrogen and progesterone. When fasting blood samples were taken from women with endometriosis associated infertility, it was found that there was a positive correlation between the levels of estrogen and progesterone and eNOS protein levels.\textsuperscript{74} NO activates cyclooxygenase-2 (COX-2); subsequently, prostaglandins such as prostaglandin E\textsubscript{2}...
Endometriosis and Ovarian Malignancy

It has been suggested that epithelial ovarian cancer (which accounts for 90% of all cancers) shares a common inflammatory pathophysiology with endometriosis, or could even derive from endometriosis (Figure 37.4). 76

It is clear from what has preceded that the presence of ectopic endometrial tissue is associated with oxidative stress, increased local inflammation reactions, involving elevated number of macrophages and increased secretion of cytokines and chemokines. 77 These cytokines, as discussed earlier, often upregulate or downregulate their own secretion and can sometimes induce unregulated cell divisions, differentiation or apoptosis that resemble those of malignant cells. 78

Another supporting mechanism is that of iron and heme induced oxidative stress, which has been demonstrated to cause genetic damage, even in cells of histologically normal endometriotic tissue. And DNA

are increased and cause aromatase levels to rise as well. 75

The resultant estrogen increase stimulates further eNOS gene expression in a positive feedback loop (Figure 37.3). 74

Targeting to treat women with elevated NO levels will not only help endometriosis but associated infertility. Wang et al, studied the effects of administration of gonadotropin-releasing hormone agonist (GnRH-a) (goserelin or leuprolelin acetate) on levels of NOS expression in women with endometriosis associated infertility. It was found that eNOS levels were diminished within the endometrium, but only significantly during the early proliferative stage and the early and middle secretory stages. 74 Peroxynitrate was also found to be diminished in both eutopic and ectopic endometrium once treated with GnRH agonists of buserelin or nafarelin. 72 Adding selective inhibitors of eNOS to the GnRHa therapy may also prove to be favorable in reducing the positive feedback loop of estrogen and progesterone on eNOS, and as a result lessen endometriotic implantation and improved pregnancy rates.

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damage or loss of heterozygosity as a result of oxidative stress are known to be part of carcinogenesis.\textsuperscript{79}

Further more, endometrioma and ovarian cancer have shown similar molecular genetic modifications, such as PTEN (phosphatase and tensin), p53 and bcl genes mutations, theorizing the existence of a genetic continuum between those pathologies.\textsuperscript{78} This theory is supported by epidemiology, pathology, anatomy, and clinical data.

Few epidemiologic controlled studies have assessed the prevalence of ovarian cancer among endometriosis patients. However, the evidence available shows that endometriosis could be a risk factor for ovarian cancer, with up to a 5 fold increased risk\textsuperscript{80} and at least 1.7 fold after adjustment for other risk factors.\textsuperscript{81}

On the other hand, it has been demonstrated that endometriosis sometimes exhibits atypical architectural or cytological characteristics, like invasiveness or adherence, that resemble malignancy, and that 25\% of the cases presenting with atypical ovarian endometriosis further develop ovarian cancer.\textsuperscript{82} Patients with epithelial ovarian cancer have been confirmed to have a higher prevalence of endometriosis than the general population.\textsuperscript{78} This is particularly true of the clear cell carcinoma and the endometroid carcinoma subtypes of ovarian cancer, which represent together up to 75\% of endometriosis-associated ovarian carcinoma.\textsuperscript{83} These tumors were curiously found to have a left-sided predominance (similar to endometriosis) and were also found to present at an earlier stage and to have better survival rates than the general population of ovarian cancers.\textsuperscript{84}

References


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