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Endometriosis
A Comprehensive Update

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Chapter 1
Introduction to Endometriosis

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1.1 Definition

According to Webster's New World/Stedman's Concise Medical Dictionary, Endometriosis (En'do-me-tri-o'sis) is "ectopic occurrence of endometrial tissue, frequently forming cysts containing blood."

The word Endometriosis is derived from the Greek words *endon*, meaning "within," metra, meaning "uterus," and *osis*, meaning "abnormal or diseased condition." Endometriosis is a complex yet common debilitating gynecological disease where the functional endometrial glands and stroma, which are normally part of the innermost lining of the uterine cavity (the endometrium), are present outside the uterine cavity. These locations include, but are not limited to, the ovaries, fallopian tubes, pelvic peritoneum, gastrointestinal tract, bladder, recto-vaginal septum, and less commonly, the pericardium and pleura [1].

It is a highly prevalent disease among women of reproductive age [2]. The disease is estrogen dependent [3] and has a chronic inflammatory component.

1.2 Classification of Endometriosis

In 1921, Sampson first classified ovarian hemorrhagic cysts—he described them as endometrial, stromal, corpus luteal, and follicular. Based on the histologic appearance, he also staged the endometrial hematomas [4]. Since then, numerous classification systems have been created based on the histologic appearance, anatomic location, size, and extent of endometrial tissue growth.

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1.2.1 ASRM Classification (Stage 1 to Stage 4)

According to the American Society for Reproductive Medicine (ASRM) endometriosis can be classified as minimal (stage I), mild (stage II), moderate (stage III), or severe (stage IV) depending on the extent of tissue growth [5]. Revised for the third time in 1996, it is the most commonly used system to classify endometriosis. Each occurrence of endometriosis is assigned to one of these stages based on a point value system that ranks certain attributes of the disorder. These components include: whether the endometriosis is superficial or deep, whether the posterior cul-de-sac is partially or completely obliterated and, lastly, whether the adhesions that form around the ovaries and fallopian tubes are flimsy or dense. Also, the morphology of the endometriotic lesions is recorded as red, red-pink, clear, white, yellow-brown, black, or blue.

Limitations of ASRM Classification: However, despite providing a simple means of documenting the extent of endometrial lesions, the ASRM staging system only weakly parallels pain symptoms [5, 6] and the risk of infertility. In addition, staging reproducibility is limited due to observer bias [7]. Visual discrepancies are common and can depend on the timing of laparoscopy. Further, documentation and staging during laparoscopy may differ from those made during laparotomy [8].

Although this system accurately assesses the placement and degree of endometriosis, it fails to convey the probability of achieving pregnancy following treatment [9]. While the ASRM's current classification system is helpful to surgeons who require standard terms to discuss this disease, it still has significant limitations. Namely, the depth of the endometrial tissue does not always correspond to pain levels, but this is not expressed in the diagnostic scale [10]. While it is evident that infertility may be a direct consequence of endometriosis, a specific causal link has yet to be established [11].

Newer classification systems include the EFI (endometriosis fertility index)—a clinical tool that is used to assess fertility outcomes for women with endometriosis-associated infertility who have undergone surgical staging for their disease. EFI rates the predicted prognosis in order to tailor the most suitable treatment plan [7]. The EFI combines the score of the patient's medical history and her surgical factors into a combined EFI score [12]. This index was validated by several studies [13].

1.3 Disease Burden: Prevalence of Endometriosis Worldwide and in North America

The exact prevalence of endometriosis may never be known because a laparoscopic procedure needs to be performed in order to establish a definitive diagnosis [14]. Moreover, some women remain asymptomatic and often go undiagnosed [5, 15, 16].

However, it is estimated that it affects 6–10 % of reproductive aged women [17]. The ASRM reports that 24–50 % of infertile women may have endometriosis along with 20 % of women with chronic pelvic pain. This prevalence increases to 50 % in infertile women with a normal menstrual cycle and whose partner has healthy sperm [18]. Current estimates report that seven million women in the United States and more than 70 million women around the world have the disease. [19] Although hysterectomy was a relatively common treatment for endometriosis, in the United States as of 2010, hysterectomy rates due to endometriosis declined by 65 % as compared to 1998 [20]. Approximately 10–25 % of women who choose ART (assisted reproductive technology) have endometriosis. Additionally, ovarian endometriomas are present concomitantly in 17–44 % of these females [21, 22].

According to studies done by Eskenazi et al, among women who underwent laparoscopy for complaints of pelvic pain 25 % had endometriosis and 20 % of women evaluated for infertility had endometriosis [23]. The prevalence of endometriosis rises to 20–50 % in women with infertility [24].

1.4 Symptoms and Signs of Endometriosis

Symptoms vary widely but commonly include considerable pelvic pain (40–50 %), dysmenorrhea (58–80 %), dyspareunia (40–50 %), dysuria (1–2 %), dyschezia (1–2 %), gastrointestinal discomfort (1–2 %), and decreased libido.

Two thirds of women with pelvic endometriosis experience the aforementioned symptoms. In addition, women with this disease are more likely to have cyclical leg pain than those without endometriosis, however, there is no change in the intensity of pain between the two groups [25]. The main cause of pain in patients with endometriosis is the compression and the infiltration of the nerves by the lesions. Nerves in the ectopic endometrium are stimulated by inflammatory factorsperipheral sensory (afferent) nerve fibers sense and transmit pain stimuli to the central nervous system to do an afferent function. Other nerves release substance P, calcitonin, gene-related peptide, tachykinins, and nitric oxide to do an efferent function. This function is essentially the cause of neurogenic inflammation and it also increases local vascular permeability.

Infertility is also a common finding in patients with endometriosis [26]. In addition to its unpredictable nature, symptoms commonly associated with endometriosis also present in other gynecological diseases, leaving several cases of endometriosis misdiagnosed or overlooked [27]. This uncertainty significantly contributes to the average delay of 7–8 years from symptom onset to confirmed diagnosis [28]. Differential diagnoses include pelvic congestion syndrome, chronic pelvic pain, pelvic inflammatory disease (PID), and irritable bowel syndrome (IBS) [27].

1.5 Disease Background

1.5.1 Historical Perspectives

In 1690, Daniel Schoen, a German physician, published '*Disputatio Inauguralis Medica de Ulceribus Ulceri*', in which he wrote about what is now known as endometriosis. Further, in 1774, a Scottish physician described the symptoms of the disease, stating that, "In its worst stages, this disease affects the well-being of the female patient totally and adversely, her whole spirit is broken, and yet she lives in fear of still more symptoms such as further pain, the loss of consciousness and convulsions." In the eighteenth century, a woman was likely thought to have hysteria when she had endometriosis-like symptoms, and these were blamed for infertility. In 1860, an Austrian pathologist, Karl Freiherr Von Rokitansky, gave the first histological description of the disease followed by researcher Thomas Cullen who used the term 'adenomyomas,' which we now call endometriomas. Cullen also wrote about their similarity to the mucous membrane of the uterus.

In the nineteenth century, Sampson coined the term "Endometriosis" and postulated the theory of retrograde menstruation, which brought further attention to the disease. Initially, treatment was mainly surgical. In the mid-1900s, physicians began using hormonal treatment with large doses of estrogen, which invariably caused severe side effects.

1.6 Key Points and Summary

Endometriosis is a complex, yet common debilitating gynecological disease seen in reproductive age women. It affects more than 70 million women worldwide and seven million women in the United States. The ASRM classifies the disease as minimal, mild, moderate and severe in Stages I–IV, respectively. The disease predominantly causes pain and infertility. Sampson coined the term "endometriosis" in the nineteenth century.

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Chapter 2
Predisposing and Protective Factors of Endometriosis

Sajal Gupta, Avi Harlev, Ashok Agarwal, and Elizabeth Pandithurai

2.1 Predisposing Factors

2.1.1 Early Menarche, Late Menopause, Low Parity

A number of risk factors are associated with endometriosis, including early menarche (age $\leq$ 11 years), short menstrual cycles (<26–27 days), menorrhagia (bleeding > 7 days or 80 mL), [1], nulliparity, low birth weight [2] and obesity [1, 3, 4] have been related to higher risks of endometriosis. Most risk factors are associated either with elevated estrogen levels or prolonged menstruation. This strengthens the estrogen dependence of endometriosis and the association between menstruation and endometriosis.

2.1.2 Hormonal Factors: High Estrogen and Low Progesterone

Patients with endometriosis often have high levels of estrogen and low levels of progesterone. The presence of the steroid hormone imbalance enhances the disease severity. Imbalance caused by estrogen excess and a lack of progesterone has been hypothesized to exacerbate endometriosis. Estrogens activate estrogen receptors inside endometriotic cells and regulate their gene expression. The four different types of natural estrogen are estradiol (E2), estrone (E1), estriol, and estetrol. Estradiol (E2) is secreted by the ovary and is the predominant type of circulating estrogen during the reproductive years. Estradiol levels increase around ovulation. Estradiol directly reaches the endometriotic tissue implants in the pelvic region and acts on the estrogen receptors present within them, which thereby increases endometriotic tissue survival. Endometriotic tissue implants contain the enzyme

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aromatase, which converts androgens to estrogens, thereby increasing local estrogen concentration and enhancing the growth of endometriotic lesions.

Endometriotic tissue implants also contain receptors for progestins and androgens [5]. The hormone progesterone (P4) is responsible for the development of secretory endometrium, embryo implantation and regulation of various genes. It also suppresses estrogen receptor α [6]. Progesterone is known for its growth limiting action. It can inhibit and even reverse estrogen-induced endometrial growth in human endometrium. Progesterone acts through its receptors PR-A and PR-B. A truncated variant of PR-A isoform also acts as a repressor for PR-B function [7]. Studies have shown down-regulation of PR-B in ectopic endometrial lesions in women with endometriosis compared to their eutopic endometrium [7, 8]. Eutopic endometrium is resistant to progesterone. The enzyme 17 β-hydroxysteroid dehydrogenase type 2 (17 βHSD2) inactivates the conversion of estradiol to estrone in response to progesterone in the eutopic endometrium. However, estradiol levels are enhanced in endometriotic tissue due to the lack of progesterone [9]. The progesterone resistance seen in women with endometriosis can be attributed to the absence of the stimulatory PR-B isoform and the presence of the inhibitory PR-A isoform in the endometriotic tissue [7].

Prostaglandins are locally produced, hormone-like compounds that contribute to the symptoms of endometriosis. In particular, higher levels of prostaglandins E2 and F2α are present in the endometriotic tissues of women with endometriosis. High levels of these prostaglandins induce pain. Prostaglandin F2α has vasoconstrictive properties, and increased levels cause excessive uterine contractions leading to the dysmenorrhea seen in women with endometriosis [10].

The enzyme aromatase also plays a role in the pathophysiology of endometriosis. It converts androgens in the peripheral tissue to estrogen. Aromatase is highly expressed in the eutopic endometrium of women with endometriosis and positively correlates with the severity of dysmenorrhea experienced in women with endometriosis [11]. Elevated levels of aromatase mRNA levels were observed in women with ovarian endometrioma [12].

2.1.3 Exposure to Environmental Agents Such as Dioxins

Evidence suggests that women who were exposed in utero to synthetic estrogens such as diethylstilboesterol (DES) and potent environmental toxins had a higher incidence of endometriosis [4].

Growing evidence suggests a possible link between endometriosis and exposure to environmental pollutants. Some environmental pollutants contribute to the pathogenesis of endometriosis and include dioxins, polyhalogenated aromatic hydrocarbons, organochlorine pesticides, phthalates, and bisphenols. Dioxins mainly exert their action through the binding and activation of aryl hydrocarbon receptor (AhR). AhR exists in tissues throughout the body including eutopic and ectopic endometrium. The mechanism by which they cause endometriosis includes a combination

of growth factor activation, gene regulation, immunosuppression, and altered estrogen signaling pathways.

2.1.4 Immunological Dysregulation

CD4 T cells are divided into type1 (Th1) and type 2 (Th2) helper T cells. Th1 cells secrete interleukin IL-2, IL-12 and interferon γ while type2 (Th2) cells secrete IL-4, 5, 6, 10, and 13 [13]. In women with endometriosis, Th2 helper cells in the peritoneal fluid suppress cell-mediated immunity by increasing IL-4 and IL10 secretion in the peritoneal fluid [14]. As a result, there is decreased T cell cytotoxicity, allowing endometrial cells to implant in the peritoneum.

Upon extra-uterine implantation, ectopic endometrial tissues release cytokines, activate macrophages, and suppress phagocytosis within the peritoneum [15]. Endometriosis induces a chronic inflammatory state. Elevated cyclooxygenase-2 (COX-2) levels stimulate local activity of aromatase [2]. The peritoneal fluid of endometriosis patients is known to contain elevated levels of cytokines, growth factors, T cells, B cells, and macrophages [16, 17] along with reduced natural killer (NK) cell activity [15]. Ectopic implants also establish a constant blood supply through angiogenic growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) and insulin like growth factor (IGF) [18, 19]. Because endometriosis is a hormonally dependent disease, sites of ectopic implantation are sensitive to estrogen, which fuels the growth of endometrial cells [20].

As mentioned earlier, normal physiologic mechanisms utilize the recruitment of immune cells such as macrophages, NK cells, and lymphocytes to expel excess menstrual tissue and endometrial cells outside the uterine cavity [21]. However, since retrograde menstruation is a common occurrence in most women, those with endometriosis may have a dysregulated immune response [2]. An aberrant immune surveillance mechanism is a plausible cause for the survival of ectopic tissue [22] Abnormal cell-mediated immunity (CMI, particularly defective functioning of NK cells), may allow for the persistence and implantation of ectopic endometrial tissue, as was first demonstrated in 1991 by Oosterlynck et al. [23] Further, endometriosis may develop from an impaired ability of NK cells to scavenge autologous endometrial cells [17].

ICAM-1, an immunoglobulin involved in cell adhesion, has been detected in ectopic endometrial tissue. Because it is normally present in the endometrium, expression of ICAM-1 in ectopic endometrium and implants may contribute to defective NK cytotoxicity and allow ectopic cells to evade detection by the immune system [24]. The cytotoxicity of NK cells may also be inhibited by endometrial secretion of the s-ICAM-1 (soluble) receptor in peritoneal fluid and its subsequent binding to lymphocyte presenting LFA-1 [25, 26]. Binding of s-ICAM-1 to leukocyte-related ligands causes leukocyte-cell communication to falter, thus weakening natural immune responses [27]. Within the peritoneal fluid of women with endometriosis, Th2 helper cells have been shown to hamper CMI by stimulating

release of IL-4 and IL-10 [13, 14]. This decreased cytotoxicity of T-lymphocytes to autologous endometrial cells may also allow for peritoneal implantation of endometrial cells [28]. These mechanisms of ectopic endometrial tissue escape from the body's normal defense systems and its subsequent survival and implantation outside the uterus can be triggered by persistent retrograde menstruation [29]. As such, dysfunctional immunity likely contributes to the disease.

Endometrial cells, through retrograde menstruation, implant in the pelvis. There they gain access to the peritoneal cavity and lead to an inflammatory reaction followed by angiogenesis, adhesion, neuronal infiltration and oxidative stress [30]. However, almost 95 % of women have retrograde menstruation, and roughly only 10 % of them develop endometriosis [12]. This can partially be explained by differences in the eutopic endometrium and also in the peritoneal fluid among women with and without the disease [31]. An immune dysfunction is one of those differences. Lymphocytes T and NK cells have been described as down-regulated in women with endometriosis, and lymphocyte B is associated with autoantibody production. Mier-Cabrera et al. [32] showed that the cytotoxic response is diminished because of a change in T cell functions and not because of a quantitative difference in the number of cells.

The transcriptional factor NF-kB also seems to play an important role in the pathogenesis of the disease, as it increases inflammation, invasion and angiogenesis and decreases apoptosis of endometriotic cells [33]. The accumulation of iron, likewise, is related to endometriosis and induces the chronic activation of NF-kB [34].

Peritoneal macrophages also seem to play an essential role in the pathogenesis of the disease. Their phagocytic capability is reduced in women with endometriosis [35]. Interleukin-1 (IL1), secreted by peritoneal macrophages, promotes inflammation, cell growth, angiogenesis, and cell adhesion. IL 1 is up-regulated in the peritoneal fluid of women with endometriosis and as a result, IL-1, IL-8, TNF-alpha and IFN-gama are elevated. These interleukins stimulate peritoneal macrophages in women with endometriosis [36]. The peritoneal macrophages release prostaglandin E2, responsible for pelvic pain, and VEGF, responsible for neovascularization.

Apoptosis is another important underlying causative factor because eutopic endometrium of women with endometriosis may also have an anti-apoptotic capacity [30].

2.1.5 Genetic Predisposition and Epigenetic Alterations

This disease has a polygenic inheritance. Knowledge about the genetic predisposition of the disease can help us in the diagnosis of endometriosis and also to assess its severity. In 1981, Simpson et al. studied 123 probands who were histologically proven to have endometriosis. They found that among the 123 probands, 10 of them had mothers who were affected by endometriosis; 5.9 % of their female siblings over the age of 18 years had endometriosis. The study also found that

61 % of probands with an affected first degree relative had severe endometriosis when compared to the 23 % of affected probands without an affected first degree relative [37].

Endometriosis has also been strongly linked with heredity, especially between monozygotic twins [38] and first-degree relatives [1, 39, 40], and several identified genetic polymorphisms seem to increase the risk for disease [41].

In addition, women who have first-degree family members with endometriosis appear to have about seven and ten times the risk of developing the disease than those without affected relatives. Daughters and especially sisters of patients with endometriosis are considered to have a significantly higher risk for the disease [1, 39]. Despite numerical discrepancies regarding the risk and prevalence of endometriosis among relatives, several studies show that there is a clear hereditary pattern [1, 42–44].

Endometriosis results from interactions between genetic and environmental factors [45, 46]. The probability of inheriting these genetics factors is 51 % [47]. To define the genes related to endometriosis, several studies were conducted using GWAS (Genome-wide association studies) or DNA mapping technology [47–49]. In familial linkage, this disease does not appear to be inherited by a simple Mendelian heredity, and the inheritance pattern is most probably polygenic/multifactorial [49].

Some evidence suggests that chromosomes 7 and 10 are linked with endometriosis [50].

Epigenetic alterations: Other underlying causes are epigenetic variations. Kawano Y et al. studied a tumor suppressor gene, CCAAT/enhancer-binding protein (C/EBP-alpha), and found that in endometriotic women, it is silenced by histone de-acetylation. As a result, there is increased proliferation of endometrial tissue and decreased apoptosis. Quantitative RT-PCR was designed to assess C/EBP-alpha mRNA expression. Immunohistochemical staining was done for C/EBP-alpha protein. C/EBP-alpha knockdown was developed with small interfering RNA (siRNA), and quantitative RT-PCR was performed to evaluate the mechanisms of C/EBP-alpha [51].

Epigenetic changes in transcription factors SF-1 and ER-β also contributes to the pathogenesis of endometriosis. These transcription factors are overexpressed in endometriotic stromal cells due to decreased promoter methylation leading to increased estradiol production, decreased estradiol inactivation and increased progesterone resistance. These epigenetic changes occur due to genetic and environmental factors that cause changes in DNA methylation [16].

2.1.6 Persistent Inflammatory Status

It is believed that the initial trigger of the immune system is the presence of constant irritation in the form of endometrial cells in the peritoneal cavity, thus leading to persistent inflammation. Inflammation is the basic and primary response to an

infection, irritation, or injury in the body [52]. An inflammatory response increases blood flow and initiates the non-specific immune system to send the necessary defense mechanisms (i.e., macrophages, leukocytes, cytokines etc.) to the infection site, increasing local blood flow. This in turn leads to increased swelling and redness in the inflamed area as well as a release of cytokines from the injured epithelial cells [52].

2.2 Protective Factors

Some of the protective factors for endometriosis include parity, oral contraceptive use, NSAID use, tubal ligation, hysterectomy, and prolonged breast feeding. Endometriosis is associated with pelvic inflammation. Studies show that there is elevated local inflammatory activity in women with endometriosis. Procedures such as tubal ligation and hysterectomy bisects the connection between the upper and lower genital tracts and prevent environmental inflammants from reaching the ovarian epithelium, consequently preventing chronic inflammation.

Parity, prolonged breast feeding, and use of NSAIDs and oral contraceptives all suppress ovulation. Whenever there is ovulation, irritation and inflammation occur. Levels of pro-inflammatory cytokines are elevated after ovulation including TNF-α, interleukin-1 and interleukin-6. The cytokines augment cell proliferation, oxidative stress, and vascular permeability and increase levels of leukotrienes and prostaglandins [53].

Given the dependence of endometriosis on estrogen, factors that decrease estrogen in the body such as prolonged amenorrhea (often encountered in female athletes) and exercise [3] demonstrate protective effects. Additionally, increased parity and lactation have exhibited protection against disease occurrence [4]. Women who take combined oral contraceptives are at a decreased risk of developing endometriosis [54].

2.3 Key Points and Summary

The risk factors associated with endometriosis include early menarche, late menopause and other conditions where there are increased episodes of ovulation. During ovulation, secretion of estradiol increases, leading to increased cell proliferation and survival of endometriotic tissue implants. The disease responds to high estrogen and low progesterone levels. The disease has a polygenic inheritance and genetic predisposition with increased incidence of disease seen in first-degree relatives and in monozygotic twins. Exposure to environmental pollutants, immunological dysregulation, persistent inflammatory status, and/or epigenetic alterations also increase the disease risk. Conditions that suppress ovulation indirectly decrease estrogen levels and reduce disease risk.

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Chapter 3
Theories on Endometriosis

Sajal Gupta, Avi Harlev, Ashok Agarwal, and Elizabeth Pandithurai

3.1 Sampson's Theory

There are several theories as to how endometriosis develops, but the most widely accepted one is Sampson's theory. First hypothesized in 1927, Sampson's theory [1] states that three elements are required to cause endometriosis: retrograde menstruation, the presence of viable cells within the retrograde menstruation, and the implantation of these viable endometrial cells, which continue to grow and form peritoneal lesions [1].

Retrograde menstruation refers to the regression of menstrual blood backwards through the fallopian tubes into the peritoneal cavity, with subsequent attachment and implantation of endometrial fragments [2]. Several studies conducted over the last six decades have suggested that retrograde menstruation occurs in most females [3–5].

According to Sampson's theory, some part of the endometrial lining refluxes back through the fallopian tubes into the peritoneal cavity during menstruation. Here, the endometrial cells can attach to local tissues and form their own nerve endings and blood supply. Although most women of reproductive age have some amount of retrograde menstrual flow, [6] their immune systems are usually able to clear the implanted cells and prevent their growth. When this does not occur, however, the patient develops endometriosis. Up to 20 % of women diagnosed with idiopathic infertility have endometrial implants as seen via laparoscopic examination [7].

Many factors that have yet to be studied further may play a role in the development of endometriosis, such as a damaged immune system, genetics, or exogenous factors [8]. There are many points that can be argued in favor of Sampson's theory. The site of occurrence of several observed peritoneal endometrial or endometriotic lesions corresponds to a tubal reflux pathway. Also, endometrial cells recovered post menstruation are viable and have the capacity to grow rapidly. These cells also have integrins on their surface that allows them to attach to the peritoneal cavity. In

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addition, the endometrium can produce certain angiogenic factors that enable the creation of neo-angiogenesis.

3.2 Coelomic Metaplasia

The oldest alternative theory to retrograde menstruation is coelomic metaplasia. Coelomic metaplasia describes the ability of normal cell derivatives of primitive parietal peritoneum to transform into endometrial tissue [9]. The metaplasia theory is used to explain endometriosis in females with absence of menstruation, such as those who are prepubescent or have a history of total abdominal hysterectomy, in premenopausal women, and in rare cases of endometriosis in males [11–13].

The theory of coelomic metaplasia is based on the fact that the ovaries and Mullerian ducts are derived from the coelomic epithelium. This epithelium may undergo metaplastic transformation to form tissue much like that of the endometrium.

The coelomic epithelium is a common ancestor to both peritoneal and endometrial cells, and it may transform into the latter by means of chronic inflammation [7]. The metaplasia theory is viable because it can explain the presence of endometriosis in the absence of menstruation, such as in men who undergo estrogen therapy for prostate cancer, pre-menarche women, and post-menopausal females [13, 14]. However, there are many points that argue against the idea. If the metaplasia theory is true, endometriosis would be possible without the presence of an endometrium, such as in women with a congenital absence of the uterus or in healthy males through the potential of peritoneal metaplasia. Coelomic metaplasia would then also be expected to occur anywhere in the body where tissue derived from the coelomic epithelium is found. Hence, most scientific institutions continue to cite the retrograde menstruation theory instead [7].

3.3 Embryonic Rest Theory

This theory proposes that the presence of cells of Mullerian origin within the peritoneal cavity can be induced to form endometrial tissue when subjected to the appropriate stimuli [15].

This hypothesis could account for the presence of endometriosis of the rectovaginal septum as well as in any location along the migration pathway of the embryonic Mullerian system. Furthermore, this theory could account for the presence of rare endometriosis in men because the male embryo initially develops female-specific embryological structures that regress with activation of the male genome. This theory remains speculative, as it would require the assumption that these embryological rests persist to adulthood. Again, this theory remains unproven and purely hypothetical.

3.4 Lymphatic and Vascular Metastasis Theory

Endometrial tissue is usually spread through the fallopian tubes. However, the presence of endometrial tissue in remote locations may be explained by possible transport through the vasculature and lymph nodes [1, 16]. Because endometriosis most commonly occurs in the ovary, spread through the lymph nodes is likely. Results from many studies support the dissemination of endometrial tissue to distal locations in the body via detection of endometriosis in atypical locations suggesting hematogenous and/or lymphatic transport of endometrial tissue.

This theory may explain why endometriosis can be found in areas outside the peritoneal cavity such as the pleura and pericardium [17].

3.5 TIAR (Tissue Injury and Repair) Theory

The TIAR theory postulates that endometriosis is caused by trauma. Either a normal chronic peristalsis or a state of hyper-peristalsis caused by an event during reproductive life leads to microtraumatizations. These microtraumatizations lead to tissue injury and repair, increasing production of local estrogen. Estrogen acts as a positive feedback for the hyper-peristalsis, resulting in self-perpetuation of the disease. This auto traumatization, together with high motility, allows endometrium to implant outside the uterine cavity [18].

3.6 Quinn's "Denervation-Reinnervation" Theory

According to Quinn, endometrial cells are deposited outside the uterine cavity as a result of nerve injuries. These injuries cause denervation and can occur in response to some process, such as straining during defecation or vaginal delivery, leading to an alteration of fundo-cervical polarity. The ectopic endometrial cells from the retrograde menstruation adhere to the injured tissue in the peritoneal cavity and uterosacral ligaments. Re-innervation then occurs causing pelvic pain [19].

3.7 Stem Cell Theory

According to this theory, stem cells in shedding endometrium may play a role in the pathogenesis of early onset endometriosis [20], possibly due to retrograde neonatal uterine bleeding. Some of the elements that support this hypothesis include the manifestation of overt vaginal bleeding in 5 % of neonates, which is attributed to the fact that during later stages of pregnancy, the endometrium of the fetus transforms

in to a decidualized layer that desquamates after birth. The hypothesis is further supported by the presence of functional obstruction in the endocervical canal in neonates, which leads to the regurgitation of endometrial cells into the peritoneal cavity. These endometrial cells later implant and survive long term as endometrial stem cells/progenitor cells. This is similar to the theory of retrograde menstruation proposed by Sampson explaining the pathogenesis of endometriosis in adolescent and adult population [21].

3.8 Key Points and Summary

The pathogenesis of endometriosis is multi-factorial. There are several theories including retrograde menstruation, coelomic metaplasia, lymphatic and vascular metastasis, embryonic rest, TIAR, Quinn's denervation-re-innervation theory, and stem cell theory. The most widely accepted theory is retrograde menstruation proposed by Sampson in 1927.

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Chapter 4
Oxidative Stress and Endometriosis

Sajal Gupta, Avi Harlev, Ashok Agarwal, Alaa Al Safaar, Akshay Gupta, and Garrett Hack

4.1 What Is Oxidative Stress?

Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and antioxidants.

Many studies confirmed a correlation between ROS production and endometrial tissues outside the uterine cavity [1]. ROS have some important physiological functions but an excessive amount might affect leukocytes, granulosa cells, endothelial cells, folliculogenesis and embryo development [2]. This will lead to a lower quality oocyte and embryo in women with endometriosis [3].

4.2 Role of Macrophages, Interleukins in Causing Oxidative Stress

Retrograde menstruation leads to the deposition of erythrocytes, macrophages, apoptotic endometrial cells, and other menstrual remnants into the peritoneal cavity [4], possibly creating an oxidatively stressed environment. Studies by Ota et al. [5] and Khorram et al. [6] reported elevated levels of catalase and eNOS respectively, in females with endometriosis [5, 6]. In the pro-inflammatory setting of endometriosis, an abundance of oxidants can encourage growth of endometrial cells and increase chemo-attractant activity, thereby promoting disease development [7]. Normal physiologic mechanisms utilize the recruitment of immune cells, such as macrophages, NK cells, and lymphocytes, to expel excess menstrual tissue and endometrial cells outside the uterine cavity [8]. However, since

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retrograde menstruation is a common occurrence in most women, those with endometriosis may have dysregulated immune responses [9]; evasion of immune surveillance mechanisms is a plausible cause for the survival of ectopic tissue in these patients [10].

Abnormal cell-mediated immunity (CMI), defective functioning of NK cells in particular, may allow for the persistence and implantation of ectopic endometrial tissue, as was demonstrated in 1991 by Oosterlynck et al. [11]. Further, endometriosis may develop from an impaired ability of NK cells to scavenge autologous endometrial cells [12].

ICAM-1, an imunoglobulin involved in cell adhesion, has been detected in ectopic endometrial tissue. Because it is normally present in the endometrium, its expression in ectopic endometrium and implants may contribute to defective NK cytotoxicity and allow ectopic cells to evade detection by the immune system [13].

Inhibited cytotoxicity of NK cells may also result from endometrial secretion of the s-ICAM-1 (soluble) receptor in peritoneal fluid and its subsequent binding to lymphocytes presenting lymphocyte function-associated antigen 1 (LFA-1) [14, 15]. Binding of s-ICAM-1 to leukocyte-related ligands causes leukocyte-cell communication to falter, which weakens natural immune responses [16].

Within the peritoneal fluid of women with endometriosis, Th2 helper cells have been shown to hamper cell-mediated immunity by stimulating release of IL-4 and IL-10 [17, 18]. This decreased cytotoxicity of T-lymphocytes to autologous endometrial cells may also allow endometrial cells to implant in the peritoneum [19].

These mechanisms of ectopic endometrial escape from the body's normal defense systems and its subsequent survival and implantation outside the uterus can be triggered by persistent retrograde menstruation [20]. As such, dysfunctional immunity likely contributes to the development of endometriosis.

In one study, researchers investigated the formation of endometrial tissues and reported that the percentage of neutrophils in the peritoneal fluid was not directly associated with the development of endometriosis [21]. However; the same study concluded that there was an increase in the concentration of human neutrophil peptide (HNP1-3) in the peritoneal fluid (Fig. 4.1). It also found a strong correlation between the percentage of HNP1-3 and the recruitment of CD3 T cells. T cells stimulate local immune reactions, which consequently leads to the formation of endometrial tissue [21].

Several studies have discussed the origin of endometriosis based on the most obvious feature that is associated with it—the presence of the inflammatory reactions. There are several inflammatory cytokines that are present in the endometrium, such as, interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), and TNF [22]. These cytokines activate peritoneal fluid leukocytes. Therefore, cytokines play a role in initiating the formation of endometrial tissues [17]. The spread of these tissues result from a cell-cell and cell-tissue bindings that are caused by the activation of the transcriptional factor kB and the activator protein 1 by various free radicals such as nitric oxide and hydrogen peroxide [23]. The pro-inflammatory cytokines play a key role in the recruitment of phagocytic cells which are noted to produce ROS [24].

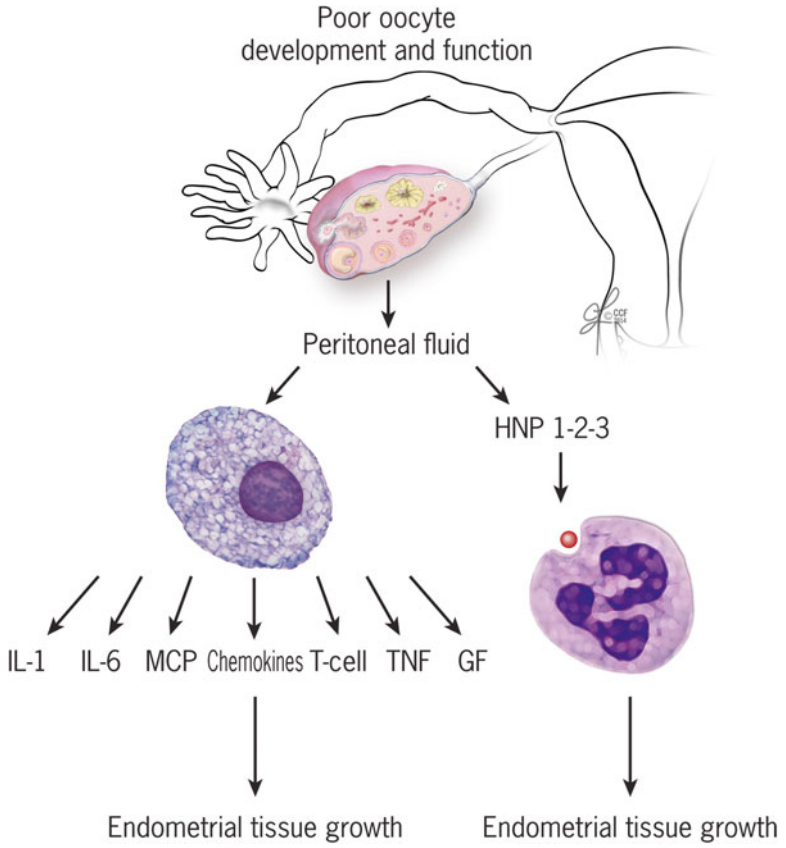

Fig. 4.1 Retinoid levels in endometriosis. *HNP1* human neutrophil peptide, *MCP* monocyte chemotactic protein, *TNF* tumor necrosis factor, *GF* Growth factor, *IL* Interleukin

Heme and iron are pro-oxidant components that enter the peritoneal cavity during retrograde menstruation, contributing to the oxidative stress recorded in the peritoneal cavity of women with endometriosis [25]. Some studies have reported higher levels of oxidative stress markers in the peritoneal cavity of women with endometriosis [24].

Carvalho et al. studied the total antioxidant capacity in endometriotic patients and concluded that a decreased level of DNA repair activity contributes to the progression of the disease [26]. ROS is also associated with cell adhesion [25].

The peritoneal fluid of women with endometriosis contains increased levels of white blood cells and macrophages, and increased activation of these macrophages release higher levels of pro inflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor necrosis factor alpha (TNF-α). These cytokines, in turn, stimulate other cytokines and chemokines like Interleukin-8 (IL-8) and RANTES (regulated upon activation, normal T-cell expressed and secreted) [12].

RANTES potentially attracts and activates macrophages, T-lymphocytes and eosinophils [27]. IL-8 promotes angiogenesis. Both IL-6 and TNF-α promote proliferation, adhesion of endometrial cells, and formation of new blood vessels [12]. IL-1, TNF-α and IL-6, also induces the expression of the enzyme aromatase and 17β-hydroxysteroid dehydrogenase levels in endometriotic lesions [28].

4.3 Mechanistic Pathway Connecting Oxidative Stress and Endometriosis

The passage of iron, apoptotic endometrial tissue, and desquamated menstrual cells into the peritoneal cavity through retrograde menstruation induces inflammation. Elevated levels of pro-inflammatory factors triggers the immune system, particularly recruiting and activating granulocytes and macrophages [29]. These cells significantly elevate ROS. Several studies have noted the association between the elevated ROS markers and endometriosis [25].

4.4 Nitric Oxide and Endometriosis

Nitric oxide (NO) is a free radical gas that is soluble in lipids. NO forms during the transformation of L-arginine to L-citrulline under the effect of the nitric oxide synthase (NOS) enzyme [30]. There are three forms of NOS enzymes: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). These different forms of enzymes help in the production of nitric oxide (NO). Minute amounts of NO are necessary for various functions such as optimal ovarian function and oocyte implantation [6] but high levels of NO can affect gametes, embryos, and fertility [30]. Moreover, one study suggested that there is an association between fertility and NO levels. Fertilization usually occurs in the ampulla of the oviduct, and the oviduct lumen is adjacent to the peritoneal cavity. Normally, endometrial peritoneal cavity macrophages migrate to the lumen of the oviduct where they increase NO production. This is considered the main reason for reduced fertility in women with endometriosis—iNOS expression.

Research also suggests that iron overload-induced macrophage apoptosis leads to overproduction of NO. Elevated levels of NO might play a significant role in the further establishment and growth of endometriotic lesions [31].

4.5 Is Oxidative Stress a Cause or Effect of Endometriosis?

Oxidative stress is strongly correlated with the presence of endometrial tissues outside the uterine cavity. There is a growing body of evidence discussing the generation of oxidative stress both locally and systemically in patients with endometriosis.

Oxidative stress can generate inflammation in the peritoneal cavity through various pathways. Oxidative stress modulatory genes play essential roles in initiating inflammatory reactions and forming new nerve fibres and blood vessels. Researchers are still questioning whether the use of antioxidants, specifically vitamins C and E, significantly improves the number of pregnancies. Therefore, more original, laboratory based research is necessary.

Several clinical trial studies produced varying results regarding the effect of fruits, vegetables, and meat intake on endometriosis. More studies are recommended to determine the best dietary interventions that will help decrease the risk of endometriosis.

4.6 Measurement of Oxidative Stress Markers

1. Most antioxidants in the body contain organic compounds called thiols. These compounds defend against free radical-induced DNA damage. In one study, thiol levels were significantly lower in 67 women with pelvic endometriosis who underwent laparoscopy than in 41 women without pelvic endometriosis who underwent tubal ligation, suggesting that oxidative stress is likely present in women with endometriosis [32].
2. 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the most important biomarkers of DNA damage caused by free radicals and is a likely marker of carcinogenesis. Isoprostanes are isomers of prostaglandins that are formed during the per-oxidation of arachidonic acid in cell membranes by free radicals. They are considered reliable markers of oxidative stress. A study found that 8-OHdG and 8-isoprostane levels were higher in the peritoneal fluid of patients with endometriosis than in fluid from the reference groups. A statistically significant positive correlation was found between 8-OHdG and 8-isoprostane levels in peritoneal fluid ($R = 0.3$; $P < 0.01$). Women with severe endometriosis experienced higher oxidative stress and had higher levels of free radical-induced DNA damage, and hence higher levels of 8-OHDG and 8-isoprostane levels [3].
3. A prospective study was conducted in Brazil to compare the serum markers of oxidative stress in infertile patients with those from endometriosis and infertile patients without endometriosis in order to find a correlation between the levels of these markers and the stage of the disease. The serum markers assessed in the study included malondialdehyde, glutathione, hydroxyperoxide and vitamin E levels. Blood samples were collected, and the levels of these markers were assessed using spectrophotometry and high performance liquid chromatography and compared with the control group. The study showed a positive association between the levels of hydroxyperoxide and the stage of the disease and a negative association between the levels of vitamin E and glutathione levels and no correlation between the stage of the disease and serum malondialdehyde levels in infertile women with

endometriosis when compared to the control group. All these results indicate that people with severe endometriosis have high levels of oxidative stress [33].

4. A cross sectional study was conducted on 66 women of reproductive age who were undergoing laparoscopy. Among them, 45 had histologically and laparoscopically proven endometriosis and 21 women did have not have endometriosis and served as controls. Their serum levels were measured for oxidative stress markers such as heat shock protein (HSP70), HSP70b′, thioredoxin (TRX), and ischemia-modified albumin (IMA). Researchers found that mean serum HSP70b levels were higher in the patients with endometriosis than the controls (0.178 ng/mL, SD 0.103, and 0.135 ng/mL, SD 0.014, respectively). The other markers—heat shock protein 70, IMA, and TRX—did not vary between women with endometriosis and the controls [34].

4.6.1 The Function of Thioredoxin (TRX) and Thioredoxin-Binding Protein-2 (TBP-2)

Redox regulators such as TRX help prevent cellular damage caused by oxidative stress. They also play roles in cell proliferation, apoptosis, and control of transcriptional factors such as AP-I and function in fetal growth and blastocyst implantation.

The function and the expression of TRX can be regulated by binding with TRX binding protein-2 (TBP-2) or the endogenous inhibitor vitamin D3 up regulated protein [35]. Moreover, an excessive amount of TBP-2 can lead to growth suppression apoptosis that results in the inhibition of TRX activity [36]. Furthermore, unregulated TRX activity that is caused by oxidative stress can induce the implantation of endometrial cells [37]. These changes can affect its normal expression and that of its binding protein: TBP-2. This results in a reduction of TBP-2 expression and an increase in TRX levels in endometrial cells during the late-secretory and menstrual phases. This can lead to the ectopic growth of endometrial tissues that result in endometriosis. Down-regulation of TBP-2, which functions as antitumor agent, can lead to the unregulated growth of endometrial cells or cancerous cells. Examples of cancers that can be caused by the down-regulation of TBP-2 are bladder cancer, colon cancer, prostate cancer, and breast cancer [38].

Apoptosis can alter homeostasis in human endometrium. The presence of endometrial cells outside the uterine cavity is facilitated by a reduction in apoptosis and an increase in cell proliferation during the late-secretory and menstrual phases. Endometriosis is an estrogen-dependent disease and thus, the expression of TBP-2 is affected by estrogen levels. TBP-2 can inhibit blastocyst implantation and embryo development. Any alteration in TRX and TBP-2 expressions will activate a transcription factor such as NF-kB, which is associated with the formation of macrophages and inflammatory cytokines. This, in turn, leads to the formation of inflammatory tissues, thus resulting in endometriosis [37].

4.7 Role of Antioxidants as a Defense System Against Oxidative Stress

Antioxidant systems play a role in cellular homeostasis as they help neutralize excess ROS. Examples of enzymatic antioxidants are superoxide dismutase, catalase and glutathione reductase. Examples of non-enzymatic antioxidants are vitamin E, vitamin C, glutathione and taurine [39]. In one study, vitamin E and vitamin C antioxidant therapy reduced chronic pelvic pain in 43 % of patients with endometriosis [40]. Antioxidants can be present in various percentages whether combined or independent [41]. Antioxidants play an important role in the inhibition of oxidative stress-induced damage and the reduction of the pelvic pain in patients with endometriosis. Patients with endometriosis have low levels of antioxidants including superoxide dismutase and glutathione peroxidase in their peritoneal fluid compared with healthy women. Both of these antioxidants are key components in neutralizing free radicals [42]. Diet is an essential source of antioxidants in the human body. Women who consume small amounts of fruits and vegetables have a higher risk of developing endometriosis [43].

4.7.1 Vitamins E, A, C and Melatonin as Anti-Oxidants

Many studies have examined the role of vitamin E supplementation in women diagnosed with endometriosis. Malondialdehyde (MDA) is a biomarker that measures the extent of lipid peroxidation. Oral supplementation with vitamin E lowered levels of lipid hydroperoxide (LOOH) and MDA in the peripheral blood in patients with endometriosis [44] and also lead to a non-significant increase in pregnancy rates. A third of the women who were provided the vitamin E supplementation conceived within 5 months [44].

4.7.1.1 The Role of Vitamin A

Vitamin A is an anti-oxidant that plays an important role in cell growth, immunity, hematopoiesis, and reproduction. It is also important in ovarian follicular growth, oocyte quality, and steroidogenesis [45]. High levels of vitamin A have a negative effect. All-trans retinoic acid (ATRA), which is the active form of vitamin A, plays a fundamental role in oocyte development, and women who have low levels of ATRA can develop inflammation, thus leading to the proliferation of endometrial cells outside the uterine cavity. One factor that may explain why some women develop endometriosis moreso than others is low retinoid metabolism in follicular fluid. This initiates aberrant expressions of ARTA regulated genes, cytokines, matrix metalloproteinases, tumour necrosis factor-B, IL-6, IL-11, several integrins, bax, and fas ligand. In turn, the aberrant expression stimulates the growth and the migration of cells and decreases cell death. These are some of the features that initiate the growth of the endometrial cells (Fig. 4.2).

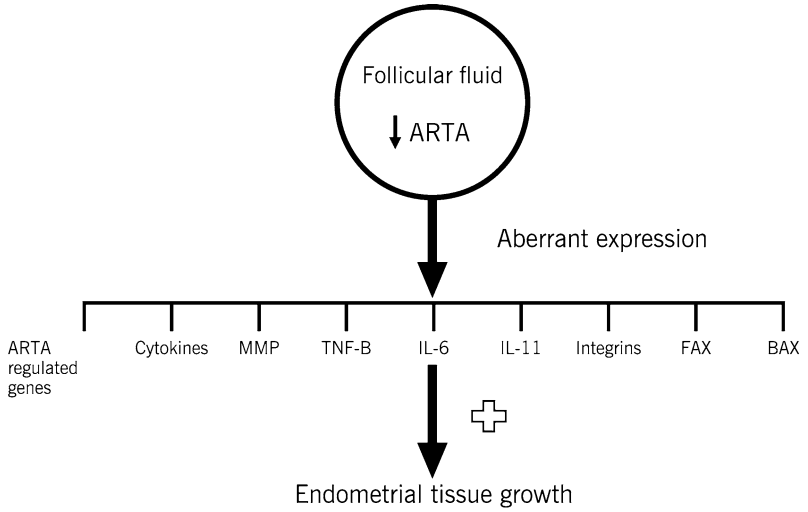

Fig. 4.2 Formation of endometrial tissues

4.7.1.2 The Role of Vitamin C

Vitamin C is a water-soluble antioxidant that neutralizes the hydroxyl ion, superoxide ion, and nitrogen radicals. Vitamin C is needed to convert the oxidized vitamin E into its original form. The original recycled form of vitamin E can neutralize ROS [46].

Eight weeks of oral vitamin E and C supplementation significantly reduced levels of inflammatory markers such as RANTES, interleukin-6 (IL-6), and monocyte chemotactic protein (MCP-1) in the peritoneal cavity of women with endometriosis [40]. The study results also showed that after the supplementation with vitamins C and E, pelvic pain during menstruation declined in 43 % of the women [40].

4.7.1.3 Role of Melatonin as an Antioxidant in Endometriosis

Melatonin is an analgesic, antioxidant and anti-inflammatory agent. It has a reverse effect compared with brain-derived neurotrophic factor (BDNF) (Fig. 4.3). Administration reduced pelvic pain caused by inflammation in 39 % of women with endometriosis, even during menstruation, and improved sleep quality [47]. That study also found that the women who used melatonin required fewer analgesics.

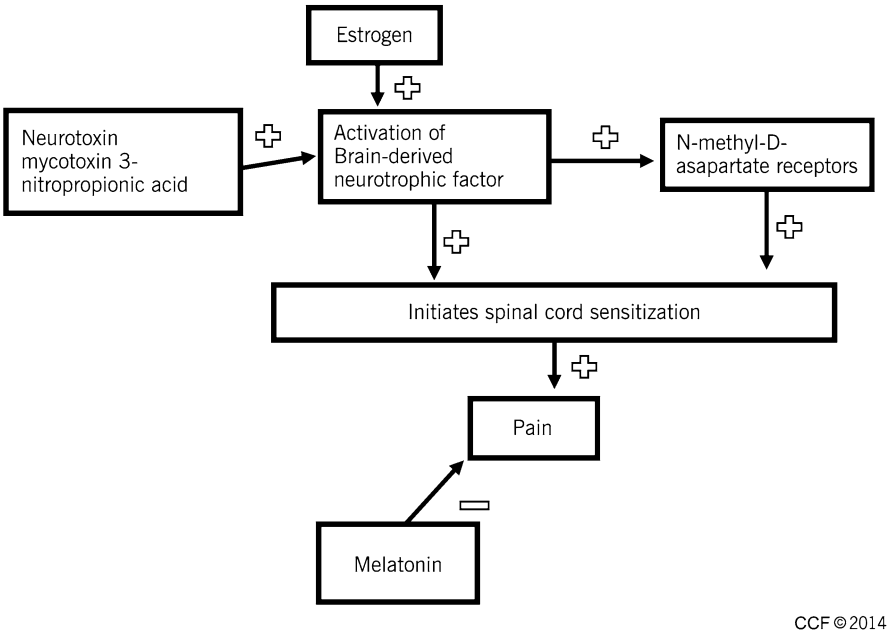

Fig. 4.3 Effect of melatonin in endometriosis patients

Melatonin may reduce pain by inhibiting luteinizing hormone (LH) levels, thus obstructing ovulation and increasing progesterone levels. This can explain the antioxidative effect of melatonin in decreasing the pelvic pain that is caused by endometriosis [47].

4.7.2 Studies on Antioxidant Rich Diet in Endometriosis Patients

Diet plays an essential role in reducing the effects of endometriosis. For example, foods that contain fish oil have high levels of polyunsaturated fatty acids (PUFAs), which are rich in omega-3 fatty acids (FAs). These reduce the inflammatory reactions that are present in the endometrium. A study done on mice found that adding fish oils to their diet decreased the size of the lesions caused by endometriosis [48].

However, other diet components have the opposite effect of fish oils, such as palmitic acid, which contains both saturated and trans-unsaturated fats. These components increase the risks associated with endometriosis.

There are varying viewpoints on the effects of fruit and vegetables on endometriosis. Some researchers believe that a dietary rich in fruits and vegetables may increase the risk of endometriosis [49]. On the other hand, Parazzini et al. [43]

found that the intake of fruit and green vegetables can decrease the effects caused by the disease. Due to the different opinions regarding the effect of fruits and vegetable on endometriosis patients, more studies need to be performed.

The intake of red meat such as ham and beef may increase the effects of endometriosis [50]. The effects of smoking and alcohol are still controversial. For more explanation about diet and endometriosis, Table 4.1 lists studies outlining different nutrition sources and their effects on endometriosis.

Table 4.1 Dietary intake and the risk of endometriosis

Author/year of publication	Dietary intervention	Subjects studied/methodology	Results/effects on endometriosis
Savaris AL1, do Amaral VF, 2011	Intake of fiber, vitamins B1 and B9	Women with endometriosis and a control group. Women with endometriosis were divided based on two criteria: site affected: (peritoneal, ovarian or rectovaginal septum) and the stage of the disease: stage I (minimal), stage II (mild), stage III (moderate) and stage IV (severe)	The study found that women with endometriosis had higher intake of fiber compared to the control group. This indicates that high fiber can increase the risk of endometriosis
		The intake of food was determined by a 24 h food recall method which the patients provide information on their food intakes during a 3 days period	
		Other data were also measured, such as, anthropometric data, body density, fat percentage, weight/height ratio, and antioxidant capacity	
Trabert B1, Peters U, De Roos AJ, Scholes D, Holt VL, 2010	Dairy, fruits, vegetables, whole grain, legumes, red meat, poultry, fatty fish, non-fatty fish, and seafood	Control study of women in the reproductive age and in the perimenopausal stage is at higher risk of developing endometriosis. All of the women were interviewed for demographics, employment, prior medical history, menstrual history, pregnancy history, contraceptive method, hormone use, tobacco and alcohol use, and family and personal history of endometriosis. The study excluded women who were diagnosed with endometriosis, non-English speaking women and also the women who had hysterectomy and bilateral oophorectomy. There were also questions about the intake some food types, like, fruits, vegetables, and fish. Other questions were about food purchasing and preparation	Increased total fat consumption was associated with decreased endometriosis risk. However, Increased β-carotene consumption and fruit were associated with increased risk of endometriosis. The study concluded that other food types are not related to the development of endometriosis

(continued)

Table 4.1 (continued)

Author/year of publication	Dietary intervention	Subjects studied/methodology	Results/effects on endometriosis
Parazzini F, Chiaffarino F, Surace M, et al.	Fruits, vegetables, and red meat	Women who have endometriosis and a control group. A questionnaire was given to both groups about their socio-demographic life, personal characteristics, gynaecological and obstetric history, and oral contraceptive use. The women were also questioned about the frequency of food intake specifically food that include retinoids, carotenoids, coffee, and alcohol	Intakes of milk, liver, carrot, cheese, fish, whole-grain food are not really related to increasing the risk of endometriosis. However, high intake of fruit and vegetables will decrease the effects that are caused by endometriosis. The study also mentioned that red meat can increase the risk of endometriosis

4.8 Key Points and Summary

An imbalance between anti-oxidants and ROS has a significant correlation with the presence of endometrial tissues outside the uterine cavity. Growing evidence suggests that oxidative stress plays a role both locally and systemically in women with endometriosis through many pathways. In the future, we need larger original randomized control studies in order to evaluate the role of antioxidants in preventing the damage caused by oxidative stress and methods to improve the rates of pregnancy in endometriosis patients.

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Chapter 5
Role of Iron in the Pathogenesis of Endometriosis

Sajal Gupta, Avi Harlev, Ashok Agarwal, Sheila Gokul, and Deepika Kumaresan

5.1 Iron: Background

Iron can accumulate in the peritoneal fluid, macrophages, and endometrial lesions in women with endometriosis. The process begins as erythrocytes are phagocytized by macrophages, releasing hemoglobin into the peritoneal fluid where it can form a complex with haptoglobin or be catalyzed by hemeoxygenase-1 to produce free iron. The free iron can be taken up by iron storage and transporting proteins such as ferritin and transferrin, which can lead to iron accumulation within macrophages or lesions. Iron overload can lead to the dysregulation of genes that code for molecules specific to endometriosis and oxidative stress.

5.2 Iron Regulated Genes

Iron overload in endometriosis affects the proliferation of ectopic endometrial tissue and progression of the disease through the regulation of several important genes involved in iron metabolism and oxidative stress. A number of the genes regulated by iron are specific to endometriosis, and some of these genes overlap with those involved in certain mechanisms of oxidative damage. Gene expression, such as the targets of heme and iron-mediated signaling, are affected by iron. Endometriosis-specific genes that are regulated by iron levels includes the ones that encode for proteins such as proteases, adhesion molecules, signal molecules, transcription factors, hormones, cytokines, pro-inflammatory factors, growth factors, and molecules involved in the cell cycle, stress, and detoxification systems [1]. Other iron-regulated genes that are not specific to endometriosis but overlap with those that are endometriosis-specific include stress response genes, detoxification genes, genes

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involved in fibrosis, cell cycles, growth factors, signaling, transcription, adhesion, and inflammation, along with hormones and proteases [1]. Iron is also an important regulator of iron storage proteins, such as ferritin, that are involved in the accumulation of iron in endometriosis [2].

Iron is an absolute requirement for proliferation, as iron-containing proteins catalyse key reactions involved in oxygen sensing, energy metabolism, respiration, folate metabolism and DNA synthesis. It has been shown that subunit R2 of the ribonucleotide reductase gene contains iron-specific regulatory elements and is upregulated by blood supplementation [3]. Iron-deprived cells are unable to proceed from the G1 to the S phase of the cell cycle [4].

Iron is also able to act with ROS to affect the expression of OS response genes, including those encoding for hemeoxygenase and other detoxification enzymes. The upregulation of NF-κB and the stimulation of COX-2 and prostaglandin F2α, which are involved in inflammation, may affect the regulation and expression of OS response genes [5–7]. In addition to the regulation of HO-1 and ferritin, iron also regulates the expression of genes encoding for molecules released by macrophages in order to protect cells from lipid peroxidation and other free radical induced damage [8]. Iron may also mediate the progesterone block seen in women with endometriosis through the down regulation of hormone receptors, progesterone responsive genes, and consequently progesterone-regulated genes. The dysregulation of genes in both ectopic and eutopic endometrial tissue by iron may also be a factor in female infertility related with the disease [9].

5.3 Retrograde Menstruation and Iron Levels in Endometriosis

In humans, hemoglobin (Hb) in red blood cells contains about 30 mg Fe/kg, which is roughly two thirds of the total iron content in the body [10]. Iron is a byproduct of the breakdown of heme by HO-1. Levels of free iron and iron bound by proteins in the peritoneal cavity are often increased because of the increased levels of erythrocytes in endometriosis and subsequent degradation and accumulation in endometrial lesions and peritoneal fluid. Increased iron levels have been reported in women with endometriosis. High levels of iron and iron-storing molecules within endometrial lesions and the peritoneal fluid may be used as a potential marker for endometriosis, although it is unlikely that these markers will be used for diagnosis of the disease or will be used to replace the golden standard of laparoscopy for diagnosis.

Studies have shown that heavily iron-laden macrophages can be found in the peritoneal fluid of women with endometriosis (Fig. 5.1) [11]. These significantly higher levels of siderophages, or iron-storing macrophages, are indicative of iron accumulation and iron overload as well endometriosis [12, 13].

Another by-product of heme degradation by HO-1 is bilirubin, a powerful antioxidant. Bilirubin has also been found in higher levels in the macrophages of women

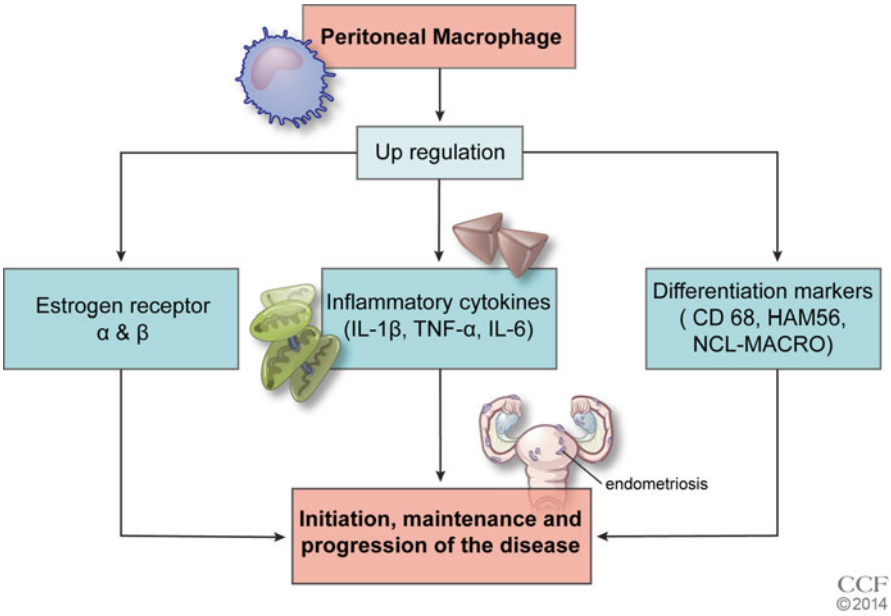

Fig. 5.1 Role of peritoneal macrophages in inflammation

with endometriosis [12]. Iron storage in macrophages also correlates with amounts of iron overload in the peritoneal fluid. Higher iron levels have been detected in the peritoneal fluid of patients with endometriosis; these iron levels have also been shown to correlate with the severity of the disease [14, 15].

Not only is free iron from heme degradation a source of increased iron levels in the peritoneal fluid of women with endometriosis, but macrophages are also able to release ferritin, which may add to the higher iron concentrations found in endometriosis patients [16, 17].

Transferrin, which ensures iron transport, also contributes to iron overload in the pelvic cavity. Transferrin can be incorporated into and expressed by ectopic endometrial cells in cases of iron overload. In endometriosis, peritoneal macrophages have more transferrin receptors than normal [18]. Studies have shown an association between increased levels of both ferritin and transferrin in women with endometriosis versus women without the disease, both in the peritoneal fluid and in peritoneal macrophages [11, 19]. High iron levels in cystic fluid and carcinomas have also been associated with endometriosis and possible carcinogenesis within the cysts due to the effects of iron and OS [20].

Lousse *et al.* reported that iron storage is significantly increased in peritoneal macrophages in women with endometriosis [11]. This increased iron storage correlated with iron overload in the peritoneal fluid. Van Langendonckt et al. found that iron deposits in ectopic endometrial lesions could be induced by the addition of red blood cells in menstrual effluent [21], showing that retrograde menstruation is one of the major contributors to iron accumulation in endometriosis. Defrère *et al.* reported

that iron overload enhances the proliferation of ectopic endometrial cells in endometriosis [22]. While injection of erythrocytes and menstrual effluent resulted in the proliferation of endometrial lesions, it did not lead to the initial establishment of endometriosis. This is important because significant iron overload may only occur after endometrial lesions have already been established, but not during the initial stages of the disease when refluxed endometrial tissue is first attaching to areas in the pelvic cavity. These overall increases in iron accumulation may be due to higher degradation of red blood cells from retrograde menstruation, as well as a deficiency in iron metabolism in endometriosis patients.

In retrograde menstruation, there is an abundance of menstrual reflux into the peritoneal cavity. The refluxed erythrocytes are phagocytized by peritoneal macrophages, and as a result, they release Hb into the peritoneal cavity. Haptoglobin binds free Hb in the blood to prevent oxidative damage and the hemoglobin-haptoglobin complex is usually removed (Fig. 5.2). Free Hb is also digested by hemeoxygenase-1 (HO-1), and iron is subsequently released from the heme molecules. Ferritin is one of the metabolic byproducts of hemeoxygenase-1. It sequesters iron and prevents oxidative stress. Hemosiderin is a complex of ferritin that also stores iron within macrophages. Iron is usually transferred to ferritin and hemosiderin within macrophages to prevent iron-mediated damage, inflammation, and the production of excess amounts of ROS that may lead to oxidative stress (Fig. 5.2) [23].

Iron accumulation can lead to numerous cytotoxic effects because it can disrupt the balance between free radical production and antioxidant defense, which leads to oxidative stress (OS) and, in turn, contributes to the pathogenesis of endometriosis. Therefore, iron-induced OS may trigger the chain of events resulting in the development and progression of the disease [24].

Iron overload caused by retrograde menstruation leads to accumulation of somatic mutations through Fenton reaction-mediated OS. The development of endometriosis is triggered by epigenetic disruption of gene expression along with environmental changes. There are three phases for development of endometriosis: genetic inheritance from parents, epigenetic modification in the female offspring, and iron overload, which is subjected to modulation later in life [1].

However, with an abundance of menstrual reflux with an overwhelmed peritoneal disposal system or along with a defective disposal system, iron levels exceed the sequestration capability of the macrophages, leading to iron overload. There is a resultant iron overload in the peritoneal environment, which in turn permits attachment and growth of the endometrial cells or fragments [25] and, ultimately, iron toxicity [23]. Iron overload may also occur when endometrial lesions bleed. This leads to the additional accumulation of erythrocytes in the peritoneal cavity [22] (Figs. 5.2 and 5.3). Iron levels in peritoneal macrophages of women with endometriosis tend to be higher than levels in macrophages from healthy women [11]. Studies have also shown that iron levels in the peritoneal fluid of women with endometriosis correlate with the severity of the disease [14, 15].

Van Langendonckt et al. showed that endometrial lesions can be induced in nude mice by injecting human menstrual tissue into the pelvic cavity, thereby mimicking menstrual reflux [21]. Defrère et al. found that while adding erythrocytes to the menstrual fluid injections did not lead to the establishment of endometrial lesions in

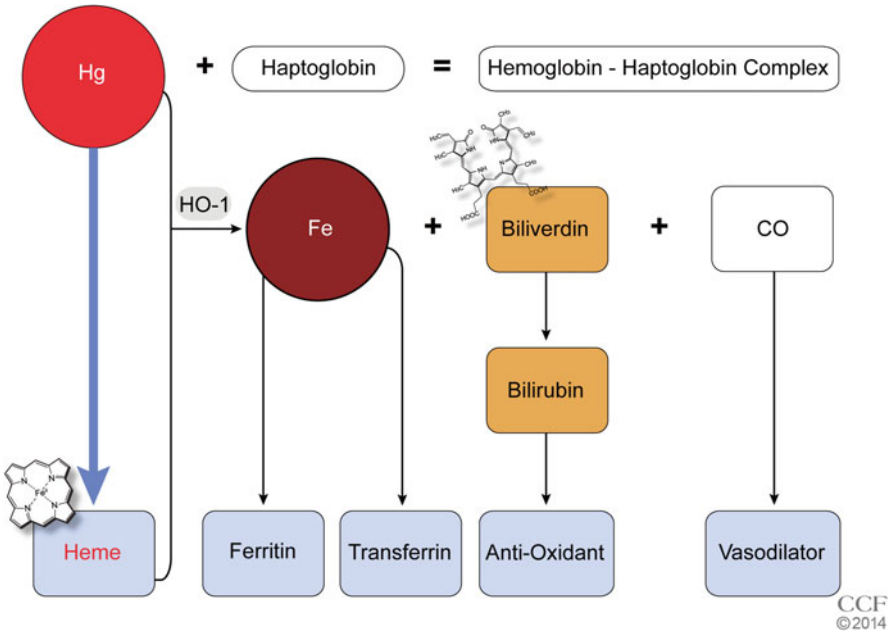

Fig. 5.2 The role of iron in the pathophysiology of endometriosis. *HO* hemeoxygenase, *CO* carbon oxide

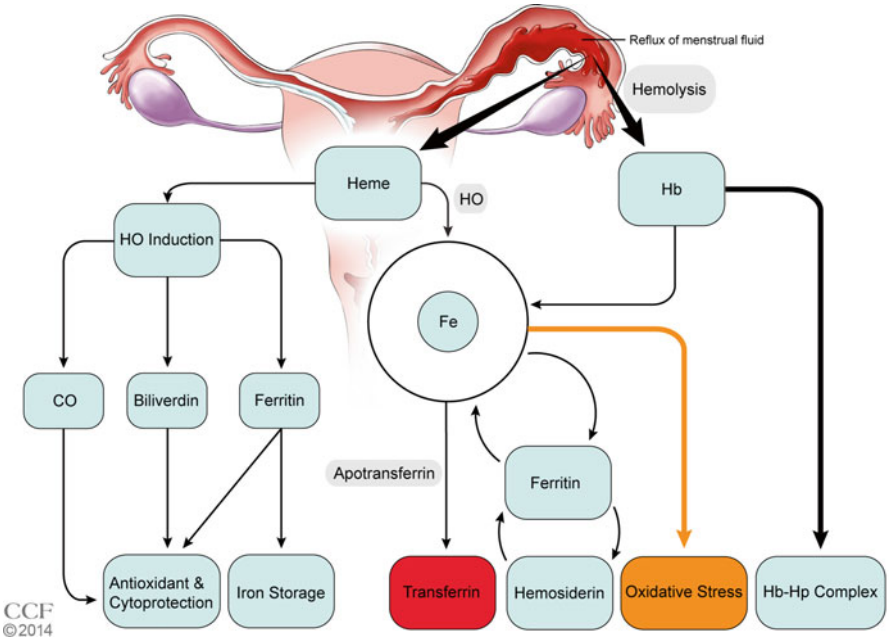

Fig. 5.3 Iron metabolism and binding in peritoneal cavity. *Hb-Hp* hemoglobin-haptoglobin, *HO* hemeoxygenase, *CO* carbon oxide

mice, it did significantly increase the proliferation of the lesions [22]. These studies help confirm that menstrual reflux and iron metabolism within the peritoneal cavity play a role in the development of endometriosis and may be factors in oxidative damage associated with the presence of endometrial lesions. A disrupted peritoneum is not a requirement for endometrial tissue invasion [26].

5.4 Iron and Oxidative Stress

Iron-induced oxidative stress plays a fundamental role in the pathogenesis of endometriosis. Oxidative stress, secondary to influx of iron during retrograde menstruation, modifies lipids and proteins, leading to cell and DNA damage. Studies have demonstrated that hepatocyte nuclear factor (HNF-1β) overexpression in endometriotic foci increases the survival of endometriotic cells under iron-induced oxidative stress conditions possibly through the activation of forkhead box (FOX) transcription factors and/or endometriosis-specific expression of microRNAs. Endometriotic cells expressing HNF-1β also display cell cycle checkpoint pathways required to survive DNA damaging events [27].

The iron-induced ROS signals can contribute to carcinogenesis via estrogen-dependent pathways leading to endometrioid adenocarcinoma (EAC) or estrogen-independent clear cell carcinoma (CCC), thus supporting tumor progression and metastasis [28].

5.4.1 Fenton Reaction

Iron toxicity is mainly related to its ability to catalyze the production of a wide variety of damaging free radical species in the Fenton reaction, leading to deregulation of cellular processes, cell dysfunction, and eventually to apoptosis or necrosis through lipid peroxidation, protein, and DNA damage [29].

Iron acts as a catalyst in the Fenton reaction and generates wide range of free radicals such as hydroxyl radicals (OH) or the peroxynitrite anion (ONOO−), which is the product of reaction between NO and superoxide anion (O2−) (Fig. 5.4).

5.5 Mechanistic Pathway of Iron Induced Oxidative Stress in Endometriosis

Endometriosis → Retrograde Menstruation → Iron → Oxidative stress.

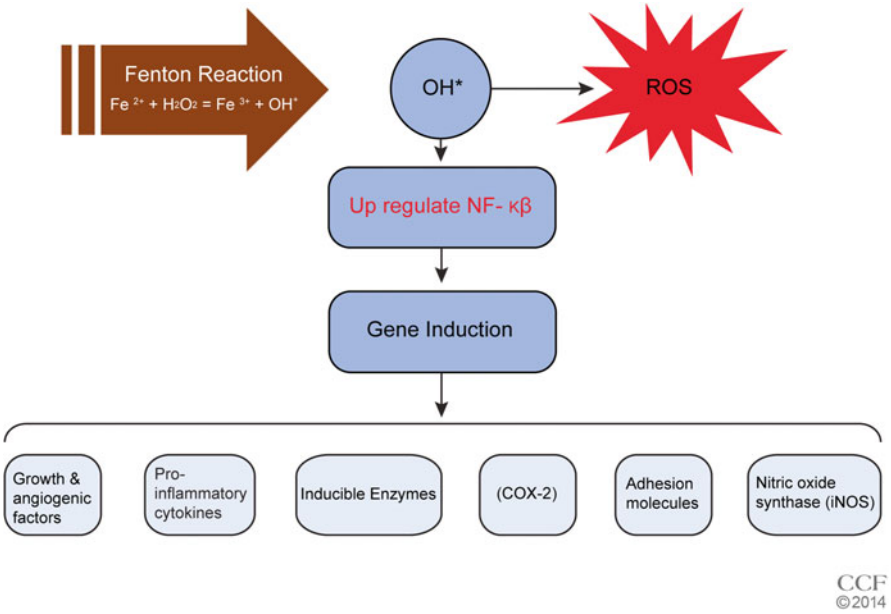

Fig. 5.4 Implications of Fenton reaction in endometriosis. *ROS* reactive oxygen species, *NF-κβ* nuclear factor kappa β, *COX* cyclooxygenase

5.6 Hemoglobin: Haptoglobin Complex, Hemopexin and Fenton Reaction

After phagocytosis of senescent erythrocytes, Hb may be catabolized by hemeoxygenase-1, or Hb may form a complex with haptoglobin. Haptoglobin is a protein that binds free Hb with a high affinity to prevent oxidative damage that can result from Hb overload [30]. The hemoglobin-haptoglobin complex can be cleared by parenchymal cells of the liver [31] although the complex may also be scavenged by peritoneal macrophages [32]. Binding of Hb by haptoglobin is a defense mechanism. Both Hb and haptoglobin can induce inflammation and oxidative stress [18, 19]. This defense mechanism still may be overloaded by Hb in the peritoneal cavity.

A Hb scavenger receptor, cluster of differentiation 163 (CD163) is expressed on monocytes and macrophages [33]. CD163 is a member of the cysteine-rich scavenger receptor family [34]. The CD163 scavenger receptor mediates endocytosis of Hb into peritoneal macrophages [34] and also mediates the degradation of the hemoglobin-haptoglobin complex [35]. The CD163-mediated uptake of iron through the hemoglobin-haptoglobin complex may explain iron accumulation within macrophages. The CD163 scavenger receptor present on macrophages also acts in coordination with haptoglobin [30, 36]. There are several scavenger receptors including CD163 and CD206 that are involved in both scavenging of hemoglobin with iron transfer into macrophages and the silent clearance of inflammatory molecules.

In endometriosis, peritoneal macrophages have been found to accumulate iron. Phagocytosis of red blood cells or endocytosis of the hemoglobin-haptoglobin complex leads to the origin of iron in macrophages. Heme, which is a product of catabolization of hemoglobin by HO, produces reactive iron. This free iron is then incorporated into macrophage ferritin or transferrin in the peritoneal fluid. Studies have reported that peritoneal macrophages in endometriosis patients exhibited high Tf receptor expression and were more likely to be saturated with Hp [18]. Endometrial lesions have also been found to synthesize and secrete Hp [37, 38]. Haptoglobin may act as an angiogenic or immunomodulatory factor [37]. This may lead to further vascularization of lesions and possible proliferation. Past studies have reported findings of increased haptoglobin in the peritoneal fluid of women with endometriosis [39]. However, other studies have reported no increase in Hp levels in endometriosis patients [40]. Haptoglobin may not be sufficient in binding Hb. Therefore, free Hb may be present in the peritoneal fluid and provide a source of heme that may become bound to hemopexin (HPX). Hemopexin is a glycoprotein that binds heme with the highest affinity of any protein. HPX scavenges heme that is released in the degradation of Hb by HO-1 and acts as another extracellular defense mechanism to protect the body from OS induced by free heme and iron molecules. Heme may cause damage through lipid peroxidation and the production of hydroxyl radicals [40]. The antioxidant capacity of HPX can also be overwhelmed by heme and iron overload in the peritoneal cavity [41].

HPX is also involved in cytoprotection and the prevention of iron-mediated damage, including the prevention of inflammation and proliferation of endometrial lesions [42]. Liver cells uptake heme and HPX in order to maintain levels of iron in the body along with Hb-Hp complexes. Hemopexin binds heme and transports it into the liver for degradation in the reticuloendothelial system. Blood serum levels of HPX have been used to indicate how much free heme is present in the blood. HPX levels in peritoneal fluid may also prove to be a marker of endometriosis. Macrophages are also able to uptake HPX-bound heme, but not in significant amounts except in cases of iron overload [23]. Studies have shown that hemopexin is significantly down-regulated in peritoneal fluid from patients with endometriosis [43].

5.7 Ferritin, Transferrin, and Hemosiderin

When heme-sequestering proteins experience iron overload, the degradation of Hb and heme by HO-1 releases free iron. Iron can be stored by the cells of endometrial lesions through the iron transporter protein transferrin within the peritoneal fluid, or the free iron can be stored in ferritin within macrophages [23]. Ferrous iron is able to catalyze the formation of ROS through the Fenton reaction, which produces hydroxyl radicals [44]. When free ferrous iron is released into the peritoneal fluid, it can be stored in ferritin, which oxidizes the ferrous iron to ferric iron and traps it within the shell of the protein [45].

Ferritin is an intracellular protein that stores and releases iron within macrophages. When ferritin does not have iron bound to it, it is called apoferritin. Because iron is redox-active, ferrous iron can be released by ROS from ferritin. Redox-active iron can lead to the generation of even higher amounts of ROS, continuing the cycle of iron release and cell damage by OS [1].

Hemosiderin is an iron-storing complex of ferritin, which also allows for iron accumulation within macrophages. The name hemosiderin can be used to describe clusters of iron deposits within cells, which may or may not include ferritin within the deposits [46]. Hemosiderin molecules may be found independently apart from ferritin in cases of iron overload [12]. Unlike ferritin, hemosiderin cannot be released from macrophages. However, hemosiderin-laden macrophages may be incorporated into endometrial lesions, resulting in the dark lesions seen in endometriosis [47].

Hephaestin is a membrane-bound protein that is essential for normal iron metabolism. It is a multi-copper ferroxidase protein that promotes the oxidation of ferrous iron to ferric iron [48]. Ferric iron can be bound to transport proteins such as transferrin and other plasma ligands [49]. When ferrous iron is oxidized by hephaestin, it can be taken up by transferrin and transported to the basolateral membrane [45]. This oxidization of iron allows the free iron to bind rapidly to transferrin and be transported to receptors on cells to prevent oxidative damage [49].

Transferrin is a blood plasma glycoprotein that binds iron and transports iron to tissues in need of iron, although it can also be incorporated into endometrial lesions in cases of endometriosis, possibly through the incorporation of iron-laden macrophages. Transferrin, ferritin, and hemosiderin are where most of the free iron in the body is stored—this helps maintain levels of iron in the body and prevent OS. Transferrin, ferritin, or hemosiderin levels within the peritoneal cavity, either in endometrial lesions or in the peritoneal fluid, may act as markers for increased iron levels and possible endometriosis.

5.8 Long-Term Consequences of Iron Overload

Iron overload is thought to cause oxidative stress, and persistent OS has been associated with carcinogenesis. Epidemiological studies support a close link between iron overload and carcinogenesis.

Iron-induced oxidative stress generates ROS, causes DNA modifications, and activates detoxification and anti-apoptotic pathways, hence contributing to cell progression from endometriosis to malignancy [28].

A combination of animal experiments and microarray analyses suggests that homozygous deletion of CDKN2A/2B is one of the major target genes involved in iron overload-induced carcinogenesis. CDKN2A/2B are the second most frequently inactivated tumor suppressing genes in human cancers, and excess iron can cause deletion of CDKN2A/2B leading to cancer [44].

5.9 Key Points and Summary

In endometriosis, iron accumulation can occur within endometrial lesions and peritoneal macrophages. Erythrocytes are phagocytized by macrophages, releasing Hb into the peritoneal fluid where it can form a complex with haptoglobin or be catabolized by hemeoxygenase-1 to produce free iron. The free iron can be taken up by iron storage and transporting proteins such as ferritin and transferrin, which can lead to iron accumulation within macrophages or lesions.

Increased iron levels have been found in the peritoneal fluid of women with endometriosis. High levels of iron have also been found in the peritoneal macrophages of endometriosis patients. Several genes involved in the development and progression of endometriosis as well as the body's response to OS are often regulated by iron. Iron overload does not appear to affect lesion establishment but may contribute to the further growth of endometriosis by promoting cellular proliferation within lesions. Iron chelator treatment could therefore be beneficial in endometriosis to prevent iron overload in the pelvic cavity and decrease cellular proliferation of lesions.

Endometriosis may be treated in the future with iron chelators such as DFO used locally as a depot to prevent the proliferation of endometrial lesions and the progression of the disease. Additional research is necessary to further examine how iron overload affects endometriosis and the possible treatments for endometriosis that may be specific to iron overload localized to the pelvic cavity.

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Chapter 6
Role of Environmental Pollutants in Endometriosis

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6.1 Introduction

Both animals and humans are exposed to toxins in the environment that may influence the onset and progression of endometriosis. Human exposure occurs mainly through ingestion of contaminated foods. Dioxins encompass a group of environmental pollutants that act as endocrine disruptors through the aryl hydrocarbon receptor and disturb the body's physiologic homeostatic mechanisms. Some have even been labeled as carcinogens. Human exposure to toxins is often unavoidable, but measures including a detailed history taken by clinicians and lifestyle changes can help detect and limit exposure and assist in the body's detoxification processes. Growing evidence suggests a possible link between endometriosis and environmental pollutants. Environmental pollutants such as dioxins, organochlorine pesticides (OCPs), bisphenols and phthalates and their association with endometriosis are highlighted in this review along, with the steps patients can take to avoid them. Even though results from studies remain contradictory, we can't overlook the positive association between environmental toxicants and endometriosis as they are disruptors of endocrine and reproductive function. The literature reviewed in this section highlights the general pathogenesis of endometriosis and proposed theories regarding its etiology. The chapter also provides an updated discussion of the implications of environmental exposure to pollutants in the development of endometriosis.

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6.2 Classification of Environmental Pollutants

1. Polyhalogenated aromatic hydrocarbons
2. Dioxin like compounds
3. Organochlorine pesticides
4. Phthalates
5. Bisphenols

6.3 Toxic Effects of Environmental Pollutants

6.3.1 Human Studies

Some studies have suggested that exposure to dioxin-like compounds is linked to the development of endometriosis in humans. Mayani et al. conducted a study in which 44 infertile women with endometriosis were evaluated. Eight tested positive for the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) in their blood compared to 1 of 35 infertile women without endometriosis [1].

6.3.2 Animal Studies

Rier et al. [2] conducted a long-term controlled study that included a group of Rhesus monkeys—the 24 monkeys exposed to dioxin had biopsy proven accumulation of dioxin in their adipose tissue. Ten years after the termination of dioxin exposure, they found 7 of the monkeys had died: 3 of them due to endometriosis and 4 of them due to other unrelated causes. The long-term effect of dioxin exposure and the development of endometriosis are known to be significant [1].

Wood DH et al., found that when female rhesus monkeys exposed to protons of varying energy levels for a minimum period of 7 years developed endometriosis when compared to non-radiated animals of the similar age group. They also correlated this finding to the level of radiation exposure that a female crew worker experienced while she was flying in a near earth orbit amidst a random solar flare event. Thus, radiation should be taken into consideration as an important factor potentially promoting the development of endometriosis [3].

6.4 Mechanism of Toxic Induction

Dioxins exert adverse effects through binding and activation of the aryl hydrocarbon receptor (AhR)—a transcription factor containing two heat shock protein (HSP) 90 molecules, one prostaglandin E synthase 3 (p23) molecule, and one X-associated

protein 2 (XAP2) molecule [4]. This binding prevents AhR from performing homeostatic functions such as tumor suppression regulation of the cell cycle [5] and cell death [6]. Upon activation by dioxin, AhR dissociates from one hsp molecule, p23, and XAP2. It then translocates from the cell's cytoplasm to the nucleus and forms a complex with aryl hydrocarbon nuclear translocator (ARNT), releasing the remaining attached hsp 90 molecule [7]. Finally, the new AhR-ARNT complex binds to dioxin-responsive enhancers (DREs) found within the promoter regions of many TCDD-responsive genes [8], particularly CYP1A1 and perhaps CYP1B1 [9]. Through persistent activation of AhR, dioxins can disrupt the normal expression of genes and enzymes. Dieldrin, endosulfan, and lindane (γ-HCH) are amongst the OCPs that have both estrogenic and anti-androgenic properties [10]. Endosulfan and Lindane are also known to block the enzyme aromatase, which mediates the production of E2 by catalyzing the peripheral conversion of testosterone to estrogen. Ingestion of dioxins can cause several adverse effects in animals and humans. As endocrine disruptors, they can impair development, hormone actions, and immune defenses in the body. The endocrine disruptors are also considered carcinogenic.

6.5 AhR Modulation and Its Impact on Endometriosis Development

AhR exists in tissues throughout the body, including both eutopic and ectopic endometrium [11]. Dioxin-AhR complexes may encourage the development of endometriosis via a combination of growth factor activation, immunosuppression, gene dys-regulation, and altered estrogen-signaling pathways [12].

Rier et al. 1993 discovered endometriosis incidentally while conducting a study on reproduction and toxicants in Rhesus monkeys. The monkeys were treated with TCDD for 4 years. Six years later, the monkeys underwent laparoscopy and most of them had endometriosis. The amount of TCDD administered to the animals was found to be in direct proportion to the prevalence and severity of endometriosis [2]. In 2001, the group reported that elevated serum levels of PCBs are responsible for the greater prevalence and severity of endometriosis seen in the monkeys [13]. This experiment demonstrated the actions of PCB congeners through AhR in promoting endometriosis.

More recently, Willing C et al. demonstrated that the AhR-ARNT complex within endometrial cells caused alterations in hsp, leading to its amplification and ultimately invasion and implantation of ectopic tissue, causing endometriosis [14]. These results support the data reported from earlier studies [15–17].

6.6 Activation of Growth Factors

Dioxin has been shown to affect tumor growth factor (TGF) alpha and beta as well as the receptors for epidermal growth factor (EGF) and insulin growth factor (IGF). Induction of cytokines such as IL-1beta and IL-6 has also been demonstrated by dioxins [18, 19].

The effects of varied levels of TCDD exposure were measured 13 years later by Rier S et al. 2001. Exposure over a 4-year period was found to be associated with elevated TNF-α, which may influence the development of endometriosis [13]. Serum levels of IFN-gama and TNF-α are increased in women with endometriosis [20, 21]. Along with IL-6 and interferon-γ, TNF-α is important for the function, proliferation, and apoptosis of endometrial cells. Increased serum levels of dioxins and altered functions of leukocytes were also observed to disturb the regulation of growth factors, thus precipitating endometriosis in the animals [13].

Endometriosis is also associated with chronic inflammation, and several pro-inflammatory cytokines have been detected in the peritoneal fluid of affected women. Chronic ovarian inflammation was observed in rats in response to dioxins and PCB126. The study also linked uterine inflammation with dioxins and PCB153. It has been suggested that PCB153 may have estrogenic actions and thus could compete with estrogen for ER binding [22]. The combination of agonistic and antagonistic effects of DLCs on estrogen might account for the disrupted epithelial differentiation seen in endometriosis [23].

6.7 Gene Dys-Regulation

Interestingly, a study by Tsuchiya et al. [9] found that women with elevated serum dioxin TEQ levels had a statistically significant lower risk for. All 138 subjects were confirmed to have endometriosis by laparoscopy, and the role of cytochrome P450 (CYP) gene polymorphism was examined for its potential to protect against dioxin- and PCB-induced endometriosis. One particular gene polymorphism, *CYP1A1 462Val*, was found in association with a significantly decreased risk of endometriosis in women with higher serum TEQ. No association, however, was found between serum PCB TEQ, advanced endometriosis, and the *CYP1B1 Leu432Val* allele. Gene polymorphisms such as CYP1A1 and CYP1B1 were found to have a direct effect on how dioxins and DLCs affect the female body. It is the most recently published work relating specific CYP gene polymorphisms to dioxins and their effects on endometriosis [9]. Nevertheless, further studies must be conducted to validate this connection.

Several genes have been suggested to be involved in endometriosis. Amongst these are CYP1A1, COX-2, CYP19, ER-α, ER-β, PR A and B, and c-*fos* [24, 25]. In a recent study, Van Ede KI et al. 2010 reported a dose-dependent elevation of uterine CYP1A1 mRNA secondary to activated AhR at day 3 and a significant 2.5-fold increase of COX-2 as well as an increase in ER-β mRNA. Of further significance is the sharp elevation of the c-*fos* proto oncogene at a dose of just 0.5 μg TCDD/kg body weight and subsequent dose-dependent *reduction* in levels at 25 μg TCDD/kg; this finding has also been documented by another group [26]. At day 14, PR A/B was significantly increased, but levels of COX-2 mRNA were unchanged. These

data indicate the possibility of TCDD-induced altered endometrial expression of genes linked to the development of endometriosis.

Estrogen metabolism involves conversion of estrone (E1) to estradiol (E2) by 17β hydroxysteroid dehydrogenase (17β-HSD) type I [27]; this process is catalyzed by CYPA1A. By demonstrating the induction of CYPA1A by dioxins and PCBs, the results of Tsuchiya et al. 2007 and Van Ede KI et al. 2010 suggest consequent post-exposure surges of estradiol production. Logically, this would promote the onset and maintenance of endometriotic lesions, which exclusively express activity of 17β-HSD type I [28]. Also, Lai et al. noted that most genes induced by TCDD had three or more DREs (Dioxin-responsive element) [18]. Moreover, the CYPA1A gene contains 14 DREs (Dioxin-responsive element) in the 5′→3′ orientation and is very sensitive to TCDD induction [29]. It can be considered a prototype of the TCDD-inducible gene [18].

The glutathione S-transferase (GST) enzymes are essential in protecting cells against both toxin-induced and oxidative damage. GSTM1 may contain a particular deletion polymorphism, which may be altered by dioxins [30]. GSTM1 is one of the phase II detoxification enzymes that participate in a protective mechanism against toxic environmental pollutants. GSTM1 deletion polymorphism leads to the lack of detoxification and results in endometriosis.

Expression of the *GSTM1 null* mutation has been associated with endometriosis in some studies [30, 31], but others [32, 33] have failed to make the same link. To assess the role of PCBs on the *GSTM1 null* mutation and development of endometriosis, Roya R et al. 2009 studied a population of 199 infertile women. Their results confirmed a significant association between *GSTM1 null* mutation and endometriosis that had been reported in earlier studies [34]. Additionally, they observed that increased PCB levels were correlated with more severe disease [35]. Roya's group were amongst the first to demonstrate that in addition to expressing higher degrees of the GSTM1 polymorphism, women with histories of higher PCB exposures may also suffer from more severe disease.

6.8 Immunosuppression

Dioxin exposure decreases leukocyte phagocytic function, preventing the elimination of menstrual debris, and thus, may play a key role in establishing endometriotic implants from retrograde menstruation [8]. Another important dioxin-related immunosuppressive effect is inhibited—T-lymphocyte function and NK cell activity in the plasma and peritoneal fluid. Through the activation of AhR, endometriosis may be triggered by increasing interleukin levels, tissue remodeling, and activating cytochrome P-450 enzymes. TCDD-activated AhR can also disrupt normal cell function by stimulating NF-κ, resulting in altered immune responses and uncontrolled proliferation of cells [36] (Fig. 6.1).

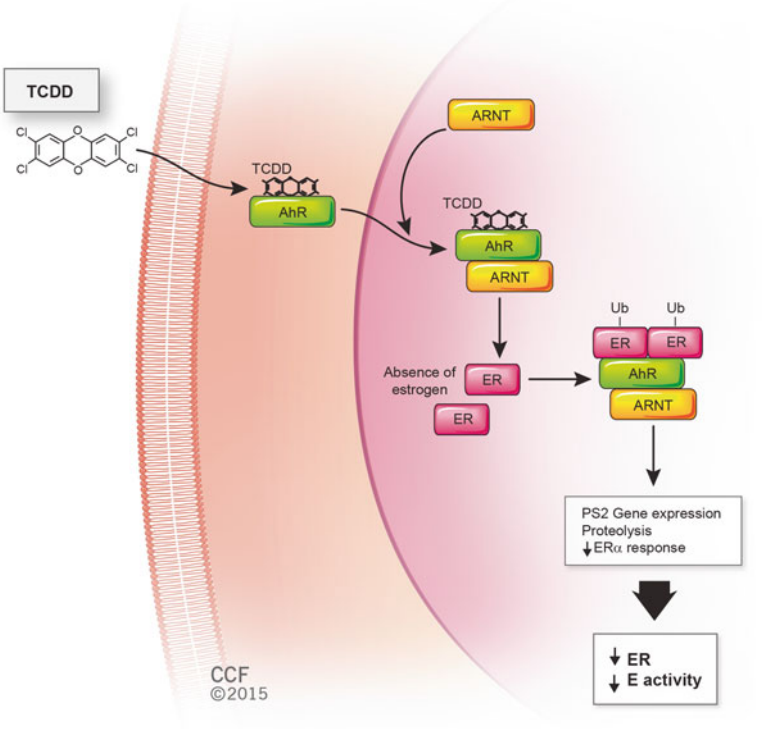

Fig. 6.1 TCDD induced AhR receptor modulation. *TCDD* tetrachlorodibenzo-p-dioxin, *ARNT* aryl hydrocarbon nuclear translocator, *ER* estrogen receptor, *AhR* aryl hydrocarbon receptor, *E* estrogen

6.9 Altered Hormone Signaling Pathways

TCDD has been shown to disturb the activities of both estrogen and progesterone [36]. As a known antagonist of estrogen, TCDD was determined to alter expression of CYP1A1 and CYP1B1 in human endometrial cells [37]. Because endometriosis is estrogen dependent, the disruption of normal estrogen function has the potential to influence the disease [8]. Therefore, the toxicity of dioxins and DLCs largely depends on the body's estrogen content. While estrogen-activation of ERs prevents AhR complex expression, failure of estrogen to ignite these receptors leaves them open for activation by the dioxin-activated AhR-ARNT complex [38] (Fig. 6.1).

Concentrations of estrogen and ERs decrease in response to dioxin exposure, and thus, dioxins are often believed to exhibit anti-estrogenic properties. On the contrary, an increase in the incidence and extent of endometriosis has been demonstrated in monkeys [2] and growth of ectopic lesions have been seen in mice and rats [39, 40] both in response to dioxin exposure. It can be clearly understood that TCDD disrupts the human reproductive system by acting as an estrogen antagonist and also

causing imbalances in progesterone distribution [36]. Other etiologic pathways of endometriosis may depend on TCDD exposure, other than the estrogen and progesterone disruption [36].TCDD activates an inflammation-like pattern [41], which explains the development of endometriosis. Also, TCDD and dioxin-like PCBs affect gene expression by using AhR expressed in both endometrial and immune cells and disrupt endocrine signaling [36, 42].

In 2004, Kitajima M et al. conducted a study on mice after surgically inducing endometriosis. Four weeks of exposure to TCDD did not seem to enlarge lesion size but instead diminished the size of epithelial and stromal masses. Treatment with estrogen alone resulted in the enlargement of epithelial and stromal cells mass within the lesions, indicating that estrogen is an important factor for the growth of these lesions. When TCDD was administered short term, its anti-estrogenic effects reduced ectopic lesions and cell mass [43].

Increases in local estrogen production by heightened ER activity can promote estrogen-dependent diseases such as endometriosis. Elevated expression of mRNA of aromatase, an enzyme essential to estrogen synthesis, has been noted to increase estrogen levels and promote endometriosis. Heightened expression of aromatase may be propelled by environmental factors such dioxins and results in reduced levels of 17β HSD type II. This creates an environment of elevated local estrogen, making the local peritoneal environment a prime location for survival of ectopic endometrial stromal cells. Studies have reported that progesterone hinders endometrial growth and causes regression of endometriosis with treatment [12]. The endometrium of women with the disease, however, demonstrates a decreased response to progesterone. Because TCDD inhibits progesterone, it often exacerbates endometriosis, especially when combined with estrogen [44]. Endometrial dysfunction has been linked to progesterone resistance in endometriosis patients. Progesterone action through progesterone receptors (PR) is necessary for female reproductive functions in humans and other mammals [15]. During pregnancy, progesterone exposure has been shown to protect against endometriosis.

In endometriosis, the matrix metalloproteinases (MMP) are involved in degrading the extracellular matrix, a process important to the development and invasion of endometriotic lesions [44]. Suppression of MMP by progesterone is required to up-regulate TGF-β, resulting in diminished growth of ectopic endometrial tissue [45]. In their study on nude mice, Nayyar et al. 2007 demonstrated that significant decreases in progesterone-mediated expressions of TNF-β2 and PR-B in response to TCDD exposure resulted in a more severe presentation of endometriosis [15]. The group concluded that TCDD can decrease progesterone levels via expression of TGF-β2. This decrease in progesterone-propelled ectopic growth of surgically placed endometrial tissue leads to endometriosis. Exposure of mice to TCDD both in utero and during reproductive maturation decreased PR-A and PR-B, predisposing them to endometriosis [15].

Bruner-Tran et al. 2010 were unable to find a definite correlation between endometriosis and TCDD exposure in cynomolgus monkeys and rodents. However, since TCDD has both estrogenic and antiestrogenic properties, reduced lesion size and spontaneous abortion are plausible outcomes of exposure. As such, the group reported that rodents exhibited infertility and an inability to maintain pregnancy secondary to TCDD exposure in utero and pre-puberty [46].

6.10 Prevention and Management

Human exposure to environmental pollutants is often inevitable, however, strategies have been suggested in an effort to limit exposure, maximize elimination from the body, and implement lifestyle changes (Fig. 6.2). These strategies may prove useful to limit the toxic effects of environmental contaminants in human populations.

Persistent toxicants remain as residuals in human body long after the first exposure. This is probably the most important obstacle that can inhibit the progress of detoxification since many of the environmental toxicants have a long half-lives. Genis et al. [47] analyzed the sweat of 20 individuals and found some phthalates metabolites. Hence, induced perspiration may be a way to eliminate potentially toxic metabolites. Exercising may be another way to eliminate these toxins since it induces sweating. However, some studies emphasize that excretion rates do not change depending on how perspiration occurs (e.g., infrared sauna, dry or wet regular saunas or exercise) [48].

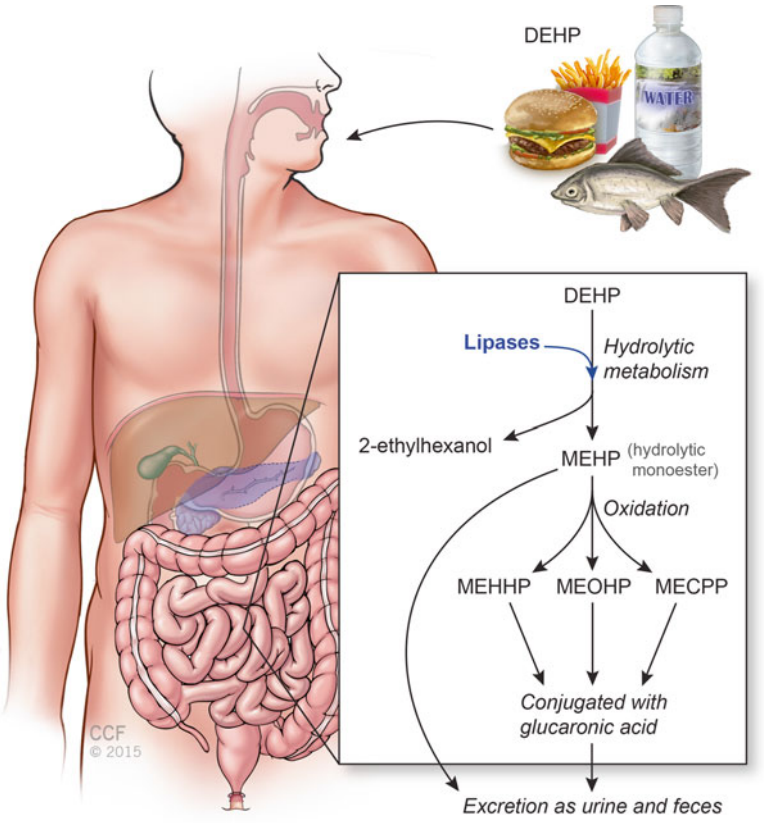

Fig. 6.2 Pthalate metabolism. *DEHP* di-(2-ethylhexyl)phthalate, *MEHP* mono-(2-ethylhexyl) phthalate, *MEOHP* mono-(2-ethyl-5-oxohexyl) phthalate, *MEHHP* mono (2-ethyl-5-hydroxyhexyl) phthalate, *MECPP* mono(2-ethyl-5-carboxypentyl) phthalate

There is also an effort to limit the exposure by restricting the use of these chemicals. Some nations, including the United States, have banned the use of some environmental toxicants like organochlorine pesticides, mostly DDT. Moreover, lifestyles changes like diet modification can be helpful. This include vitamin and mineral supplementation to replenish depleted body induces, increased fiber intake to limit body absorption of toxic compounds and promote elimination; and avoidance of certain types of foods known to have high accumulations of environmental toxins [49].

Conventional and novel treatment options for endometriosis are discussed elsewhere; this section will focus on disease prevention strategies and management options for endometriosis as they relate to human contamination with environmental toxins. Currently, no cure exists for the disease. As such, goals of management are mainly to provide relief from pain and restrict progression of the disease. When appropriate, efforts are also made to preserve or restore fertility through medical or surgical therapy. In general, combination oral contraceptives (COCPs), gonadotropin-releasing hormone (GnRH) agonists, progestational agents, danazol and aromatase inhibitors are commonly used in the medical treatment of endometriosis for patients who wish to conserve fertility.

As mentioned before, dioxins have been shown to up-regulate expression of P-450 aromatase, leading to increased estrogen synthesis within endometriotic tissue [50]. This is the rationale for the use of aromatase inhibitors to manage endometriosis. After administration, endometriotic lesions have been noted to regress, thus, signifying the need for aromatase in patients with persistent endometriotic lesions [51].

6.11 Key Points and Summary

The role of dioxins in the development of endometriosis remains controversial. Thus far, research has produced varying results, contributing to this uncertainty. Over time, experiments on animals have culminated in the development of correlations between environmental toxins and of endometriosis in mammals. These studies, including those on primates, have propelled current exploration of a possible similar association in humans.

Similarities between dioxin characteristics and risk factors for endometriosis have been noted. For example, dioxins are lipophilic with long half-lives and accumulate in high fat tissues. Obese females have large amounts of adipose tissue; thus, the more fatty tissue present in the body, the longer it will take dioxins to be eliminated from the body. Given that obesity is a risk factor for endometriosis, a potential link exists between lipophilic compounds such as dioxins and endometriosis. Due to their accumulation in lipids, dioxins can be largely excreted in breast milk and eliminated from the body through lactation, which is believed to be a protective factor against the onset of endometriosis.

Epidemiological studies on endometriosis in association with environmental pollutants have been quite inconsistent in both animal and human studies. Although copious studies investigating the involvement of environmental toxins in endometriosis

have produced promising results, no study has demonstrated a direct link nor confirmed a causal relationship between the two. Case-control studies can prove valuable under certain conditions. In addition to a large sample size, controls should be laparoscopically confirmed to be free of endometriosis. Since endometriosis tends to run in families, clinicians should aim to identify family members confirmed or suspected to have endometriosis when taking a family history.

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Chapter 7
Endometriosis and Ovarian Cancer

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7.1 Introduction

Recent studies have provided much evidence to support the theory that endometriosis is actually a neoplasm and that affected cells can undergo malignant change. Women with endometriosis have a higher risk of certain types of ovarian cancer than the general female population [1]. Approximately 2.5 % of ovarian endometriosis turns malignant, but this may be an underestimation [2]. Studies have shown that Type 1 ovarian cancers such as clear cell carcinoma (CCC) and endometrioid adenocarcinoma (EAC) are associated with endometriosis [3]. Together, these carcinomas have been termed endometriosis-associated ovarian cancer (EAOC). The prevalence of endometriosis in ovarian cancer includes 39 % for clear cell carcinoma, 21 % for endometrioid carcinoma, and 3 % for serous and mucinous carcinoma [4].

Endometriosis and EAOC share a possible similar origin and etiology, and have defective immuno surveillance. Both the conditions are estrogen dependent and their growth is promoted by increased levels of estrogen and decreased levels of progesterone. They are both stimulated by steroid hormones and have common pathogenic mechanisms such as inflammation, persistent oxidative stress, hormonal alterations, retrograde menstruation, familial predisposition, and genetic alterations.

7.2 Sampson's Theory of Malignant Transformation

There are several theories as to how endometriosis develops, but the most widely Accepted is Sampsons's theory. The possibility of malignant transformation of endometriosis to EAOC was also first suggested by Sampson in 1925 [5]. The theory states that malignant transformation of endometrial cysts, which is believed to be a

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major factor concerning EAOC, must arise from pre-existing benign endometrial tissue that has not invaded from another unrelated source [6]. The benign and the carcinomatous tissue must be present in the same ovary and the tissue should resemble endometrial stroma and have epithelial glands [6]. This theory is supported by numerous studies that have found endometriosis in more than 50 % of clear cell and endometrioid cancers but in less than 10 % of serous cancers [4, 7–9].

7.3 Ovarian Cancers Associated with Endometriosis

Malignant transformation of endometriosis occurs most commonly in the ovaries, less commonly in rectovaginal septum, vagina and colon and more rarely in the abdominal wall [10]. Studies have reported that endometriosis involves, multiple genes and environmental factors that also contribute to its malignant transformation [11]. Pathological studies have found a sequence of steps in the transformation of normal endometriotic cyst epithelium to atypical endometriosis leading to invasive cancer. Tanase et al. reported a case in which the patient's disease advanced from a benign endometriotic cyst to atypical endometriosis and finally to endometrioid adenocarcinoma within 10 years and suggested that cases of atypical endometriosis need to be followed up diligently for long periods because of the possibility of malignant transformation [12].

7.4 Genetic Alterations Common in Endometriosis and EAOC

A number of factors have been associated with the malignant transformation of endometriosis into EAOC: oncogenic activation, deletion or loss of function of tumor suppressor genes, loss of heterozygosity (LOH) at numerous chromosomes, and OS [13]. Different cell system (cycle) components such as cyclins and cyclin dependent kinases (Cdk) may affect the pathogenesis of EAOC. Various techniques such as fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH), and conventional genomics were used to determine the extent of genomic mutations in women with endometriosis or EAOC (Table 7.1).

Accumulating evidence suggests that various epigenetic aberrations exist in endometriosis like in ovarian cancer. Some genes like HOXA10 and progesterone receptor B (PR-B) are hypermethylated (down-regulated) and estrogen receptor beta (ER-β), steroidogenic factor-1 (SF-1) and aromatase is hypomethylated (over-expressed) in eutopic endometrium (epigenendo). Growing evidence shows that microRNAs that play a key role in regulating gene expression and deregulated in cancer, are also involved in endometriosis such as ER-α, ER-β, PR and transforming growth factor β (TGF-β).

Table 7.1 Genetic alternations in endometriosis compared to EAOC

Genetic alteration seen in endometriosis	Genetic alteration seen in EAOC
PTEN Deletion: Present in endometrial cyst (Sato N et al.)	*PTEN Deletion*: Present in 40 % of CCC of ovary (Tan D S and S. Kaye)
HNF-1 β upregulation: Seen during oxidative stress conditions (Shigetomi et al.)	*HNF-1 β upregulation*: Over expression seen in CCC of ovary (Kobayashi, H et al.)
KRAS activation: Seen in endometriosis adjacent to ovarian cancer (Otsuka et al.)	*KRAS activation*: Overexpression present in EAOC (Dinulesc et al.)
ER β expression: Expression of ER-β was significantly higher in ectopic endometriotic stromal cells (Xue et al.)	*ER β expression*: SRAP causes decreases ER-β expression in CCC (Lin K et al.) Upregulation of ER-β in EAOC (Lai et al.)
ARID1A/BAF250 loss: Present in atypical endometriosis (Wiegan et al.)	*ARID1A/BAF250 loss*: Loss of BAF250 in 73 % of OCC and 50 % of endometrioid with ARID1A mutation (Wiegan et al.)

Number of cell signaling pathways and gene mutations are involved in the pathogenesis of malignant transformation of endometriosis into endometrioid and clear cell carcinoma. Gene mutations include loss of ARID1A/BAF 250a expression, KRAS activation, inactivation of PTEN [14]. Increased SRAP [15], changes in ER-beta expression, upregulation of HNF-1b [16] microsatellite instability [17]. Mutations of CTNNB1 are seen in 16–53.3 % of cases of endometrioid adenocarcinoma. Mutations in PIK3CA are present in 20–40 % of cases of clear cell carcinoma of the ovary [3] (Table 7.1).

7.5 Mechanistic Pathways Underlying Conversion of Ovarian Endometriosis to EAOC

Oxidative stress is known to be involved in a number of pathological conditions, including atherosclerosis, neurodegeneration, cancer and aging. It is also associated with endometriosis and its conversion to EAOC. It causes genetic alterations by inducing stress responsive genes. Some of the genetic alterations include DNA hypo-methylation, telomere shortening, chromosomal aberration, microsatellite instability.

High quantities of iron found within the endometriotic cysts play a vital role in the transformation of endometriosis to EAOC. Free iron due to retrograde menstruation in endometriosis causes persistent oxidative stress and modification of proteins and lipids in the cells, generating free radicals and hypoxia. This leads to extensive DNA damage [18], loss of heterozygosity, and decreased DNA repair contributing to carcinogenesis [19] (Fig. 7.1).

Recent studies have shown HNF-1β overexpression in endometriosis, including both the inflammatory and atypical lesions and within the foci of endometriotic cells. Over expression of hepatocyte nuclear factor-1 (HNF-1 beta) contributes to

the formation of clear cell carcinoma under stressful conditions such as oxidative stress. Oxidative stress causes hypo-methylation, which may lead to HNF-1 beta activation and genomic instability, which are evident in clear cell carcinoma of ovary [16] (Fig. 7.1).

Yamada et al. [20] proposed three major processes by which iron induces oxidative stress in endometriosis leading to EAOC (Fig. 7.1).

1. Oxidative stress causes DNA modifications like chromatin remodeling, histone modification, and gene product activation/inactivation contributing to the initiation of EAOC.
2. Iron-induced oxidative stress activates detoxification and anti-apoptotic pathways through the over expression of HNF-1β, which is involved in the promotion of clear cell carcinoma of the ovary.
3. Iron-induced generation of ROS creates an environment that supports the formation of new blood vessels, growth, invasion, and migration of cancer cells through an estrogen-dependent (EAC) or estrogen-independent mechanism (CCC) [20].

SRAP (Steroid Receptor Activator Protein) is a protein that enhances estrogen receptor-beta expression. The activation of estrogen receptor-beta increases endometrial cellular apoptosis, because it augments pro-apoptotic gene expression. Methylation of DNA suppresses pro-apoptotic gene activity by altering the chromatin structure and plays a role in the pathogenesis of ovarian cancer. Lin et al. observed decreasing levels of estrogen receptor beta expression when endometriosis of the ovary progress to atypical endometriosis, which then leads to ovarian clear cell carcinoma [15, 21] (Fig. 7.2). Previous studies have reported decreased levels of ER-beta m-RNA expression in estrogen dependent tumors like breast, ovarian and prostate cancers which highlights on the evidence that loss of ER-beta expression may be involved in carcinogenesis [21]. Lin k et al. [15], in their study speculated that during the malignant transformation of endometriosis to clear cell carcinoma, SRAP (Steroid Receptor Activator Protein) acts as a co-repressor, hypermethylates the promoter region of ER-beta (Estrogen Receptor-beta), suppressing gene activity and contributing to clear cell carcinoma of the ovary.

Estrogen receptor-beta is a tumor suppressor gene and therefore reduces the growth of tumor cells. It reduces the expression of genes involved in the cell cycle such as cyclin D. It decreases the proportion of cells in the S phase of the cell cycle, which is the synthesis phase where the replication of DNA occurs. However, Estrogen receptor beta increases the proportion of cells in the G2-M Phase, which is the cell cycle check point where DNA damage is assessed before the cell enters the mitotic phase. ER-β indirectly acts on ER-α and hence, inhibits cell proliferation. Whenever there is a decrease in ER-β expression, there is reversal of all these mechanisms and cell proliferation increases, leading to carcinoma [22] (Figs. 7.3, 7.4 and 7.5).

Endometriosis and endometrioid ovarian carcinoma cells are estrogen dependent and predominantly positive for estrogen receptor beta unlike clear cell carcinoma, which has low ER expression. Endometriotic stromal cells have higher levels of ER-beta expression due to deficient methylation. These increased ER-beta levels

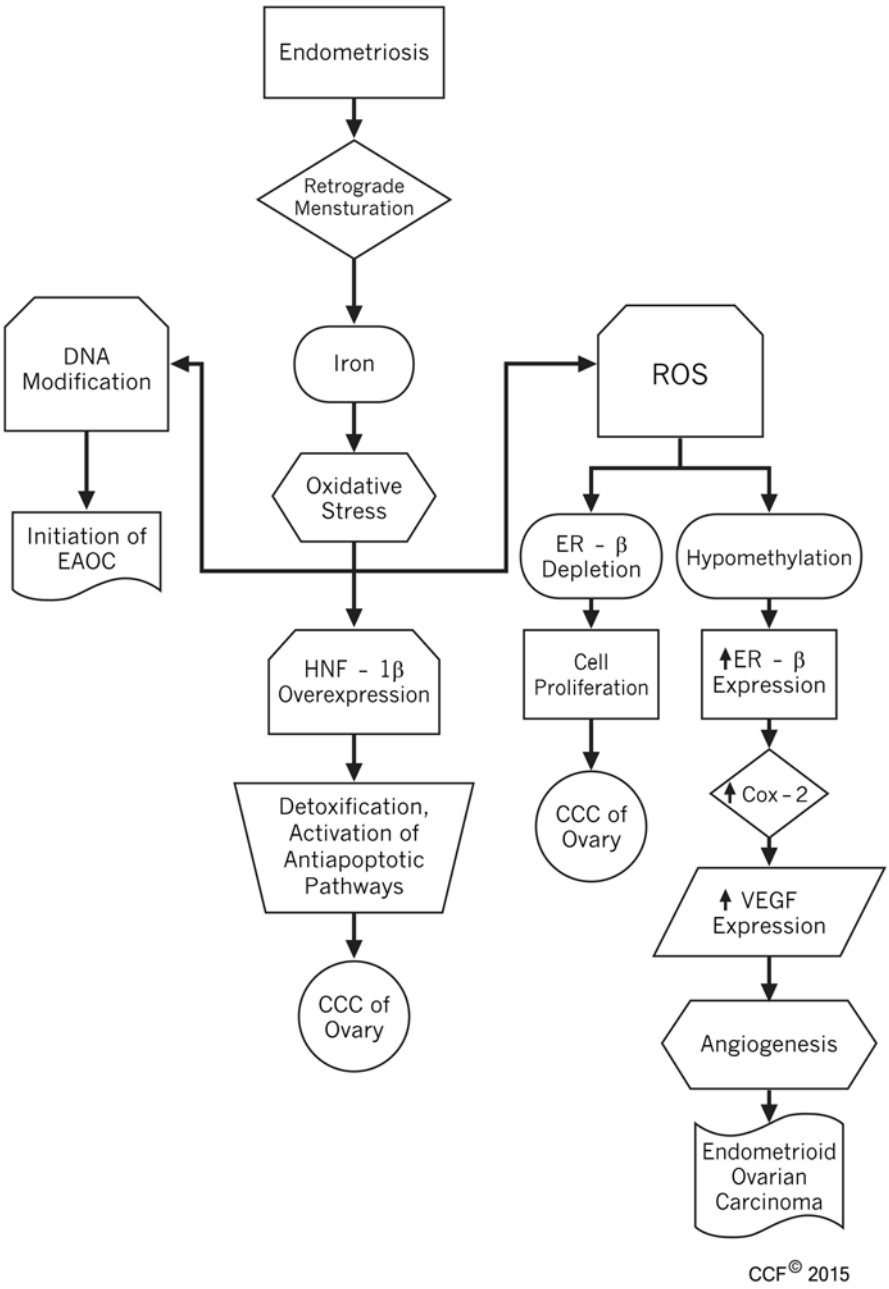

Fig. 7.1 Role of oxidative stress in malignant transformation of endometriosis to EAOC. *EAOC* endometriosis-associated ovarian cancer, *HNF* hepatocyte nuclear factor, *VEGF* vascular endothelial growth factor, *CCC* clear cell carcinoma, *ER* estrogen receptor

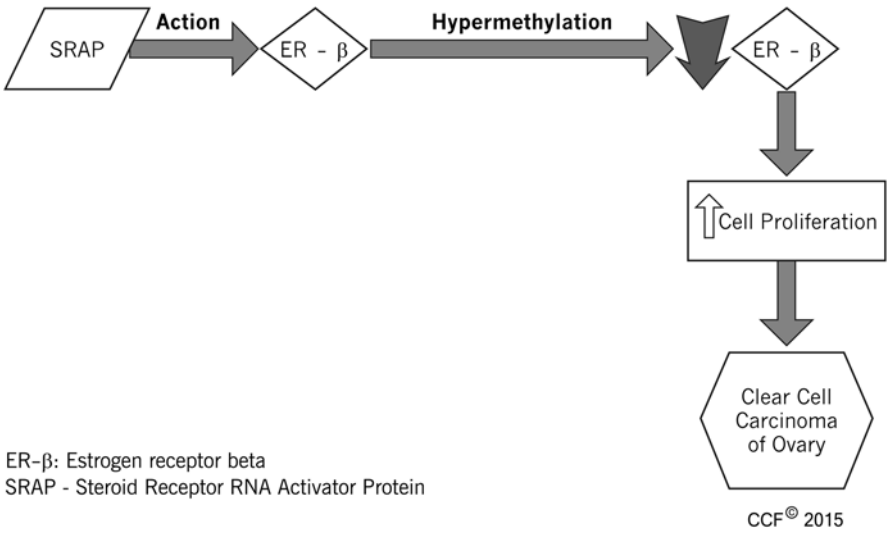

Fig. 7.2 Pathogenic mechanism leading to clear cell carcinoma of the ovary-increased SRAP action on ER-beta expression

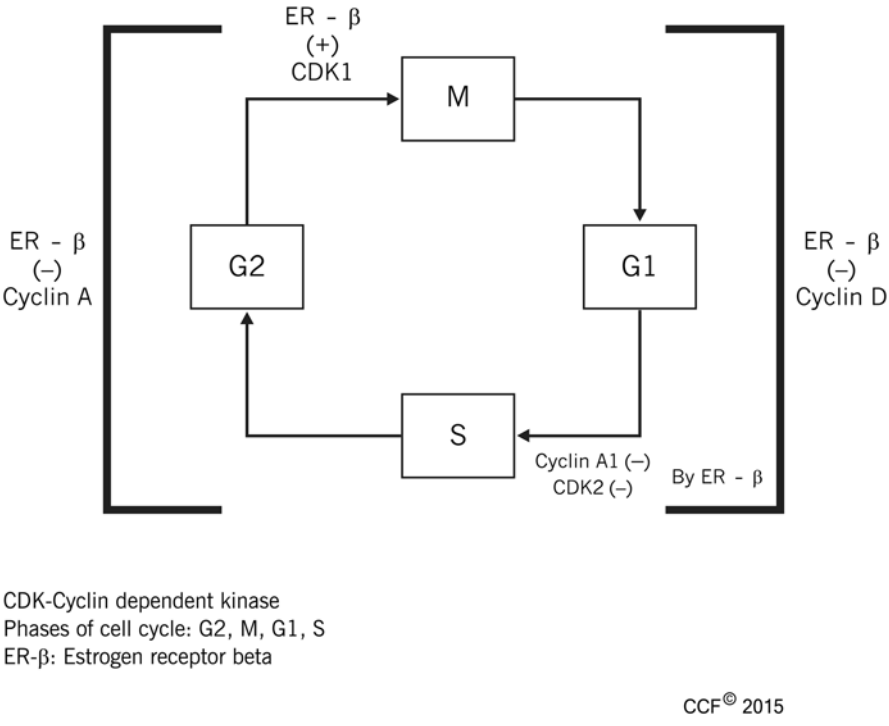

Fig. 7.3 ER-β action on cell cycle

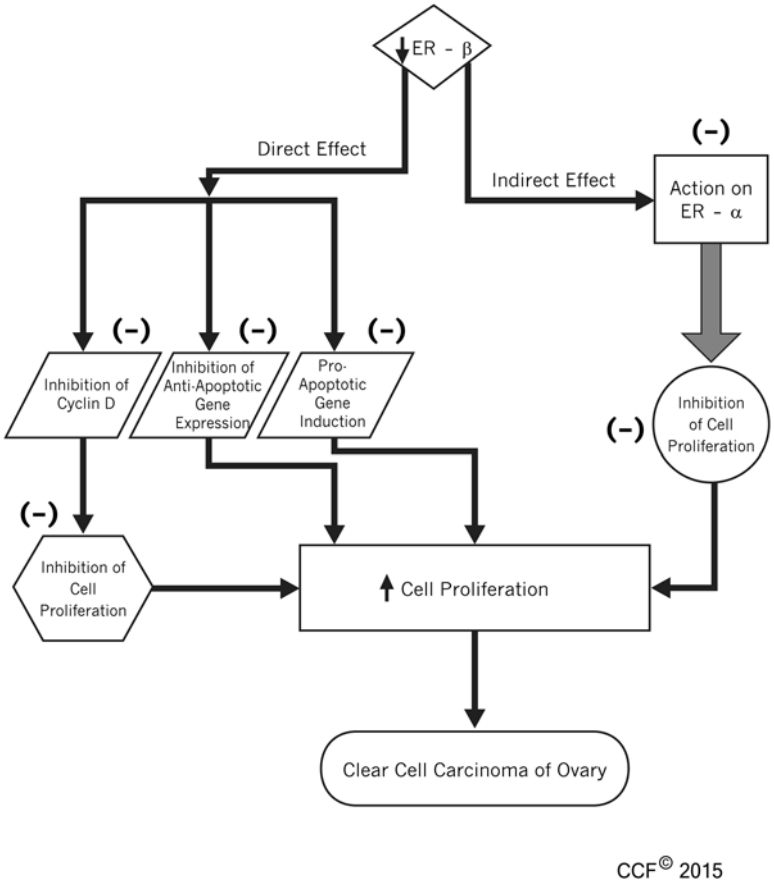

Fig. 7.4 Impact of ER-β expression in the pathogenesis of clear cell carcinoma of ovary

increase cyclooxygenase 2 [cox-2] and decrease progesterone receptor (PR) and ER alpha. Interaction with the estrogen receptor enhances the transcription of genes and promotes the synthesis of specific RNAs and proteins. Increased COX-2 levels are linked to endometriosis and also early malignant transformation (Fig. 7.5).

7.5.1 PI3K/AKT-Pathway

The PI3K (phosphatidylinositol 3-kinase) pathway is a significant pathway in the development of many cancers including breast, colon, and ovarian. PI3K has three classes among which class 1A is mostly involved in the development of cancer.

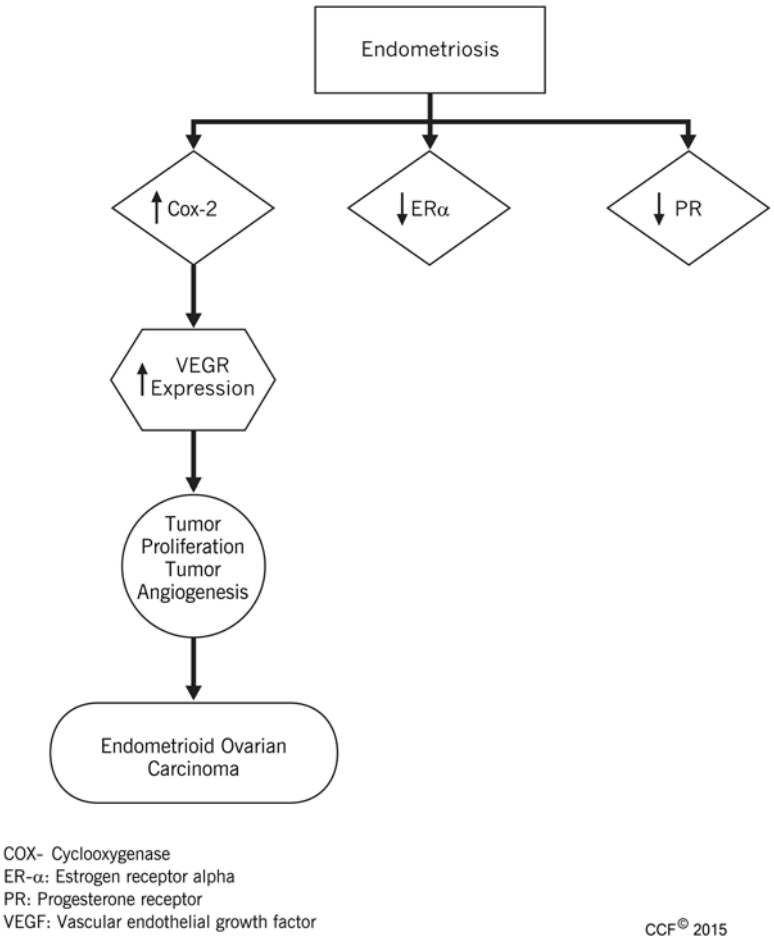

Fig. 7.5 Role of Cox-2 in the malignant transformation of endometriosis to endometriod carcinoma

The activation of PI3K/AKT pathway is more commonly achieved by activating its receptor tyrosine kinase (RTK). Genetic alterations such as mutation in tumor suppressor gene PTEN (Phosphatase and TENsin homolog) due to LOH (loss of heterozygosity) also activates the PI3K pathway, which contributes to the malignant transformation of endometriosis to EAOC [17]. Activation of phosphatidylinositol 3-kinase (PI3K) leads to activation of AKT, a serine-threonine protein kinase, which in turn activates mTOR (mammalian target of rapamycin), thus increases cell proliferation and reduces apoptosis leading to EAOC (Fig. 7.6).

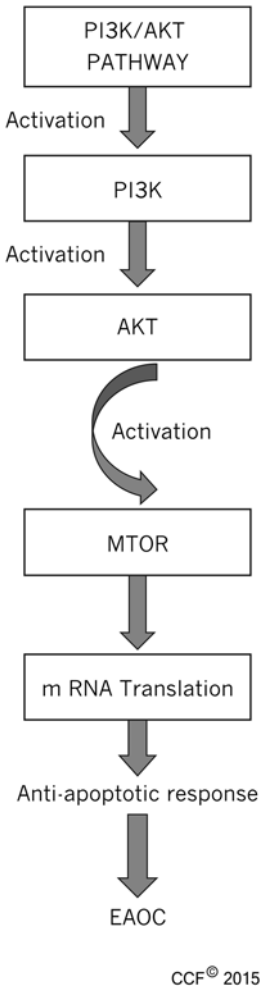

Fig. 7.6 P13/AKT pathway activation in EAOC. *PI3K* phosphatidylinositol 3-kinase, *MTOR* mammalian target of rapamycin

7.6 Preventive Measures and Screening Options

Although endometriosis increases the risk of ovarian cancer, the long-term risk can be reduced by hysterectomy without oophorectomy, oral contraceptives, aspirin and breast feeding [23]. All of these preventive measures suppress ovulation, which increases inflammation in the pelvic region [23]. Consequently, it has been shown that persistent inflammation leads to an increased risk of cancer.

In particular, oral contraceptives are extremely effective because they counteract the pituitary gonadotropin hypothesis. The pituitary gonadotropin hypothesis states that the continuous presence of elevated luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in ovarian cysts stimulate trapped epithelial cells, cause inflammation and play a primary role in the malignant transformation of such

tissue to ovarian cancer [23]. Thus, oral contraceptives keep LH and FSH levels low through the negative feedback system involving the secretion of estrogen. If estrogen levels are high, as induced by oral contraceptives, then the hypothalamus, and, consequently, the pituitary gland, will secrete less LH and FSH, cutting off the gonadotropin supply necessary for epithelial cells trapped in ovarian cysts to grow. The best choice of oral contraception is one that combines both estrogen and progestin. This would suppress levels of both gonadotropins and eliminate the risk factors associated with unopposed estrogen [23].

Progesterone has been shown to counteract some of the biological effects of estrogen and increase endometrial cell apoptosis, thus making it preferable to estrogen-based medications. Oral contraceptives, of course, are not a viable treatment option for women who wish to become pregnant. In that case, other options must be explored. Nonetheless, oral contraception is a non-invasive treatment that may bring relief and reduce the risk of cancer in many women afflicted with endometriosis. A patient's risk for developing ovarian cancer is significantly reduced after hysterectomy without oophorectomy. One reason may be that the procedure removes the pathway connecting the lower and upper genital tract, preventing inflammants from reaching the ovaries [23]. Thus, removing the constant inflammation lowers the risk of ovarian cancer because the immune system is not activated, which prevents the tissues from entering an OS state, which in turn prevents the tissue from entering an OS state and causing DNA damage. However, unlike the treatment proposed with oral contraceptives, a hysterectomy is not an option for many women, including those of reproductive age who wish to preserve their fertility. Additionally, hysterectomy is an invasive and complicated medical procedure that carries a risk of infection and surgical and post-operative complications. Thus, a hysterectomy is a much more radical form of treatment as opposed to an oral contraceptive, and it therefore should only be considered in severe cases.

Another viable option for reducing the risk of ovarian cancer in women with endometriosis is treatment with non-steroidal anti-inflammatory drugs (NSAIDS) [24]. Because these drugs are anti-inflammatory, they have a dual advantage for patients—they not only help reduce the primary inflammation in the pelvic region initially caused by the endometriosis, but they also may prevent the carcinogenesis of epithelial tumors by inhibiting cyclooxygenase-2, which inhibits the aromatase enzyme, reducing estrogen levels [24]. Therefore, NSAID therapy is a realistic treatment option for endometriosis patients. It is a simple and low-cost treatment that can be taken daily. Similarly, NSAIDs appear to have beneficial effects on both inflammation and ovarian cancer. It is important to note that this treatment option does not suppress ovulation, making it an acceptable option for women of reproductive age.

Finally, breastfeeding has also been shown to decrease inflammation in patients with endometriosis and ultimately prevent ovarian cancer by reducing estradiol levels. Therefore, for women who have recently given birth and have been diagnosed with endometriosis, breast feeding is a viable option that can reduce both inflammation and the risk for developing ovarian cancer.

Because ovulation is a natural process that causes inflammation, suppressing it can control both inflammation and reduce the risk of ovarian cancer. However, it

should be remembered that in patients with endometriosis, the immune response is altered, leading to high levels of cytokines such as Interleukin 1, Interleukin 6 and TNF [23]. Thus, treatment becomes more *difficult*, and responses to treatment vary accordingly. The high levels of cytokines released in endometriosis patients are known to stimulate the proliferation of endometrial cells as they contain receptors for IL-6 and TNFα [25]. The presence of such cytokines also leads to persistent inflammation in the pelvic region. In fact, inflammatory cells have been found near ovarian tumors and are thought to help break down the extracellular matrix and allow tumors to invade, allowing ovarian cancer to metastasize [23].

Thus, the preventive care mentioned above would help women lower their risk for ovarian cancer mainly by suppressing ovulation. Treatment now centers on the idea that suppressing ovulation decreases the amount of inflammation, thus reducing the production of ROS and minimizing the immune system response. Together, this lowers the risk for ovarian cancer in patients with endometriosis, leading to an overall positive improvement in quality of life.

However, ovulation suppression is not an option in women who are interested in maintaining their fertility or becoming pregnant. In these cases, antioxidant therapy may be considered. The goal is to regain a balance between ROS and antioxidant levels in the afflicted patient. This would help eliminate the detrimental effects of immune system by removing the main trigger—inflammation.

7.6.1 Screening

CA-125 is a highly sensitive biomarker for ovarian cancer, but its specificity is poor. Studies show that tissue expression of HE4 (human epididymis protein 4) is higher in malignant epithelial ovarian tumors than in benign ovarian tumors. In support to other studies [26–29], Huhtinen et al., found that levels of HE-4 in patients with ovarian and endometrial cancer were increased; this was not true in patients with endometriosis of the ovary. On the other hand, patients with ovarian endometriomas and advanced endometriosis had higher levels of serum CA-125. It has also been observed that concentrations of HE-4 increase with age whereas CA-125 levels do not [30]. The accuracy of using HE4 to distinguish ovarian cancer from other benign gynecological diseases such as endometriosis was found to be to be superior than serum CA125 [31]. Measuring both HE-4 and Serum CA-125 levels will provide a more reliable method of screening of patients with ovarian endometriosis and ovarian cancer and will help clinicians in differentiating the two conditions. It may also be useful during follow up to assess the malignant transformation of advanced endometriosis.

Among patients with an ultrasound-detected ovarian mass, increased levels of serum HE4 and CA125 would suggest the presence of ovarian cancer whereas higher levels of CA125 without elevated HE4 levels would suggest advanced endometriosis or ovarian endometrioma or other benign conditions. Also, normal serum levels of CA-125 and higher levels of HE-4 point towards the presence of either ovarian cancer or other type of cancer, such as endometrial cancer [30].

Studies have shown that plasma MiRNA profiling can be used as a biomarker to distinguish patients with endometriosis and ovarian cancer from normal healthy individuals [32, 33]. The golden standard for diagnosing endometriosis is laparoscopy. It allows direct visualization of lesions with histological confirmation. However, there is always a risk associated with surgery, so physicians prefer to use alternative methods. The most commonly used, non-invasive technique for screening (for both endometriosis and EAOC) is a combination of ultrasonography and laboratory testing for serum CA-125 [34]. While CA-125 has been used as a biological marker, it also has a high false positive rate among women [35]. Thus, HE4 can be used in conjunction with US and CA-125 [28, 29, 36]. It is important to note that this new marker is more sensitive to EAOC and the false positives associated with advanced stage endometriosis are considerably lower. Huhtinen et al. showed that the combination of CA 125 and HE4 was 94 % accurate in distinguishing women with cancer from those with endometriosis [30]. Together, CA-125, HE4 and US are commonly used to detect endometriosis without the need for laparoscopy. Doppler ultrasonography, trans-rectal ultrasonography, computerized tomography and magnetic resonance imaging (MRI) are the other noninvasive diagnostic approaches. MRI may be reliable for vaginal and extra pelvic localizations [37].

It has been also suggested that MRI is useful in showing malignant transformation within an endometrioma. The presence of one or more contrast material-enhanced mural nodules within a cystic mass, enlargement of the endometrioma and the disappearance of shading within the mass on T2 weighted images is suggestive of malignant transformation [38].

7.7 Key Points and Summary

In conclusion, numerous novel treatment options are beginning to be recognized and should be made available to women with endometriosis to decrease pelvic inflammation and reduce the risk of ovarian cancer. Possible preventative options are breast feeding in post-partum women and oral contraceptive pills for women who do not desire pregnancy. With the knowledge of the various pathways and pathologic mechanisms that underlie endometriosis and EAOC, novel biomarkers can possibly be identified for the early diagnosis of endometriosis. Physicians can use them to follow up patients with an established diagnosis of endometriosis to monitor the early events involved in the malignant transformation to EAOC and thereby prevent them from progressing to malignant disease. Combined screening with serum levels of CA-125, HE4 and US is recommended in current literature for possible noninvasive and specific diagnosis. As chronic inflammation is one of the most important underlying pathogenesis of endometriosis and its progression to EAOC, NSAIDs can be used. Further studies should evaluate whether anti-oxidant therapy can normalize levels of ROS and combat oxidative stress.

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Chapter 8
Endometriosis: Impact on Patient Quality of Life

Sajal Gupta, Avi Harlev, Ashok Agarwal, Nathan Reynolds, Tahir Beydola, and Namariq Haroun

8.1 Introduction

Two thirds of women with pelvic endometriosis experience chronic pelvic pain (CPP), dysmenorrhea, dyspareunia, dysuria, dyschezia and pain in musculoskeletal regions such as the thighs and lower back [1]. Due to the stress caused by infertility and pain, endometriosis interferes with quality of life by negatively impacting social, sexual and professional aspects. The disease is reported to be associated with depression, anxiety, high trait anxiety and high levels of stress. This section explores the effects of endometriosis and treatment on quality of life.

8.2 Measures of Quality of Sexual Life

The Derogatis Sexual Functioning Inventory is composed of 254 items and ten subtests: Information, Experience, Drive, Attitude, Symptoms, Affect, Gender Role, Fantasy, and Sexual Satisfaction subtests [2]. The 10-item sexual satisfaction subtest served as the basis for the 14-item sexual functioning questionnaire used by Ferrero et al. [3]. Some studies have reported the global sexual satisfaction Index scores of endometriosis patients. This Index is a global measure of the Derogatis Sexual Functioning Inventory. The global sexual satisfaction index represents quality of sex life on a nine-point scale from 0 (could not be worse) to 8 (could not be better) [4].

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8.3 Pain Related Measures

Visual analogue scales (VAS) have been extensively used to assess the different types of pain endometriosis patients experience. The simplest form of a VAS for pain is a straight line anchored by word descriptors at each end ('no pain' at one end and 'very severe pain' at the other end). The patient marks a point on the line that they feel depicts the severity of their pain.

Recently, a new pain measure was developed specifically for endometriosis patients. This new measure is called the Endometriosis Pain and Bleeding Diary, which is a 17-item daily electronic instrument filled out by patients [5]. The content of this measurement includes the assessment of intermittent and continuous pelvic pain and intermittent and continuous dysmenorrhea and dyspareunia. This measure was developed to assess improvements related to the treatment of endometriosis in clinical settings, which helps clinicians make decisions regarding treatment. The endometriosis pain and bleeding diary demonstrated at least acceptable internal consistency and acceptable test-retest reliability and validity [5].

8.4 Measures to Assess Depression

Depression measures used in endometriosis research include the Beck Depression Inventory (BDI–II). The BDI–II is one of the most extensively used measures in psychology. It is used to reflect depression symptoms that include somatic and vegetative symptoms as well as cogitation and effectiveness. The BDI–II demonstrated construct validity and reliability [2, 6, 7].

8.5 Measures to Assess Anxiety

Anxiety measures used in endometriosis research include the Hamilton Anxiety Rating Scale (HAM-A) and the State-Trait Anxiety Inventory (STAI). The HAM-A is a 14-item scale completed by the clinician based on the patient's symptoms. This measurement is used to evaluate psychic and somatic anxiety, which are psychological distress and somatic complaints that are related to anxiety, respectively. The reliability and concurrent validity of the HAM-A was shown to be sufficient despite that criticism against its poor outcomes in terms of the differentiation between anxiolytic and antidepressant effect or somatic anxiety and side effects [8, 9].

The STAI is used to show the presence and severity of anxiety symptoms and is also a widely used measure of anxiety. The inventory includes 40 items; 20 assess the state anxiety and 20 assess trait anxiety. The STAI showed reliability, concurrent validity and construct validity [10].

8.6 Measures Related to Professional Life

The Global Study of Women's Health used the Work Productivity and Activity Impairment Questionnaire to assess professional life in endometriosis patients [11]. Six items of this questionnaire ask respondents to answer questions about the different aspects of their professional life in the last 7 days [11]. The Work Productivity and Activity Impairment Questionnaire demonstrated construct validity and reproducibility [12].

8.7 Key Points and Summary

Endometriosis is a debilitating disease due to the pain and infertility it causes. Literature studies investigating various quality-of-life measures have shown that the disease negatively impacts social, sexual, and professional aspects of life. The disease is also associated with depression, high levels of anxiety and high levels of stress. Endometriosis undoubtedly negatively impacts quality of life.

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Chapter 9
Diagnosis of Endometriosis

Sajal Gupta, Avi Harlev, Ashok Agarwal, Julia Ellis-Kahana, and Caroline Cirenza

9.1 Introduction

Because endometriosis is a chronic condition, a diagnosis is not usually made expeditiously, and the diagnosis is typically delayed from the onset of symptoms by several years [1]. Women usually dismiss severe menstrual discomfort as being a normal part of their menstrual cycle. In addition, a cross-sectional study on the diagnostic experience of women with surgically diagnosed endometriosis found that prior to diagnosis, 63 % of women had their symptoms dismissed. Alarmingly, 60 % of the respondents in this study revealed on the questionnaire that their physician did not validate their concerns when seeking treatment [1].

Once again, the discomfort and pain traditionally associated and expected with menstruation lengthens the time that it takes for women to approach physicians about their gynecological concerns. From examining the narrative interviews of women with endometriosis-associated pelvic pain, it was found that women delay their diagnosis and course of treatment by believing that their pain is a natural biological side effect of being female [2].

9.2 Gold Standard: Invasive Diagnostic Technique

A definitive diagnosis is achieved by laparoscopy and histological biopsy [3, 4].

This allows direct visualization of lesions with histological confirmation [5]. Obviously, there are definitive risks associated with it since it is an invasive procedure.

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9.3 Existing Diagnostic Techniques

Other methods are available but with less specificity, sensitivity, and accuracy. The most commonly used are trans-vaginal ultrasound (TVS) and magnetic resonance imaging (MRI). The ultrasound is more often used due to its lower costs when compared to MRI. These two modalities are both good options for the diagnosis of ovarian endometriosis (80–90 % sensitivity and 60–98 % specificity) [6]. Nevertheless, these techniques are limited when detecting implants outside the peritoneum, profound adhesions and infiltrations. Doppler ultrasonography is also a tool that can be used to help in the diagnosis of ovarian endometriosis; the blood flow changes according to the presence or absence of endometriomas [7].

9.4 Need for Non-invasive Diagnostic Technique

In the last few years, studies have been conducted to find a biomarker of disease to avoid the need for laparoscopy and make an earlier diagnosis. Several proteins have been studied among women with and without endometriosis. A good biomarker must have high specificity, sensitivity, and be affordable. Moreover, reproducible results must be obtained, and proteins that are common between women of different ages, nationalities and cultures should be used. Also, it is important to note that some proteins are differentially expressed depending on the phase of the menstrual cycle and disease stage [8]. A protein can even be differently expressed among the three types of endometriosis (peritoneal, ovarian and recto-vaginal) [9]. Therefore, a biomarker can be specific to endometriosis in general or for only one (or two) types of the disease [10]. However, a biomarker could not only be used to make a diagnosis but also to help to understand the mechanisms of endometriosis, to follow its progression and to assess the effectiveness of treatment [11]. Biomarkers could even be used to predict recurrence, as the incidence of recurrence is more than 40–45 % after 5 years of treatment [12].

When referring to non-invasive diagnosis, urine, plasma, serum, peritoneal fluid, follicular fluid and even menstrual fluid can be considered. Menstrual fluid can be taken via aspiration, through the vaginal posterior fornix or from the cervix during speculum examination. Trans-cervical biopsy of the endometrium is considered a semi-invasive procedure, but is also useful. Differences can be found between eutopic endometrium among women with and without endometriosis [13]. The collection of endometrium tissue is simple and minimally painful; a Pipelle (Cooper-Surgical, Trumbull, CT, USA) or Endosampler (Surgimed-MLB, Newtown, PA, USA) suction curettes can be used [14].

The collection of abdominal serous liquid can be made by transvaginal cul-de-sac aspiration [15]. Symptoms of endometriosis are unspecific and present in many other pelvic diseases. Therefore, women without endometriosis and who do not require surgery would benefit from a non or semi-invasive diagnosis technique. It would also be good for women with endometriosis who have normal ultrasound

results. Any such technique should not be used in women without symptoms as there are no related benefits of treating women with asymptomatic endometriosis.

Making the diagnosis via laparoscopy is invasive and the accuracy depends on the experience and ability of the surgeon [16]. Currently, there are no molecules that have been proven to be useful as biomarkers to diagnose endometriosis [10].

9.5 Peripheral Biomarkers for Endometriosis

A biomarker is a biological component such as a protein, miRNA or gene, whose concentration is altered according to the presence of a specific disease or outcome [13]. For it to be useful, the biomarker concentration must be specifically related with the studied disease. A predictive algorithm possibly could be constructed with different biomarkers and could be used to diagnose endometriosis.

Biological functions, protein abundance and availability are some criteria that can be used to choose a protein to be validated [17].

Validation of a protein is a complex process and important phase of the process of finding a biomarker. As stated before, biomarkers can change within the menstrual cycle phase, endometriosis stage and location of ectopic implantation. Common causes for lack of validation are a limited number of cases and or control patients, limited reproducibility, no identification of protein peaks and lack of robust statistical approaches. There is no agreement about the number of samples that must be evaluated in order to validate a biomarker. Fasbender et al. proposed that the number of samples used to validate a biomarker must be at least equal the number of samples used when the protein is first found to be differently expressed in the disease. Also, they recommend that the sample distribution should reflect the prevalence of the disease. Standardized operating procedures and clinical phenotyping protocols must also be created [18].

9.5.1 Biomarkers: Peripheral Blood

Many diseases can be diagnosed with a blood test. Blood tests are easy conduct and cause little to no pain. Many are inexpensive. Turgut et al. studied the relationship between copper (Cu) and ROS in endometriosis stages III and IV [19]. Ceruloplasmin (Cp), which carries 95 % of the total Cu of the serum, serum paraoxonase-1 (PON-1) and malondialdehyde (MDA) were measured in women with endometriosis along with the total antioxidant status (TAS) and total oxidant status (TOS) in which study [19]. Cu and Cp were shown to be significantly elevated. Also Cu and Cp were associated with TOS, showing that Cu and Cp might play a role in the development of oxidative stress in the disease. Additionally, high levels of TOS and low levels of TAS were observed along with PON-1 down-regulation. As for MDA, no significant differences were found. MDA results were confirmed by Prieto et al. [20].

Hong et al. [21] used 2-DE, Western blotting and mass spectrometry to study protein expression in the serum of women with endometriosis. They found that G antigen family B1 protein was increased. This protein plays a role in the progression of the androgen-insensitive phenotype, so it may affect estrogen indirectly. As endometriosis is an estrogen-dependent disease, this protein may have a relationship with the pathogenesis of the disease. Levels of beta-actin, a cytoskeletal protein that plays a role in cellular motility, were increased in the serum of women with endometriosis.

Cyclin A1 plays a role in cellular proliferation, but its relationship with endometriosis needs to be studied further. In one study, it was differently expressed in the serum of endometriotic women [10].

Fibrinogen beta-chain peptide, identified by MALDI-TOF/TOF MS, was found to be decreased in the plasma of women with endometriosis. Fibrinogen is a blood-borne glycoprotein, and some of its cleavage products regulate cell adhesion [22]. Therefore, it may play a role in the pathogenesis of endometriosis.

Glycodelin is a protein derived from the endometrium and plays a role in angiogenesis, immunosuppression and contraception [23]. It was found to be over-expressed in the plasma of women with endometriosis, which suggests that it may play a role in the disease.

Vascular endothelial growth factor (VEGF) stimulates neovascularization. It was found to be significantly increased in women with endometriosis, showing a possible role in promoting angiogenesis and allowing the implantation of ectopic tissue [22]. FasL was also over-expressed in the serum of women with endometriosis, showing once again that the immune system plays a role in the disease [24].

9.5.1.1 Proteomic Profiling of Endometriosis

Proteomics Technology

To use the proteomic technique, the analyzed proteins must be pure, single proteins. Thus, the first step is to denature, purify and solubilize the samples.

Protein Separation

Two-Dimensional Gel Electrophoresis (2DE) is used to separate proteins from big complexes. It is a commonly used method, although its sensitivity and reproducibility are limited. Proteins are separated in the first dimension by isoelectric focusing (IEF) and in the second dimension by SDS PAGE. They are then visualized using either fluorescent dyes or stains [8].

Difference Gel Electrophoresis (DIGE) was developed to overcome the limitations of 2DE. It is a more efficient and reliable tool [8]. Up to three different proteins are labeled using mass- and charge-matched and fluorescent dyes [25]; these proteins then undergo 2D gel electrophoresis. Therefore, is more sensitive and accurate. Protein expression among different samples can be compared, and differences as small as 10 % can be detected. It also permits the identification of various proteins at the same time [8].

Protein Identification

Mass spectrometry (MS) separates proteins according to their mass-to-charge ratio. It is fast and can be used for small samples. It produces peak intensities that characterize the mass-charge (m/z) ratio of each peptide in the mixture of proteins [8]. Knowing the ion charge, the mass can be calculated. MALDI is the name for matrix-assisted laser desorption. It is an ionization solid phase technique and is used as a first scan of the protein component. ESI (electrospray ionization), a liquid phase tool, can also be used [8].

TOF (time-of-flight) is one of the most commonly used mass analyzers. SELDI (surface-enhanced laser desorption ionization) is also an ionization tool in MS used for analyzing proteins. MALDI-TOF-MS is a variation of MALDI. However, it is time consuming to perform, vulnerable to human error and has not yet been proved efficient at studying proteins with a high-molecular-weight [8].

LC-MS (liquid chromatography-mass spectrometry) is a highly specific and sensitive technique that is used to separate and identify mass proteins. It is useful when the proteins are mixed with another chemical substance. There are two different approaches: data-independent and data-dependent experiments. The data-independent experiments are good for complexes mixtures, when the data-dependent may not be able to sequence all the proteins [8]. Therefore MS can be used to identify peptides, sequence proteins, identify post-translational modifications, characterize multi-protein complexes and analyze protein structure [8].

The next step is to identify the proteins by searching databases. The identified masses are compared to previously identified ones [8]. Swissprot and UniProt are two such databases.

Confirmation of Identified Proteins

Western blotting is used to validate the proteins that have been identified. It has a high sensitivity and specificity. It is an analytical technique in which specific proteins can be detected. It uses gel electrophoresis to separate the extracted proteins by their mass. The proteins are then transferred to a membrane containing specific antibodies to the studied protein. Immunoblotting blot analysis, Western analysis and immunohistochemistry can also be used for this purpose [8].

9.6 Potential Biomarker for Non-invasive Diagnosis

9.6.1 Biomarkers: Peritoneal Fluid (Pf)

Many studies in the literature have looked at the importance of peritoneal fluid in the development and evaluation of endometriosis, showing that it contains many differentially expressed proteins compared to women without endometriosis (Figs. 9.1 and 9.2).

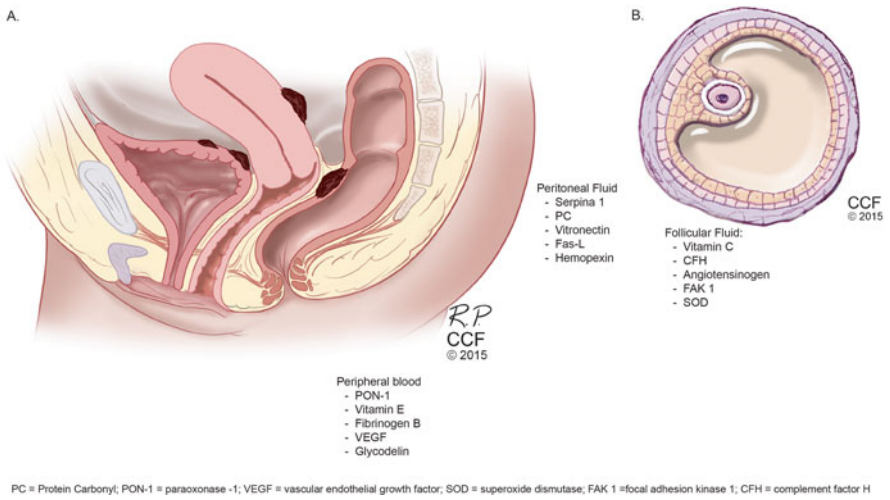

Fig. 9.1 Proteomic profiles in peritoneal fluid, follicular fluid and peripheral blood in endometriosis

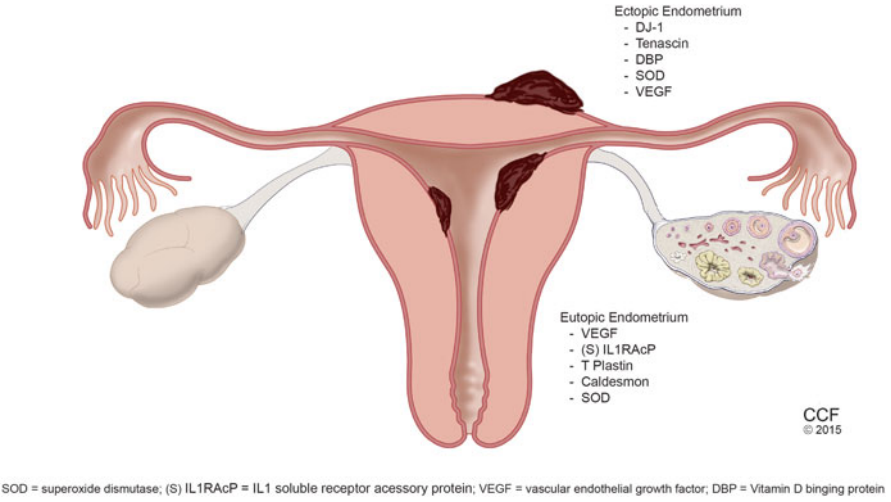

Fig. 9.2 Proteomic profiles of eutopic and ectopic endometrium

Wolfler et al. [26] studied the expression of proteins in the peritoneal fluid of women with endometriosis distinguishing between ovarian endometriosis (OE) and peritoneal endometriosis (PE). 2DE was performed. Hemopexin, which is related to the excretion of iron and helps prevent oxidative damage, was found to be down-regulated in OE and PE, showing that there is either a state of oxidative stress with

anti-oxidants being consumed or a lower antioxidant capacity in the peritoneal fluid of endometriotic women. Further investigation is needed to elucidate the relationship of hemopexin with the pathophysiology of the disease.

Haptoglobin also plays a role in the excretion of iron. Nonetheless, it was found to be up-regulated in PE and OE. This result is the opposite of what was expected for a protein that prevents oxidative stress. Its function in promoting endometriosis requires further investigation.

Vitronectin is a protein that promotes migration, adhesion and invasion. It was found to be up-regulated in PE and OE, which suggests that it may play a role in endometriosis.

Complement component 4A, a part of the immune system, was found to be down-regulated in OE and PE. As the immune system of women with endometriosis has been shown to be 'deficient', this protein may be a factor for the differences seen between a normal immune system and one in an endometriosis patient.

SERPINA1 is an important blood-born serine and is present in inflammatory and infectious disease. It was found to be up-regulated in PE and OE, in accordance to what is expected as endometriosis is an inflammatory condition. However, further evaluation needs to be done to clarify its relationship with the disease [26] (Figs. 9.1 and 9.3).

Vitamin E-binding protein afamin is a protein that binds to Vitamin E, a non-enzymatic antioxidant. Vitamin E levels were previously reported to be significantly lower in the peritoneal fluid of women with endometriosis [27]. This finding correlates to a state of oxidative stress caused by high consumption of antioxidants. Seeber et al. studied the levels of Vitamin E and Afamin using ELISA. Although the levels of vitamin E in peritoneal fluid were not altered in their study, levels of afamin were significantly increased and correlated to the levels of Vitamin E [28]. Nonetheless, Wolfler et al. found afamin to be up-regulated in OE and not in PE. The role afamin plays in endometriosis requires further elucidation [26].

Carvalho et al. studied oxidative stress in the peritoneal fluid of women with endometriosis [29]. 8-hydroxy-2-deoxyguanosine (8-OhdG) and protein carbonyl (PC), both markers of oxidative stress damage, were measured. 8-oxoguanine glycosylase 1 (OGG1), a DNA repair glycosylase marker of antioxidant activity was also analyzed. Immunohistochemistry was used to assess 8-OhdG and OGG1 and the colorimetric assay for PC. PC and 8-OhdG levels were significantly higher in patients with endometriosis. OGG1 levels were significantly decreased in all patients, mainly in stages III and IV. Another study using the chromatography electrochemical technique confirmed these findings [30]. Receiver-operating characteristic (ROC) curves were made to predict the chance of having endometriosis. 8-OhdG had the highest rate for predicting endometriosis (86 %). A model to predict the chances of having the disease was designed using these three proteins. A concordance index of 0.87 was achieved.

CA-125 is the most well-known biomarker of endometriosis. However, its concentration in peritoneal fluid did not differ between a healthy control group and women with endometriosis [28].

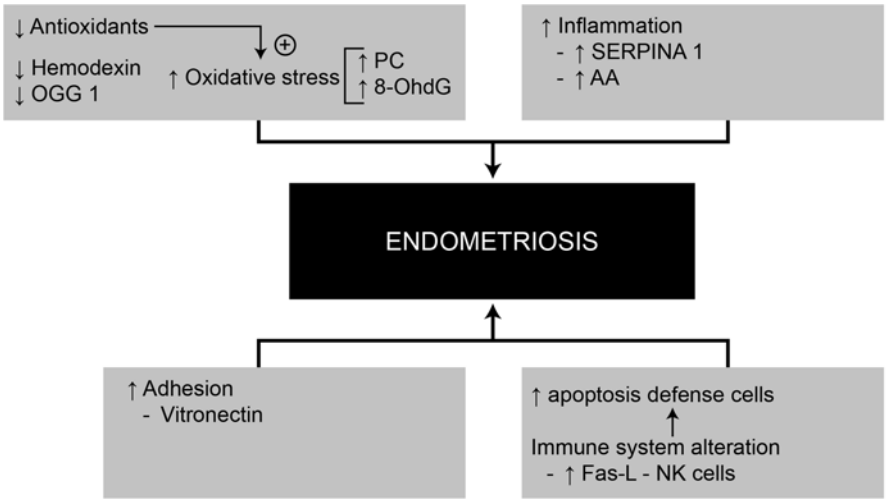

Fig. 9.3 Impact of differential protein expression in peritoneal fluid in endometriosis

Serum amyloid protein A (SAA) is an inflammatory marker that is produced when levels of TNF-alpha, IL-1, IL-2 and IL-6 are high. Its relationship to inflammation raises a possible association with endometriosis [31]. It was shown to be over-expressed in women with endometriosis, with a sensitivity of 66.7 % and a specificity of 62.1 % [15]. This finding is consistent with the inflammation present in the disease. However, inflammatory markers are not specific for endometriosis.

FasL is a protein that binds to Fas, activating apoptosis. Its levels were found to be increased in the peritoneal fluid of women with endometriosis, mainly in those with moderate to severe disease [32]. It was measured by the Soluble Fas Ligand Enzyme-Linked Immunosorbent Assay. This finding was correlated to increased apoptosis of Fas-bearing immune cells, impairing scavenger activity and, therefore, leading to conditions conducive to the implantation of ectopic endometrium [33].

To summarize, proteins related to oxidative stress, alterations in the immune system, inflammation and adhesion were found to be associated with endometriosis. Therefore, we can infer how these proteins are related to endometriosis. Higher levels of migration can help the endometrial cell to move from their original site to the peritoneal cavity. An alteration in the immune system can lead to an impaired clearance of retrograde menstruation cells, allowing the implantation of these cells outside the uterine cavity. These endometrium cells are related to a state of oxidative stress and inflammation. Further investigation is needed to determine if the lower levels of antioxidants are a cause or a consequence of endometriosis. There are also increased levels of cell motility and adhesion, which allow the development of endometriosis.

9.6.2 *Biomarkers: Eutopic Endometrium*

Carvalho et al. studied the importance of the eutopic endometrium in the development of pelvic endometriosis [29]. Although the morphology of the cells in eutopic endometrium of women with and without the disease is similar, there are some differences in the biochemistry, function and genetics among these cells. These differences may be one of the factors contributing to the development of endometriosis.

Evidence suggests that the density of nervous fibers in women with endometriosis is increased, although it is not known if this is correlated with the disease or with pelvic pain [18]. Thereby, some neural transmitters appear to be increased in endometriotic women. NT-4/5 and brain-derived neurotrophic factor proteins were found to be over-expressed in women with endometriosis [34]. PGP9.5 immuno-active nerve fibers were suggested to predict endometriosis with sensitivity of 98 % and specificity of 83 % [35]. A combination of PGP9.5, vasoactive intestinal peptide and substance P was also studied and was shown to have a 95 % sensitivity and 100 % specificity [36].

VEGF was also found to be up-regulated in eutopic endometrial tissue, mainly in the late secretory phase and during menstruation of women with endometriosis [37] (Fig. 9.2).

Ren et al. studied the effect of ischemic precondition (IPC) in endometriotic women. The researchers hypothesized that the endometrium becomes slightly ischemic during the early and middle secretory phase, mimicking an IPC response. They also found that this IPC lead to an increase in VEGF expression and a decrease in apoptosis, therefore facilitating angiogenesis and implantation of endometrial cells [38]. Additionally, there seems to be a relationship between oxidative stress and VEGF. Schafer et al. showed that an increase in ROS levels leads to an increase in VEGF levels [39].

Annexin V plays a role in proliferation and cell mobility. It was up-regulated in the eutopic endometrium of women with endometriosis, showing a possible relation to the implantation of endometriotic tissue. T plastin plays a role in cell locomotion and maintenance of cellular architecture. It was reported to be up-regulated in the eutopic endometrium of women with endometriosis [36]. Further investigation about its correlation with the pathophysiology of the disease is needed, but it may play also play a role in implantation of endometrial cells outside the uterine cavity. Both of these proteins were studied using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and their increase show they might influence the migration of endometrial cells outside the uterine cavity.

Caldesmon is a protein that binds actin, inhibiting ATPase activity. In non-muscle cells, it inhibits motility and phagocytosis. Meola et al. studied the expression of the CALD1 gene, which encodes caldesmon, and the levels of caldesmon in eutopic and ectopic endometrium. Real-time PCR and Western blot analysis and immunostaining to determine cellular localization were used. No variation among the cells was found in the immunostaining. Caldesmon levels were found to be lower in the eutopic endometrium of women with endometriosis [40]. The study included

patients with other gynecologic diseases, which is a large limitation. However, the findings showed that this protein may play a role in the disease. Once it is diminished, it cannot bind to actin and, thus, is unable to inhibit cell motility.

Stephens et al. used a proteomic approach to study differently expressed proteins in eutopic endometrium [17]. Glucocorticoid receptor subunit alpha (GCR), a protein that can bind progesterone, was found to be highly expressed among endometriotic women. Because endometriosis is associated with progesterone resistance, it may play a role in the pathophysiology of the disease.

Heat shock protein (HSP) is a chaperone protein that accumulates in cells under stressful conditions. It plays a role in (un)folding and transporting proteins, controlling the cellular cycle, counteracting the effect of oxidative stress and modulating apoptosis [8]. It was found to be increased in endometriosis, suggesting an increased oxidative stress in endometrial cells of women with endometriosis.

Superoxide dismutase (SOD), an enzymatic anti-oxidant, and peroxide, which forms as a result of oxidative stress, were both up-regulated in eutopic endometrium. SOD may be high because it is compensating for the OS [41].

Thioredoxin is an anti-oxidant protein and is involved in apoptosis and cellular proliferation. Thioredoxin binding protein 2 (TBP-2) regulates thioredoxin and promotes apoptosis when a cell has a high level of oxidative stress. There were no significant differences in the levels of TRX or TBP in endometriosis. However, the ratio of TRX to TBP and TRX/TBP was increased in endometriotic lesions, As a result, the anti-oxidant capacity is decreased while cell proliferation is increased, [42] suggesting that these proteins may be related to the development of endometriosis. The methodology of the study included real-time PCR and immunohistochemistry in endometrial tissue.

IL1 soluble receptor accessory protein, (s)IL1RAcP, inhibits secretion of IL1. It was significantly down-regulated in eutopic endometrium of women with endometriosis, at the mRNA and the protein levels in the secretory phase and in glandular and surface epithelial cells. The membrane-bound IL1 receptor accessory protein, (mb)IL1RAcP, was not correlated with the presence of the disease, neither at the mRNA nor the protein levels [43]. IL1 decoy inhibitory receptor, IL1R2, was significantly decreased in eutopic endometrium of women with endometriosis. These associated findings show that there is an imbalance in IL-1 production. As IL-1 is associated with the capacity of endometrium implantation, these results are in accordance with the pathophysiology of the disease [44].

Some apoptotic molecules were shown to be differently expressed. Bcl-2, an anti-apoptotic protein, is over expressed in stromal cells in proliferative eutopic endometrium. Additionally, Bax, a pro-apoptotic protein, was absent in proliferative endometrium and increased in the endometrium of endometriotic women [45].

In the eutopic endometrium, a large amount of proteins reflecting various processes were identified. Alterations in the immune system, oxidative stress and inflammation markers along with high levels of cell migration, motility, proliferation and adhesion were once again observed. However, in this biological window, there were some new findings. A progesterone resistance was identified. Furthermore, a higher density of nervous fibers was found, and some neural transmitters were found

to be highly expressed. Nonetheless, this finding needs further investigation in order to elucidate whether the nerve fibers are related to the disease or to pelvic pain. It may be a factor that contributes to the chronic pain seen in endometriotic women.

9.6.3 Biomarkers: Follicular Fluid (FF)

Follicular fluid contains secretions from the ovarian follicles and is an ultra-filtrate from the blood plasma. It contains many elements such as proteins, hormones and enzymes and therefore, it is a reliable biological fluid that can be studied for biomarkers and to discover the underlying causes of the disease. Lo Turco et al. [46] compared the follicular fluid between three groups of women; healthy controls (C), endometriotic women who achieved pregnancy (E.P) and endometriotic who did not achieve pregnancy (E.NP) Proteins were separated by 2DE and compared and identified by LC-ESI-MS-MS (Refer Fig. 9.4). Serum albumin was significantly down-regulated in the E.P and E.PN groups. It is a protein whose functions are binding to DNA, copper and fatty acids. It also has antioxidant activity. Therefore, low levels may indicate the presence of oxidative stress. This finding is in accordance to the oxidative stress pathophysiology of endometriosis.

Complement Factor I is typically a serum protein and also a glioma and lung-cancer protein. It was first found in follicular fluid and down regulates complement activation. The complement system is composed of proteins that, when activated, kill invasive pathogens and destroy non-self-molecules (Fig. 9.4). Complement Factor H, which has similar functions, was up-regulated in the E.P group. This highlights the importance of an altered immune system in the pathogenesis of endometriosis. Angiotensinogen, a growth factor, was found to be highly expressed in the E.P and E.NP groups, showing that this protein may contribute to the proliferation of ectopic tissue. Vitronectin, an integrin-binding protein and also a component of the extracellular matrix protein, was found to be overexpressed in the E.NP group [46]. It may play a role in the adhesion process of endometriosis (Fig. 9.4). Focal adhesion kinase 1 is a protein found in adhesion sites of cells and is associated with cell migration and survival [47]. It was highly expressed in the E.P group, showing that it may play a role in the development and progression of the disease, as adhesion is one of the main characteristics of endometriosis. Kininogen-1 protein, which participates in blood coagulation, was found to be increased only in the E.NP group [46]. Jarkovska et al. showed that there is a link between Kininogen-1 protein and VEGF. Therefore, this increase may contribute to adhesion and neovascularization—two common processes in endometriosis [48].

Fas antigen in NK-cells were found to be highly expressed in the peritoneal fluid of women with endometriosis. It suggests that the elimination of NK cells provides allows ectopic endometrium to survive. This finding underlies the importance of a deficient immune system in the development of endometriosis [49].

Prieto et al. compared levels of OS markers among infertile women with endometriosis and infertile women due to other conditions. Vitamin C, a non-enzymatic

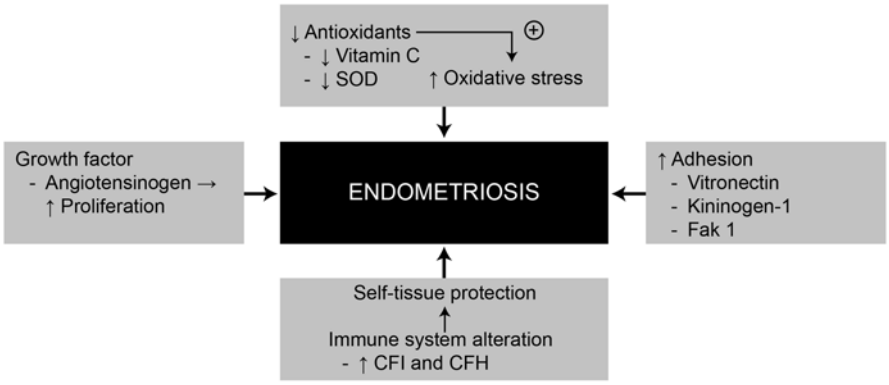

Fig. 9.4 Differential proteomic profiles of follicular fluid in endometriosis

anti-oxidant, was decreased in the follicular fluid of women with endometriosis. Superoxide dismutase, on the other hand, was decreased in the plasma of endometriotic women [20].

9.6.4 Biomarkers: Ectopic Endometrium

Investigating the protein expression of ectopic endometrial tissue may lead to the discovery of some biomarkers and, also, lead to a better understanding of the pathophysiology of this enigmatic disease.

The CALD1 gene, which encodes the protein caldesmon, was analyzed as a potential biomarker to diagnose endometriosis (Fig. 9.2). Endometrial tissue from eutopic and ectopic endometrium was obtained from women with endometriosis and levels of CALD1 gene and caldesmon protein were determined by PCR, western blot and immunostaining. The results found that they were increased in the endometriotic tissue. It was found that the protein caldesmon can predict endometrial dysregulation in women with endometriosis [40].

DJ-1 is a protein that plays a role in cell adhesion, mainly to collagen type IV, migration, proliferation, and invasion and protects against oxidative stress-mediated apoptosis (Fig. 9.2). Ray et al. studied these effects by knocking down DJ-1 expression in endometriotic cells and over-expressing it in normal endometrial cells. Cells were transfected with siRNA that specifically targets the DJ-1 gene. Also, adenoviral vector was used for expressing DJ-1-GFP fusion to over-express the protein. They also evaluated levels of DJ-1 by using SDS-PAGE. It was found to be up-regulated in ectopic endometrium. The authors concluded that high levels of DJ-1 expression could play a part in endometriosis, possibly by stimulating endometrial cell survival, proliferation, migration, and invasion [50].

In accordance to the increased adhesion process in endometriosis, focal adhesion kinase expression concentration was found to be increased in ovarian endometriotic tissue [51].

Tenascin is a component of the extracellular matrix (ECM), which is important in cell migration, adhesion and proliferation. Western blotting showed that it was over-expressed in endometriotic tissue. It may play a role in the migration and implantation of endometrial tissue outside the uterine cavity. Other components of the ECM—laminin, fibronectin, collagen IV and vitronectin—were also analyzed in the same study but no significant results were found [52].

Donnez et al. reported increased VEGF levels in the ectopic tissue of women with endometriosis, which increased sub-peritoneal vascularity enhanced implantation and survival of endometrial tissue [37].

Vitamin D-binding protein, (DBP) is usually present in the serum. It binds to Vitamin D in a very specific way; it is a chemotactic factor for neutrophils, monocytes and fibroblasts, is a precursor of macrophage-activating factor (MAF) and acts as an actin scavenger protein. It was found to be highly expressed in the ectopic endometrium of women with endometriosis and contributes to disease progression. It may play a role in the scavenger function of macrophages and in the survival and implantation of endometrium outside the uterine cavity [53]. SOD and peroxide were also measured. Their levels were higher in ectoptic tissue than in eutopic endometrium, showing an increased OS condition [41].

9.7 Key Points and Summary

To summarize, we have highlighted the importance of an altered immune system in the pathophysiology of endometriosis. The immune system is deficient in eliminating cells from retrograde menstruation, allowing them to implant and proliferate outside the uterine cavity. High levels of oxidative stress with lower levels of antioxidants are present. Some proteins in the extracellular matrix were altered and some were not, emphasizing the need for further investigation. EMC proteins are responsible for some processes such as cell invasion, migration, adhesion and proliferation—all important mechanisms in the development of endometriosis. Application of proteomics technology to find a potential biomarker or a panel of biomarkers which can be validated for clinical use in endometriosis is emerging.

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Chapter 10
Management of Endometriosis

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Treatment of endometriosis is broadly classified into pharmacological and surgical methods. Because the etiology of the disease is not well established, none of the currently available treatments can prevent or cure endometriosis. Rather, treatment is aimed mainly at providing symptom relief or improving fertility rates [1]. Therefore, one should consider how treatment options affect pain levels and infertility when investigating whether endometriosis treatment improves quality of life.

Medical therapy is usually started as an empirical treatment, mainly proposed as a temporary aid for pain management [2]. The effect of pharmacological treatment on fertility is minimal. The surgical approach aims to address both pain and fertility. Surgical treatment is the treatment of choice for ovarian endometriomas, mostly due to the ineffectiveness of pharmacological therapy in these cases. Nevertheless, ovarian surgery reduces the ovarian reserve and its long-term implications are not yet well-known [3, 4].

10.1 Pharmacological Treatment

Several pharmacological agents including oral contraceptives, danazol, GnRH agonists, progestogens, anti-progestogens, non-steroidal anti-inflammatory agents and aromatase inhibitors have been used to treat endometriosis [5]. In many cases, chronic pelvic pain, a major endometriosis symptom, is the reason for the initiation of empirical treatment even before endometriosis is diagnosed [2]. The following section will summarize the pharmacological treatments for endometriosis.

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10.1.1 Hormonal Therapies

10.1.1.1 Oral Contraceptives

Combined estrogen-progestogen contraceptive pills are commonly used to control endometriosis-related pelvic pain and dysmenorrhea [6]. These agents were initially used to maintain a "pseudo pregnancy regimen" to relieve symptoms [5]. Cyclical use of oral contraceptives is the only treatment for endometriosis that permits regular uterine bleeding [7]. However, because dysmenorrhea is a major symptom of endometriosis, the regular use of oral contraceptives has a limited advantage. Continuous use is more effective at addressing pelvic pain [8, 9].

Harada et al. [10] conducted a multi-center, placebo-controlled, double-blind randomized trial to assess the use of oral contraceptives in the treatment of dysmenorrhea. Pain was measured using verbal rating scales (VRS) and visual analogue scales (VAS). Ultrasonic examination was used to diagnose endometriomas. The authors noted that dysmenorrhea significantly improved in the oral contraceptive group. The volume of endometrial tissue and the average diameter of the endometriomas decreased significantly more so in the oral contraceptive group than in the placebo group. Nevertheless, side effects of oral contraceptives, mainly irregular uterine bleeding and nausea, were significantly increased in the oral contraceptive group. Although oral contraceptives have long been used to treat endometriosis related pain, their effectiveness is debatable [5].

10.1.1.2 Progestogens

Progestogens help alleviate pain either by inducing decidualization and atrophy of endometrial tissue [4] or suppressing matrix metalloproteinases—enzymes that play a central role in the development and implantation of ectopic endometrium [11]. Several progestogens have been evaluated in the treatment of endometriosis such as medroxyprogesterone acetate [12], norethisterone [13], and newer progestins such as dienogest [14]. Progesterones can be taken orally or via a levonorgestrel-releasing intrauterine device—both are effective at providing pain relief [15, 16]. Oral progesterones are highly effective at treating symptomatic endometriosis and relatively inexpensive, but they can cause significant side effects, which negatively impact quality of life [15].

Petta et al. [17] investigated the effect of a levonorgestrel-loaded intrauterine device (IUD) on chronic pelvic pain and uterine bleeding. Six months after the insertion of the IUD, most patients reported a significant decrease in chronic pelvic pain, and 70 % of the patients reported no bleeding. Lockhat et al. [15] reported promising results concerning pain symptoms and disease staging after intrauterine-administered levonorgestrel use. At 3 and 6 months after the IUD insertion, a significant decrease in pain scores was noted. Moreover, the proportion of patients with moderate or severe dysmenorrhea decreased from 96 % of patients pre-treatment to 68 % at 3 months post-insertion ($p = 0.001$) and to 50 % at 6 months post-insertion ($p < 0.001$). The mean number of days per month during which patients experienced pain decreased from 15 ± 6.9 to 10.7 ± 8.7 days after the 6-month therapy ($p < 0.05$) [15].

10.1.1.3 GnRH Agonists

GnRH agonists bind to pituitary receptors and have a longer half-life than native GnRH. GnRH agonists induce down-regulation of the pituitary-ovarian axis and as a result, lead to hypoestrogenism [4]. Hypoestrogenism, in turn, induces amenorrhea and endometrial atrophy [11]. On the other hand, GnRH agonists induce menopausal symptoms such as hot flushes, vaginal dryness, decreased libido, mood swings, headache, and bone mineral depletion [18, 19]. GnRH agonists can be delivered via a daily nasal spray or with daily or monthly subcutaneous injections [7].

Petta et al. [17] investigated 37 endometriosis patients treated with a GnRH agonist for 6 months. The pain score decreased significantly after the first month of GnRH analogue treatment with further reduction in those who completed the 6-month therapy. It is also worth noting that GnRH analogue treatment reduced bleeding more than the IUD-administered progestin treatment.

10.1.1.4 Danazol

Androgens are steroid hormones that promote male secondary sexual characteristics [20]. Danazol is one androgen that is commonly used for endometriosis treatment. It is a derivative of 17α-ethinyltestosterone, which inhibits steroidogenesis and the LH surge, thereby increasing free testosterone levels [11]. Due to the increase in androgen levels, hirsutism, acne, and deepening of the voice are potential side effects [20]. When comparing danazol and high-dose medroxyprogesterone acetate in the treatment of endometriosis, Telimaa et al. [21] reported that a slight increase in resolution of peritoneal implants in patients receiving danazol compared to patients receiving medroxyprogesterone acetate. Compared to placebo, both danazol and medroxyprogesterone acetate significantly improved pelvic pain.

Rotondi et al. [22] compared a group of endometriosis patients who underwent danazol therapy for 24 weeks with a group of patients who underwent GnRH analogue therapy for the same period. The authors reported comparable results between the groups regarding endometriosis growth and symptoms during treatment. However, symptoms recurred after treatment in both groups, although symptom severity was lower than at admission. Danazol had androgenic side effects such as weight gain, acne, and edema.

10.1.1.5 Aromatase Inhibitors

Aromatase is an enzyme that converts steroidal precursors into estrogens. The estrogens cause ectopic tissue to grow, leading to the onset of pelvic pain [23]. The inhibition of aromatase reduces estrogen levels [24]. Only a small number of studies have assessed the use of aromatase inhibitors as a treatment for endometriosis [25]. The main concern in using these agents is osteopenia and osteoporosis, which can result in bone fractures [26]. The blockage of estrogen production in premenopausal women increases FSH levels, which may lead to ovarian follicular cysts [27]. Therefore, in

premenopausal women, both an "add-back therapy" and oral contraceptive use are advised with aromatase inhibitors. This combination therapy significantly decreases abdominal and pelvic pain and diminishes endometriotic lesions at second-look surgery [27, 28].

10.1.2 Analgesics

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be effective in the treatment of primary dysmenorrhea [29, 30]. These agents have been investigated extensively and are widely used for pain relief; they have an added desired anti-inflammatory effect in endometriosis [31]. Despite being the most commonly used medications for pelvic pain, their efficacy in treating endometriosis-associated pain is not conclusive [32].

10.1.3 Melatonin

As discussed in previous chapters, anti-oxidants can be used in the treatment of endometriosis to prevent disease progression. Melatonin, a broad spectrum antioxidant secreted from the pineal gland, is a scavenger of free radicals [33]. Animal studies have already shown that it significantly reduces the size of endometrial lesions [34, 35]. In a recent randomized, double-blind, placebo-controlled trial, Schwertner et al. [36] reported a significant decrease in endometriosis-related pelvic pain and an improvement in sleep quality.

10.2 Surgical Treatment of Endometriosis

The aims of surgical treatment are to remove endometriomas, relieve pain and improve fertility rates via adhesiolysis [37]. Laparoscopy is the preferred surgical approach for the treatment of endometriomas [38]. Laparoscopy is associated with shorter hospitalizations, more rapid recovery, less use of analgetic agents [39], decreased costs [40], and less damage to a patient's ovarian reserve when an ovarian procedure is carried out subsequently [38].

10.2.1 Ovarian Endometriomata Surgical Management

Several surgical techniques are available to manage ovarian endometriomas including aspiration, ablation, and ovarian cystectomy. A combination of these procedures may be used as well.

Endometrioma removal (either by excision or ablation) was more effective than non-invasive techniques at reducing pain [41]. When comparing excisional cytoreductive surgery with ablative surgery, Hart et al. found that excision was more advantageous than ablation in the management of ovarian endometrioma particularly, in regards to endometrioma relapse and pain relief [42]. Other studies showed similar results in terms of pain relief after surgical intervention [43]. The previous consensus on endometriosis management recommended laparoscopic excision over laparoscopic ablation [37, 41]. The biggest concern about ovarian cystectomy for endometriosis patients is a reduced ovarian reserve [44]. The effect of different surgical approaches on fertility will be discussed later in this chapter.

10.2.2 Ovarian Endometriomata Aspiration

A surgical approach is typically applied prior to IVF/ICSI cycles. With this technique, a trans-vaginal ultrasound guided ovarian cyst aspiration is performed on an outpatient basis [45]. Aspiration is considered a relatively safe procedure [46].

The main potential advantage of aspiration is that it leaves the pseudocapsule of the cyst intact, thereby preserving follicles in the ovarian tissue. Moreover, compared to laparoscopy, this procedure is less invasive. Nonetheless, recurrence of ovarian endometriomas is invariably higher because the ectopic endometriotic tissue is not resected [47]. To address this problem, a sclerosing agent can be used during aspiration [46, 48].

10.2.3 Deep Endometriosis Surgical Treatment

Deep endometriosis refers to endometriotic lesions that penetrate the retroperitoneal space by at least 5 mm leading to severe symptoms. Several treatments have been used in patients with deep disease to relieve those symptoms. Of them, the surgical approach is still debatable. A careful partial resection of the lesions may result in unsatisfactory symptom management and a complete resection is associated with larger potential for complications involving the urinary, reproductive and gastrointestinal systems [37]. The practice of using a surgical laparoscopic approach in cases of endometriosis is well established. Nevertheless, the ideal management of deep infiltrating endometriosis is still unknown [49].

10.3 Treating Endometriosis to Improve ART Outcomes

10.3.1 Introduction

Infertility and subfertility are commonly associated with endometriosis [50]. Many mechanisms have been proposed to explain the association between endometriosis and infertility in women such as inflammation, decreased ovarian reserve, difficulty

of oocyte retrieval in severe endometriosis, poor egg quality, and decreased implantation rates [50]. Although medical and surgical treatment options can reduce symptom severity, their effect on infertility is limited [51–53]. A negative correlation between decreased pregnancy rates and increased severity of endometriosis has been reported as well [50]. Thus, these factors may be overcome by the use of assisted reproductive techniques [53, 54].

A study by Gupta et al. reported that patients with ovarian endometriomas had a decreased response to ovarian stimulation during IVF treatment cycles [55]. In a systematic review and meta-analysis that looked at the effect of surgical treatment on IVF outcomes in patients with endometriomas, Tsoumpou et al. [56] concluded that surgical management of endometriomas does not significantly increase IVF pregnancy rates or the ovarian response to ovarian stimulation compared with no treatment. The authors advised using a practical approach to manage endometriomas prior to IVF cycles, suggesting that treatment be based on endometrioma size; patients with small endometriomas (less than 3 cm) do not require any preliminary measures and can start IVF cycle. In cases of larger (3–5 cm) endometriomas, a GnRH agonist treatment for 3 months prior to the IVF cycle is advised. For endometriomas larger than 5 cm, laparoscopic ovarian cystectomy is recommended [56].

10.3.2 Endometrioma Aspiration

Pabuccu et al. [47] investigated whether aspiration of ovarian endometriomas prior to controlled ovarian hyper-stimulation (COH) would improve the outcome of intracytoplasmic sperm injection (ICSI). They reported that the duration of COH was longest in the non-aspirated group. Nevertheless, the clinical pregnancy rates and implantation rates were similar among the four factions of patients: those who had aspiration at the beginning of COH, those who did not have aspiration, those with a history of ovarian surgery but no current endometriomas, and those with tubal factor infertility. Essentially, aspiration of the ovarian endometriomas did not improve the number of follicles or diminish the dosage of gonadotropins needed or the number of mature oocytes. It also did not increase the rate of implantation. Most importantly, since all groups had comparable clinical pregnancy and implantation rates, the authors concluded that aspiration and previous resection of endometriomas (1–6 cm in diameter) is not of value prior to IVF cycles [47].

Suzuki et al. [57] evaluated the outcome of IVF in patients diagnosed with endometriosis in the presence of an ovarian endometrioma. They compared three groups of patients (50 women in each). The first group consisted of patients who underwent aspiration and examination of the aspirated fluid to verify the presence of endometriosis. The second group was composed of patients who did not have endometriomas but had endometriosis diagnosed laparoscopically. The last group was composed of women with tubal factor infertility. The results revealed that ovarian endometrioma did not affect the quality of embryos or lower pregnancy rates but it did negatively impact the quantity of viable oocytes. Additionally, the number of mature

oocytes retrieved in the first group of patients who had one affected and one normal ovary were comparable, thus demonstrating that endometriosis did not impact oocyte development [57].

Aspiration of ovarian endometrioma during oocyte retrieval may also be considered. However, this procedure may place the patient at a higher risk for ovarian or pelvic infection [58, 59]. Also, if chocolate cyst fluid comes into contact with the ooctyes, it may contaminate them. These are potential theoretical complications, however, that have not been supported by a definitive RCT as large-scale RCTs that examine women affected by ovarian endometriosis do not exist. Essentially, this type of RCT would be extremely difficult to conduct as endometrioma-affected women only represent a minority of patients who seek IVF.

10.3.3 Endometrioma Ablation

Endometrioma ablation is another surgical option. During an ablation procedure, the internal cyst wall is destroyed with bipolar coagulation or a CO2 laser following drainage. This is one of the most frequently used techniques. This procedure has favorable results in terms of IVF outcomes and is also advocated because it is thought to cause less anatomic damage and disruption than cystectomy [60].

Donnez et al. [61] investigated the effect of laparoscopic ablation of ovarian endometrioma on the ovarian response to stimulation. Specifically, they vaporized the internal cyst wall using a CO2 laser. The study group consisted of 85 patients who failed to become pregnant for 1 year following the ablation, at which point IVF was performed. A control group of 289 patients with tubal factor infertility who also underwent IVF was included. The clinical pregnancy rate was similar between the two groups. The two groups were statistically comparable across a number of important variables, including number of ampoules used for stimulation, the number of follicles aspirated, the number of follicles >15 mm in diameter, the number of mature ooctyes aspirated, E2 peak levels, fertilization rate, number of embryos/cycle, number of transferred embryos/cycle, implantation rates, and ongoing pregnancy rates.

10.3.4 Ovarian Cystectomy

Besides ablation, cystectomy is another surgical approach for endometrioma management. The effectiveness of cystectomy as a surgical technique is still controversial. Although cystectomy is known to have the lowest recurrence rates, it is also reported to have a negative impact on ovarian reserve and ovarian responsiveness to hormonal stimulation. The emergent pattern from the literature is that while cystectomy may reduce ovarian response, this deficiency is compensated for through increased ovarian stimulation [62], which results in overall cumulative pregnancy rates that are similar to those of other techniques [63].

Performing laparoscopic cystectomy prior to an IVF cycle did not improve the number of oocytes retrieved, the number of mature oocytes, fertilization rate, and clinical pregnancy rate [64]. Although laparoscopic cystectomy did not damage ovarian reserve or function, it also did not help patients achieve pregnancy in a significant way. Because cystectomy of ovarian endometriomas prior to IVF does not improve pregnancy rates, there is no incentive for the patient to undergo this procedure [64].

In one study, drainage was cited as being more advantageous than ablation of ovarian cysts in regards to dysmenorrhea, dyspareunia, chronic pelvic pain, recurrence of the ovarian endometriomas, and ability to achieve spontaneous pregnancy [42]. However, this analysis did not include women who underwent IVF after surgery.

Previously [65], Canis et al. performed a retrospective study to assess ovarian response during IVF cycles after laparoscopic cystectomy. All of the ovarian endometriomas were >3 cm. Of note, the pregnancy rates during the first cycle of IVF for Group A (endometrioma >3 cm), Group B (no endometrioma), and Group C (tubal infertility) were 35.9 %, 31.2 %, and 30.5 %, respectively. When comparing the three groups, the number of oocytes and embryos attained were similar, demonstrating that laparoscopic cystectomy is a beneficial method to treat ovarian endometriomas. Nevertheless, reduced ovarian reserve following ovarian cystectomy was shown in other studies [66]. Kahyaoglu et al. compared two groups of patients: 22 women who underwent laparoscopic cystectomy and cauterization before IVF and 22 women with tubal factor infertility who proceeded directly to IVF. Fewer follicles and oocytes were retrieved in the cystectomy endometrioma-operated group. The clinical pregnancy rate in the endometrioma group exceeded that of the tubal factor group by 9 %, with rates of 45 % and 36 %, respectively. When comparing the operated and contralateral normal ovary, the normal ovary produced more mature follicles for retrieval than the affected one [66].

While it is evident that cystectomy does in fact have a negative effect on ovarian function [67], a new technique that combines cystectomy by stripping with ablation through a CO2 laser may have favorable results in preserving ovarian reserve [68]. Although this is a laparoscopic modification of traditional cystectomy, it is effective and has immense potential for future development. With this integrative approach, 80–90 % of the cyst is excised and the remaining 10–20 % of the endometrioma wall is vaporized. The ablated portion is proximal to the ovarian hilum and thus, vaporization is the preferred technique for this location as it is contains the vasculature most prone to damage in the ovary [68].

In order to increase IVF success rates, women with ovarian endometrioma may first undergo treatment to minimize the presence of the disease. Although laparoscopic surgery has long been considered the first-line treatment for minimal and mild (stage I and II) endometriosis [62], a new approach claims that surgery may not be beneficial in terms of pregnancy rates and disease management if the ovarian endometriomas are <3 cm in diameter [69]. Nevertheless, it is noteworthy that for patients who do not require IVF and want to procreate naturally, surgery may be their best option as Donnez et al., 2004 reported a postoperative pregnancy rate of 50 % [63].

While the surgical technique that yields the best results for IVF is still being debated, a careful review of recent studies shows that laparoscopic removal of ovarian endometriomas that are less than <3 cm in diameter does not increase pregnancy

rates naturally or with IVF and may in fact cause irreparable damage to the affected ovary. Surgery should be considered only when the cysts are large and painful, when medical therapy fails or once malignancy cannot be excluded [70].

10.4 Recent Advances in Management of Endometriosis

10.4.1 Introduction

More recent research has focused on conservative management of endometriosis rather than surgical approaches [71]. Numerous studies have suggested that ovulation plays a crucial role in the pathogenesis of endometriosis and medical management that inhibits ovulation alleviates the symptoms of endometriosis and decreases the rate of recurrence [2].

Endometriosis is a chronic and long-term disease; repeated therapy is needed to treat the symptoms and to limit its recurrence. However, the side effects of long-term therapy also should be considered. The primary aim of medical management is to stop the growth of the endometriotic lesions and to control symptoms, hence improving the patient's quality of life. Hereby, some of the advances in the management options will be briefly viewed by groups.

10.4.2 Hormonal Agents

10.4.2.1 Selective Estrogen Receptor Modulators

Because endometriosis is an estrogen-dependent disease, selective estrogen receptor modulators (SERM) may be beneficial as a treatment. Bazedoxifene is a SERM that effectually antagonizes estrogen-induced uterine endometrial stimulation without disrupting the necessary estrogenic effects in bone or the central nervous system [72]. These advantages make it an excellent treatment. Indeed, one animal study showed that it decreased the size of endometriotic implants and levels of endometrial proliferation markers [72].

10.4.2.2 Selective Progesterone Receptor Modulators

Because endometriotic lesions contain progesterone receptors [73], treatment with selective progesterone receptor modulators (SPRM) has been suggested to deactivate the ectopic endometrium lesions. Animal studies showed that SPRMs reduced endometrial thickness; adding progesterone to the treatment regimen prevented undesirable transformation of the endometrium, and most of the glands remained tubular [74]. Indeed, Chwalisz et al. [75] reviewed two randomized, placebo-controlled studies that reported a significant reduction in pain symptoms and a dose-dependent correlation to bleeding complaints.

10.4.3 Non Hormonal Agents

10.4.3.1 Anti Inflammatory Agents: TNF-Alpha Inhibitors

Inflammation is known to be a major part of endometriosis genesis and development [76]. Anti-inflammatory agents may be beneficial in reducing endometriosis symptoms and disease progression even without suppressing ovulation as other agents do. Animal studies showed that anti-TNF alpha agents reduced endometrial lesions size [77, 78] and number [79] and endometriosis-induced infertility [80]. Lu et al. [81] recommended studying anti-TNF alpha to assess whether it offers a suitable management option for women with endometriosis.

10.4.3.2 Statins

These commonly used agents inhibit cholesterol production, lowering levels of cholesterol- a well-established ischemic heart disease risk factor. By yet poorly understood mechanisms, statins are known to inhibit proliferation in several biologic systems [82]. Animal studies showed that high-dose statin treatment significantly regressed endometriotic implants [83, 84]. Statins were also shown to exhibit anti-inflammatory [85] and anti-oxidative [83] activities, which are desirable in the treatment of endometriosis.

10.4.3.3 Apoptotic Agents: Metformin

Metformin is a commonly used oral hypoglycemic agent from the biguanide family that is used to treat type II diabetic patients. In addition to being an insulin sensitizer, metformin modulates the inflammatory response and inhibits sex steroid production [85]. Animal studies showed that it significantly reduced the size and volume of endometriotic lesions [86, 87].

10.4.3.4 Anti-angiogenic Agents: Dopamine Agonist

Because the endometrium requires a large blood supply, it is assumed that angiogenesis is essential for endometrial lesion development [88]. Hence, researchers hypothesized that inhibiting angiogenesis would be beneficial in the treatment of endometriosis. Indeed, animal studies showed decreased activity and proliferation of endometriotic lesions after dopamine agonist treatment [88]. Moreover, dopamine agonist treatment was more effective than GnRH agonist therapy in promoting lesion regression [89].

10.4.3.5 Antioxidants: Epigallocatechin-3-Gallate

Epigallocatechin-3-gallate is a major component of green tea. It has a high antioxidant capacity and has been recognized as an effective treatment for different tumors [90]. Because it inhibits cell proliferation, promotes apoptosis and has antioxidative effects, researchers have studied it as a possible treatment for endometriosis. Animal studies showed that it significantly suppressed angiogenesis in endometrial tissue without affecting blood vessel development in ovarian follicles [91] and reduced the size and activity of the endometrial implants [92]. The authors concluded that Epigallocatechin-3-gallate has the potential to be an effective treatment by inhibiting the formation of new endometriotic lesions [91].

10.4.3.6 Intraperitoneal Treatment with Local Anesthetics

Lignocaine is a commonly used local anesthetic agent that has anti-inflammatory and anti-arrhythmic properties. The peritoneal cavity of women with endometriosis is subjected to a local inflammatory reaction [93]. The peritoneal endometriotic lesions in women with endometriosis also have larger numbers of nerve fibers than normal peritoneum [94]. The inflammatory substances released by macrophages in the peritoneal cavity of women with endometriosis stimulate these nerve endings and cause significant pain, which contributes to the dysmenorrhea reported by these women. A double-blind, randomized controlled trial conducted in Sweden reported that perturbation (flushing) of the uterine cavity and fallopian tubes with lignocaine significantly reduced pain symptoms and can be used as a non-hormonal treatment option for women with dysmenorrhea caused by endometriosis [95, 96].

10.4.4 Surgical Techniques Robotic Surgery

As mentioned above, laparoscopy removes endometrial implants and scar tissue, reduces pain and aids fertility. Laparoscopy is considered the gold standard for treating mild and moderate stages of endometriosis [49]. Advanced endometriosis involves neighboring organ systems, which can greatly complicate a surgical procedure.

Robotic-assisted laparoscopic techniques have been shown to be useful in the treatment of extensive endometriosis and may prove useful in the treatment of urinary tract endometriosis [97]. Robotic surgery is a relatively safe technique, especially in high risk groups such as obese women [98].

10.5 New Frontiers in Management of Endometriosis

10.5.1 Endometriosis Diagnosis

At present, the gold standard for endometriosis diagnosis is histologic inspection of an endometrial lesion obtained via laparoscopy. However, a surgical diagnosis is hazardous. Either a late diagnosis of the disease is achieved, resulting in potentially preventable complications due to the progressive nature of the disease, or an empirical symptom-based treatment is initiated, possibly targeting the wrong disease. For that reason, a better diagnostic tool is necessary [99].

In a systematic review, May et al. [100] identified more than 100 biomarkers in the literature. Unfortunately, no single biomarker or combination of biomarkers has clearly been proven to be accurate [100]. Further investigation is still required.

10.5.2 Endometriosis Treatment

10.5.2.1 Stem Cells

Stem cells have the ability to self-renew by undergoing innumerable cell divisions. They also have the potential to differentiate into specialized cell types. The mucosa of the human endometrium has a basal and functional layer. The basal layer is permanent and provides cells for regeneration each month. The functional layer is shed every month during the menstrual cycle. Human endometrium contains epithelial and mesenchymal stem/progenitor cells, which play a vital role in the regeneration of endometrial tissue during the menstrual cycle, after parturition and after surgical resection. Endometriotic lesions in women with endometriosis have a clonal origin and are initiated by retrograde movement of the shedded endometrial stem/progenitor cells [101]. The origin of ovarian endometrioma from stem cells rather than from ovarian surface epithelium [102] further enhances the role of stem cells in the pathogenesis of endometriosis Targeting these endometrial stem/progenitor cells may prevent the development of endometriotic lesions in women with endometriosis.

10.5.2.2 Gene Therapy of Endometriosis

Gene therapy is defined as the transfer of genetic material (DNA or RNA) into target cells in order to cure or relieve disease symptoms [103]. The genetic material can be delivered using a viral vector or with a non-viral technique [103]. The lack of a real cure for endometriosis makes gene therapy a potential treatment or preventive option.

Animal models of gene therapy with angiogenesis inhibitors were found to be very effective in the treatment of endometriosis [104]. Other studies reported promising results and advances in gene therapy [105, 106]. Further studies are required before gene therapy can be applied in a clinical setting.

10.6 Key Points and Summary

Although endometriosis has been recognized and treated for many years, no treatment can cure the disease. Not knowing the etiology of the disease plays a major role in our inability to cure it. Therefore, treatment today mainly focuses on relieving pain and improving fertility.

Current research is focusing on treating different suggested etiologies such as oxidative stress. New horizons such as gene therapy and stem cell therapy are being developed in order to find a cure.

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Chapter 11
Concluding Remarks

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Endometriosis is a common and complex debilitating gynecological disease seen in reproductive age women; it affects more than 70 million women worldwide and seven million women in the United States. The main symptoms are pain and reduced fertility.

Although it has long been recognized, its etiology is still unknown. Nonetheless, its risk factors are well established and include early menarche, late menopause, and other conditions that determine estrogen levels. The disease is known to respond to high estrogen and low progesterone levels. It has a polygenic inheritance and genetic predisposition with increased incidence in first-degree relatives and in monozygotic twins. Exposure to environmental pollutants, immunological dysregulation, persistent inflammatory status and epigenetic alterations also increase the disease risk. Conditions that suppress ovulation indirectly decrease estrogen levels and reduce the disease risk.

The pathogenesis of endometriosis is most likely multifactorial. There are a number of theories—retrograde menstruation, coelomic metaplasia, lymphatic and vascular metastasis, embryonic rest, TIAR, Quinn's denervation-re-innervation theory, and stem cell theory—but they only partially explain the pathogenesis of endometriosis. The most widely accepted theory is retrograde menstruation proposed by Sampson in 1927.

An imbalance between antioxidants and ROS significantly correlates with the presence of ectopic endometrial tissue. Growing evidence suggests that oxidative stress plays a role, both locally and systemically, in women with endometriosis through many pathways. Future randomized control studies are required to evaluate the role of antioxidants in preventing the damage caused by oxidative stress and methods to improve pregnancy rates in endometriosis patients.

Part of the pathogenesis of this disease is the accumulation of iron within the endometrial lesions and peritoneal macrophages. Erythrocytes are phagocytized by macrophages, releasing Hb into the peritoneal fluid, where it can form a complex with haptoglobin or be catabolized by hemeoxygenase-1 to produce free iron. The free iron can be taken up by iron storage and transporting proteins such as ferritin and transferrin, which can lead to iron accumulation within macrophages or lesions.

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Indeed, increased iron levels have been found in the peritoneal fluid of women with endometriosis. High levels of iron have also been found in the peritoneal macrophages of endometriosis patients. Several genes involved in the development and progression of endometriosis as well as the body's response to oxidative stress are often regulated by iron. Iron overload does not appear to affect lesion establishment but may contribute to the further growth of endometriosis by promoting cellular proliferation within lesions. Iron chelator treatment could therefore be beneficial in the treatment of endometriosis to prevent iron overload in the pelvic cavity and decrease cellular proliferation of lesions.

Endometriosis may be treated in the future with iron chelators such as DFO used locally as a depot to prevent the proliferation of endometrial lesions and the progression of the disease. Additional research is necessary to further examine how iron overload affects endometriosis and the possible treatments for endometriosis that may be specific to iron overload localized to the pelvic cavity.

The role of dioxins in the development of endometriosis remains controversial. Thus far, research has produced varying results that contribute to this uncertainty. Over time, experiments on animals have shown that exposure to environmental toxins is correlated with endometriosis in mammals. These studies, including those on primates, have propelled researchers to explore possible similar associations in humans. Similarities between dioxin characteristics and risk factors for endometriosis have been noted. Due to their accumulation in lipids, dioxins are largely excreted in breast milk and eliminated from the body through lactation, which is believed to be a protective factor against the onset of endometriosis.

Epidemiological studies on endometriosis in association with environmental pollutants have been quite inconsistent in both animal and human studies. Although copious studies investigating the involvement of environmental toxins in endometriosis have produced promising results, no study has demonstrated a direct link nor confirmed a causal relationship between the two. Since endometriosis tends to run in families, clinicians should aim to identify family members confirmed or suspected to have endometriosis when taking a family history.

Numerous novel treatment options are beginning to be recognized and should be made available to women with endometriosis to decrease pelvic inflammation and reduce the risk of ovarian cancer. Possible preventative options are breast feeding in post-partum women and oral contraceptive use for women who do not desire pregnancy.

With the knowledge of the various pathways and pathologic mechanisms that underlie endometriosis and EAOC, novel biomarkers can possibly be identified for the early diagnosis of endometriosis. Physicians can then use them to guide follow up visits and monitor the early events involved in the transformation of endometriotic tissue to EAOC and thereby prevent it from progressing into malignant disease.

Combined screening with serum levels of CA-125 and HE4 along with ultrasound is recommended in the current literature for possible noninvasive and specific diagnosis. Because chronic inflammation is one of the most important underlying factors in the pathogenesis of endometriosis and its progression to EAOC, NSAIDs

can be used. Further studies must be performed to evaluate whether anti-oxidant therapy can be used to reduce ROS levels and combat oxidative stress.

Endometriosis negatively affects social, sexual, and professional aspects of life. The disease has been shown to be associated with depression and high levels of anxiety and stress. The medical and surgical treatments that are currently available help improve quality of life. Yet, there is a need for more efficient treatments that result in lower recurrence rates.

The surgical approach is still the gold standard for the diagnosis of endometriosis. However, surgery is invasive, and the accuracy of diagnosis depends on the skill of the surgeon. Newer non-invasive diagnostic tools such as biomarkers are needed to provide an earlier and more accurate diagnosis as well to assess disease progression and treatment efficacy.

Although there are many treatments for endometriosis, none can completely resolve or cure the disease. Not knowing the etiology of the disease plays a major role in our inability to cure it. Therefore, treatments mainly focus on relieving pain and improving fertility.

New research is focusing on different potential etiologies such as oxidative stress with promising results. New horizons such as gene therapy and stem cell therapy are being developed in an effort to find a cure.