Chapter 9
Diagnosis of Endometriosis

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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.


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 complex 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

Wolf 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, Wolff 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].
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 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, 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.

IL-1 soluble receptor accessory protein, (s)IL1RAcP, inhibits secretion of IL-1. 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 IL-1 receptor accessory protein, (mb)IL1RAcP, was not correlated with the presence of the disease, neither at the mRNA nor the protein levels [43]. IL-1 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
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 ectopic 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.

References


